Restek ADVantage News #2 : 2006-2015

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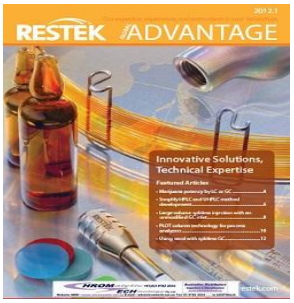

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see ADVantage_News.htm for Updates

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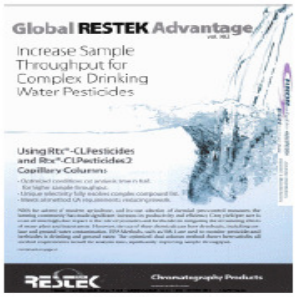

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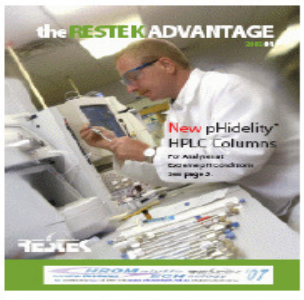

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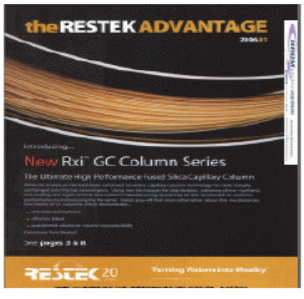

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Restek ADVantage News

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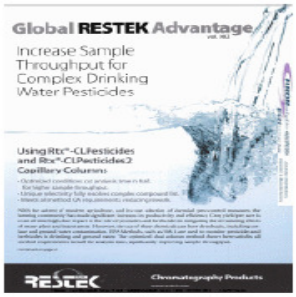

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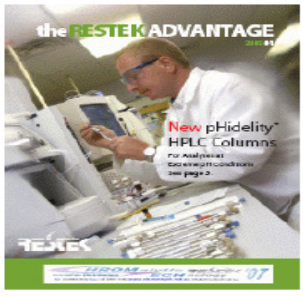

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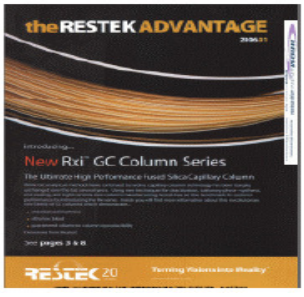

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Our expertise, experience, and enthusiasm is your Advantage.
2015.1

Make Method Development Faster, Easier, and More Reliable with Restek

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In This Issue

About Restek Corporation

Chromatography is what Restek does, and chromatography is who we are. We are an independent, international, and diverse team of employee-owners not bound to a specific brand of instrument or geographic region. We live and breathe phase chemistry, peak separations, resolution, and inertness because while chromatography may be a necessary tool in your business, it is our business. And it is a business that we directly serve across 100+ countries and six continents with unrivaled Plus 1 service, applications, and expertise.

From LC and GC columns to sample prep, reference standards to accessories, Restek is your first and best choice for chromatography.

Restek is *Pure Chromatography.*

www.restek.com

Reflections from the Bench

So... method development! Exciting? Scary? Frustrating? Rewarding?

How about "all of the above"? I've experienced all four—and more—over my years in R&D and product marketing.

Method development is the thrill of creation. The challenge of problem solving. The excitement of discovery. But, let's face it: sometimes it's the frustration of just wanting to get the job done. It's the reality that something is not working, and the spotlight is on you to fix it.

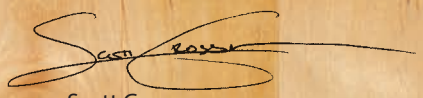
However you look at it, method development comes with a great deal of responsibility. Many of us are developing methods for very important applications in our own industries. So, building an accurate, precise, and robust method that doesn't require a third arm and a lucky rabbit's foot is vital. I appreciate this even more now as the supervisor of our Quality Control Department, where we develop our own methods to make sure Restek® products perform as you need them to—every time you use them.

Whether you are in the emerging field of medical cannabis testing (see page 18), on the hunt for Vitamin D in blood (see page 16), or looking for some ways to continue improving methods you already have (look inside for articles on choosing LC silica particles, GC carrier gasses, and GC inlet liners), you should find something in this issue of the *Advantage* to make your job easier.

When I came to Restek eight years ago, I found that my method development game progressed in leaps and bounds by virtue of being surrounded by skilled colleagues who both knew and loved the challenge of creating outstanding chromatographic methods. They were sincerely invested in my success, and let me tell you, having coworkers like that is awesome.

And as much as they were willing to help me then, my colleagues and I are eager to lend you a hand now. Chromatography is what we do, and we love sharing it!

Best regards,



Scott Grossman
Quality Control Technical Supervisor

You Have Opinions... and We Want Them

We chemists are an opinionated bunch, so the odds are good that you have some thoughts about the *Restek® Advantage.* Love it? Hate it? Want to see something different in the next issue? Maybe you have a response to one of our technical articles? Whatever you have to say, let's hear it! E-mail your comments to advantage@restek.com and you may even see them in an upcoming issue.

Questions from You

Our technical specialists field an astounding variety of questions from our customers.

Q: Some Restek GC columns have an "MS" in the name. What exactly is an MS column?

A: An "MS" designation indicates a Restek® column is mass spec grade and that we test it specifically for low-bleed performance. One reason for using a GC-MS is to achieve low detection limits; however, column bleed can have an impact on your system's detection limit. Column bleed will create an elevated background, which decreases the signal-to-noise ratio. If the signal-to-noise ratio is lower, detection limits become elevated. A low-bleed, MS column is ideal for sensitive detectors, like a mass spec. Restek MS columns may not be required with other detectors (e.g., FID, ECD, NPD, etc.), but they can be used and provide a good low-bleed option.

When conducting GC-MS analyses, one should always opt for an MS column, if available. If a column that is not designed for GC-MS must be used in a GC-MS, there are a few things you can do to minimize the potential for bleed. Try using a thin film column. Also, keep the transfer line temperature at least 20 °C below the maximum temperature of the column. Finally, use the lowest possible oven temperature, avoiding the column's maximum temperature. If bleed does occur, one will likely need to clean the source a little more frequently.

In addition to columns with the "MS" designation, Restek offers several GC columns that do not have the MS suffix but that are specifically designed with low bleed performance for use in a GC-MS. These columns are method or application specific (e.g., Rtx®-1614, Rxi®-PAH, and Rtx®-PCB columns).

If you ever have questions regarding column selection, contact Restek's Technical Service team at support@restek.com or 800-356-1688 ext. 4.

- Chas Simons
Technical Service Manager

Q: How can I make a clean cut on my fused silica or metal column using a scoring wafer?

A: Column cutting is an activity that is done routinely in any GC lab, but it is important that it be done correctly in order to obtain a proper seal in a press-fit connector. To make an optimal connection, the end of the column must be cut square at a 90° angle. Ceramic scoring wafers are among the simplest tools one can use to obtain a clean, square cut.

To cut a fused silica (Rxi®, Rtx®) column, pinch it against your fingernail and draw the smooth edge of the ceramic wafer gently along your nail in one direction, leaving a slight scratch on the column. Then, tap or push the column lightly with your finger until it breaks. If the end piece does not fall off, bend it in the opposite direction until it does. It is very important to use a smooth edge of the wafer when cutting fused silica; if you use a rough edge, the polyimide will be damaged and that will cause problems when coupling the column to the connector. Once the cut has been made and confirmed to be square, clean the column with lab tissue and methanol, or methylene chloride, and then immediately push the column into the connector to make the seal. If the seal has been made properly, a dark ring will be visible all around the end of the column where it meets the connector.

In addition to cutting fused silica columns, a ceramic scoring wafer can be used to cut metal MXT® columns. For this, use the rough edge of the wafer and use a sawing motion to create a scratch on the metal. Note that after breaking off the end there will be a scratch on the outside of the column that may give a non-ideal connection when using a direct injection or PTV type liner.

Column cutting with a ceramic scoring wafer is a simple task that—when done correctly—allows a good connection to be obtained. For illustrations and further discussion, visit **www.restek.com/ADV1511**

- Jaap de Zeeuw
International GC Specialist

Wrestling with a question of your own?

Call 1-800-356-1688, ext. 4, or e-mail **support@restek.com** today!

Hot Topics

Click to Quickly Translate Methods and Calculate Flows

Fresh from winning a 2014 TASIA (The Analytical Scientist Innovation Awards), the new *EZ*GC® method translator and flow calculator makes it simple to switch carrier gases, to change column dimensions or detectors, or to optimize a method for greater efficiency and shorter analysis times. Simply enter your original method specifications to receive a full set of translation conditions that provide similar chromatography. Results include oven program and run time as well as average velocity, flow rate, splitless valve time, and other parameters—all in an easy-to-use, single-screen interface.

Available for online use or download, these free tools are the latest addition to the *EZ*GC® method development suite, already well known for the analyst-favorite *EZ*GC® chromatogram modeler.

Save yourself hours of calculations, guesswork, and trial-and-error: Make the award-winning *EZ*GC® suite your go-to resource for method development.

Turn to page 8 to see it in action and then try it yourself at
www.restek.com/ezgc

Fortify or Calibrate for 203 Pesticides by GC-MS/MS with this Single Restek® CRM Kit

GC-MS/MS is the technique of choice for analyzing pesticide residues in many fruits, vegetables, botanicals, and herbals like tea, ginseng, ginger, Echinacea, and dietary supplements. And Restek's new GC-MS/MS pesticide reference standards kit contains over two hundred compounds pulled from the food safety lists of the FDA, USDA, and other global agencies.

This stock, comprehensive set joins the 204-compound LC-MS/MS kit in Restek's lineup of world-class certified reference materials (CRMs) for multiresidue pesticide analysis. Formulated and quantitatively tested for maximum long-term stability, both kits feature detailed support documentation and a free optimized method; the downloadable XLS files include conditions and transition tables.

No more long nights or weekends in the lab. No more custom standards. Restek's food safety chemists can help you make quick work of getting the accurate results you need.

www.restek.com/gc-multiresidue

Get Raptor™ Speed, Efficiency, and Ruggedness in 2.7 and 5 µm C18

Raptor™ LC columns launched with the time-tested Restek® Biphenyl and the acid-resistant ARC-18 phases on 2.7 µm particles. Now, this new species of column has grown to include 5 µm particles and a general-purpose C18 phase.

Every LC lab has a cache of C18s, but while the chemistry may be similar, every C18 is not created equal. The traditional end-capped Raptor™ C18 offers the highest hydrophobic retention of any Raptor™ phase, and it is compatible with a wide range of mobile phases (pH 2–8). This new phase offers consistently excellent data quality in less time across myriad reversed-phase applications, matrices, and compound classes. When you need a general-purpose LC column, don't just grab any C18. Choose the speed, efficiency, and long-lasting ruggedness of the new Raptor™ C18 SPP LC column.

Like the C18, all Raptor™ phases are now available on both 2.7 and 5 µm particles. Raptor™ 5 µm particles provide the benefits of SPP without the significant increase in pressure. Their improved efficiency and sensitivity help you easily and significantly speed up existing methods on systems that simply cannot handle smaller 2.7 µm core-shell particles. To increase sample throughput and productivity on your existing 400-bar HPLC system, 5 µm Raptor™ columns are a perfect choice. (See page 6 for more information on choosing between 2.7 and 5 µm Raptor™ particles.)

Experience Selectivity Accelerated with Raptor™ SPP LC columns.
www.restek.com/raptor

Rxi®-1301Sil MS GC Columns Provide the Selectivity you need without the Bleed

Cyano stationary phases provide more retention of polar compounds than 5-type columns; however, they are prone to high bleed and poor robustness. New Rxi®-1301Sil MS GC columns from Restek offer true cyano phase selectivity along with the highest thermal stability in the industry, which ensures you get dependable, accurate MS results and increased uptime.

In addition to providing both stable 1301 selectivity and the lowest bleed/highest temperature limits available, the Rxi®-1301Sil MS column is designed to provide maximum inertness. Each column is tested with a QC mix that includes both acidic and basic probes to ensure inertness across multiple compound classes. Greater column inertness improves peak shape and response, ensuring more accurate quantitative results.

Try this top-performing, 1301-type column today and improve the performance of existing methods for solvents, glycols, and other

Restek Signs On with Aegis to Benefit Veterans

In its second year, the Aegis Sciences Foundation's N2N (short for Natchez to Nashville) charity bike tour covered a blistering 444 miles—from Mississippi to Tennessee—in just four days, and Restek was proud to be a sponsor of this great event.

The Aegis Sciences Foundation was established in 2013 by our valued partner Aegis Sciences Corporation, a forensic toxicology and health-care sciences laboratory in Nashville. It is dedicated to supporting local communities with a particular focus on youth education, military veterans, and healthy living.

Proceeds from the last N2N—which exceeded $80,000—went to Team Red, White, and Blue. The national non-profit Team RWB has a mission to enrich the lives of America's veterans and to connect them to their communities through physical and social activities.

For information about the 2015 N2N, visit **www.biken2n.com**

Photo courtesy of Kelsey Morris, Aegis Sciences Corporation

ChromaBLOGraphy

Topical and Timely Insights

ChromaBLOGraphy is where Restek's renowned experts go to share their thoughts on current trends along with best practices and troubleshooting tips. Better yet, you have the opportunity to weigh in yourself.

Here's a look at some of our latest posts:

- *Peak Capacity in Capillary GC*
- *Alternate GC Carrier Gas: Helium to Nitrogen*
- *Another Cup of PAH Tea Please!*
- *How Dirty Are You? Part 4…Manual Syringe Rinsing*
- *Lab Hack: Quickly Reducing GC Inlet Pressure*
- *Need Help Finding the Correct Ferrule to Install Your GC Column?*

Join the discussion at **blog.restek.com** today!

Meet with Us Face-to-Face

Whether you want to talk through a nagging chromatographic issue, set up a one-on-one meeting, or just see our latest analytical solutions, an industry conference is a great place to connect with Restek. Here are a just a few of the stops on our 2015 schedule; visit **www.restek.com/events** for a full list.

2015 Events Calendar

TCEQ ETFC | May 5–6 | Austin, TX, U.S.
LAPRW | May 10–13 | Santiago, Chile
ISCC GCxGC | May 17–21 | Fort Worth, TX, U.S.
ASMS | May 31–June 4 | St. Louis, MO, U.S.

HPLC | June 21–25 | Geneva, Switzerland
ISSS 2015 | June 30–July 3 | Ljubljana, Slovenia

EnviroAnalysis | July 11–17 | Banff, AB, Canada
NEMC | July 13–17 | Chicago, IL, U.S.
NACRW | July 19–22 | St. Pete Beach, FL, U.S.
PRChem | July 28–31 | San Juan, Puerto Rico

Lab Africa | August 4–6 | Johannesburg, South Africa
INEF | August 4–6 | Toronto, ON, Canada
Dioxin | August 23–28 | São Paulo, Brazil

Did you know?

The Philae comet lander contained four different phases of Restek® MXT® GC capillary columns! Try these rugged columns in your most demanding applications: **www.restek.com/mxt**

Raptor™ LC Columns

Selectivity Accelerated

The Effects of LC Particle Choice on Column Performance:

Fully Porous Particles (FPP) vs. Superficially Porous Particles (SPP)

By Sharon Lupo, Ty Kahler, and Paul Connolly

- Switch from FPP to SPP for faster, more efficient analyses on existing instrumentation.
- Substitute Raptor™ 5 µm SPP columns for current FPP columns on traditional LC systems.
- Upgrade to Raptor™ 2.7 µm SPP for larger analyte lists on systems that can sustain higher pressures.

The fully porous particles (FPP) used in traditional LC columns are just that—fully porous—so mobile phase permeates the entire silica particle as it travels through the column. As an alternative, newer superficially porous particles (commonly referred to as SPP or "core-shell" particles), like those used in Restek's Raptor™ LC columns, feature a solid, impermeable core enveloped by a thin, porous layer of silica. As a result, SPP columns offer a greatly decreased diffusion path and reduced peak dispersion.

By comparing the performance of Raptor™ SPP LC columns to traditional FPP LC columns, it is easy to understand why you should switch to superficially porous particles. When you do switch, choose the Raptor™ SPP LC particle that is best for your intended experimental conditions and instrument capability.

Why Switch from FPP to SPP LC Columns?

By switching your 3 or 5 µm FPP column to a Raptor™ 5 µm SPP LC column of similar dimension, you gain greater efficiency, reduced system pressure, and dramatically faster analyses (Figures 1 and 2), as well as more sensitivity—all without changing instrumentation.

Certain assays may require some degree of method development to achieve optimal results, but whether you are developing new assays or looking to improve existing methodologies, Raptor™ 5 µm LC columns are compatible with most assays and offer an excellent way to increase performance over 3 or 5 µm FPP columns without extra cost or labor.

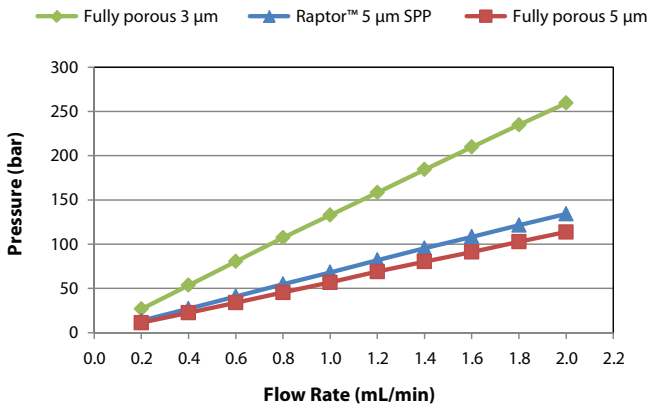
How to Choose between Raptor™ 2.7 vs. 5 µm SPP LC Columns

In addition to 5 µm, Restek's Raptor™ SPP LC columns are also available in 2.7 µm diameter particles, giving you flexibility to select the most appropriate particle size for your specific assay.

Raptor™ 5 µm diameter particle columns display low backpressure as well as good efficiency and sensitivity. These columns can be substituted into existing methods to increase analysis speed on traditional LC systems, especially those with pressure limitations (i.e., maximum operating pressure of 400 bar) and a larger amount of system volume. Raptor™ 5 µm SPP is an ideal LC particle choice for fast assays containing fewer analytes.

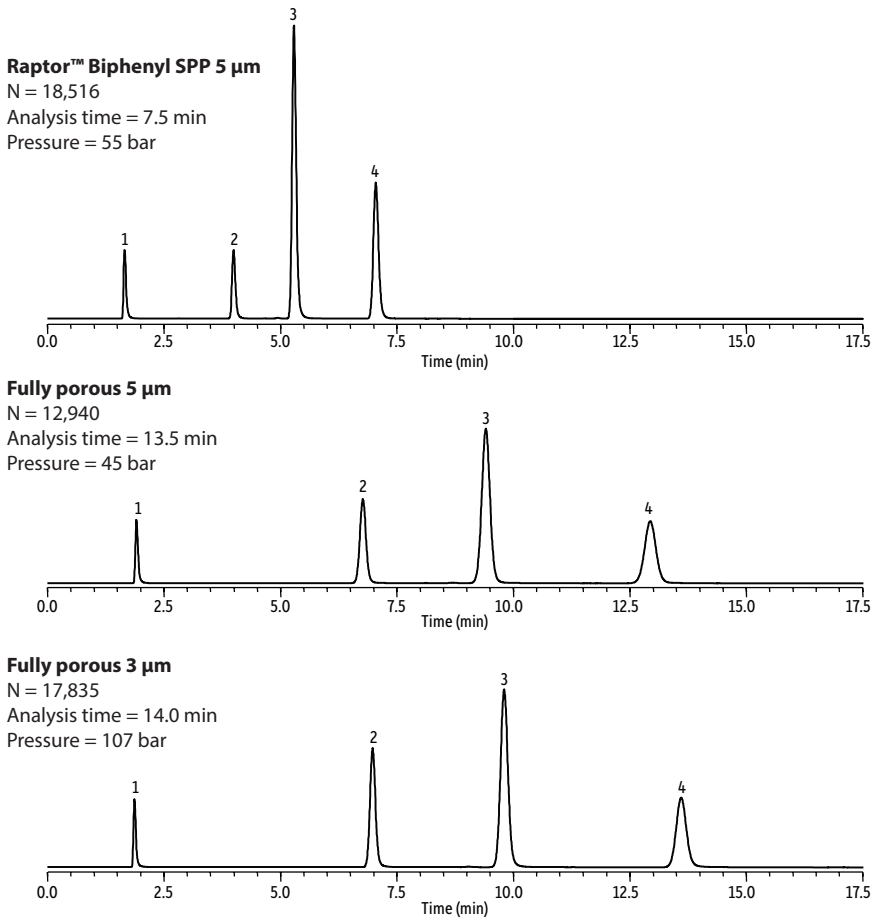
Raptor™ 2.7 µm diameter particle columns exhibit greater efficiency and sensitivity than the 5 µm, but the operating pressures are somewhat higher. Since extra-column peak broadening is most pronounced with short, small-diameter columns packed with small particles, 2.7 µm columns are best suited for instrumentation with reduced system volume that does not exceed pressures of 600 bar. Raptor™ 2.7 µm SPP is the right LC particle choice for larger analyte lists that require additional peak capacity.

Figure 1: Switch from a 3 µm FPP column to a Raptor™ 5 µm SPP to cut backpressure in half.



Column Dimensions: 150 mm x 4.6 mm ID; Temp.: 30 °C; Mobile Phase: water: acetonitrile (45:55)

Figure 2: Increase efficiency and decrease analysis time, with lower pressure, by switching from FPP to Raptor™ SPP columns.



Peaks	Conc. (mg/mL)
1. Uracil	0.02
2. Benzene	3.0
3. Naphthalene	0.5
4. Biphenyl	0.06

Column
Temp.: 30 °C
Sample
Diluent: LC Reversed Phase Test Mix #1 (cat.# 35005)
Methanol:water (75:25)
Inj. Vol.: 2 µL
Mobile Phase
A: Water
B: Acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.8	45	55
20	0.8	45	55

Detector DAD @ 254 nm
Instrument HPLC
Notes All columns were 150 mm x 4.6 mm ID. Values for efficiency (N) were calculated from the Biphenyl peak.

LC_GN0556

Experience *Selectivity Accelerated*

Whether 2.7 or 5 µm diameter particles are better for your application, rugged Raptor™ SPP LC columns can give you the increased speed and resolution you have been looking for. Experience *Selectivity Accelerated* by visiting **www.restek.com/raptor** and ordering your Raptor™ SPP LC columns today. You can also contact your local Restek® representative (**www.restek.com/contact-us**) to set up an in-depth consultation.

More on SPP and FPP

Read more on our work comparing SPP and FPP or 2.7 and 5 µm SPP columns:

Look under "Resources" at
www.restek.com/raptor

Helium to Hydrogen:

Optimize for Speed or Match Your Original Compound Retention Times with Restek's *EZ*GC® Method Translator

By Jack Cochran and Jaap de Zeeuw

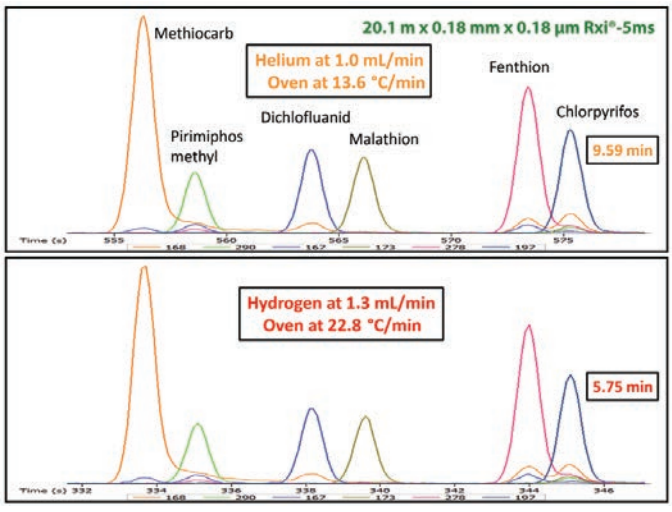
- Improve throughput by translating your GC method from slower helium to faster hydrogen carrier gas.
- Substitute expensive helium GC carrier gas with hydrogen and get the same chromatogram with translation.
- Improve MS detectability by using hydrogen at a lower flow rate without sacrificing separations.

When discussing the conversion of GC methods from helium to hydrogen carrier gas, generally the focus is on speed as hydrogen has a higher optimal flow rate than helium and can be used to achieve faster run times without sacrificing separation efficiency. While speedier analysis times offer the attraction of improved productivity, there are times when matching the original compound retention times is more important (for example, to make calibration updates or new method validation easier). Regardless of whether the goal is faster analyses or maintaining the original compound retention times, proper method translation is critical for success. The new *EZ*GC® method translator/flow calculator is an easy-to-use tool that ensures proper conversion from helium to hydrogen for either speed-optimized or matched retention time scenarios.

Increase Sample Throughput with Faster Separations

Obtaining faster GC run times so more samples can be analyzed in a day is often the driving force behind converting from helium carrier gas to hydrogen. With proper method translation, this can be an easy way to improve productivity and reduce dependence on expensive and increasingly scarce helium. The conversion requires a faster GC oven program rate for hydrogen versus helium to maintain the same chromatographic elution pattern for the compounds of interest. For example, when translating a GC-MS pesticides analysis from helium to hydrogen, the conditions for the original method using helium were simply entered into the *EZ*GC® method translator and the software returned a translated method. This translated method uses a faster flow rate and oven ramp rate. As shown in Figure 1, the translated method yielded a very comparable chromatographic separation with no elution order changes in nearly half the time.

Figure 1: Get the same separation in nearly half the time by using Restek's *EZ*GC® software to properly convert instrument conditions when switching from helium to hydrogen carrier gas.



Maintain the Original Retention Times for Easier Calibration Updates and Method Revalidation

In the second scenario, where the goal is to maintain not just the same peak elution order but also the same retention times as closely as possible, the method conversion is based on using approximately the same linear velocity for both gases, which is best done by matching the holdup time of the new hydrogen carrier method with the helium holdup time from the original method. Here, the *EZ*GC® method translator is used in custom mode and the holdup time (and/or linear velocity) for hydrogen is set to match that of helium (Figure 2). This means the GC column is operating below the optimum flow rate for hydrogen carrier gas, but an advantage is gained in being able to use exactly the same GC oven program from the original helium method. Figure 3 demonstrates that this approach gives essentially the same retention times as were obtained when using helium—with no noticeable loss in separation even though hydrogen is used at a sub-optimum flow. This technique of matching the linear velocities and holdup times for

Figure 2: To quickly determine conditions for hydrogen that will maintain the retention times obtained when using helium, simply match the method holdup times in the *EZ*GC® program's custom mode.

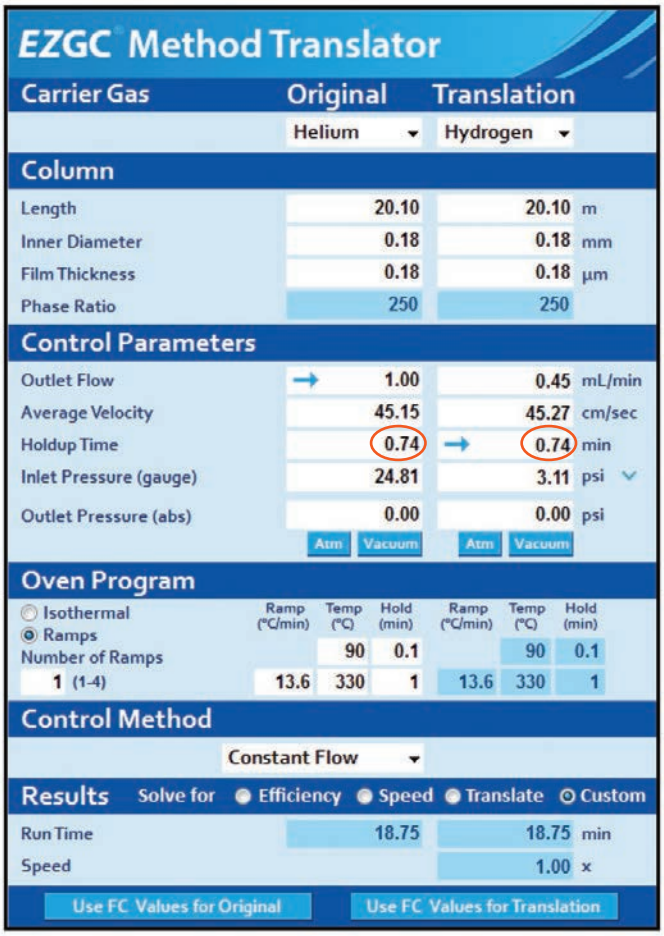
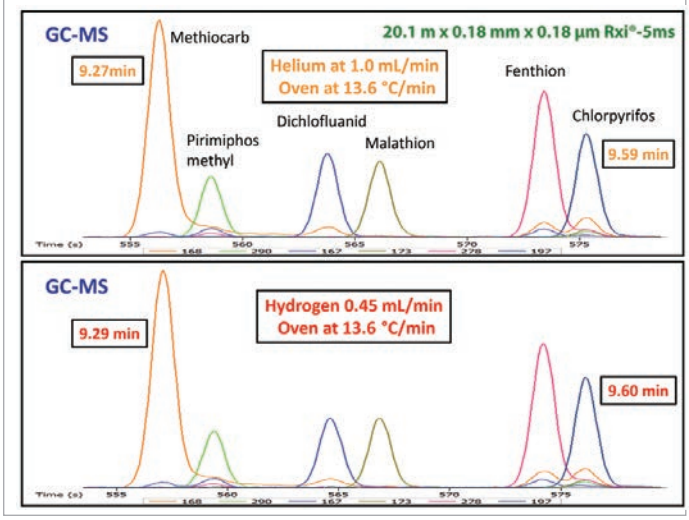


Figure 3: Get the advantage of switching to hydrogen, without having to reset retention time windows. Use the *EZ*GC® method translator/flow calculator to establish conditions that give the same retention times as your original method.



helium and hydrogen when switching carrier gases can be used to some advantage with GC-MS, where hydrogen is not easily pumped and a higher (optimum) flow would lead to a more drastic detectability loss. In addition, confirmation of method performance is simpler as the oven program and retention time windows do not change. This approach should allow easier entry for labs making the switch from helium to hydrogen carrier gas for GC.

How to Choose a GC Inlet Liner:
Simplify Selection Based on Injection Type

By Scott Adams

Choosing the correct GC inlet liner is critical in assuring that the desired amount of sample is transferred onto the column in an efficient manner, without negatively impacting the target compounds. However, liners come in many configurations that differ in geometric design, volume, base material, deactivation, and the presence or absence of packing material. With so many choices available, how do you choose the liner that's best for your application? Fortunately for the user, finding the proper GC inlet liner can be greatly simplified by basing the decision on injection type.

Split Injections

A split injection is used when the compounds of interest in your sample are of relatively high concentration or when low limits of detection are not necessary to achieve. As the name implies, the injection is split so that a manageable amount of sample is transferred onto the GC column. Split injections are accomplished by high flow rates through the inlet, with some flow (and sample) going to the GC column and some going out the split vent. Since there is a high flow rate, the time that the sample actually spends within the inlet is minimal. In order to efficiently and reproducibly get a representative amount of sample onto the analytical column, the inlet must vaporize and mix the sample quickly.

Sky® Precision® liner with wool for Agilent® GCs

Sky® Cyclo liner for Agilent® GCs

Two liners are suggested for split injection based on their ability to vaporize and mix the sample. The first is the Sky® Precision® split liner with wool. This liner contains deactivated glass wool that is held in place by dimples on the inside of the liner. The wool enhances vaporization and mixing of the sample by increasing surface area, and it also wipes the syringe needle during injection to increase repeatability. The wool is deactivated *in situ*, making for a very inert liner that works well for the majority of split injection applications. However, if your sample interacts negatively (e.g., compound degradation or adsorption) with wool, then a Sky® Cyclo liner is recommended for split injections. This highly inert liner is also treated with Sky® deactivation, but it does not contain wool. Instead, the bottom third of the liner contains a corkscrew of glass, which increases the interior surface area and assists with sample vaporization and mixing.

Splitless Injections

A splitless injection is used when the compounds of interest are present at lower levels. With this technique, the split vent is closed at the start of the injection and all of the flow passing through the inlet is directed through the column for a programmed period of time. The split vent is then opened to flush out any remaining solvent vapor. In a proper splitless injection, 99% of the compounds of interest will be transferred onto the GC column.

Sky® single taper with wool for Agilent® GCs

Sky® single taper without wool for Agilent® GCs

As with a split injection, two liners are recommended for use with splitless injection. The first is the Sky® single taper liner with wool on the bottom. The single taper at the bottom of the liner limits the interaction of the target analytes with the metal inlet seal and helps direct or focus the sample to the head of the column. The wool catches the injected sample and provides a place from which it can vaporize, while also trapping nonvolatile "dirt" that can contaminate the GC column. Again, the wool is treated *in situ* with Sky® deactivation, creating a very inert liner, which often is needed for trace-level analysis. This liner is a good choice for the majority of splitless injections. However, if your target compounds degrade or adsorb on wool, a Sky® single taper liner without wool is recommended instead.

Programmable Temperature Vaporization (PTV) Injections

PTV injections differ from split and splitless injections in that with PTV the sample is injected into a cold inlet. The inlet is then programmed to increase in temperature, often vaporizing the solvent to vent, and then programmed to further increase in temperature to vaporize the compounds of interest and introduce them onto the analytical column.

A number of different manufacturers offer PTV inlets, and liners for these inlets will vary depending upon the geometry of the inlet. Certain features that almost all PTV liners have include a small inner diameter and baffles or dimples on the inner surface of the liner. These baffles/dimples increase the inner surface area of the liner, providing more space for the sample to adhere as well as enhancing the heat transfer from the inlet to the sample as the temperature of the inlet is increasing. When choosing a PTV liner, look for your specific inlet manufacturer, then select a liner with Sky® deactivation and a small inner diameter that contains at least one baffle or dimple.

By basing liner choice on injection type, you can quickly identify the inlet liner style that will work best for your application. For more on liner selection, including recommendations for gas samples and direct injections, visit **www.restek.com/ADV1512**

tech **tip**

Correct installation of Sky® inlet liners is quick and easy. Simply orient the liner so the column installs toward the "R" on the Restek logo

Optimizing an Agilent-Style Splitless Inlet

for Concurrent Solvent Recondensation–Large Volume Splitless Injection (CSR-LVSI)

By Chris Rattray and Jack Cochran

Large volume injection (LVI) can be quite advantageous when analyzing trace-level compounds because the increased amount of analyte introduced onto the column significantly improves detectability. This approach can work well for clean matrices like drinking water; however, a special injection port, such as a programmable temperature vaporization (PTV) inlet, is generally required. Since PTV involves the expense of a specialized inlet and is limited to applications with large differences between the boiling points of the solvent and target analytes, Restek's chemists have been developing applications using concurrent solvent recondensation–large volume splitless injection (CSR-LVSI) in a completely unmodified Agilent-style inlet as an alternative.

> CSR-LVSI gives you the sensitivity of large volume injection without the expense of a specialized PTV injection port.

Building on the work of chemists at Thermo Scientific [1,2], Restek's applications laboratory has successfully demonstrated that CSR-LVSI can be used without any modification to an Agilent-style splitless injection port for a variety of analyses, including polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPH), EPA Method 8270 semivolatiles [3], and brominated flame retardants [4], as well as many organochlorine, organonitrogen, and organophosphorus pesticides. You can configure your instrument for these and other CSR-LVSI analyses using the basic setup illustrated in Figure 1.

Setting up for CSR-LVSI Success

CSR-LVSI is very similar to a standard splitless injection that incorporates solvent focusing; the primary difference being that a large uncoated (but deactivated) precolumn is used to provide enough surface area for the large solvent volume to evenly wet and maintain a mechanically stable film. (Table I gives some starting points for precolumn dimensions based on injection volume.) This recondensation step requires that the GC oven be set at or below the pressure-adjusted boiling point for the solvent during the duration of the solvent transfer. Unlike a splitless injection, you cannot begin the oven temperature program immediately after completing solvent transfer; evaporative cooling prevents the segment of column holding the analytes of interest from heating with the GC oven, so all the transferred solvent must be evaporated first. This yields a very narrow analyte band at the head of the analytical column, which results in the sharp, symmetrical peaks needed for accurate trace-level analysis.

Example Application: Lower Detection Limits for Volatile Drinking Water Contaminants

When using a PTV inlet, the solvent-venting, analyte-concentrating step requires a relatively large difference in boiling points between solvent and solute (>100 °C) in order to prevent analyte loss to the split vent. This rules out using LVI with a PTV-type injection port for volatile analytes. CSR-LVSI does not share this disadvantage. In fact, it is the only way to further lower detection limits for non-purgeable organic compounds like 1,4-dioxane and tetrahydrofuran. Recent work in our laboratory achieved low ppt levels for these drinking water contaminants, as well as several nitrosamines, which are an emerging class of contaminants [5,6]. While CSR-LVSI allows accurate quantification at very low levels, there is a trade-off in that increasing the injection volume increases the analysis time (by approximately 1 minute for every 10 µL injected) because the solvent must evaporate completely before starting the oven temperature program. Figure 2 shows the time offset seen in the same analysis using 10 µL and 50 µL injections. Note that when calculating the splitless hold time for the CSR-LVSI injection, we used the same value recommended by the *EZ*GC® flow calculator for both injections.

While the CSR-LVSI approach results in a moderate increase in analysis time, it allows lower detection limits for important drinking water contaminants. Using the setup described here, the CSR-LVSI technique can be applied when greater sensitivity is needed for compounds in clean matrices without the expense of a PTV inlet.

Read the full application at **www.restek.com/ADV1513**

Figure 1: How it Works: The CSR-LVSI Setup.

1. Clean, interference-free extracts from samples are produced using Resprep® SPE cartridges.
2. A fast autosampler injection with liquid band formation is used to make large volume injections.
3. The liquid sample enters a 4 mm Sky® inlet liner containing deactivated quartz glass wool at the bottom. The wool is critical since it acts as a "solvent reservoir." It also enhances vaporization and improves injection-to-injection reproducibility.
4. Rapid solvent evaporation occurs in the hot inlet, causing a pressure surge and a high rate of flow onto an Rxi® retention gap (precolumn), which is attached to the analytical column using a press-fit connector.
5. Because the starting oven temperature is below the boiling point of the solvent, solvent recondensation occurs in the retention gap at the same rate that evaporation occurs in the inlet, driving the rapid transfer of material to the column and preventing backflash.
6. Higher boiling point solutes transfer to the retention gap after the solvent transfer, and are trapped by the recondensed solvent film.
7. After total sample transfer to the retention gap, the oven temperature ramp evaporates the solvent, focusing the analytes into a narrow band prior to analysis on the analytical column.

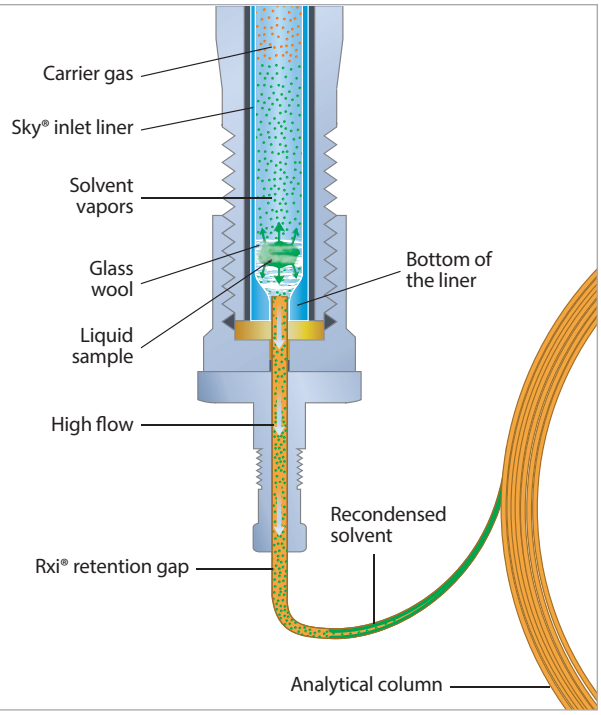


Table I: Starting points for CSR-LVSI method optimization.

Starting Parameters for Dichloromethane Injection Volumes		
Injection Vol. (µL)	Precolumn (m x mm ID)	Wool in liner (mg)
≤ 12.5	5 x 0.25[b]	5[a]
≤ 25	5 x 0.53	5
≤ 50	10 x 0.53	10
250	30 x 0.53[c]	10

[a]Standard single taper liner with wool, [b]an Integra-Guard® column may be suitable, [c]30 m segments of guard columns may require a custom order

References

[1] P. Magni, T. Porzano, *Concurrent Solvent Recondensation Large Sample Volume Splitless Injection*, J. Sep. Sci. 26 (2003) 1491.

[2] Patent No: US 6,955,709 B2.

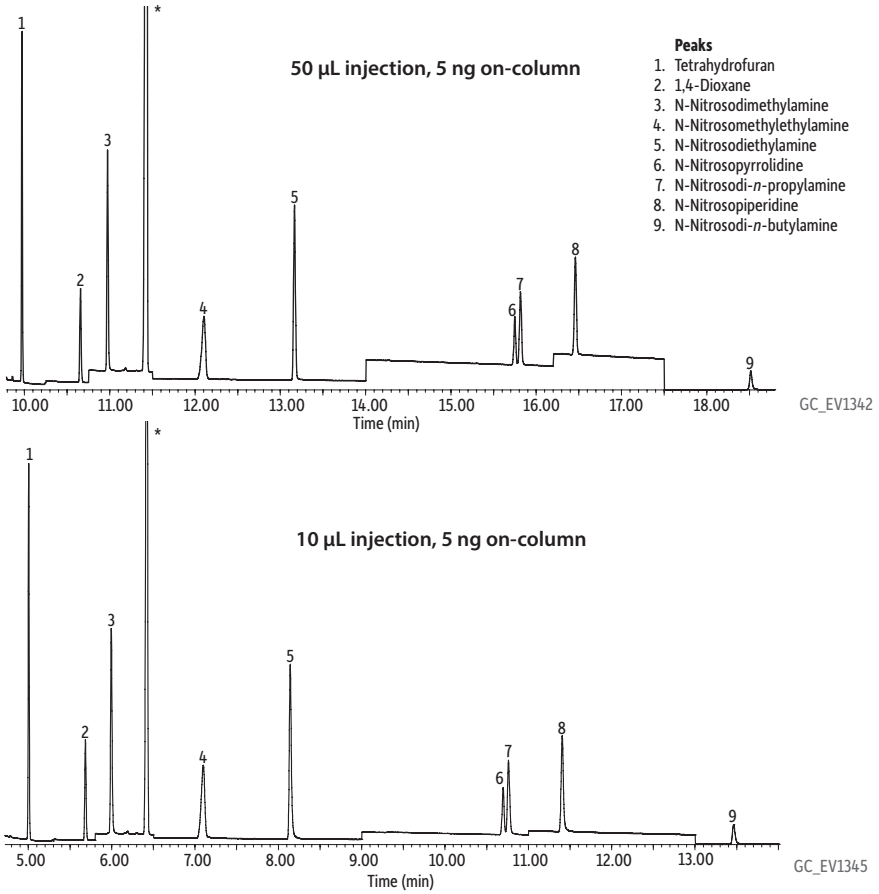
[3] J. Cochran, *The Solvent Effect in Concurrent Solvent Recondensation Large Volume Splitless Injection with Methylene Chloride – EPA Method 8270 Semivolatiles*, ChromaBLOGraphy, Restek Corporation, 2011 http://blog.restek.com/?p=1902 (accessed March 2, 2012).

[4] M. Misselwitz, J. Cochran, *Large Volume Splitless Injection Using an Unmodified Split/Splitless Inlet and GC-TOFMS for Pesticides and Brominated Flame Retardants*, Application Note EVAN1331-UNV, Restek Corporation, 2011.

[5] C. Rattray, J. Cochran, C. English, *Lowering Detection Limits for 1,4-Dioxane in Drinking Water Using Large Volume Injection in an Unmodified Splitless GC Inlet,* Application Note EVAN1548-UNV, Restek Corporation, 2012.

[6] C. Rattray, J. Cochran, *Combined Determination of 1,4-Dioxane and Nitrosamine Contaminants in Drinking Water Using a Single SPE Cartridge and Concurrent Solvent Recondensation–Large Volume Splitless Injection (CSR-LVSI) With EI GC-MS* , Application Note EVAN1922A-UNV, Restek Corporation, 2014.

Figure 2: While large volume injections extend analysis times, using CSR-LVSI for drinking water contaminant analysis provides good sensitivity without the expense of a PTV inlet.



Column: Rxi®-5Sil MS, 30 m, 0.25 mm ID, 1.00 µm (cat.# 13653) using Rxi® guard column 10 m, 0.53 mm ID (cat.# 10073) with BGB P/N: 2553LD; **Sample:** 1,4-Dioxane (cat.# 30287), Nitrosamine calibration mix, Method 521 (cat.# 31898), Tetrahydrofuran (THF) (cat.# 30414); Diluent: Dichloromethane; Liner (for CSR-LVSI): Custom Sky® single taper with 15 mg quartz wool; Liner (for standard injection): 4 mm Sky® single taper w/wool (cat.# 23303.5); Inj. Temp.: 275 °C; Purge Flow: 100 mL/min; **Oven:** (for CSR-LVSI): 35 °C (hold 1.5 min) to 50 °C at 50 °C/min (hold 7.1 min) to 320 °C at 11.12 °C/min (hold 1.5 min); Oven: (for standard injection): 35 °C (hold 1.5 min) to 50 °C at 50 °C/min (hold 2.02 min) to 320 °C at 11.12 °C/min (hold 1.5 min); **Carrier Gas:** He, constant flow; Flow Rate: 5.08 mL/min; **Detector:** MS; Mode: SIM; Transfer Line Temp.: 320 °C; Analyzer Type: Quadrupole; Source Temp.: 230 °C; Quad Temp.: 150 °C; Ionization Mode: EI; **Instrument:** Agilent 7890A GC & 5975C MSD. **Notes:** For SIM program and other conditions, visit www.restek.com and enter GC_EV1342 and GC_EV1345 in the search.
*Toluene contaminant

New GC Method for Polycyclic Aromatic Compounds

in Yerba Mate Tea Combines Simplified Prep and Improved Accuracy for EFSA PAH4 and EFSA PAH8 Compounds

By Jack Cochran, Julie Kowalski, and Amanda Rigdon

- Fast, simple modified QuEChERS extraction and silica cartridge SPE cleanup extend column lifetime and reduces inlet maintenance.
- Novel Rxi®-PAH GC column selectivity ensures separation and accurate reporting of EFSA PAH4 and other key PAHs.

Traditionally, yerba mate tea, which is brewed from loose *Ilex paraguariensis* leaves and stems, has been especially popular in Argentina, Brazil, Paraguay, and Uruguay. More recently, the popularity and economic importance of mate tea has grown worldwide, due in part to its reputation of providing numerous health benefits. Unfortunately, a high incidence of esophageal cancer has been found in populations with high mate tea consumption, indicating a possible link between mate tea and cancer [1,2]. Since mate tea contains relatively high levels of toxic polycyclic aromatic hydrocarbons (PAHs), accurate analysis of these compounds is becoming increasingly important. Currently, monitoring efforts are focused on two analyte lists recommended by the European Food Safety Authority (EFSA): EFSA PAH4 (benzo[a]pyrene, chrysene, benz[a]anthracene, and benzo[b]fluoranthene) and EFSA PAH8 (all PAH4 analytes plus benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, dibenz[ah]anthracene, and benzo[ghi]perylene).

Due to the complexity of the botanical matrix, testing methods for mate tea often use exhaustive sample preparation, including supercritical fluid extraction, pressurized fluid extraction, and gel permeation chromatography. In addition, isobaric compounds also make PAH analysis difficult because, since isobars cannot be distinguished by mass spectrometry, accurate reporting depends on being able to obtain chromatographic separations. Given these challenges, our goal was to develop a robust, yet simple, sample preparation method for PAHs in tea. As shown here, we paired this sample preparation approach with a highly selective GC column and both TOFMS and MS/MS analyses to produce accurate quantitative data for critical PAHs—including isobaric compounds—in a short analysis time.

Speedy Sample Preparation Saves Time and Removes Matrix Interferences

QuEChERS sample preparation methods are a desirable alternative because they are quick and easy, but still provide quality results. The QuEChERS approach was originally designed for pesticide residues in fruit and vegetables, but modifications such as those used here have been developed to expand it beyond the original scope. Compounds such as PAHs, veterinary drugs, and persistent organic pollutants have been testing using QuEChERS methods in difficult commodities like tea, spices, and tobacco. The procedure used here (see sidebar), was much less time- and labor-intensive than traditional sample preparation methods for tea, and it effectively removed chlorophyll and other nonvolatile materials that can quickly foul GC inlets and columns (Figure 1). Not only was this approach fast and effective in removing matrix interferences, but it also can save labs time and money by reducing inlet maintenance and extending GC column lifetime.

Unique Rxi®-PAH Column Prevents Coelutions and Ensures Accurate Reporting

An Rxi®-PAH column was chosen for this analysis because its novel selectivity separates all priority compounds, including the EFSA PAH4 subset as well as benzo [b], [k], and [j] fluoranthenes (Figure 1). During method development, accuracy was assessed based on the recovery of 30 PAHs fortified at 500 ng/g in mate tea samples. In addition, incurred PAH levels were determined in an unfortified tea sample. Samples were analyzed by both GC-MS/MS and GC-TOFMS and results using both techniques were quite similar for the EFSA PAH4 compounds.

Overall, the modified QuEChERS method used here effectively produced good quantitative data for PAHs in mate teas. As shown in Table I, satisfactory recoveries (72-130%) were obtained for the 500 ng/g fortified sample and concentrations ranging from 7 ng/g to 540 ng/g were determined in the unfortified sample. The selectivity of the Rxi®-PAH column separated all isobars and allowed us to report accurate values without bias from coeluting compounds. For example, this method effectively separated triphenylene and chrysene, which are

among the most difficult PAHs to separate. Other notable PAHs that coelute on most GC columns include benzo[b]fluoranthene/benzo[j] fluoranthene and dibenz[a,c]anthracene/dibenz[a,h]anthracene; all these compounds were separated and accurately reported using an Rxi®-PAH column and the Restek® methodology described here.

Visit www.restek.com/ADV1514 for a complete presentation of the data summarized here.

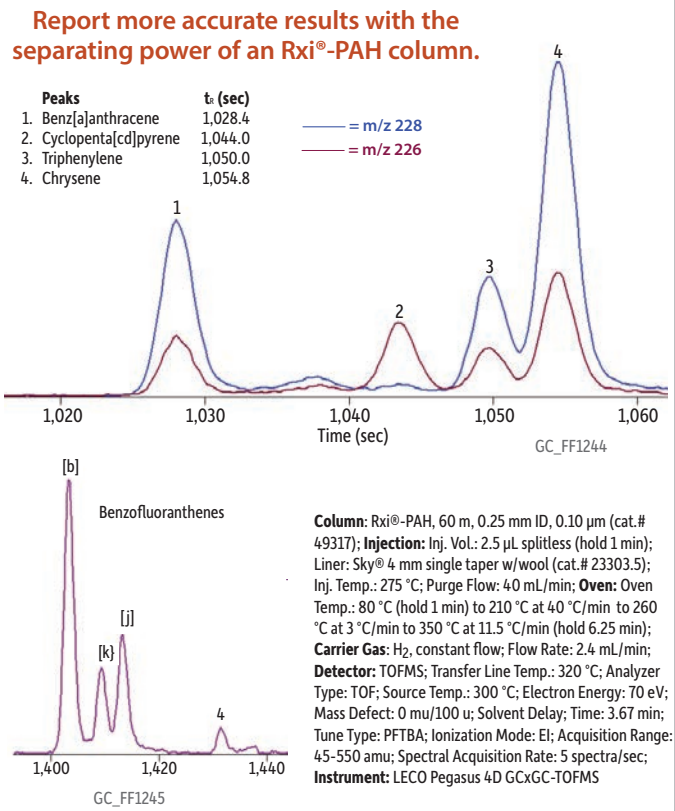
References

[1] A.P. Dasanayake, A.J. Silverman, S. Warnakulasuriya, *Mate Drinking and Oral and Oro-pharyngeal Cancer: A Systematic Review and Meta-analysis,* Oral Oncol 46 (2010) 82.

[2] D. Loria, E. Barrios, R. Zanetti, *Cancer and Yerba Mate Consumption: A Review of Possible Associations,* Rev Panam Salud Publica 25 (2009) 530.

Figure 1: Chlorophyll and other nonvolatiles will quickly foul GC inlets and columns, but they can be removed easily and reliably with this modified QuEChERS method.

Figure 2: The Rxi®-PAH column separates isobaric PAHs, allowing unbiased quantification of critical compounds that coelute on most GC columns.



Column: Rxi®-PAH, 60 m, 0.25 mm ID, 0.10 µm (cat.# 49317); **Injection:** Inj. Vol.: 2.5 µL splitless (hold 1 min); Liner: Sky® 4 mm single taper w/wool (cat.# 23303.5); Inj. Temp.: 275 °C; Purge Flow: 40 mL/min; **Oven:** Oven Temp.: 80 °C (hold 1 min) to 210 °C at 40 °C/min to 260 °C at 3 °C/min to 350 °C at 11.5 °C/min (hold 6.25 min); **Carrier Gas**: H_2, constant flow; Flow Rate: 2.4 mL/min; **Detector:** TOFMS; Transfer Line Temp.: 320 °C; Analyzer Type: TOF; Source Temp.: 300 °C; Electron Energy: 70 eV; Mass Defect: 0 mu/100 u; Solvent Delay; Time: 3.67 min; Tune Type: PFTBA; Ionization Mode: EI; Acquisition Range: 45-550 amu; Spectral Acquisition Rate: 5 spectra/sec; **Instrument:** LECO Pegasus 4D GCxGC-TOFMS

Fast, Simple Sample Preparation for PAHs in Mate Tea

Modified QuEChERS Extraction

1. Homogenize dry tea into a powder.
2. Soak 1 g tea powder in 10 mL water for 10 min in an FEP centrifuge tube.
3. Add 10 mL hexane:acetone (1:1) and vortex 30 min.
4. Add Q-sep® QuEChERS unbuffered salts (cat.# 23991), shake 1 min, and then spin for 5 min in a Q-sep® 3000 centrifuge.
5. Evaporate 2 mL of extract down to 1 mL, then adjust final volume to 2 mL with hexane. Perform this step twice.

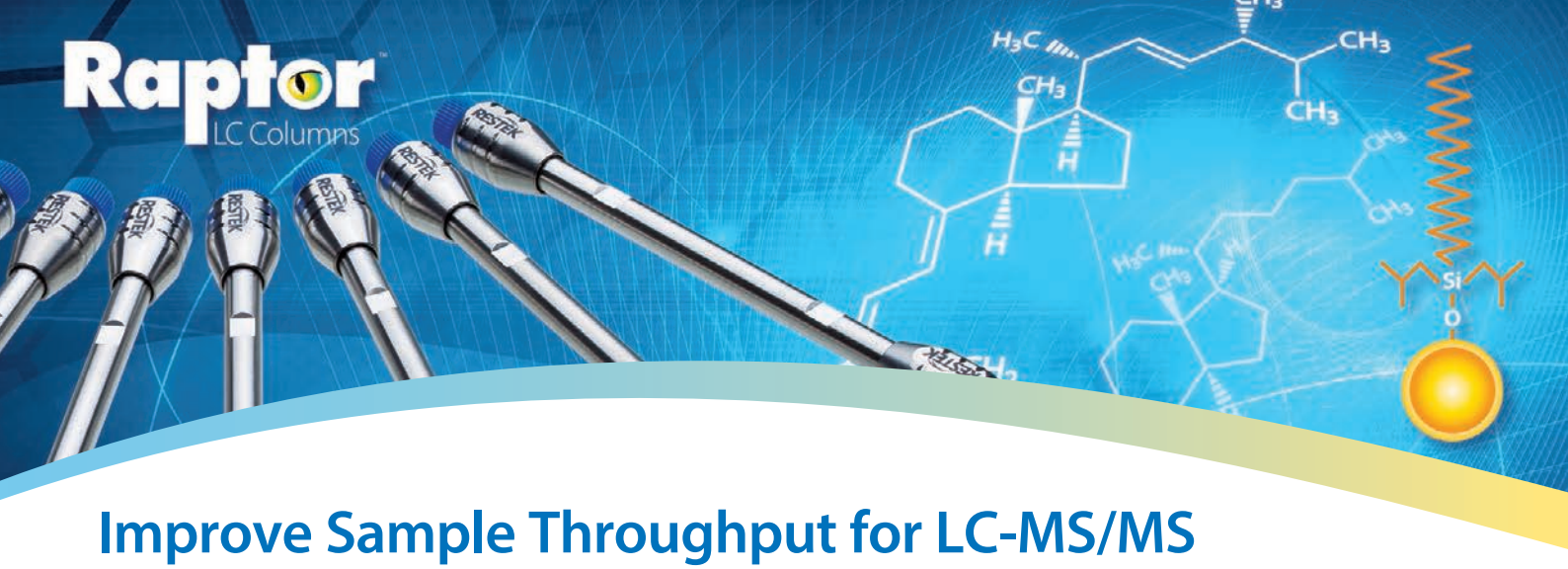
Silica SPE Cleanup

1. Rinse Resprep® SPE cartridges (3 mL, 0.5 g silica; cat.# 24036) with 3 mL methanol followed by 3 mL acetone.
2. Condition cartridges with 3 mL hexane:methylene chloride (1:1), followed by 6 mL hexane.
3. Load 1 mL of extract onto cartridge and elute with 5 mL hexane:methylene chloride (7:3).
4. Evaporate to 1 mL.

Table I: The simplified PAH method developed by Restek produced good quantitative results for both fortified and unfortified tea samples.

PAH	% Recovery (500 ng/g Fortified Tea)	Unfortified Tea Sample (ng/g)
Naphthalene	90	93
Acenaphthylene	110	42
Acenaphthene	99	8
Fluorene	110	25
Phenanthrene	81	540
Anthracene	130	58
Fluoranthene	72	270
Pyrene	74	290
Benzo[c]phenanthrene	75	14
Benz[a]anthracene	81	66
Triphenylene	80	28
Chrysene	82	120
5-Methylchrysene	76	ND
Benzo[b]fluoranthene	92	49
Benzo[k]fluoranthene	96	21
Benzo[j]fluoranthene	89	25
Benzo[a]fluoranthene	97	11
Benzo[e]pyrene	89	44
Benzo[a]pyrene	100	55
Perylene	94	14
Dibenz[a,c]anthracene	100	7
Indeno[1,2,3-cd]pyrene	110	52
Dibenz[a,h]anthracene	98	12
Benzo[ghi]perylene	88	94
Dibenzo[a,e]pyrene	93	ND
Coronene	86	130

ND = not detected



Improve Sample Throughput for LC-MS/MS Analysis of Vitamin D Metabolites in Plasma

With a New Raptor™ ARC-18 Column

By Shun-Hsin Liang, Sharon Lupo, Frances Carroll, Ty Kahler, and Paul Connolly

- Separate target analytes in just minutes for faster sample throughput.
- Report accurate results with confidence based on validated method performance.
- ARC-18 column endures low-pH mobile phases without sacrificing retention or peak quality.

Vitamin D deficiency has been linked to an increased risk for many chronic diseases including diabetes, heart disease, autoimmune diseases, and some cancers. Vitamin D exists in two forms: vitamin D2 and vitamin D3. While vitamin D3 is an endogenous nutrient that the human body can synthesize, vitamin D2 must be obtained from dietary sources, such as dairy products and fish. These parent compounds undergo metabolism to form 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. For accurate determination of vitamin D levels in the blood, it is important to distinguish between these metabolites and to separate them from major matrix interferences.

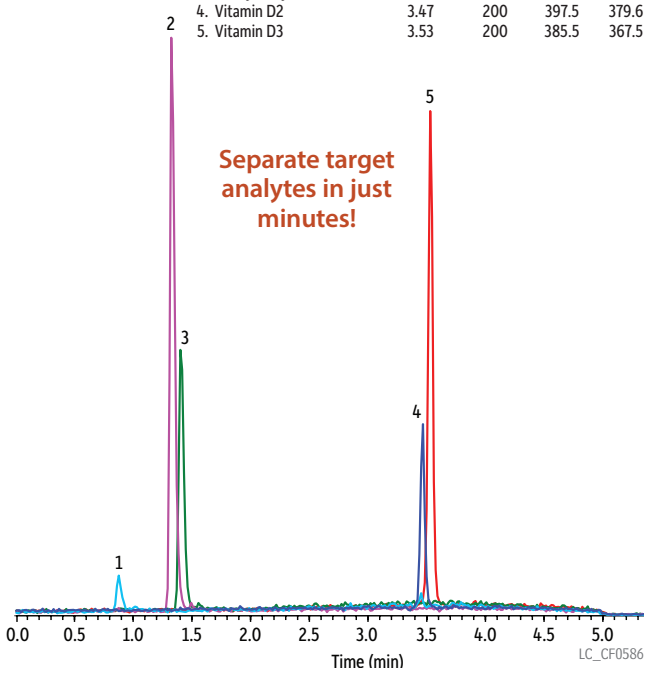
Separating fat-soluble vitamins by LC can be quite time-consuming, taking up to 20 minutes or longer by some methods. However, the new Raptor™ ARC-18 LC column can analyze these difficult compounds using reversed-phase chromatography (RPC) in less time than traditional columns, which helps increase sample throughput and overall lab productivity. In the method developed here, the Raptor™ ARC-18 column combines the speed of superficially porous particles (SPP) with the resolution of highly selective USLC® technology to produce a simple and accurate method for the determination of vitamin D metabolites in plasma.

Fast Analysis Times Improve Productivity

The Raptor™ ARC-18 column was selected for this method because its resolving power allows accurate determination of both forms of vitamin D as well as the metabolites. It was also chosen because it performs well with the low pH mobile phases used to promote ionization in MS detection. Prior to evaluating the method with fortified samples, the suitability of the Raptor™ ARC-18 column for the analysis of vitamin D metabolites was established using a neat standard solution. As demonstrated in Figure 1, all compounds were separated with an analysis time of less than 4 minutes, while the metabolites specifically targeted here eluted in less than 2 minutes. This allows reliable quantitative data to be generated quickly, so sample throughput can be increased.

Figure 1: The Raptor™ ARC-18 column makes quick work of analyzing vitamin D and metabolites by LC-MS/MS.

Peaks	t_R (min)	Conc. (ng/mL)	Q1	Q3
1. 1,25-Dihydroxyvitamin D3	0.88	200	399.4	381.5
2. 25-Hydroxyvitamin D3	1.33	200	401.5	383.5
3. 25-Hydroxyvitamin D2	1.41	200	413.5	395.5
4. Vitamin D2	3.47	200	397.5	379.6
5. Vitamin D3	3.53	200	385.5	367.5



Column: Raptor™ ARC-18 (cat.# 9314A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Temp.: 40 °C; **Sample:** Diluent: Methanol; Conc.: 200 ng/mL; Inj. Vol.: 5 µL; **Mobile Phase:** A: 0.1% Formic acid + 5 mM ammonium formate in water B: 0.1% Formic acid + 5 mM ammonium formate in methanol; **Gradient (%B):** 0.00 min (90%), 4.00 min (100%), 4.01 min (90%), 6.00 (90%); **Flow:** 0.5 mL/min; **Detector:** ABSCIEX API 4000™; Ion Source: TurbolonSpray®; Ion Mode: ESI+; **Instrument:** Shimadzu UFLCXR

Table I: Excellent results for method accuracy and precision provide confidence in data quality.

	Low Fortification (5 ng/mL)			Mid Fortification (25 ng/mL)			High Fortification (100 ng/mL)		
Analyte	Conc. (ng/mL)	Accuracy (%Recovery)	Precision (%RSD)	Conc. (ng/mL)	Accuracy (%Recovery)	Precision (%RSD)	Conc. (ng/mL)	Accuracy (%Recovery)	Precision (%RSD)
25-Hydroxyvitamin D2	5.4	107.3	10.7	25.3	101.1	3.9	101.5	101.5	1.6
25-Hydroxyvitamin D3	4.5	92.6	8.5	25.6	102.4	0.3	107.1	107.1	1.4

Table values are averages of replicate samples.

Good Accuracy and Precision Ensure Reliable Results

In order to evaluate method accuracy and precision in matrix, replicate charcoal-stripped rat plasma samples were fortified at 5, 25, and 100 ng/mL with 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. Quantitation was performed using calibration standards ranging from 1 to 150 ng/mL that were prepared in 4% human albumin in PBS solution. Eight calibration concentrations were used for 25-hydroxyvitamin D2 and seven were used for 25-hydroxyvitamin D3. Both the fortified samples and standards were extracted using a simple liquid-liquid extraction method with 25-hydroxyvitamin D3-d6 as the internal standard. Visit www.restek.com/ADV1515 for the full sample preparation procedure.

Linearity was evaluated and good response curves were obtained for both metabolites (Figure 2). Using 1/x weighting, the correlation coefficients (r) were 0.9992 (25-hydroxyvitamin D2) and 0.9989 (25-hydroxyvitamin D3), and the deviations were ≤10% for both compounds. Blanks and fortified samples were also analyzed to evaluate accuracy and precision. Since the extracted blank plasma samples contained 25-hydroxyvitamin D3 (Figure 3), blank values were subtracted from fortified samples to improve quantitative accuracy. As Table I shows, excellent results for accuracy and precision were obtained for both compounds at all three fortification levels, with an overall range of 92.6-107.3% recovery for accuracy and 0.3-10.7 % RSD for precision.

Summary

Designed specifically for use on LC-MS/MS systems, the Raptor™ ARC-18 column is the cornerstone of this high-throughput LC-MS/MS method for analysis of vitamin D metabolites in plasma. This new column from Restek delivers the fast analysis times needed to improve sample throughput and lab productivity along with the accurate, precise performance needed to ensure data quality.

Figure 2: Good linear response was achieved for vitamin D metabolites using the Raptor™ ARC-18 column.

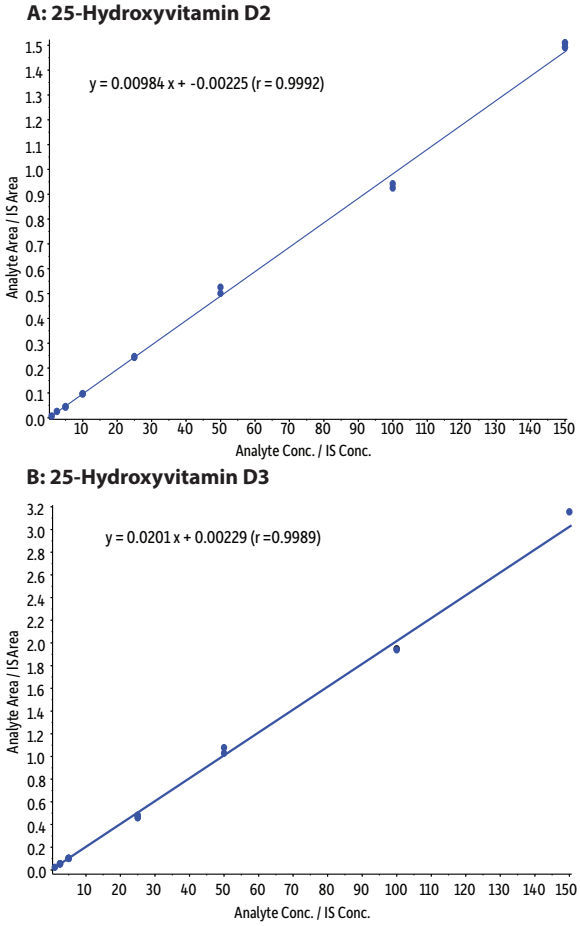
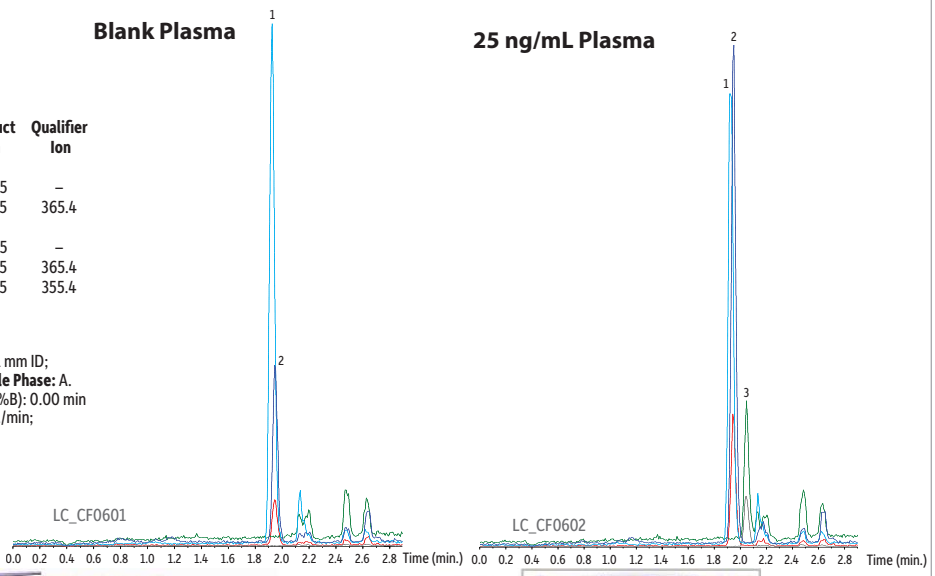
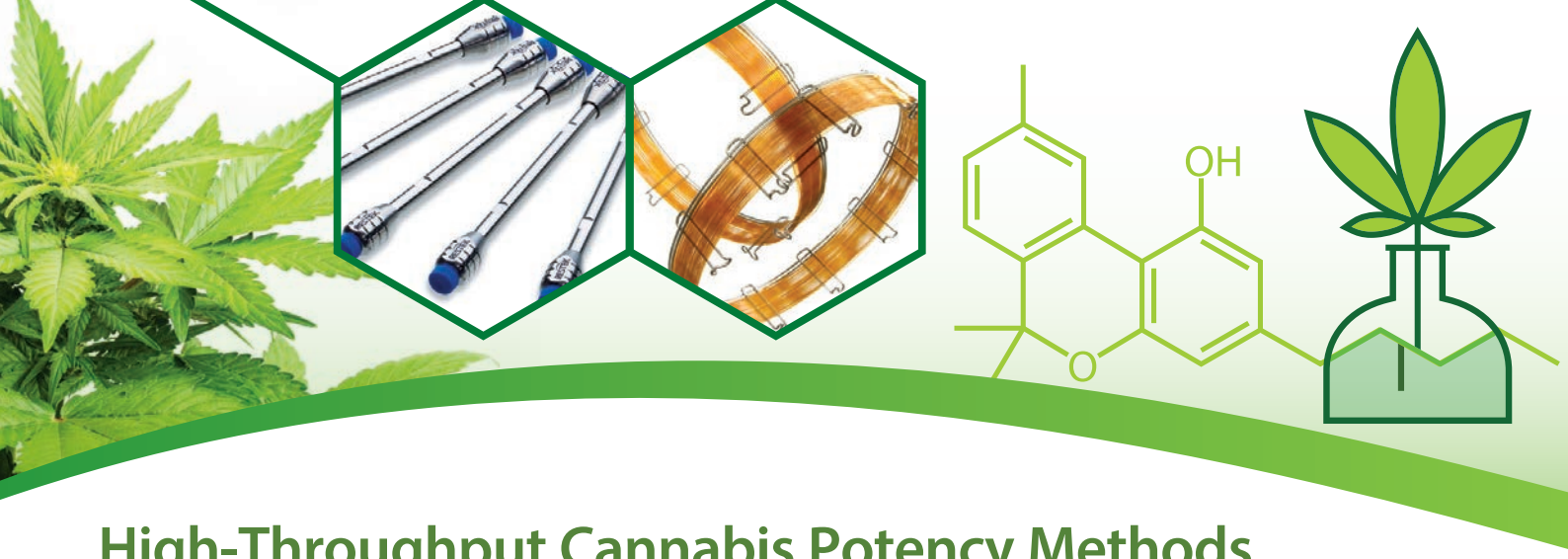


Figure 3: Good separation of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 from matrix components ensures more accurate results.



Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Qualifier Ion
Blank Plasma					
1. 25-Hydroxyvitamin D3-d6	1.93	25	407.3	389.5	–
2. 25-Hydroxyvitamin D3	1.95	unknown	401.3	383.5	365.4
25 ng/mL Plasma					
1. 25-Hydroxyvitamin D3-d6	1.92	25	407.3	389.5	–
2. 25-Hydroxyvitamin D3	1.95	25	401.3	383.5	365.4
3. 25-Hydroxyvitamin D2	2.05	25	413.3	395.5	355.4

Column: Raptor™ ARC-18 (cat.# 9314A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 40 °C; Inj. Vol.: 5 µL; **Mobile Phase:** A. 0.1% Formic acid in water, B. 0.1% Formic acid in methanol; Gradient (%B): 0.00 min (85%), 3.00 min (96%), 3.01 min (85%), 5.00 min (85%); Flow: 0.5 mL/min; **Detector**: MS/MS; Ion Mode: ESI+; **Instrument:** HPLC.



High-Throughput Cannabis Potency Methods for LC and GC Produce Results Quickly

without the Cost of New Equipment

By Frances Carroll, Jack Cochran, and Amanda Rigdon

As medical cannabis becomes more frequently prescribed, demand is growing for analytical testing services to perform potency testing to determine the levels of therapeutic compounds in cannabis products. While interest in terpene profiling and pesticide residue analysis is also increasing, accurate potency testing remains the cornerstone of every medical cannabis lab, and it is critical that this testing be carried out in the most efficient way possible. Cannabis potency testing can be performed reliably using either LC or GC methodologies. However, in cases where separate quantification of the acid forms of cannabinoids (e.g., delta-9-tetrahydrocannabinolic acid A [THCA] and cannabidiolic acid [CBDA]) is required, LC is the most viable quantitative option. Rules for quantification of cannabinoids for potency testing vary by state, and the choice of technique is determined by both these regulations and by existing laboratory constraints. This article will outline LC and GC approaches to potency testing. Restek has been committed to helping medical cannabis labs establish sound analytical practices from the beginning of this emerging industry through its recent years of rapid growth. Here we provide products and methodology for accurate, high-throughput potency testing by LC and GC so that you can improve productivity and get more done in a day, regardless of current instrumentation.

Whether you are testing potency by LC or GC, Restek has the products and expertise to get you accurate results quickly so you can analyze more samples per day.

Analyze Cannabinoids at UHPLC Speed without Investing in New Equipment

Instrumentation is one of the largest investments made when starting a new medical cannabis testing lab. In setting up potency testing, higher throughput is attractive in order to get the most out of your instrument investment. However, the cost of a UHPLC instrument is significantly more than that of a conventional HPLC instrument. Now, you can get UHPLC performance out of any HPLC instrument using Restek's Raptor™ line of HPLC columns. The superficially porous particles used in these columns allow for faster flow rates and higher efficiency than conventional fully porous particles, without the high backpressure of sub-2 µm particles used with UHPLC instruments.

As shown in Figure 1, Restek has developed a fast analysis (3.8 min analysis [7 min total cycle time]) of cannabinoids that can be performed on any LC instrument. By utilizing Raptor™ column technology, you can obtain UHPLC speed without the capital investment. Also, we specifically chose simple, fast, and easy-to-prepare mobile phases that can be directly transferred to LC-MS if you ever need to switch due to regulation changes. Raptor™ columns enable you to keep your start-up capital available while at the same time building a flexible and fast analytical foundation.

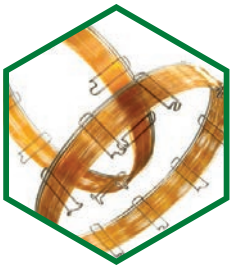
Rxi®-35Sil MS GC Column Provides Baseline Separations for More Accurate Reporting

GC instruments are the workhorses of labs in many industries, and reliable, used instruments can be purchased at a very reasonable cost. In cases where separate quantification of cannabinoid acids is not required, GC is often the technique of choice for cannabis potency testing. Restek has developed a method for cannabis potency testing using the Rxi®-35Sil MS column, due to its ruggedness and selectivity. All columns in the Rxi® family have high thermal stability, making them very rugged, which results in a longer lifetime and reduced consumables costs. In addition, the high phenyl content selectivity of the Rxi®-35Sil MS column provides much better separation of cannabichromene (CBC) and cannabidiol (CBD) than what can be achieved using traditional 5-type columns. Using cost-effective hydrogen carrier gas, all cannabinoids are baseline separated in a very fast analysis. Additionally, by consolidating quantification into only the neutral forms of cannabinoids, the need for expensive cannabinoid acid standards is eliminated.

Acknowledgement

The Ferguson Township Police Department supplied seized marijuana and oversaw sample handling. Frank Dorman at The Pennsylvania State University assisted with sample extraction.

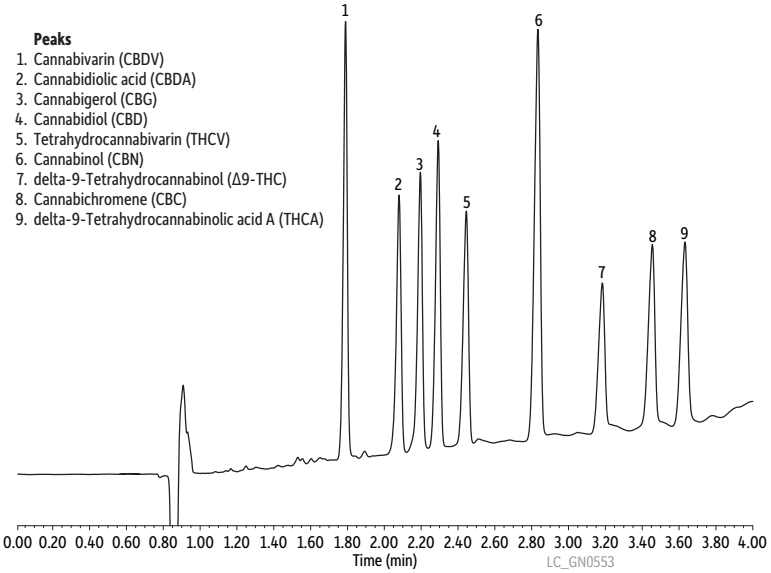
Whether you are using LC or GC for cannabis potency analysis, Restek can provide the products and expertise you need to obtain accurate results quickly. Use the methods shown here for analyzing the full spectrum of acid and neutral cannabinoids using LC with minimal capital investment, or get extremely fast, reliable, cost-effective results for neutrals only by using GC. In addition to the methods and columns recommended here, Restek offers the most comprehensive selection of cannabinoid-related certified reference materials (CRMs), manufactured and QC tested in our ISO-accredited laboratories. Visit www.restek.com/cannabis for the products, expertise, and methodology that ensure confidence in results and compliance with changing regulations.



tech **tip**

To see how the Rxi®-35Sil MS outperforms traditional 5-type columns, access our full technical article at **www.restek.com/ADV1516**

Figure 1: Raptor™ LC columns give you fast analysis times for cannabinoids without the expense of UHPLC equipment.

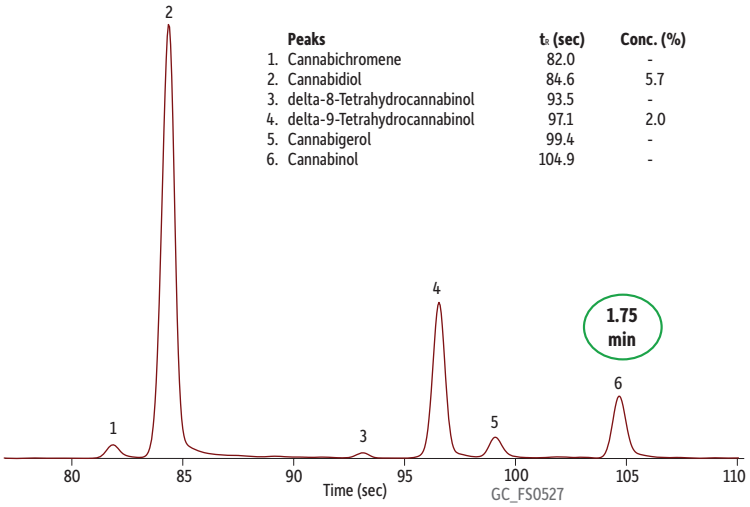


Column	Raptor™ ARC-18 (cat.# 9314A65)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	2.7 µm
Temp.:	50 °C
Sample	Cannabidiolic acid (cat.# 34094) Cannabigerol (cat.# 34091) Cannabidiol (cat.# 34011) Cannabinol (cat.# 34010) delta-9-Tetrahydrocannabinol (THC) (cat.# 34067) Cannabichromene (cat.# 34092) delta-9-Tetrahydrocannabinolic acid A (THCA) (cat.# 34093)
Diluent:	50:50 Methanol:water
Conc.:	50 µg/mL
Inj. Vol.:	5 µL
Mobile Phase	
A:	0.1% Formic acid in water
B:	0.1% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	1.5	25	75
4.00	1.5	0	100
4.01	1.5	25	75
7.00	1.5	25	75

Detector	UV/Vis @ 220 nm
Instrument	HPLC

Figure 2: Determine critical cannabinoids in minutes by GC using an Rxi®-35Sil MS column.



Peaks	t_R (sec)	Conc. (%)
1. Cannabichromene	82.0	-
2. Cannabidiol	84.6	5.7
3. delta-8-Tetrahydrocannabinol	93.5	-
4. delta-9-Tetrahydrocannabinol	97.1	2.0
5. Cannabigerol	99.4	-
6. Cannabinol	104.9	-

Column	Rxi®-35Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13820)
Sample	Cannabis sample 94
Injection	
Inj. Vol.:	1 µL split (split ratio 10:1)
Liner:	Sky® 4 mm Precision® liner w/wool (cat.# 23305.5)
Inj. Temp.:	250 °C
Split Vent Flow Rate:	25 mL/min
Oven	
Oven Temp.:	225 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.9 min)
Carrier Gas	H_2, constant flow
Flow Rate:	2.5 mL/min
Detector	FID @ 350 °C
Constant Column + Constant Make-up:	50 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	40 mL/min
Air flow:	450 mL/min
Data Rate:	20 Hz
Instrument	Agilent/HP6890 GC

Get Reliable PLOT Column Performance with Less Downtime for Maintenance by Switching to **Virtually Particle-Free Rt®-Silica BOND Columns**

By Corby Hilliard and Amanda Rigdon

- Keep your instruments running longer. Fewer particle obstructions mean less maintenance and more reproducible retention times.
- Water minimally impacts retention, allowing the analysis of water-containing samples without thermal conditioning between analyses.
- Versatile column is ideal for many applications including hydrocarbons, halogenated compounds, and sulfur gases.

Porous layer open tubular (PLOT) columns are very useful to GC analysts working on a wide variety of applications, and their unique selectivity makes them particularly good for separating gaseous compounds without cryogenic cooling. However, traditional PLOT columns are hampered by the characteristic instability of the porous layer that coats the inside of the column. With most PLOT columns, particles are shed from this layer and create significant problems because they form obstructions inside the column that alter flow and cause retention-time instability. In addition, particle buildup makes frequent maintenance necessary as jets become obstructed, valves are damaged, and detectors are contaminated.

In contrast, new Rt®-Silica BOND columns from Restek are exceptionally robust due to optimized manufacturing and deactivation steps that greatly reduce particle release. These proprietary techniques result in an extremely stable porous layer. As shown in Figure 1, the Rt®-Silica BOND column shows no visible shedding of particles or peeling of the coating layer. In comparison, the non-Restek® PLOT column in the figure exhibits uneven coating as well as areas where the particles have completely detached from the column wall. The exceptional stability of Rt®-Silica BOND columns—in combination with their high loadability, inertness, and consistent selectivity—make these columns the best choice for the analysis of light hydrocarbons, sulfur gases, and halocarbons.

Minimize Downtime with Virtually Particle-Free PLOT Column Performance

The nearly particle-free nature of Rt®-Silica BOND columns can be demonstrated by a particle-generation experiment in which a column is temperature- and pressure-ramped multiple times. Changes in temperature cause changes in pressure, which can result in particle shedding with conventional PLOT columns. The free particles generate large spikes when they hit the flame ionization detector (FID), which interferes with quantification. Figure 2 shows that no particle spikes were generated when this experiment was carried out on a brand new Rt®-Silica BOND column (Figure 2). The highly stable nature of an Rt®-Silica BOND column improves lab productivity by greatly reducing the particle shedding that can interfere with quantification and result in more frequent maintenance to replace obstructed FID jets and damaged valves.

Figure 1: Traditional non-Restek® PLOT columns (middle) have an uneven coating of particles that can shed, fouling instrument parts. Rt®-Silica BOND columns (top) have a very fine porous layer with no visible particles and look very similar to wall-coated open tubular columns (bottom).

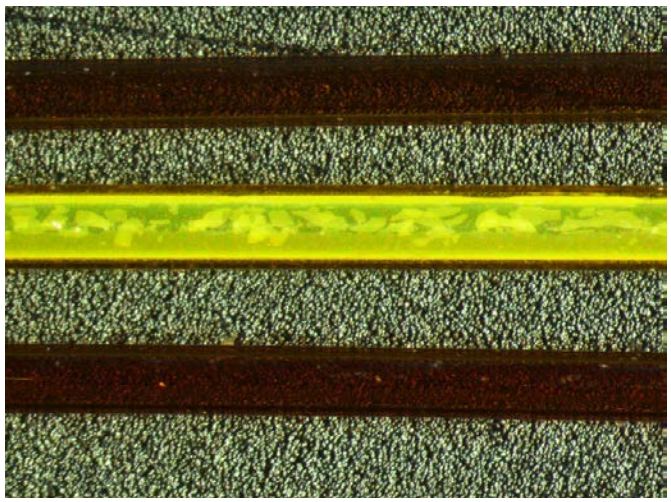
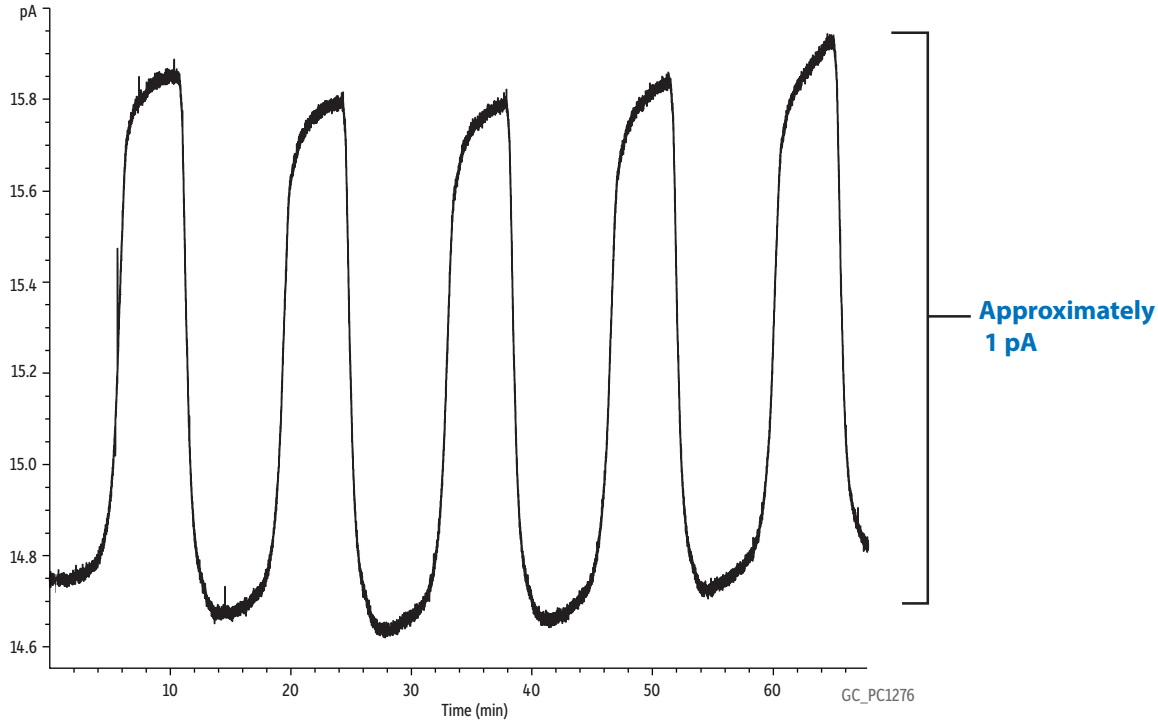
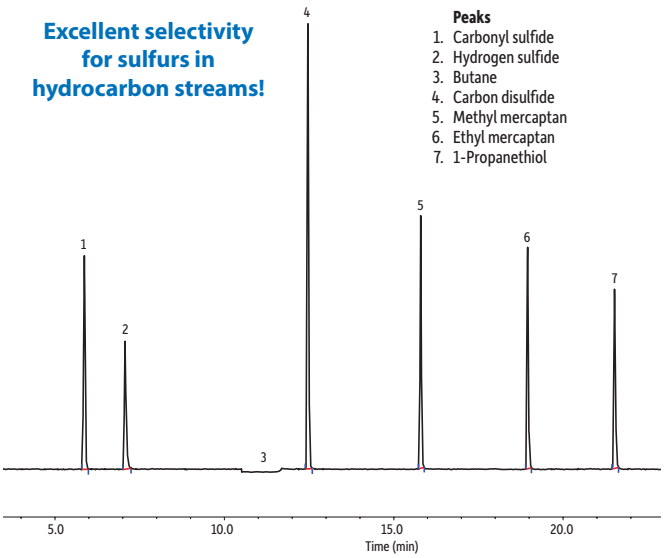


Figure 2: The Rt®-Silica BOND PLOT column shows no large particle spikes, even with temperature and pressure variation.



Column: Rt®-Silica BOND, 30 m, 0.32 mm ID (cat.# 19785); **Injection:** split (split ratio 35:1); Liner: Sky® 2.0 mm ID straight inlet liner (cat.# 23313.1); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 50 °C to 250 °C at 35 °C/min (hold 5 min) to 50 °C at 70 °C/min; **Carrier Gas:** He, constant flow; Linear Velocity: 114 cm/sec; **Detector:** FID @ 260 °C; Make-up Gas Flow Rate: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 400 mL/min; Data Rate: 10 Hz; **Instrument:** Agilent 7890A GC

Figure 3: Sulfur Compounds in Butane.



Column	Rt®-Silica BOND, 30 m, 0.32 mm ID (cat.# 19785)
Sample	
Conc.:	6 ppm in 100% butane
Injection	sample valve
Sample Loop Vol.:	250 µL
Inj. Temp.:	250 °C
Oven	
Oven Temp.:	40 °C (hold 5 min) to 200 °C at 10 °C/min (hold 8 min)
Carrier Gas	He, constant flow
Flow Rate:	2 mL/min
Detector	PFPD from OI Analytical @ 250 °C
Element Mode:	sulfur
Instrument	Thermo Trace GC
Notes	This valve/loop injection employed a split injection technique. Split flow was set to 40 mL/min.
Acknowledgement	Chromatogram courtesy of Jean-Louis Brix and Joeri Vercammen (Global Analyser Solutions, Belgium)

Versatile Column for Many Applications

The new Rt®-Silica BOND column combines the retention, capacity, and selectivity of traditional PLOT columns with virtually particle-free performance and outstanding water resistance. Since water has only a minimal impact on retention, water-containing samples can be analyzed without thermal conditioning between analyses. The bonded silica surface provides excellent retention for light hydrocarbons, permanent gases, and halocarbons, allowing for easy analysis of impurities in light hydrocarbon streams. In addition to light hydrocarbon analysis, the Rt®-Silica BOND column is especially selective for sulfur compounds. Figure 3 illustrates the good separation of sulfur compounds that can be achieved in butane.

In conclusion, the Rt®-Silica BOND column gives you the retention and capacity you need from PLOT columns, along with good water resistance and virtually particle-free operation. This provides the selectivity and stability necessary for the highly reproducible analysis of hydrocarbons, sulfur gases, and halogenated compounds. For additional applications, visit **www.restek.com/ADV1517**

Rt®-Silica BOND Columns (fused silica PLOT)

Description	temp. limits	cat.#
15 m, 0.32 mm ID	-80 to 260 °C	19784
30 m, 0.32 mm ID	-80 to 260 °C	19785
60 m, 0.32 mm ID	-80 to 260 °C	19786

Innovators in Chromatography

A continuing series of guest editorials contributed by collaborators and internationally recognized leaders in chromatography.

The Role of Selectivity in Liquid Chromatography Method Development

By Kevin A. Schug, Ph.D.

Dr. Schug is an Associate Professor and Shimadzu Distinguished Professor of Analytical Chemistry in the Department of Chemistry and Biochemistry at The University of Texas at Arlington. He specializes in the application of modern sample preparation, chromatography, and mass spectrometry techniques for trace qualitative and quantitative determinations from complex mixtures. He is also active in drug discovery, protein analysis, and environmental assessment.

The name of the game in chromatography is the separation of chemical compounds. The resolution of one analyte from another in a chromatographic separation is determined by three main factors: efficiency, selectivity, and retention. The interplay of these is described by the master resolution equation,

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_2}{1 + k'_2}\right) \quad (1)$$

where N is the number of theoretical plates (a measure of efficiency), α is selectivity, and k'_2 is the capacity factor (or retention factor) for the later eluting peak of the analyte pair of interest. Incidentally, in some forms of the master resolution equation, an average capacity factor k'_{avg}, calculated from the retention of both analytes, is used in the third term. As we are largely considering a pair of closely eluting analytes, the difference between k'_2 and k'_{avg} would be minimal. The magnitude of contributions of each of the three terms in Equation 1 to resolution varies, but the maximization of each term (without the complete disregard of the other two) will help yield the separation of analytes of interest ($R_s \geq 1.5$ is the target value for baseline separation).

Here, we focus on the selectivity term. Selectivity is defined in Equation 2 as

$$\alpha = \frac{k'_2}{k'_1} \quad (2)$$

It is the ratio of capacity factors for two chromatographic peaks. Conceptually, a capacity factor is the ratio of the amount of time an analyte spends in the stationary phase to the amount of time it spends in the mobile phase. Since all analytes spend the same amount of time in the mobile phase (equal to the dead time t_0), selectivity is the ratio of the amount of time the later eluting analyte spends in the stationary phase relative to that of the earlier eluting analyte. While the mobile phase composition in liquid chromatography can be varied to encourage an overall greater or lesser retention, the primary factor controlling selectivity is the ability of the stationary phase to differentially interact with each analyte. The primary means to alter selectivity in a chromatographic separation is to change the stationary phase or the mode by which analytes interact with the stationary phase.

While different separation modes (e.g., reversed phase [RP], hydrophilic interaction [HILIC], aqueous normal phase [ANP], normal phase [NP], etc.) can be used to affect the ways that analytes interact with a given stationary phase, we confine ourselves here to discussions on RP separations. Virtually every chemistry student has experience in RP separations—most likely focused on generic separations using an octadecylsilyl (C18-bonded silica gel) bonded phase. The first thing to note is that all C18 phases are not created equal. Changes in the underlying support chemistry, the way bonded groups are attached to the support, and the ways potentially deleterious interactions with residual silanol groups are shielded, significantly affect the retention of different analytes. For example, amine-containing compounds often exhibit significant tailing in chromatograms if they can interact with silanol groups. The strategy is to induce a uniform dominant interaction mode between the analyte and the stationary phase so that nicely symmetrical peaks are observed. For a typical C18 phase, the dominant interaction is induced by the hydrophobic effect. Significant differences in the hydrophobic content in chemical structures allow the C18 phase to exert selective interactions with each analyte and, assuming adequate retention and good efficiency are maintained, chromatographic resolution will result.

Complex mixtures will contain a multitude of chemical compounds that possess variable physicochemical properties. Oftentimes, the chromatographer is concerned with the qualitative and quantitative speciation of multiple analytes from a single class (e.g., polyphenols, drugs and their metabolites, steroids, etc.). If each compound has a different molecular weight, one might be able to bypass the need for chromatographic resolution of all components of interest by using a selective detector, such as a mass spectrometer. However, a mass spectrometer cannot directly differentiate compounds that have the same mass, and many analytes in a class of compounds may simply be isomers, which have the same elemental formula. While it is possible to use some tandem mass spectrometry approaches to differentiate coeluting isobaric compounds, the most reliable means by which to differentiate them for speciation would be to chromatographically resolve them prior to detection. A generic C18 phase may not provide sufficient selectivity to accomplish this task.

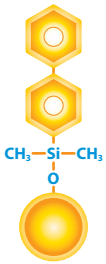
Those who move beyond college course-based laboratory exercises will quickly learn that there are other stationary phases available to impart additional selectivity in reversed-phase separations. Recent moves to alter support chemistries, including the use of superficially porous particles, have a major impact on efficiency of separations. However, to impact changes in selectivity, more important are changes in the chemistry of moieties bonded to these supports. Different manufacturers offer a milieu of alternatives that can range from the incorporation of polar units imbedded in the C18 chain or the bonding of different functional units all together. A favorite question I ask my senior-level instrumental analysis class is, "How can a cyano-bonded phase be used in both NP and RP separation modes?" The cyano phase is ideal for NP separations where a polar stationary phase is paired with a nonpolar mobile phase. However, in reversed-phase mode, this polar phase can impart vastly different retention interactions to more polar analytes compared to a C18 phase. This can cause large changes in elution order for a mixture of analytes because the cyano group provides a vastly different selectivity, and it is still effective for use in RP mode with a polar mobile phase. Similarly, use of phases that incorporate polar groups embedded somewhere along a C18 chain enable hydrogen-bonding interactions to assist in selective retention of different compound classes. Care should still be taken that these interactions are uniform and do not impart poor peak shape due to non-uniformity of chromatographic separations (similar to silanol effects), but for certain classes these additional interaction sites can be the difference between separation or coelution. Available now are also biphenyl phases which, in the presence of the right mobile phase, exert pi-interactions that can improve selectivity and retention for aromatic analytes. Interestingly, a biphenyl phase will exert these interactions in the presence of an aqueous methanolic mobile phase, but in the presence of acetonitrile, which itself has a strong pi-character, the phase will behave more like a C18. The change in selectivity can be quite dramatic.

The chromatographer's toolbox is ever expanding. Sometimes this can be overwhelming. Manufacturers have given different generic (and sometimes difficult to interpret) names to the different stationary phase supports and bonded phases they use to create their products. Luckily, they also spend a great deal of time and effort providing educational materials to guide the choice of the proper phase for different applications. Even so, one should always go back to the master resolution equation to reason the underlying fundamentals that will eventually yield separation of target compounds of interest. Chemists and biochemists will never stop creating new chemical compounds, and we are still figuring out the chemical diversity provided by nature. Thus, analytical chemists will always have a job in characterizing new analytes or determining their presence in various systems. It is a good thing that there are a lot of choices in the tools that one can use to accomplish these tasks.

Raptor™ LC Columns

SPP speed. USLC® resolution.
A new species of column.

The Raptor™ Suite of Innovative Reversed-Phase Columns



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- Allows the use of simple, MS-friendly mobile phases.
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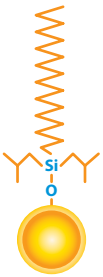

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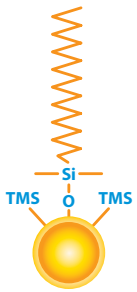
- Well-balanced retention profile.
- Endures low-pH mobile phases without sacrificing retention or peak quality.



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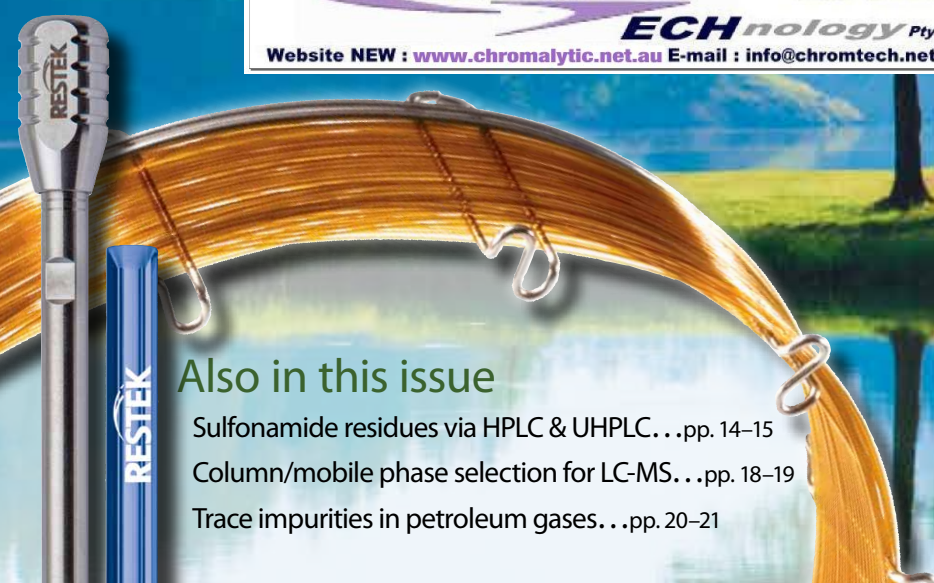
New Approaches for Increasing Analytical Sensitivity

- 1,4-dioxane at 5.0 ppt in water via large volume injection in an unmodified splitless GC inlet…pp. 6–7
- Lower detection limits without dilution using extended calibration range for semivolatiles…pp. 8–9
- QuEChERS with LC-MS/MS and GCxGC-TOFMS for comprehensive pesticide residue testing…pp. 12–13



Also in this issue

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Restek Connections

In This Issue

About Restek Corporation

A leading innovator of chromatography solutions for both LC and GC, Restek has been developing and manufacturing columns, reference standards, sample preparation materials, accessories, and more since 1985. We provide analysts around the world with products and services to monitor the quality of air, water, soil, food, pharmaceuticals, chemicals, and petroleum products. Our experts enjoy diverse areas of specialization in chemistry, chromatography, engineering, and related fields as well as close relationships with government agencies, international regulators, academia, and instrument manufacturers.

Reflections from the Bench

One night, I stopped by the Restek Innovations Laboratory to grab something from my office and stood for a moment in the dark. Looking out over the sea of LED lights and listening to the whine of pumps and cooling fans that is so familiar to GC and LC chemists around the world, I was reminded of my time working in an environmental lab. When the work was done and the instruments were up and running, I would shut off the lights and reflect for a moment on the day. But, my trip down memory lane was interrupted by the sound of an autosampler moving a vial into position—most likely Chris Rattray's instrument running a calibration curve for 1,4-dioxane by LVSI (page 6) or a semivolatile analysis with an extended calibration range (page 8). After all, with the aid of autosamplers, the lab never sleeps. Case in point, this *Advantage* is packed full of data generated at all hours of the day and night.

Our latest issue brings you a wide breadth of applications, like the ones mentioned above, produced by dedicated, passionate chemists like yourself. Julie Kowalski, Sharon Lupo and Amanda Rigdon use LC-MS/MS techniques for work ranging from pesticide analysis to therapeutic drug monitoring. Rick Lake and Ty Kahler help you find the best LC-MS column, then use it to analyze sulfonamides. If you use a GC, Scott Grossman will shatter your perceptions of injection ports. We also explore matrix effects in complex samples both with a guest editorial and with Jack Cochran's and Julie Kowalski's discussion of pesticide recoveries using LC-MS/MS and GCxGC-TOFMS.

There's something for everyone in this *Advantage*. We hope it helps you reach that place where you can turn the lights off and enjoy the ambience of the laboratory.

Cheers!

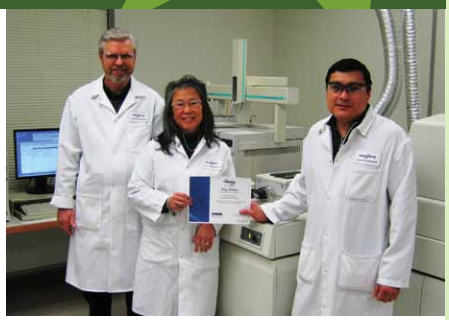
Chris English
Laboratory Manager,
Innovations Group

You Have Opinions... And We Want Them

We chemists are an opinionated bunch, so the odds are good that you have some thoughts about the *Restek Advantage*. Love it? Hate it? Want to see something different in the next issue? Maybe you have a response to one of our technical articles? Whatever you have to say, let's hear it! Email your comments to advantage@restek.com and you may even see them in an upcoming issue.

Another Restek Success Story:

Maxxam Analytics Group Receives Award After Switching to the Rtx®-Dioxin2 Column



Maxxam Analytics' HRMS team (left to right): Owen Cosby, Kay Shaw, and Angel Guerrero.

Maxxam Analytics recently presented a Kaizen award to their High Resolution Mass Spectrometry (HRMS) Department at the Mississauga laboratory in Ontario. The award recognized process improvements made possible by switching to a Restek Rtx®-Dioxin2 column to increase instrument capacity.

The Mississauga lab analyzes drinking water for 2,3,7,8-TCDD only using EPA Method 1613. They had been analyzing these short-list samples on the same instrument used for full-list PCDD/PCDF and PCB congeners, which limited their capacity. Maxxam had also confirmed the presence of 2,3,7,8-TCDF using a different column on another instrument. Since the Rtx®-Dioxin2 column provides isomer specificity for both 2,3,7,8-TCDD and 2,3,7,8 TCDF and has high temperature stability, the HRMS group explored using it for both 2,3,7,8-TCDD and 2,3,7,8-TCDF.

"Using the Rtx®-Dioxin2 column… we shortened run times, reduced instrument downtime and column changes, and increased instrument capacity for our full-list samples."

-Owen Cosby, Maxxam Analytics

By moving to an Rtx®-Dioxin2 column (cat.# 10758), they optimized the TCDD-only analysis and reduced run time from 50 to 30 minutes! (EPA 1613 requires a minimum retention time for the labeled 1,2,3,4-TCDD of 25 minutes, so results were close to ideal.) The analysis time for the TCDF confirmation analysis was not significantly reduced, but run cycle time was decreased by taking advantage of the column's 340 °C thermal stability, resulting in lower estimated detection limits and less bleed compared to the columns they had used previously. In addition, the higher maximum programmable temperature allows analysts to use high-temperature holds and reduce the potential for carryover contamination.

Since the lab was able to run both the TCDD-only and TCDF confirmation analyses on the Rtx®-Dioxin2 column, they were able to use the same instrument for both analyses, allowing more full-list dioxin and PCB samples to be analyzed on the other instrument. Learn more about Rtx®-Dioxin2 columns at **www.restek.com/dioxin2**

Do you have a Restek success story to share?

E-mail **advantage@restek.com** or call your Restek representative!

Questions From You

Our Technical Service specialists field an astounding variety of questions from our customers. Today's featured topic is a Restek innovation that extends the life of your inlet seal: the reversible Flip Seal™ inlet seal.

Q: Are there recommended GC inlet liner types for use with Flip Seal™ inlet seals?

A: Restek recommends a 4 mm ID Sky™ single taper liner with wool (cat.# 23303.1) for splitless injections and a 4 mm ID Sky™ Precision® liner with wool (cat.# 23305.1) for split injections. The thoroughly deactivated Sky™ wool provides excellent sample homogenization during either splitless or split injection, which increases repeatability and accuracy. In addition, wool keeps liquid sample from being deposited on the inlet seal, where contact with hot metal can degrade thermally sensitive compounds, or where less volatile, higher molecular weight compounds of interest can be lost. Wool also protects the GC column from non-volatile sample "dirt," preserving the column's chromatographic performance, especially for difficult to analyze compounds.

We just released a full FAQ on the Flip Seal™ inlet seal! The answers to all of your questions can be found at **www.restek.com/flipFAQ**

- **Jack Cochran**
Director of New Business & Technology

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Restek is Expanding!

In the past year, we were fortunate enough to welcome dozens of talented employee-owners to Restek as we continue to grow and fill newly created positions. We wanted to specifically highlight a few of them here since you will likely meet them at events, talk to them on the phone, or read one of their articles in this issue. We're looking forward to working with them and developing new analytical solutions for you!

Scott Adams | GC Accessories Product Marketing Manager
Eisho Beythaji | Pacific Northwest Field Sales Representative
Paul Connolly | LC Product Marketing Manager
Chris Denicola | LC Market Research Manager
Thi Do | Southwest Field Sales Representative
Jason Herrington | Air Innovations Chemist
Tim Hines | VP of Operations
Ravindra Rane | New England Field Sales Representative
Chris Rattray | Environmental Innovations Chemist
Nancy Schwartz | Technical Service Specialist
Charles "Chas" Simons | Technical Service Manager
Trent Sprenkle | Corporate Account Representative

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Hot Topics

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Pollution, Pansteatitis & Dead Crocs

Photo by Jack Cochran

South Africa is home to an abundance of impressive wildlife, including a large population of Nile crocodiles in Kruger National Park. Unfortunately, these reptiles have recently experienced massive die-offs due to pansteatitis, which hardens body fat and renders it unavailable as an energy source during metabolism.

The problem is complex, but pollution from PCBs, pesticides, industrial chemicals, and pharmaceuticals is suspected to be a contributing factor. Using GCxGC-TOFMS, Roger Dixon of the South African Police Service recorded approximately 1,600 anthropogenic organic compounds in the waters of the Olifants River within Kruger Park. Additional stressors may include increased sediment, restricted water flow, and algal blooms related to the Massingir Dam upstream in neighboring Mozambique.

The Consortium for the Restoration of the Olifants Catchment (CROC) hopes to slow the disappearance of crocodiles from the park by improving water quality, and our own Jack Cochran is keeping close tabs on this dire situation. For links to related sites and updates, visit **blog.restek.com** and search "Kruger."

Fast, Definitive Data for BAC Testing

New Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns give you definitive data in a fast, 2-minute analysis. Optimized column selectivities guarantee baseline resolution of ethanol, internal standards, and frequently encountered interferences while robust column chemistry ensures longer column lifetime and exceptional accuracy. Every one of these new BAC columns is thoroughly quality tested, and they are ideal for dual-column confirmation required when using GC-FID.

We also now offer BAC resolution control standards with either *tert*-butanol or 1-propanol internal standard. These check mixes are used to verify the retention time for each compound normally included in a blood alcohol test as well as to confirm that the analytes are well resolved and do not interfere with one another.

New Rtx®-BAC Plus 1 and Rtx®-BAC Plus 2 columns and check mixes provide reliable, consistent results quickly, allowing increased sample throughput for blood alcohol testing.

You can find them all at
www.restek.com/bacplus

Coming Soon to a City Near You!

Tradeshows, symposia, and conferences are great ways for us to meet you face-to-face and share our latest breakthroughs. Here are some of the upcoming highlights of our 2012 event tour:

Aug 26–31 | Dioxin | Cairns, Queensland, Australia

Aug 30–31 | UKIAFT | Belfast, Northern Ireland

Sept 30–Oct 3 | AOAC | Las Vegas, NV, USA

Oct 1–5 | COLACRO XIV | Florianópolis, Santa Catarina, Brazil

Oct 7–10 | ChromSAAMS 2012 | Dikhololo Game Reserve, South Africa

Oct 16–17 | Gulf Coast Conference | Galveston, TX, USA

Nov 12–15 | EAS | Somerset, NJ, USA

Consult **www.restek.com/events** for more information and be sure to pay us a visit!

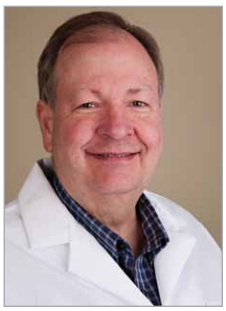
More Labs Required to Source CRMs

An increasing number of laboratories worldwide are being required to use certified reference materials (CRMs), which can only be manufactured and QC tested at an ISO-accredited lab. The U.S. Department of Defense insists on them, as do numerous other government agencies across North America, Europe, and Asia. UKAS and A2LA also mandate you use CRMs to gain ISO accreditation. In just a few years, CRM requirements have spread at an incredible rate, so if you haven't been affected yet, you may be soon.

Transitioning to CRMs doesn't need to be difficult or costly. We are proud to announce that Restek's reference standard manufacturing and QC testing laboratories in Bellefonte, PA, are ISO Guide 34 and 17025 accredited! That means you can buy the same Restek reference standards you trust for the same price while satisfying CRM regulations. And, our custom formulations are also covered!

Even if you are not required to use CRMs, you can still benefit from the outstanding product quality and customer service needed to meet strict ISO guidelines. Learn more about our quality credentials and to view certificates (including scopes of accreditation) at **www.restek.com/iso**

Brian Jones Honored With Plenary Talk at ISCC / Riva 2012



If you didn't make the trek to Italy for the 36th International Symposium on Capillary Chromatography (ISCC) / Riva 2012, you missed an enlightening talk by Restek Senior Research Chemist Brian Jones. He offered attendees a rare, behind-the-scenes look at an exciting surface science technology that holds the promise of creating well-characterized and exceptionally inert surfaces, as well as being used in many other potential applications. Still in development at Restek's R&D lab, this patent-pending technique greatly improves the chemical and physical properties of surfaces compared to current state of the art, making them better suited for tomorrow's challenges of steadily decreasing detection limits and increasing sample complexity.

We wanted not only to recognize Brian, Valerie Strom, Tom Kane, Scott Grossman, and the rest of the team for their impressive work, but also to congratulate Brian for being honored with the invitation to speak at Riva!

Restek Sponsors Multidimensional Chromatography & GCxGC Workshop

Photo by Ray Clement, Ontario Ministry of Environment

The speakers at this year's MDGC workshop.

Earlier this year, we attended the 3rd Multidimensional Chromatography and GCxGC Workshop at the Ontario Ministry of the Environment (MOE) in Ontario, Canada. Three of our chemists—Jack Cochran, Julie Kowalski, and Michelle Misselwitz—were privileged to speak due to their extensive work with GCxGC.

Initially hosted at the Centers for Disease Control (CDC) in Atlanta, Georgia, USA, this growing event serves as a means for international GCxGC experts to collaborate on cutting-edge techniques. Jack Cochran (Restek), Eric Reiner (MOE, front center in blue shirt above), Frank Dorman (The Pennsylvania State University), Jef Focant (University of Liège), and Don Patterson, Jr. (CDC) were instrumental in organizing the inaugural meeting and producing the first publication on using GCxGC-TOFMS for chlorinated dioxin and furan analysis. Since then, Eric Reiner deserves the bulk of the credit for pulling this grassroots event together. Having 150+ attendees at a word-of-mouth workshop is a testimony to the heightened interest in multidimensional separations and Eric's push for it!

For a speaker list or to request Restek's presentations from this year's meeting, go to **blog.restek.com** and search for "MOE."

Search Restek Chromatograms Online!

The chromatograms in this issue are just the beginning. Our Innovations Lab, partners, and even customers churn out a steady stream of top-notch applications that you can search and filter to find the exact chromatogram you need. Just recently, we released:

- QuEChERS Extract of Cannabis on Rxi®-17Sil MS and Rxi®-5ms by GCxGC-TOFMS (GC_FF1207)
- Therapeutic Drug Monitoring Compounds in Urine by LC-MS/MS on Ultra Biphenyl (LC_CF0535) – *Featured on page 17!*
- *p*- and *m*-Xylenes in Gasoline by GCxGC on Rtx®-DHA-150 and Stabilwax® (GC_PC1226)
- Separation of Ethanol and Aromatics from Paraffins in Gasoline with GCxGC on Rtx®-DHA-150 and Stabilwax® (GC_PC1227)
- Short-Chain Amines on Rtx®-Volatile Amine (GC_PC1243)
- TO-15 65 Component Mix on Rxi®-624Sil MS (30 m) (GC_AR1148)

You'll find these, along with hundreds of other chromatograms covering a wide range of markets, at **www.restek.com/chromatograms**

Lowering Detection Limits for 1,4-Dioxane in Drinking Water

Using Large Volume Injection in an Unmodified Split/Splitless GC Inlet

By Chris Rattray, Jack Cochran, and Chris English

- Perform large volume splitless injection with an unmodified Agilent-style split/splitless GC inlet.
- Reliably detect 1,4-dioxane down to 5.0 ppt in drinking water.
- Improve quantitative accuracy by introducing more analyte to the detector.

Global concern over the carcinogenic potential of 1,4-dioxane, along with its identification as a Group 2B compound by the World Health Organization's International Agency for Research on Cancer (IARC), has led to increased regulatory interest in this compound. For example, as part of Unregulated Contaminant Monitoring Rule 3 (UCMR3), the U.S. EPA is requiring increased monitoring of 1,4-dioxane in drinking water and has revised the $1x10^{-6}$ cancer risk assessment level* down to 0.35 µg/L. As a result, the proposed minimum reporting level (MRL) for 1,4-dioxane as part of UCMR3 is 0.07 µg/L [1].

Concurrent solvent recondensation–large volume splitless injection (CSR-LVSI), a technique described by Magni and Porzano [2,3], can be advantageous when trying to analyze trace-level contaminants in clean matrices like drinking water. Since more target compound is introduced onto the analytical column, detectability is improved; however, a specialized injection port, such as a PTV, is generally required for LVSI [4]. Building on work by chemists at Thermo Scientific, our lab has been exploring the use of CSR-LVSI with a completely unmodified Agilent-style inlet. We use a fast autosampler injection with liquid sample band formation in a liner containing glass wool, a retention gap press-fitted to the analytical column, and a starting GC oven temperature below the boiling point of the solvent (see next page for instrument setup and analytical conditions). Previously, we have successfully analyzed a wide variety of compounds, including PAHs, BFRs, organochlorine pesticides, and semivolatiles, using this technique (see blog.restek.com and enter "LVSI" in search). Here we assess its potential to lower detection limits for 1,4-dioxane in drinking water.

Evaluating CSR-LVSI With a Standard Splitless Inlet

To determine if CSR-LVSI with an unmodified split/splitless inlet was compatible with the volatile compounds in this application, linearity and interferences were assessed. Calibration curves at levels well below typical minimum detection limits displayed excellent correlations across a wide range (R^2 = 0.9998 for 1 to 1,000 pg/µL [10 to 10,000 pg on column] and R^2 = 0.9996 for 0.5 to 50 pg/µL [5 to 500 pg on column]). Calibration levels and equivalent concentrations are shown in Table I for the lowest curve, which was used to quantify recoveries from extracted drinking water samples.

While results for injected standards were quite promising, this analysis is very sensitive to interference from co-extracted material because the SIM ions are at a relatively low mass to charge ratio. Although CSR-LVSI introduces more matrix onto the column than a typical injection, no interferences for 1,4-dioxane were observed. As shown in the analysis of a fortified drinking water extract in Figure 1, 1,4-dioxane is chromatographically separated from any interferences.

Using CSR-LVSI to Lower Detection Limits

Having established that CSR-LVSI with an unmodified GC inlet is an appropriate technique, we wanted to assess its potential for lowering detection limits. The 10 µL CSR-LVSI in Figure 1 (approximately 5 pg on-column) produced a signal-to-noise ratio of 16 for the quantitation ion (m/z 88), which is above the threshold of 10. In contrast, when 1 µL of the same extract was injected, the resulting peak is barely distinguishable from the noise and the confirmation ion cannot be seen (Figure 2). Ultimately, the improved signal-to-noise ratios obtained using CSR-LVSI resulted in recoveries of 1,4-dioxane and surrogate 1,4-dioxane-d8 that were within the expected range (Table II) and that matched published method development data very well [4].

Table I: Calibration curve (0.5–50 pg/µL).

Level	Prepared Standard (pg/µL)	10 µL Injection On-Column Amount (pg)	Equivalent Concentration in 500 mL Samples (µg/L)
1	0.50	5.0	0.010
2	1.0	10	0.020
3	5.0	50	0.10
4	10	100	0.20
5	50	500	1.0

*A $1x10^{-6}$ cancer risk assessment level corresponds to the lifetime probability of one individual in an exposed population of one million developing cancer.

Figure 1: 1,4-Dioxane extracted ion chromatogram from a 10 µL CSR-LVSI of a 0.5 pg/µL fortified drinking water extract (5 pg on-column). Note that the 1,4-dioxane quantification ion (m/z 88) and confirmation ion (m/z 58) are fully separated from matrix interferences and good peak responses were obtained.

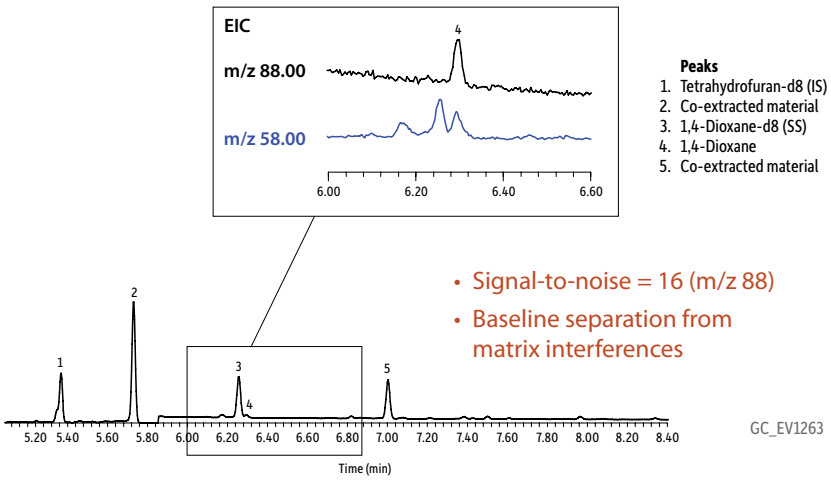


Figure 2: 1,4-Dioxane extracted ion chromatogram from a standard splitless 1 µL injection of a 0.5 pg/µL fortified drinking water extract (0.5 pg on-column). Peaks are barely distinguishable from background noise.

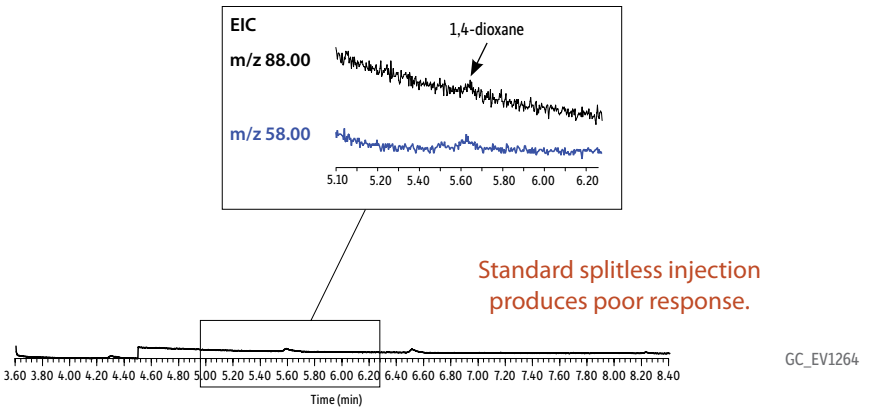


Table II: CSR-LVSI resulted in good recovery of both 1,4-dioxane and surrogate 1,4-dioxane-d8 from extracted fortified samples.

Matrix	Fortified Sample Conc. (µg/L)	Volume of Sample Extracted (L)	Theoretical Extract Conc. (pg/µL)	Recovery (pg/µL)	1,4-Dioxane % Recovery	Surrogate % Recovery
Bottled drinking water	0.0050	1.0	0.50	0.40	80	125
Bottled drinking water	0.20	0.50	10	9.2	92	102
Bottled drinking water	0.20	1.0	20	18	87	96
Reagent water	0.020	0.50	1.0	1.0	100	88
Reagent water	0.20	0.50	10	8.4	84	92
Reagent water	0.0	0.50	0.0	-	-	86

Summary

Concurrent solvent recondensation–large volume splitless injection (CSR-LVSI) with an unmodified Agilent-style split/splitless GC inlet is a viable approach for analyzing 1,4-dioxane in drinking water. While large volume injection usually involves specialized equipment, using it with a completely unmodified inlet provides a cost-effective way to meet ever decreasing detection limits.

For the complete version of this technical article, visit **www.restek.com/dioxane**

Instrument Setup for CSR-LVSI:

Column:
Rxi®-624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868) using Rxi® guard column 5 m, 0.25 mm ID (cat.# 10029) with universal angled Press-Tight® connectors (cat.# 20446-261)

Sample:
Extract of drinking water fortified at 0.5 pg/µL with 1,4-dioxane (cat.# 30287) and at 10 pg/µL with internal standard tetrahydrofuran-d8 (cat.# 30112) and surrogate standard 1,4-dioxane-d8 (cat.# 30614)

Injection:
10 µL splitless (hold 1 min); Liner: Sky™ 4 mm single taper w/wool (cat.# 23303.5); Inj. Temp.: 120 °C; Purge Flow: 80 mL/min

Oven:
35 °C (hold 1 min) to 120 °C at 12 °C/min (hold 1 min)

Carrier Gas:
He, constant flow, 1.4 mL/min; Linear Velocity: 30.556 cm/sec @ 35 °C

Detector:
MS, SIM mode

For complete conditions and SIM program, visit www.restek.com and enter GC_EV1263 in the search.

References

[1] U.S. EPA, Unregulated Contaminant Monitoring Rule 3. http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/index.cfm (accessed March 2, 2012).
[2] P. Magni, T. Porzano, Concurrent Solvent Recondensation Large Sample Volume Splitless Injection, J. Sep. Sci. 26 (2003) 1491.
[3] Patent No: US 6,955,709 B2.
[4] P. Grimmett, J. Munch, Method Development for the Analysis of 1,4-Dioxane in Drinking Water Using Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry, J. of Chromatographic Science 47 (2009) 31.

Quantify Semivolatiles Down to 0.5 ng On-Column by GC-MS

Using an Inert Inlet System and an Rxi®-5Sil MS Column to Extend the Calibration Range

By Chris Rattray

- Accurately quantify active semivolatiles down to 0.5 ng on-column using GC-MS.
- Extended linear range allows lower detection limits to be met, while minimizing dilution and reanalysis of high concentrations samples.
- Maintain critical separations with a fast 17 min analysis time.

Customers and regulatory agencies are increasingly requiring lower GC-MS detection limits for semivolatile organic pollutants. Extending the linear calibration range down below typical levels is the best way to accomplish this, while still minimizing the dilution and reanalysis of heavily contaminated samples. Analyzing semivolatiles, particularly active compounds, at sub nanogram on-column levels requires a highly inert GC system. First, an inert sample pathway results in tall, narrow peaks that improve detectability by maximizing signal-to-noise ratios. Second, the lack of reactivity reduces adsorptive losses of active analytes, which minimizes variation of the relative response factor (RRF) at low levels. As shown in the data reported here, lower detection limits for active semivolatile compounds can be achieved when the entire gas chromatographic system (liner, seal, and column) is highly inert.

Inert System Improves Response at Trace Levels

For this work, 143 semivolatiles listed in the extended EPA Method 8270, including Appendix IX compounds, were calibrated across a concentration range of 0.5-120 ng/µL. The 17-minute analysis shown in Figure 1 used an Agilent GC-MS (7890-5975C) equipped with a Siltek® deactivated EZ Twist Top® split/splitless inlet (cat.# 22178). A Sky™ inlet liner with wool (cat.# 23303), a Flip Seal™ inlet seal (cat.# 23411), and an Rxi®-5Sil MS column (30 m x 0.25 mm ID x 0.25 µm, cat.# 13623) were also used to ensure an inert sample path. The selectivity of the Rxi®-5Sil MS column separated critical isobaric pairs, such as the benzo[b]- and benzo[k]fluoranthenes, as well as aniline and bis(2-chloroethyl)ether.

The inertness of this system produces good peak shapes and responses even at 0.5 ng on-column for active compounds. This is particularly evident in a comparison of the responses of 2,4-dinitrophenol and 4-nitrophenol at different concentrations (Figure 2). While the relative decrease in 2,4-dinitrophenol response at lower concentration indicates some adsorptive loss is occurring, the peak response still exceeds method criteria by a factor of 5 (Table I).

Lower Detection Limits for Active Compounds

Chloro- and nitro- anilines and phenols are good indicators of system performance. They are highly reactive and the minimum performance criteria in the method are difficult to meet with a poorly deactivated column and liner. Tables I and II show the performance of these trou-

Table I: Nitroanilines and nitrophenols performance summary.

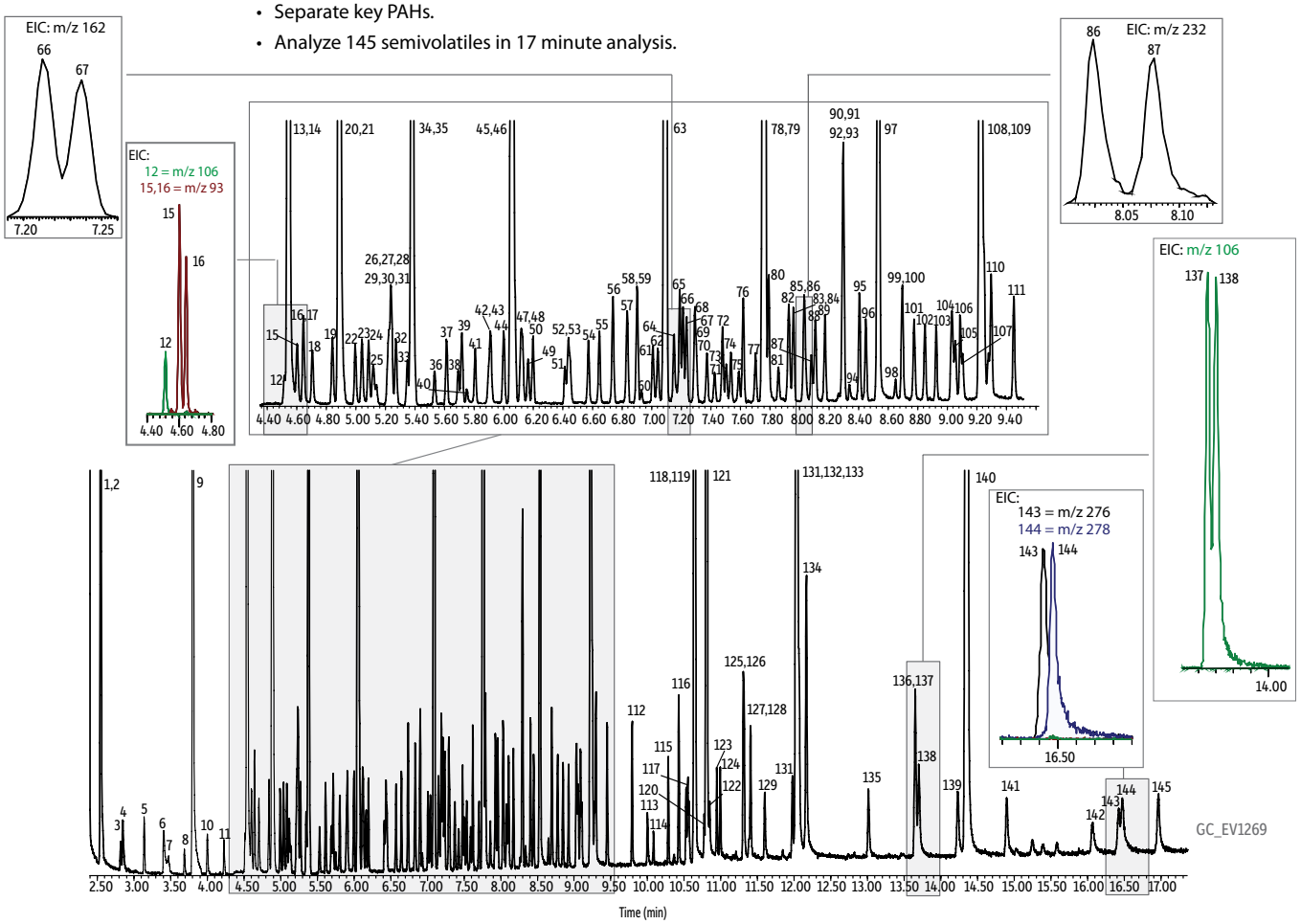
	RRF (0 .5 ng)	Minimum RF	Average RRF (0.5 – 120 ng/µL)	RRF RSD	Linear R^2
2-Nitrophenol	0.710	0.100	0.770	6.9%	0.9999
2-Nitroaniline	0.204	0.010	0.226	5.4%	0.9999
3-Nitroaniline	0.218	0.010	0.226	3.5%	0.9997
2,4-Dinitrophenol	0.055	0.010	0.176	42%	0.9992
4-Nitrophenol	0.234	0.010	0.254	8.0%	0.9914
4-Nitroaniline	0.433	0.010	0.424	3.9%	0.9995
4,6-Dinitro-2-methylphenol	0.119	0.010	0.237	28%	0.9999

Table II: Chloroaniline and chlorophenols performance summary.

	RRF (0 .5 ng)	Minimum RRF	Average RRF (0.5 – 120 ng/µL)	RRF RSD	Linear R^2
2-Chlorophenol	1.606	0.800	1.512	3.2%	0.9998
2,4-Dichlorophenol	1.157	0.200	1.155	2.9%	0.9995
4-Chloroaniline	0.468	0.010	0.456	6.3%	0.9971
4-Chloro-3-methylphenol	0.284	0.200	0.289	2.1%	0.9998
2,4,6-Trichlorophenol	0.400	0.200	0.415	4.4%	0.9999
2,4,5-Trichlorophenol	0.435	0.200	0.442	2.9%	0.9997
2,3,5,6-Tetrachlorophenol	0.327	0.010	0.377	9.3%	0.9987
2,3,4,6-Tetrachlorophenol	0.357	N/A	0.372	3.9%	0.9984
Pentachlorophenol	0.238	0.050	0.311	14%	0.9999

Figure 1: Extend the calibration range for difficult semivolatiles down to 0.5 ng on-column by using a highly inert analytical system. (total ion chromatogram of EPA Method 8270 and Appendix IX compounds)

- Quantify down to 0.5 ng on-column
- Separate key PAHs.
- Analyze 145 semivolatiles in 17 minute analysis.

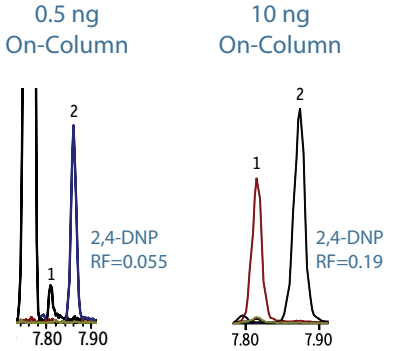


Column: Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623); **Sample**: 8270 MegaMix® (cat.# 31850), 8270 Benzidines mix (cat.# 31852), Benzoic acid (cat.# 31879), Revised B/N surrogate mix (cat.# 31888), Acid surrogate mix (4/89 SOW) (cat.# 31063), Revised SV internal standard mix (cat.# 31886), Appendix IX mix #1 (cat.# 31625), Appendix IX mix #2 (cat.# 31806); Diluent: Dichloromethane; Conc.: 0.5 µg/mL (IS/SS 20 µg/mL); **Injection:** 1 µL pulsed splitless (hold 0.59 min); Liner: Sky™ 4 mm single taper w/wool (cat.# 23303); Inj. Temp.: 270 °C; Pulse Pressure: 30 psi (206.8kPa); Pulse Time: 0.64 min; Purge Flow: 100 mL/min; **Oven:** 40 °C (hold 1 min) to 280 °C at 25 °C/min to 320 °C at 5 °C/min (hold 1 min); **Carrier Gas**: He, constant flow; Flow Rate: 1.2 mL/min; **Detector**: MS; Mode: Scan; Transfer Line Temp.: 280 °C; Analyzer Type: Quadrupole; Source Temp.: 276 °C; Quad Temp.: 150 °C; Electron Energy: 70 eV; Solvent Delay Time: 2.19 min; Tune Type: DFTPP; Ionization Mode: EI; Scan Range: 35-550 amu; Scan Rate: 5.36 scans/sec; **Instrument:** Agilent 7890A GC & 5975C MSD; **Notes:** 7890 Siltek®-treated EZ Twist Top® split/splitless injection port (cat.# 22178), Flip Seal™ dual Vespel® ring inlet seal (cat.# 23411); For peak identifications, visit www.restek.com and enter GC_EV1269 in the search.

blesome compounds at 0.5 ng on column relative to the method minimum, the average RF for the calibration range (0.5-120 ng on-column), and linearity evaluated by RRF RSD and linear regression.

Calibrations were also assessed for the full list of compounds. For the initial calibration (ICAL) as a whole to meet acceptance criteria, less than 10% of the individual compounds may have failing RSDs (or correlations, if alternative fit methods are used). When the peak response RSDs were evaluated over the entire calibration range for the full list of compounds, the average RSD was 8.7% and only 10 of the compounds tested had RSDs greater than 20%. Linearity results for both indicator and non-indicator compounds demonstrate that detection limits can be lowered for semivolatiles analysis by using a highly inert system that allows the lower end of the calibration range to be extended.

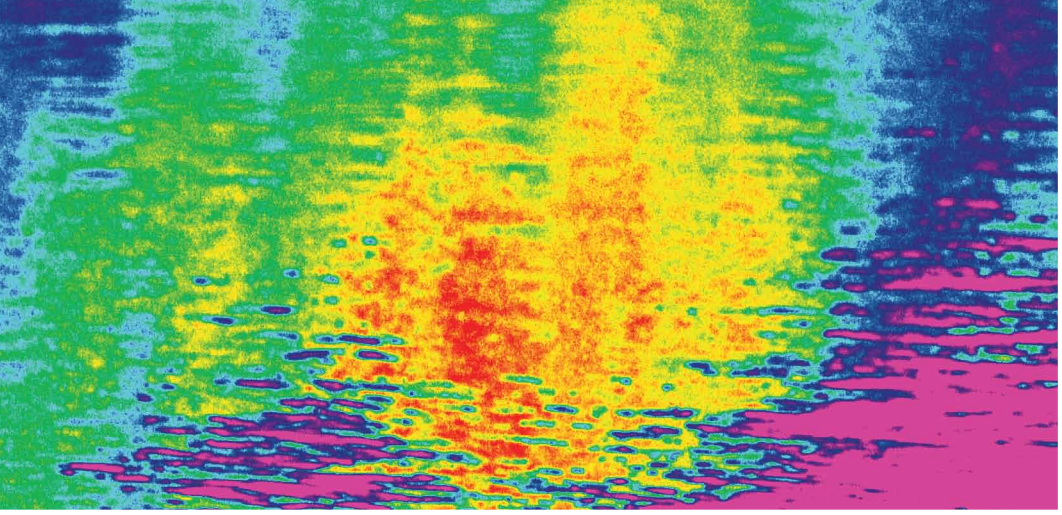
Figure 2: Response differential for 2,4-dinitrophenol and 4-nitrophenol.

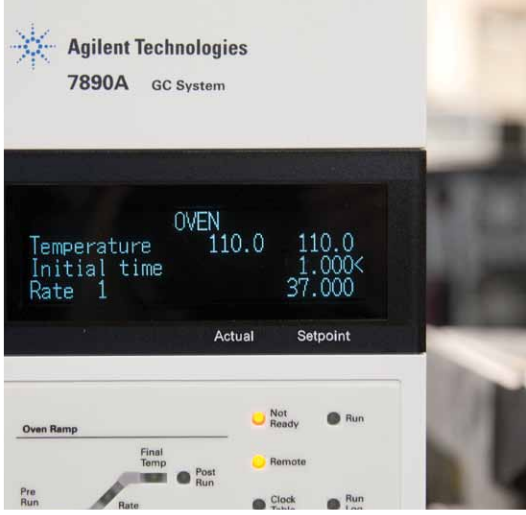


1. 2,4-Dinitrophenol (m/z 184)
2. 4-Nitrophenol (m/z 139)

See Figure 1 for analytical conditions.

For more environmental applications, visit **www.restek.com/enviro**





It's A Matter of Degrees, but Do Degrees Really Matter?

An Observation of GC Inlet Temperature Profile and Inlet-to-Inlet Temperature Variability

By Scott Grossman

- For some manufacturers, only a portion of the GC inlet is actually at the temperature setpoint; a significant thermal gradient exists both above and below this zone.
- The thermal profile of one GC inlet can vary from other similar inlets—and vary dramatically between different styles.
- Removal or damage to GC insulation can have a large effect on the inlet's thermal profile.

Injecting a liquid sample into a hot GC inlet is a dynamic and complex event. Of the many parameters that affect the success of an injection, inlet temperature is one of the most significant. Raising or lowering the inlet temperature setpoint can have a profound effect on how much sample is transferred onto the column depending on sample volatility and thermal sensitivity. But, once the inlet temperature is set, how much of the inlet is actually kept at that setpoint? Moreover, how might thermal profiles change between inlets?

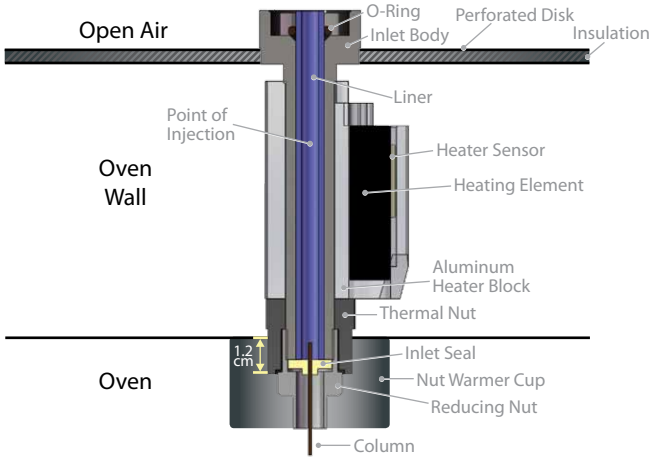
Temperature Varies Within and Between Similar Inlets

The motivation for this work came from a question about the actual temperature of an O-ring installed in an Agilent split/splitless inlet at a given inlet temperature setpoint. (See Figure 1 to identify the components of a GC inlet.) Instead of just measuring the temperature inside a liner near the O-ring's location, we used a thermocouple to measure temperature along the entire length of the liner at a constant inlet temperature setpoint of 250 °C. The resulting thermal profile confirmed that a temperature gradient exists within the inlet.*

In previous work (www.restek.com/hotseptum), we also discussed this gradient within GC inlets and noted that inlet thermal profiles can vary greatly between manufacturers, but would they vary between similar inlets from the same manufacturer? We checked another similar inlet to compare the thermal profiles and found that the second inlet exhibited a different thermal profile from the first. After measuring several more Agilent GC inlet temperature profiles, we found inlet-to-inlet variation in all cases, even in ostensibly identical inlets (Figure 2).

* For these experiments, we only measured the thermal profile of the liner inside the inlet, not the entire inlet.

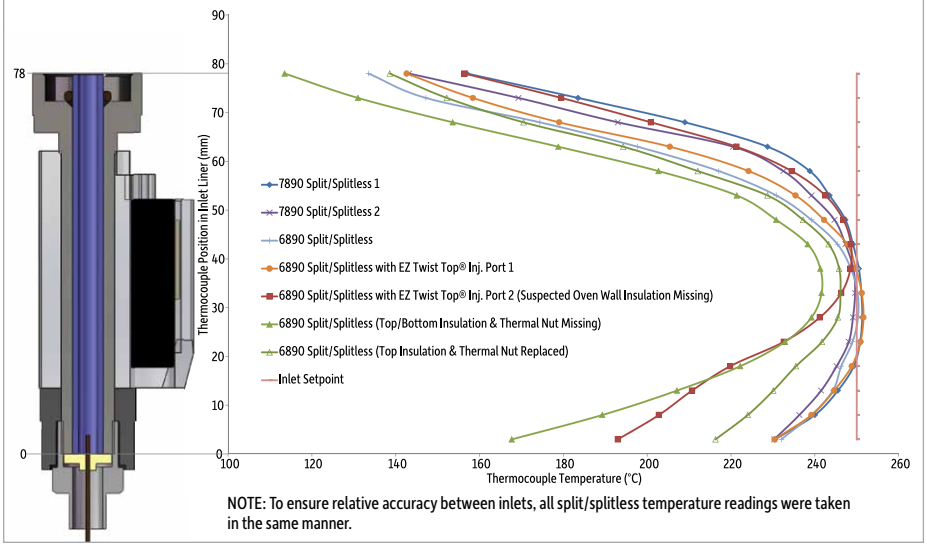
Figure 1: Considering how little of the GC inlet is actively heated by the heating element, it's no surprise a temperature gradient exists—especially if insulation is missing from the top or bottom.



Insulation is Crucial to Minimizing Temperature Variation

We did observe one split/splitless inlet with significantly lower temperatures at the top and bottom. After investigating, we discovered that the top ring of insulation, which sits just below the perforated disk of the Agilent 6890 split/splitless inlet weldment, was missing. Some of the insulation at the bottom of the inlet, along with the thermal nut, was also not installed. Simply placing insulation in the top cavity and installing the thermal nut caused the temperature of the inlet liner to more closely match the other inlets (Figure 2). This test was a valuable reminder of the need to carefully reconstruct the inlet whenever the insulation is disturbed.

Figure 2: A temperature gradient exists within a GC inlet, and temperature profiles can vary between similar inlets. These variations increase dramatically with the absence of insulation.



NOTE: To ensure relative accuracy between inlets, all split/splitless temperature readings were taken in the same manner.

Figure 3: Installing the nut warmer cup can help minimize the effects of oven temperature on the actual temperature of the inlet. (Inlet shown below was set to a constant 250 °C.)

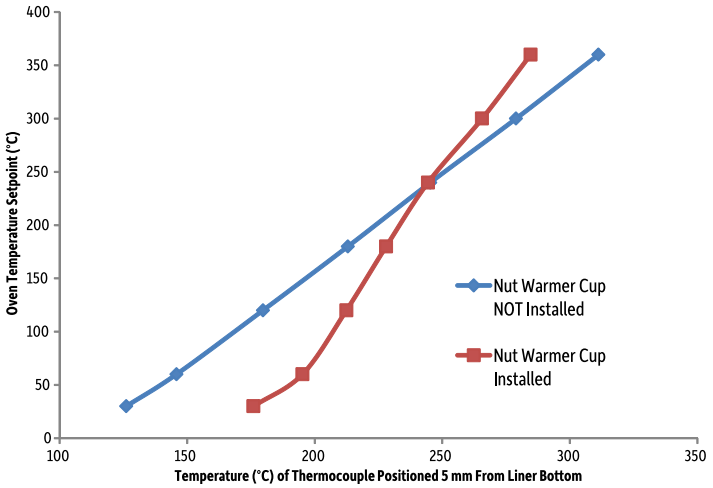
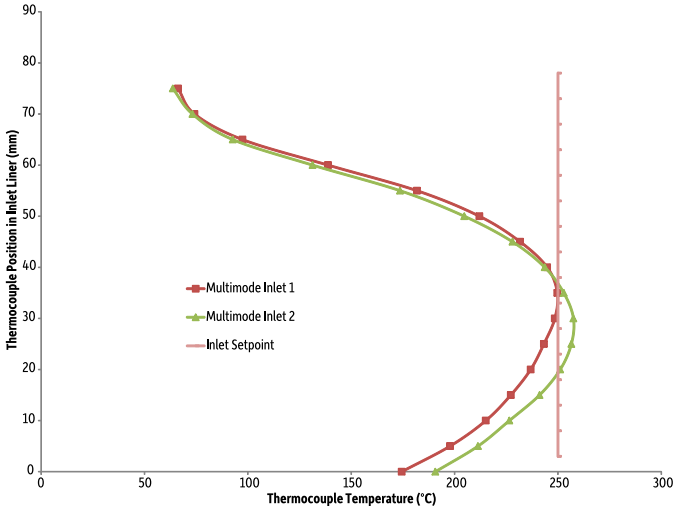


Figure 4: The Multimode Inlets (MMIs) we measured experienced almost twice the temperature drop (190 °C) of a standard split/splitless inlet between the inlet setpoint to the top of the liner.



When insulation is missing at the top of the inlet, the inlet loses heat to the open air; however, when insulation is missing at the bottom, the GC oven influences the temperature in both directions (Figure 3). Because column installation can be more challenging with the insulated nut warmer cup installed, analysts may be tempted to leave it in a drawer, but the effect on your inlet temperature can be significant.

Temperature Can Vary Drastically Between Dissimilar Inlets

The newly introduced Agilent Multimode Inlet (MMI) is said to be capable of performing both hot split and hot splitless injections like a normal split/splitless inlet. But, when we measured the thermal profiles for two MMI inlets, it was interesting to note how different the MMI thermal profiles were from a split/splitless inlet—a drop of over 190 °C from the setpoint to the top of the inlet as opposed to around 100 °C for the split/splitless inlets (Figure 4). This variation shows that changing equipment may also change your results, even if the equipment is nominally able to do the same analysis.

The Effects of Inlet Temperature Variations on Chromatography

As demonstrated here, thermal gradients exist within a single GC inlet, and temperature profiles can vary between similar, as well as between dissimilar, inlets. How do these variations affect the vaporization of a liquid sample (and, thus, the overall success of the analysis)? We answer these questions and offer details on our temperature data collection at

www.restek.com/TempEffects

Comprehensive Pesticide Residue Monitoring in Foods

Using QuEChERS, LC-MS/MS, and GCxGC-TOFMS

By Julie Kowalski[1], Jack Cochran[1], Jason Thomas[1], Michelle Misselwitz[1], Rebecca Wittrig[2]*, and André Schreiber[3]

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- One fast, simple QuEChERS extraction for a broad range of pesticides.
- Rxi®-5Sil MS and Rtx®-200 column selectivity and orthogonality promote good GCxGC separations.
- Ultra Aqueous C18 LC column retains and gives excellent peak shapes for small polar pesticides.

Pesticide residue analysis of food has traditionally been performed using GC, but there is increasing use of LC with tandem mass spectrometry (MS/MS). LC is favored for polar, less thermally-stable, less volatile, compounds. GC-MS is preferred for volatile, thermally-stable species, and pesticides that do not ionize well in electrospray or atmospheric pressure chemical ionization LC sources. With MS, complete chromatographic resolution of compounds is not always essential, as selected ions or selected reaction monitoring (SRM) transitions are used for pesticide identification and quantification. However, data quality can be improved through better retention and separation of components, especially for structurally similar pesticides and high-level matrix coextractives. In the work summarized here, we employed a comprehensive approach and analyzed QuEChERS extracts of a variety of foods for pesticides by both GCxGC-TOFMS and LC-MS/MS.

Food commodities were fortified with pesticides and processed using Q-sep™ QuEChERS extraction salts and dSPE tubes. QuEChERS (Quick–Easy–Cheap–Effective–Rugged–Safe) is a sample preparation approach developed by Anastassiades et al. [1] as a simple, rapid, effective, yet inexpensive, way to extract pesticide residues from fruits and vegetables, followed by a dispersive solid phase extraction (dSPE) cleanup of the extract. The foods chosen varied in water, fat, and pigment content, so the ruggedness of QuEChERS as well as the performance of GCxGC-TOFMS and LC-MS/MS could be assessed. Commodities tested were red bell pepper, cucumber, black seedless grape, spinach, lemon, raisin, and hazelnut. In this summary, we report data for the grape and lemon, the least complex and most complex of the matrices we assessed. Complete results are available at www.restek.com/comp-pest in the full application note.

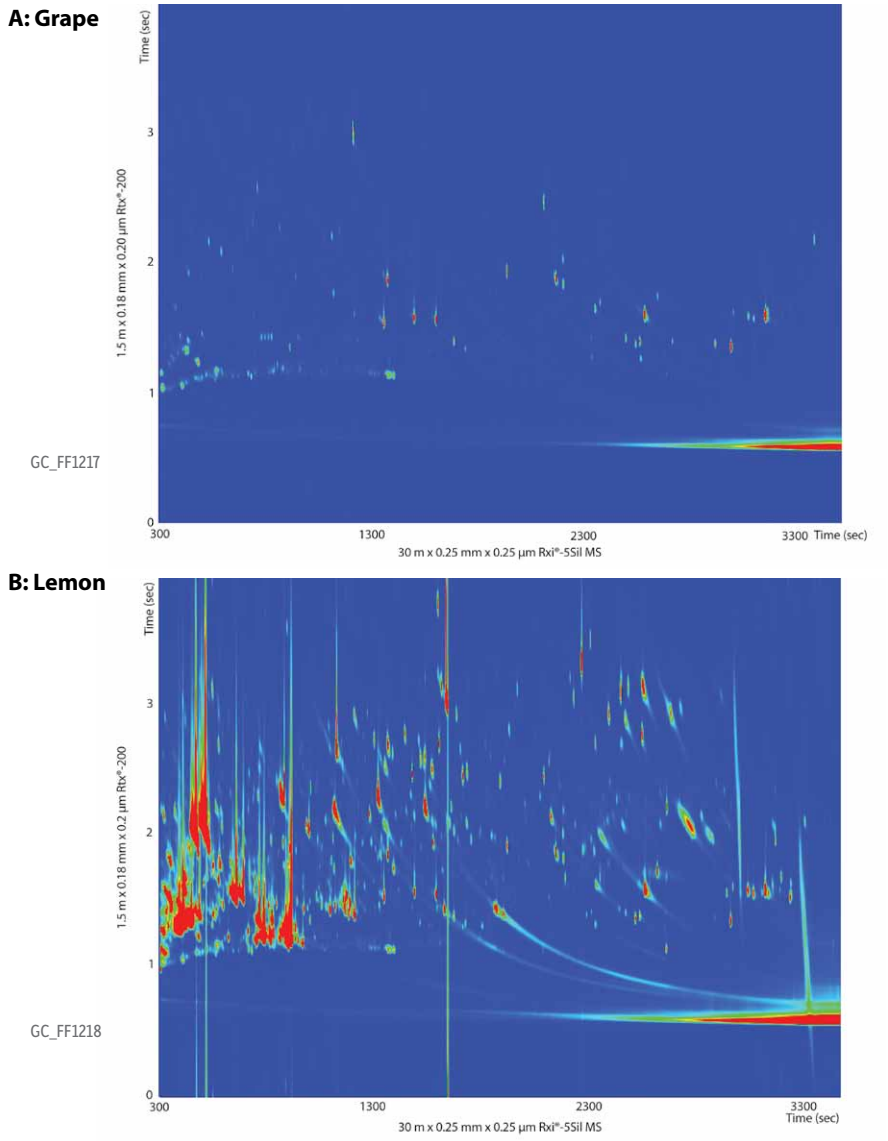
Column Selectivity and Multidimensional Techniques

We first assessed the complexity of different commodities by examining the total ion chromatogram (TIC) contour plots generated by GCxGC-TOFMS. It is clear from Figure 1 that lemon contains many more coextractives than grape, as demonstrated by the large number of intense (red) signals. While it should be possible to analyze QuEChERS grape extracts for pesticides by one-dimensional GC, multidimensional techniques (e.g., GCxGC-MS, GC-MS/MS, or LC-MS/MS) are necessary for samples as complex as lemon. Column selectivity is an important consideration in multidimensional techniques and the Rxi®-5Sil MS (cat.# 13623) x Rtx®-200 (cat.# 45001) column combination used here provided orthogonal separations that helped isolate target analytes from matrix interferences. Column selectivity is also important in LC-MS/MS methods because coelutions can be problematic if the analytes share MRM transitions. The Ultra Aqueous C18 column (cat.# 9178312) used for this work is both selective for small, polar compounds, showing good retention and peak shape, and has balanced retention for a large number of compounds that vary in physiochemical properties. More balanced retention reduces the number of MRM transitions being monitored at any point in time, and improves data quality by allowing more time to be spent on a smaller number of MRM transitions.

Evaluation of a Comprehensive Approach

Good recoveries were obtained for most pesticides in most commodities as determined by both GCxGC-TOFMS and LC-MS/MS. As shown in Table I, quantitative results for grape were excellent, but lemon proved to be a difficult matrix as demonstrated by the fact that 11 pesticides were not detected by LC-MS/MS and two pesticides had interfering compounds when using the GCxGC-TOFMS method. Given lemon's complexity, ion suppression from coelution with coextractives is likely the cause of the undetected compounds in the LC-MS/MS analysis. Similarly, coextracted matrix compounds likely caused the interference that prevented determination of propoxur and terbacil in fortified samples by GCxGC-TOFMS. While recovery results for most pesticides in most commodities demonstrate successful extract

Figure 1: GCxGC-TOFMS contour plots for grape and lemon QuEChERS extracts. The lemon extract is much more complex than the grape extract and could not be analyzed by one-dimensional GC.



Columns: Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623) and Rtx®-200, 1.5 m, 0.18 mm ID, 0.20 µm (cat.# 45001); **Samples:** Grape and lemon samples were fortified at 10 ng/g with a mixed pesticide standard solution. Snap-and-shoot internal standards (cat.# 33267 and 33261) containing the compounds specified in the EN15662 QuEChERS method were added. Samples were extracted with Q-sep™ European method extraction salts (cat.# 26236) and extracts were then cleaned with QuEChERS dSPE cleanup tubes (cat.#26230). For complete sample preparation details and analytical conditions, visit www.restek.com and enter chromatograms GC_FF1217 and GC_FF1218 in the search.

Table I: Percent recovery values for 10 ng/g fortified samples prepared using QuEChERS and analyzed by GCxGC-TOFMS and LC-MS/MS.

Pesticide	Black Grapes		Lemon	
	GCxGC	LC	GCxGC	LC
Propoxur	92	110	INT	75
Methamidophos	170	73	79	66
Acephate	73	NA	88	NA
Propham	100	50	130	ND
1-Naphthol	95	NA	110	NA
o-Phenylphenol	91	NA	100	NA
Tebuthiuron	92	90	110	42
Omethoate	68	98	100	89
Dimethoate	93	91	100	79
Prometon	96	73	110	47
Terbacil	110	NA	INT	NA
Pirimicarb	98	NA	100	NA
Metribuzin	110	76	110	58
Fuberidazole	96	85	98	ND
Carbaryl	120	150	72	14
Metalaxyl	93	81	95	52
Terbutryn	100	79	99	4
Ethofumesate	110	120	81	19
Benthiocarb	85	NA	110	NA
Cyprodinil	99	86	91	ND
Thiabendazole	110	70	83	ND
Furalaxyl	130	85	110	37
Triadimenol	110	NA	100	NA
Siduron	98	96	120	35
Imazalil	NA	70	XXX	XXX
Fludioxonil	120	NA	96	NA
Myclobutanil	130	110	100	13
Buprofezin	XXX	XXX	94	24
Oxadixyl	120	90	97	40
Mepronil	120	91	100	ND
Carfentrazone ethyl	110	150	110	74
Fenhexamid	120	51	87	ND
Propargite	110	130	100	ND
Piperonyl butoxide	110	95	110	ND
Pyriproxyfen	96	100	99	ND
Fenarimol	89	NA	100	NA
Bitertanol	92	NA	110	NA
Prochloraz	78	80	100	ND
Pyraclostrobin	110	92	61	ND
Azoxystrobin	98	86	110	30
Dimethomorph	90	98	97	25

XXX = incurred pesticides
ND = not detected
NA = not analyzed
INT = affected by interferences

cleanup using dSPE, highly complex matrices will benefit from more exhaustive sample cleanup techniques, such as cartridge SPE [2]. Incurred residues were also determined and the number of pesticides detected by each technique was comparable. However, there were some pesticides for which residue concentration could only be reported by either GCxGC-TOFMS or LC-MS/MS.

Conclusions

Use of both GCxGC-TOFMS and LC-MS/MS provides more comprehensive results for pesticide residue monitoring in food. The QuEChERS sample preparation approach using Restek Q-sep™ extraction salts and dSPE cleanup tubes worked well for a variety of pesticides and commodities. In general, good recoveries were achieved as determined by both GCxGC-TOFMS and LC-MS/MS. However, more difficult matrices like lemon may benefit from additional cleanup of sample extracts.

For the complete technical article, visit
www.restek.com/comp-pest

Acknowledgements
U.S. Food and Drug Administration/Center for Food Safety and Applied Nutrition; LECO Corporation

References

[1] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC International 86 (2003) 412.
[2] J. Cochran, J. Thomas, J. Kowalski, M. Misselwitz, R. Lake, Determining Pesticides in Dietary Supplements with QuEChERS Extraction, Cartridge SPE, and GCxGC-TOFMS, GNAN1338, Restek Corporation, 2011.

Increase Data Quality for Sulfonamide Residue Analysis by HPLC and UHPLC

Using Unique Biphenyl Column Selectivity

By Rick Lake and Ty Kahler

- Improve reporting accuracy with better selectivity and retention.
- Biphenyl column and MS-friendly mobile phases allow easy transfer between UV and MS detection.
- Maximize sample throughput by combining USLC® selectivity with UHPLC speed.

The analysis of antibiotic residues in food-producing animals is important worldwide for evaluating food safety and maintaining compliance with export regulations. Sulfonamides are a specific concern, as drugs in this antibiotic class are commonly used in feed additives for livestock in order to fight infections and maintain desired growth levels. The analysis of sulfonamides usually involves a liquid chromatographic separation and detection by either UV or mass spectrometry. In both cases, the highly selective separation produced by a Biphenyl HPLC or UHPLC column can significantly improve data quality and reporting accuracy.

Increase Accuracy With Ultra Selective Biphenyl Columns

Since selectivity is the most important factor affecting peak resolution, we chose a Biphenyl column, part of our USLC® family of phases, for this work. Due to the column's unique selectivity and high retention, we were able to develop a very effective HPLC separation of 11 common sulfonamides with complete resolution (Figure 1). Use of the Biphenyl column produced much better chromatographic data compared to results obtained from a phenyl hexyl column used under identical conditions (Figure 2). The fully resolved sulfonamide analysis obtained on the Biphenyl column allows for more consistent and accurate integration.

In addition to providing improved separation of target analytes, focusing on stationary phase selectivity when choosing the analytical column allowed us to use simple, MS-friendly mobile phases. This approach provides several advantages for sulfonamide residue analysis. First, the separation can be easily transferred from UV to MS without further method development. Second, the use of simple mobile phases saves time and money, since they are quick to prepare and do not require complex additives.

Higher Retention Reduces Matrix Interferences in MS Detection

When developing a separation for UV detection, selectivity is critical for positive analyte identification. If MS detection is used, selectivity may not be required for analyte identification, but it still may be needed for adequate sensitivity and separation from matrix interferences. Matrix interferences can play a significant role in MS analyses by lowering method sensitivity through suppressing ionization. Ion suppression in reversed phase mode often occurs with early eluting compounds, so it is good practice to retain them to a retention factor (k) of 2. In this example, we can see that the retention factor of sulfanilamide on the Biphenyl column is approximately twice as high as it is on the phenyl hexyl column (Figure 2). As a result, sulfanilamide is more susceptible to sample matrix interference if a phenyl hexyl column is used. The increased retention provided by the Biphenyl column, in combination with the MS-friendly mobile phases used here, ensure good sensitivity and allow easy method transfer between detectors.

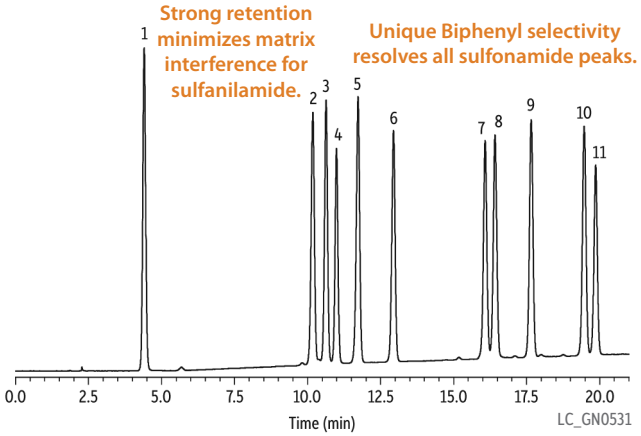
Combining USLC® Selectivity and UHPLC Speed—The Most Powerful Approach

Selectivity has the greatest influence on resolution, but efficiency is the best tool for decreasing analysis time. By optimizing column selectivity first, we can then easily transfer a robust separation to UHPLC for faster analysis. Figure 3 illustrates the power of combining USLC® selectivity with UHPLC efficiency. By using a 1.9 µm Biphenyl UHPLC column we are able to fully separate all 11 sulfonamide peaks in a fast, 8-minute analysis.

Conclusion

Focusing first on selectivity when choosing an analytical column for sulfonamide residue analysis is an easy way to improve data quality. The unique selectivity and high retention of Biphenyl columns produce complete separations and benefit both UV and MS detection. In addition, Biphenyl columns in a UHPLC format allow faster sample throughput, while maintaining good separation of target compounds.

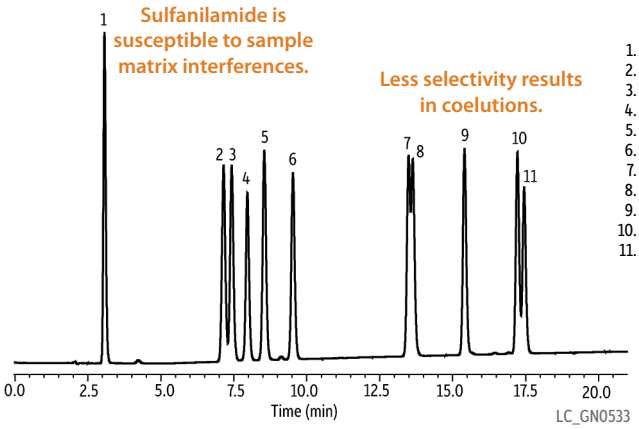
Figure 1: Due to their unique selectivity, Biphenyl columns can provide the retention and separation needed for accurate sulfonamides analysis with simple, MS-friendly mobile phases.



Peaks	RT (min)
1. Sulfanilamide	4.40
2. Sulfadiazine	10.18
3. Sulfapyridine	10.63
4. Sulfathiazole	10.99
5. Sulfamerazine	11.72
6. Sulfamethazine	12.94
7. Sulfachlorpyridazine	16.08
8. Sulfadoxine	16.42
9. Sulfisoxazole	17.65
10. Sulfadimethoxine	19.47
11. Sulfaquinoxaline	19.86

Column: Ultra Biphenyl (cat.# 9109565); Dimensions: 150 mm x 4.6 mm ID; Particle Size: 5 µm; Pore Size: 100 Å; Temp.: 25 °C; **Sample:** Diluent: 0.1% Formic acid in water; Conc.: 50 µg/mL; Inj. Vol.: 10 µL; **Mobile Phase:** A: 0.1% Formic acid in water, B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0 min (10%), 3.0 min (10%), 20.0 min (40%), 21.0 min (40%); Flow: 1.0 mL/min; **Detector:** UV/Vis @ 265 nm; **Instrument:** Shimadzu UFLCXR.

Figure 2: A phenyl hexyl column, used under identical conditions, does not provide adequate retention or selectivity for sulfonamide residue analysis.



Peaks	RT (min)
1. Sulfanilamide	3.07
2. Sulfadiazine	7.15
3. Sulfapyridine	7.43
4. Sulfathiazole	7.96
5. Sulfamerazine	8.54
6. Sulfamethazine	8.53
7. Sulfachlorpyridazine	13.49
8. Sulfadoxine	13.63
9. Sulfisoxazole	15.41
10. Sulfadimethoxine	17.22
11. Sulfaquinoxaline	17.45

Column: Waters XSELECT™ CSH Phenyl-Hexyl; Dimensions: 150 mm x 4.6 mm ID; Particle Size: 5 µm; Temp.: 25 °C; **Sample:** Diluent: 0.1% Formic acid in water; Conc.: 50 µg/mL; Inj. Vol.: 10 µL; **Mobile Phase:** A: 0.1% Formic acid in water, B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0 min (10%), 3.0 min (10%), 20.0 min (40%), 21.0 min (40%); Flow: 1.0 mL/min; **Detector:** UV/Vis @ 265 nm; **Instrument:** Shimadzu UFLCXR.

For more about the advantages of USLC® Biphenyl columns, visit **www.restek.com/uslc**

Ultra Biphenyl Columns (USP L11)

Physical Characteristics:

particle size: 3 µm or 5 µm, spherical
pore size: 100 Å
carbon load: 15%
endcap: fully endcapped
pH range: 2.5 to 8
temperature limit: 80 °C

Description	cat.#
5 µm Columns	
150 mm, 4.6 mm ID	9109565
5 µm Columns	
150 mm, 4.6 mm ID (with Trident Inlet Fitting)	9109565-700

Pinnacle® DB Biphenyl Columns (USP L11)

Physical Characteristics:

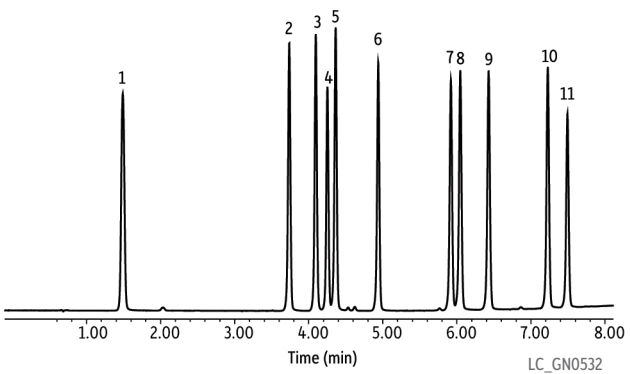
particle size: 1.9 µm, 3 µm, or 5 µm, spherical
pore size: 140 Å
carbon load: 8%
endcap: yes
pH range: 2.5 to 8
temperature limit: 80 °C

Description	cat.#
1.9 µm Columns	
100 mm, 2.1 mm ID	9409212

ordering **note**

For guard cartridges for these columns, visit our website at **www.restek.com**

Figure 3: Ultra selective analysis of sulfonamides on a unique Biphenyl column can be used in conjunction with UHPLC for higher sample throughput.



Peaks	RT (min)
1. Sulfanilamide	1.55
2. Sulfadiazine	3.74
3. Sulfapyridine	4.09
4. Sulfathiazole	4.24
5. Sulfamerazine	4.35
6. Sulfamethazine	4.91
7. Sulfachlorpyridazine	5.87
8. Sulfadoxine	5.99
9. Sulfisoxazole	6.37
10. Sulfadimethoxine	7.14
11. Sulfaquinoxaline	7.40

Column: Pinnacle® DB Biphenyl (cat.# 9409212); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 1.9 µm; Pore Size: 140 Å; Temp.: 25 °C; **Sample:** Diluent: 0.1% Formic acid in water; Conc.: 50 µg/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water, B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0 min (5%), 8 min (40%); Flow: 0.4 mL/min; **Detector:** UV/Vis @ 265 nm; **Instrument:** Shimadzu UFLCXR.

Fast, Robust LC-MS/MS Method for Quantification of Multiple Therapeutic Drug Classes Using an Ultra Biphenyl Column

By Amanda Rigdon

- Quantify 29 drug compounds from four drug classes in a fast, 5.5-minute analysis.
- Ultra Biphenyl column separates isobaric compounds for more definitive results.
- Highly reproducible retention times reduce down-time and reanalysis.

As demand for therapeutic drug monitoring rises, laboratories are under increased pressure to implement streamlined, cost-effective testing procedures. As with any high-volume application, the methods developed for therapeutic drug monitoring must be fast, robust, and easy to implement. Methods that can be used to quantify a wide variety of drug chemistries from a single analysis are particularly beneficial, as they reduce costs and save time. The objective of this work was to develop a fast, robust LC-MS/MS method for the quantification of 29 therapeutic drugs and metabolites in urine from several drug classes including opiates, benzodiazepines, tricyclic antidepressants, and anticonvulsants. Results from this partial validation indicate that the method used here produces good linearity, accuracy, and precision for most of the drugs tested in a fast, 5.5-minute analysis.

The method employed here uses a Shimadzu UFLCXR HPLC coupled to an AB SCIEX API 4000 MS/MS and a 5 µm Ultra Biphenyl (100 mm x 2.1 mm, cat.# 9109512) analytical column with a matching guard column (cat.# 910950212). The Biphenyl column was chosen for this work because of its versatility; it combines the performance of a traditional alkyl (e.g., C18) column with that of a phenyl column, and it offers excellent retention of both polar and nonpolar compounds. The adaptability of the Biphenyl phase makes it particularly useful for methods developed to analyze drugs from multiple classes. Matrix standards and samples were prepared using dilute-and-shoot methodology as described in Figure 1.

Linear Range and Sensitivity

To evaluate linearity and sensitivity, an 11-point calibration curve covering a concentration range of 1-1,000 ng/mL was prepared in matrix. Calibration curves for each compound were built from triplicate injections using either a linear or quadratic equation, depending on the

Table I: Partial validation results for 29 therapeutic drugs and drug metabolites.

Compound Name	LOQ (ng/mL)	Linearity (r)	% Accuracy at LOQ	%CV at LOQ	S/N at LOQ
Morphine	5.0	0.9995	95	5	20
Oxymorphone	5.0	0.9994	101	2	30
Pregabalin	5.0	0.9994	95	5	40
Hydromorphone	2.5	0.9993	91	1	40
Gabapentin	10.0	0.9994	98	5	10
Codeine	10.0	0.9990	109	18	50
Oxycodone	5.0	0.9989	112	10	40
Hydrocodone	5.0	0.9997	106	2	30
7-Aminoclonazepam	2.5	0.9978	85	14	50
Tapentadol	2.5	0.9993	95	7	30
Zopiclone	10.0	0.9911	102	12	20
Norbuprenorphine	25.0	0.9955	124	19	30
7-Aminoflunitrazepam	5.0	0.9993	91	12	40
Zolpidem	1.0	0.9994	96	11	200
Citalopram	2.5	0.9996	101	7	50
Fentanyl	1.0	0.9996	97	14	70
Buprenorphine	5.0	0.9996	99	2	40
Doxepin	5.0	0.9996	100	9	90
Paroxetine	5.0	0.9994	88	2	100
Promethazine	1.0	0.9997	94	12	30
Nortriptyline	1.0	0.9990	101	8	50
Amitriptyline	5.0	0.9995	92	7	100
EDDP	5.0	0.9997	91	4	200
Lorazepam	5.0	0.9994	99	13	20
Sertraline	10.0	0.9946	113	23	40
Methadone	1.0	0.9998	101	5	3
Clonazepam	2.5	0.9997	104	6	20
Flunitrazepam	1.0	0.9996	90	9	10
Diazepam	2.5	0.9994	84	6	40

response of the individual compound. All calibration curves employed 1/x weighting. As shown in Table I, good linearity was achieved with correlation coefficient values exceeding 0.999 for most compounds.

LOQs were determined by evaluating signal-to-noise ratios for the three transitions used for each compound, and values ranged from 1 ng/mL to 5 ng/mL for most compounds. Several analytes had LOQs of 10 ng/mL; only norbuprenorphine had an LOQ of 25 ng/mL, which was expected since it is a poor responder and usually requires further sample preparation. With the exception of methadone, the quantification ion for each compound had a signal-to-noise ratio of ≥10 at the LOQ, and each qualifier ion had a signal-to-noise ratio of ≥3. Because methadone was a very high responder, the first two transitions for this drug overloaded the detector at higher concentrations, so only the third transition was used for quantification. The first two transitions may be used, but detuning these transitions is recommended to reduce response and improve linearity.

Accuracy and Reproducibility

Accuracy and precision at the LOQ were assessed for each compound; acceptable ranges were considered to be 90-110% recovery and ≤15% coefficient of variation (CV). Accuracy ranged from 88% to 113% for all analytes except norbuprenorphine, which typically is not determined using a dilute-and-shoot method. Precision results ranged from 1% to 23%, and all compounds except for codeine, norbuprenorphine, and sertraline had passing results of ≤15% CV for precision (Table I).

Since retention time shifts can be a source of downtime and sample reanalysis, retention time reproducibility across multiple column lots was also evaluated. Replicate injections of a 1 µg/mL solvent standard were analyzed on three different lots of Ultra Biphenyl columns under the same conditions used for the samples. Retention times for each compound were determined and the maximum retention time variation across all three lots of analytical columns was just 0.13 minutes. This indicates retention times are stable and predictable, which minimizes the need to reset retention time windows when columns are changed.

Conclusion

Partial validation results indicate this method is suitable for the quantification of a broad range of therapeutic drugs and metabolites in urine at levels ranging from 1-1,000 ng/mL. By using a highly reproducible 5 µm Ultra Biphenyl column and the multi-drug method conditions established here, labs can reduce downtime and improve productivity.

For additional clinical/forensic articles, visit **www.restek.com/cft**

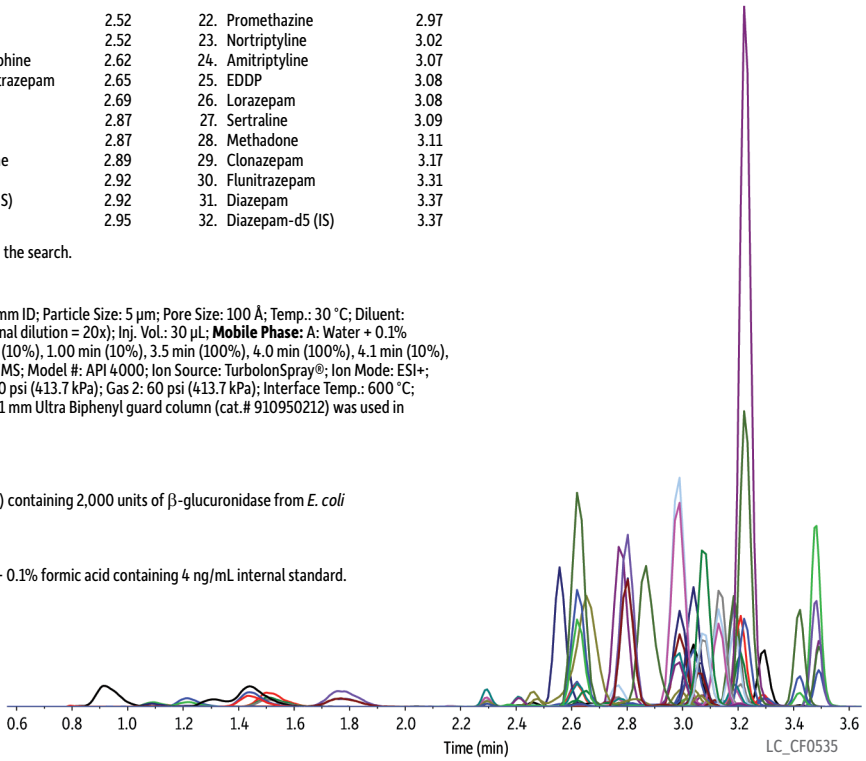
Figure 1: Analysis of 29 drug compounds and metabolites at 100 ng/mL in urine on an Ultra Biphenyl column.

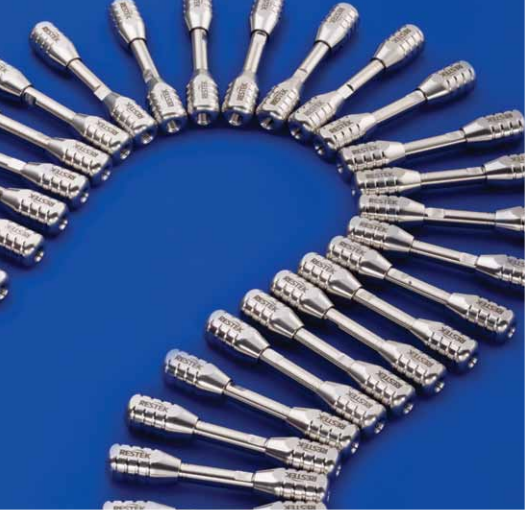
	Peaks	RT (min)						
1.	Morphine	0.95	11.	Tapentadol	2.52	22.	Promethazine	2.97
2.	Oxymorphone	1.08	12.	Zopiclone	2.52	23.	Nortriptyline	3.02
3.	Pregabalin	1.29	13.	Norbuprenorphine	2.62	24.	Amitriptyline	3.07
4.	Hydromorphone	1.34	14.	7-Aminoflunitrazepam	2.65	25.	EDDP	3.08
5.	Gabapentin	1.56	15.	Zolpidem	2.69	26.	Lorazepam	3.08
6.	Codeine	2.16	16.	Citalopram	2.87	27.	Sertraline	3.09
7.	Codeine-d3 (IS)	2.16	17.	Fentanyl	2.87	28.	Methadone	3.11
8.	Oxycodone	2.29	18.	Buprenorphine	2.89	29.	Clonazepam	3.17
9.	Hydrocodone	2.33	19.	Doxepin	2.92	30.	Flunitrazepam	3.31
10.	7-Aminoclonazepam	2.49	20.	Doxepin-d3 (IS)	2.92	31.	Diazepam	3.37
			21.	Paroxetine	2.95	32.	Diazepam-d5 (IS)	3.37

For MRM transitions, visit **www.restek.com** and enter LC_CF0535 in the search.

Column: Ultra Biphenyl (cat.# 9109512); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 5 µm; Pore Size: 100 Å; Temp.: 30 °C; Diluent: Water:acetonitrile (90:10) + 0.1% formic acid; Conc.: 100 ng/mL (final dilution = 20x); Inj. Vol.: 30 µL; **Mobile Phase:** A: Water + 0.1% formic acid, B: Acetonitrile + 0.1% formic acid; Gradient (%B): 0 min (10%), 1.00 min (10%), 3.5 min (100%), 4.0 min (100%), 4.1 min (10%), 5.5 min (10%); Flow: 0.6 mL/min; **Detector:** AB SCIEX API 4000 MS/MS; Model #: API 4000; Ion Source: TurboIonSpray®; Ion Mode: ESI+; Ion Spray Voltage: 3000 kV; Curtain Gas: 40 psi (275.8 kPa); Gas 1: 60 psi (413.7 kPa); Gas 2: 60 psi (413.7 kPa); Interface Temp.: 600 °C; Mode: MRM; **Instrument:** API LC-MS/MS. **Notes:** A 5 µm, 10 mm x 2.1 mm Ultra Biphenyl guard column (cat.# 910950212) was used in conjunction with this analysis.

Sample Preparation:
- Fortify urine at 100 ng/mL.
- To 1 mL of urine, add 1 mL of 100 mM ammonium acetate (pH = 5.6) containing 2,000 units of β-glucuronidase from *E. coli* (Sigma-Aldrich cat# G7396).
- Incubate for 90 minutes at 37 °C.
- Centrifuge at 3,000 rpm for 15 minutes.
- Dilute 100 µL of sample with 900 µL of water:acetonitrile (90:10) + 0.1% formic acid containing 4 ng/mL internal standard. (Total dilution factor = 20x)





Find the Best LC-MS Column/Mobile Phase Combination

Using a Simple Mobile Phase, USLC® Columns, and a Scouting Gradient

By Rick Lake and Ty Kahler

- Simplifying your mobile and stationary phase options will streamline method development.
- USLC® technology effectively narrows your columns options from over 600 down to four.
- A scouting gradient makes it easy to select the best column/mobile phase combination.

If we've learned anything from developing methods (and probably more from struggling with them), it's that you will generate more robust methods in less time if you start by looking at retention and selectivity. First, simplify your mobile phase; then, reduce your column options. Finally, run a scouting gradient to choose the right column/mobile phase combination based on your desired elution profile.

Reduce Your Mobile Phase Possibilities

When developing a method, the number of mobile phases you have to choose from is nearly infinite, so it's easy to become overwhelmed. What's more, using a highly customized mobile phase may not be necessary—it could even be detrimental to your data. Long story short, it's in your best interest to simplify. We advise employing a four–mobile phase system and the recommendations in Table I. When the time comes for your scouting gradient, run all four A/B combinations (e.g., A1/B1, A1/B2, A2/B1, A2/B2) and select your mobile phase based on the results.

Table I: Run these aqueous solutions and organic solvents using a four–mobile phase system and our USLC® columns to dramatically simplify mobile phase selection.

Aqueous Solutions	Organic Solvents
A1) 0.1% Formic acid in water	B1) Acetonitrile (aprotic solvent)
A2) 0.1% Formic acid and 5 mM ammonium formate in water	B2) Methanol (protic solvent)

Make the Most of the USLC® Column Set

Unlike with mobile phases, there are "only" around 600 different columns on the market. But, column phase chemistry can be so similar between product lines and even manufacturers that switching may do little to alter your results. Instead of wasting time and money running column after column with nearly identical selectivity—and getting similar results—simply plug the USLC® column set into your column-switching system. Designed with the method developer in mind, this innovative column set offers an incredible range of alternate selectivity using just four unique stationary phases. USLC® phases are so different from each other (i.e., orthogonal) that they offer selectivity and retention regardless of your target analytes.

Scout for Successful Method Development

Evaluating, or "scouting," your column/mobile phase combinations will allow you to determine which works best for your desired elution profile. To perform a scouting gradient, set your instrument to deliver a defined, linear gradient slope over a specified time. Start with the aqueous solution at 5%, and starting at time 0, begin ramping up to 95% using the flow rate and gradient time listed in Table II for your column. (If you have sample solubility issues, you can deviate from the starting or ending ratios, but be sure to keep the gradient defined and linear.) After each gradient, don't forget to equilibrate the column using the time in Table II before running the next mobile phase.

Choose Phases Based on Selectivity, Retention, and Elution Profile

When your scouting run is complete, you will have a set of 16 chromatograms (one for each column/mobile phase combination). To choose the best column/mobile phase combination, you must first calculate the ideal elution profile for each by looking at the difference in retention time between the first and last peaks (Δt) and the gradient time (t_G). A $\Delta t/t_G$ less than 0.25 would mean that an isocratic elution is feasible; a $\Delta t/t_G$ greater than 0.25 would indicate the need for a gradient.

Second, look at your peaks. The column and mobile phase combination that delivers the best retention, selectivity, and peak shape for your desired elution profile is the one you should choose for your method (Figure 1). It's that easy! For an in-depth look at the role of selectivity in reversed phase separations, check out **www.restek.com/USLCarticle**

At this point, you may find that you are already achieving complete chromatographic resolution and can continue developing your method without giving another thought to mobile or stationary phase selection. If, however, your results are less than ideal, visit **www.restek.com/USLCguide** for help fine-tuning your mobile phase.

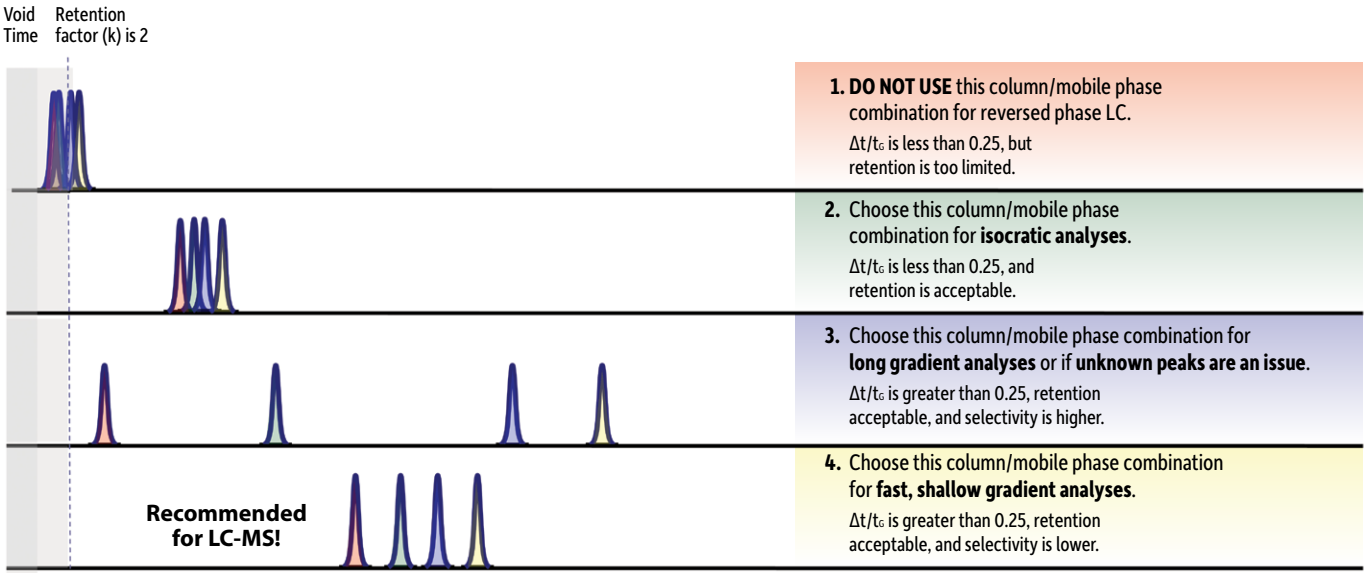
Summary

It is said that the first step is the hardest, but it can be the easiest when you start your method development by simplifying your mobile phase and focusing on selectivity and retention to choose a column/mobile phase combination based on your desired elution profile. With this dependable approach, scouting gradients and USLC® columns are a method developer's most effective tool. To learn more about LC column selectivity or the USLC® column set, visit **www.restek.com/uslc**

Table II: Use these time settings to achieve a defined, linear gradient slope that is ideal for mobile phase scouting.

Column Dimensions			Time Settings		
Column Inner Diameter (mm)	Column Length (mm)	Particle Diameter (µm)	Flow Rate (mL/min)	Gradient Time (t_G)	Post Gradient Equilibration Time (min)
2.1	30	1.9	0.6	2	1
		3	0.3	4	2
		5	0.2	6	2
	50	1.9	0.6	4	1
		3	0.3	7	3
		5	0.2	10	4
	100	1.9	0.6	7	3
		3	0.3	13	5
		5	0.2	20	8
3.0	50	1.9	1.1	4	1
		3	0.7	6	2
		5	0.4	10	4
	100	1.9	1.1	7	3
		3	0.7	13	5
		5	0.4	21	8
	150	1.9	1.1	11	6
		3	0.7	19	11
		5	0.4	31	17
4.6	50	3	1.5	6	3
		5	1.0	10	4
	100	3	1.5	13	5
		5	1.0	19	8
	150	3	1.5	19	8
		5	1.0	29	11
	250	3	1.5	32	13
		5	1.0	49	19

Figure 1: Choosing the ideal column/mobile phase combination for a method is simple if you run a scouting gradient using a four–mobile phase system and the USLC® four column set. The 16 chromatograms from your scouting run will fall into one of four categories.



Improve Trace Analysis of Polar Impurities in Petroleum Gases

Using Higher Sample Capacity Alumina MAPD Columns

By Rick Morehead, Jan Pijpelink, and Jaap de Zeeuw

- Increased sample capacity results in improved peak shape and better accuracy.
- Optimized deactivation results in highest response for polar hydrocarbons.
- Rt®-Alumina BOND/MAPD columns give more flexibility in choice of sample size.

When using PLOT columns to analyze trace impurities in petroleum gases, such as propylene, ethylene, or 1,3-butadiene, sample capacity (loadability) is an important factor in obtaining accurate data. Phase overload in adsorption chromatography results in peak tailing, which can be problematic when trace-level impurities elute near the main component where they may be obscured by the larger peak. Peak tailing can be further exacerbated by residual activity on the adsorbent surface. Using a column with higher sample capacity and an appropriate deactivation is a good strategy for reducing tailing and improving quantification accuracy for low level polar hydrocarbon impurities in volatile hydrocarbon streams.

MAPD-type alumina PLOT columns are commonly used for these applications because the selectivity and degree of deactivation of the alumina makes it very useful for separating the polar hydrocarbon analytes from the main C1-C5 components of the hydrocarbon matrix. Although selectivity is very good for these compounds, sample capacity is often a challenge, which limits the amount of sample that can be injected. Larger sample volumes can be desirable when less sensitive detectors (e.g. TCDs) are used or when trace levels of impurities, such as acetylene, propadiene, or methyl acetylene, must be detected in main hydrocarbon streams in order to prevent damage to polymerization catalysts.

Higher Retention With Good Peak Shape Yields Higher Loadability

New Rt®-Alumina BOND/MAPD columns have an improved deactivation and an increased sample capacity compared to other commercially available MAPD PLOT columns. As shown in a comparison of absolute retention times, the new MAPD column offers more than twice the retention which results in greater resolution and increased sample capacity (Figure 1). In this figure the absolute retention of MAPD columns was compared using an isothermal oven temperature of 130 °C. Note that on the Rt®-Alumina BOND/MAPD column all the C1-C5 hydrocarbons are well resolved and show perfect Gaussian peak shape.

Greater Sample Capacity Improves Accuracy

To assess sample capacity, each column was tested at the temperature shown on the manufacturer's QA protocol in order to achieve comparable retention. A range of sample volumes of a QA test mix were analyzed on each column using a 6-port sampling valve and 5 µL to 250 µL sample loops. Peak tailing was measured for the analytes that were most likely to exhibit tailing and to be sensitive to poor sample capacity in actual impurity testing. As shown in Figure 2, much less peak tailing was observed on the Rt®-Alumina BOND/MAPD column. Symmetrical peaks were obtained across a wide sample volume range, indicating that the column deactivation was highly effective and that sample capacity was greater on the Rt®-Alumina BOND/MAPD column. Linearity was also assessed, as shown in Figure 3, and excellent correlations were achieved for all target impurities across the test range.

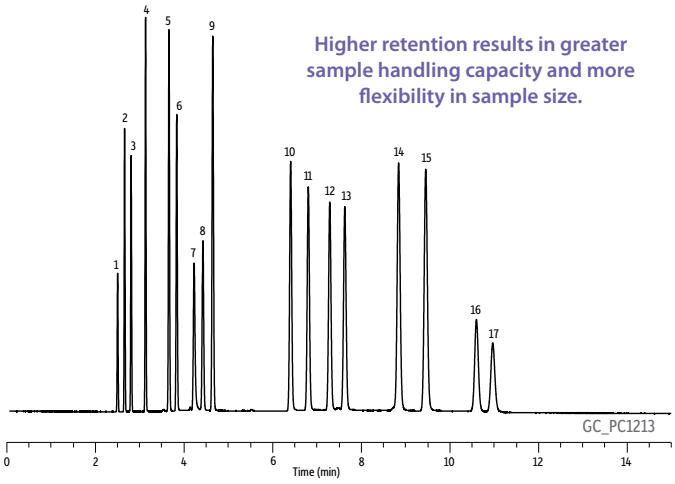
Summary

When analyzing impurities, such as acetylene, propadiene, and methyl acetylene in petroleum gases, the sample handling capacity of the analytical column is an important consideration. Rt®-Alumina BOND/MAPD columns offer higher sample capacity than other commercially available MAPD columns and are recommended for analyzing polar impurities in light hydrocarbon streams. Greater sample capacity improves data accuracy due to better peak symmetry and a wide linear range.

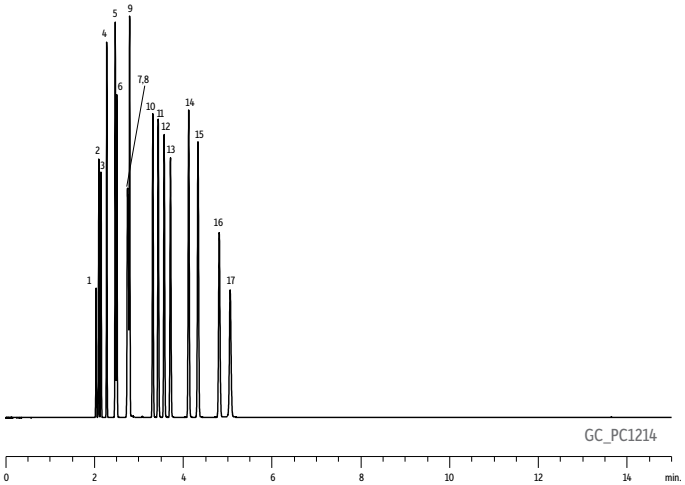
For more information on Rt®- and MXT®-Alumina BOND/MAPD PLOT columns, visit **www.restek.com/MAPD**

Figure 1: Rt®-Alumina BOND/MAPD columns have greater absolute retention than Select Al_2O_3 MAPD columns, resulting in greater sample handling capacity through increased resolution.

A: Rt®-Alumina BOND/MAPD



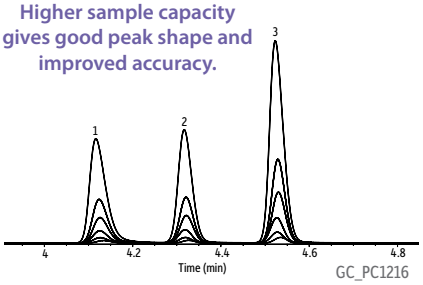
B: Select Al_2O_3 MAPD



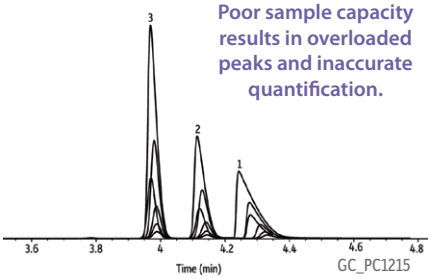
Columns: 50 m x 0.53 mm ID x 10 µm; **Sample:** PLOT column QA test mix (DCG# 547267); **Injection:** 5 µL, split, 200 °C; Split vent flow rate: 80 mL/min; **Oven:** 130 °C, isothermal; **Carrier Gas:** helium, (4.4 psi, 30 kPa); **Detector:** FID, 200 °C. **Peaks:** 1. Methane, 2. Ethane, 3. Ethylene, 4. Propane, 5. Cyclopropane, 6. Propylene, 7. Acetylene, 8. Propadiene, 9. *n*-Butane, 10. *trans*-2-Butene, 11. 1-Butene, 12. Isobutene, 13. *cis*-2-Butene, 14. Isopentane, 15. *n*-Pentane, 16. 1,3-Butadiene, 17. Methyl acetylene.

Figure 2: Higher sample capacity is also demonstrated by comparing peak symmetry. Rt®-Alumina BOND/MAPD columns produce better peak shape, even when more material is injected.

A: Rt®-Alumina BOND/MAPD



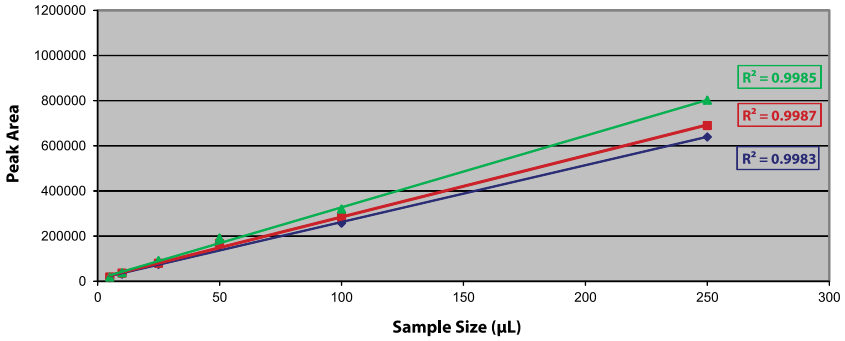
B: Select Al_2O_3 MAPD



Columns: 50 m x 0.53 mm ID x 10 µm; **Sample:** PLOT column QA test mix (DCG# 547267); **Injection:** 5-250 µL, split, 200 °C; Split vent flow rate: 80 mL/min; **Oven:** manufacturer's recommended temperature used for each column (Rt®-Alumina BOND/MAPD: 130 °C, Select Al_2O_3 MAPD: 100 °C), isothermal (hold 8 min); **Carrier Gas:** helium, (4.4 psi, 30 kPa); **Detector:** FID, 200 °C. **Peaks:** 1. Acetylene, 2. Propadiene, 3. *n*-Butane.

Figure 3: Higher sample capacity results in a wide linear range and accurate quantification, even at levels that can produce tailing and incomplete separations on other MAPD columns. (**green** = methyl acetylene, **red** = acetylene, **blue** = propadiene).

Rt-Alumina BOND/MAPD Linearity



Alumina BOND/MAPD PLOT Columns

Rt®-Alumina BOND/MAPD Columns (fused silica PLOT)

ID	df	temp. limits	30-Meter	50-Meter
0.32 mm	5 µm	to 250 °C	19779	19780
0.53 mm	10 µm	to 250 °C	19777	19778

MXT®-Alumina BOND/MAPD Columns (Siltek®-treated stainless steel PLOT)

ID	df	temp. limits	3.5" coil 30-Meter	7" diameter 11-pin cage 30-Meter
0.53 mm	10 µm	to 250 °C	79728-273	79728

tech tip

Traces of water in the carrier gas and sample will affect the retention and selectivity of alumina. If the column is exposed to water, the retention times will shorten. Alumina columns can be regenerated by conditioning for 15-30 minutes at 200-250 °C under normal carrier gas flow. Periodic conditioning ensures excellent run-to-run retention time reproducibility.

The maximum programmable temperature for Rt®- and MXT®-Alumina BOND/MAPD columns is 250 °C. Higher temperatures cause irreversible changes to the porous layer adsorption properties.

Innovators in Chromatography

A continuing series of guest editorials contributed by collaborators and internationally recognized leaders in chromatography.

Matrix Effects in Multi-Residue Pesticide Analysis When Using Liquid Chromatography-Tandem Mass Spectrometry

By Kai Zhang, Ph.D., U.S. FDA Center for Food Safety and Applied Nutrition

Dr. Zhang *is a Chemist in the Methods Development Branch of the U.S. FDA Center for Food Safety and Applied Nutrition. His research interests focus on trace analysis of various contaminants, such as pesticides and mycotoxins, in foods using LC-MS and GC-MS.*

Consumption of pesticide-contaminated food via daily diet is a major source of exposure to pesticides and poses a potential health threat to humans. It is necessary to monitor various pesticide residues in foods via multi-residue analysis procedures, because it would be impractical to develop individual analytical methods for every pesticide in suspected food commodities. The availability of liquid chromatography-tandem mass spectrometry (LC-MS/MS) has improved the selectivity and sensitivity of pesticide analysis, as well as workflow in the identification and quantification of various classes of pesticides in agricultural products. This leads to the development and use of LC-MS/MS multi-residue methods in laboratories worldwide to do consistent, targeted quantitative pesticides analysis from a single injection, providing increased sensitivity and the ability to screen a large number of target pesticides in one method.

The effect of the matrix is a phenomenon in electrospray ionization (ESI) LC-MS/MS analysis that impacts the data quality of the pesticide analysis. Matrix effects, caused by analyte and matrix component interactions, are unique to ESI-based LC-MS/MS instrumentation and present one of today's most challenging analytical issues. Matrix effects can take the form of interference or signal suppression/enhancement (when compared to a pure analytical standard) and depend on the sample matrix, target analytes, and mode of ionization. Studies of matrix effects are essential to the application of LC-MS/MS with different food

commodities. A thorough understanding of matrix effects would yield fundamental insights for different food matrices, corresponding sample preparation, and subsequent instrument performance, thus allowing major application needs (identification and quantitation) to be addressed.

Generally, there are two types of matrix effects—matrix interference and signal alteration. Matrix interference can be caused by those coeluting components in sample extracts that have similar ions in the MS/MS experiment. This type of matrix effect can lead to false positive/negative identification and can be resolved by using non-interfering MRM transitions, extensive sample cleanup, or improving the LC separation. Increased mass/charge selectivity, which can be acquired by using a high resolution accurate mass spectrometer, can help minimize matrix interference.

> The effect of the matrix is a phenomenon in electrospray ionization (ESI) LC-MS/MS analysis that impacts the data quality … and presents one of today's most challenging analytical issues.

Matrix effects may also be caused by interactions (via van der Waals, dipolar-dipolar, or electrostatic forces) between pesticides and co-extractives in the prepared sample that could suppress or enhance the ionization of a pesticide in the ESI source. This can result in a lower or higher signal, which affects the accuracy of the quantitative results. Several approaches have been used to minimize the signal suppression or enhancement resulting from the matrix components. These include extensive sample cleanup, improvement of the LC separation to avoid coelutions with matrix components, or serial dilution of the final extract, such that fewer matrix components will be injected into the analytical system. Splitting of the LC eluent flow before entering the mass spectrometer may also help eliminate matrix suppression or enhancement. Unlike the above approaches, standard addition, internal standards, or matrix-matched calibration curves are commonly used to compensate for, but not to reduce, signal suppression or enhancement.

None of the above approaches will completely eliminate matrix effects. Increased selectivity (e.g., using specific transitions or improving mass resolution/accuracy) can minimize matrix interferences, but signal suppression or enhancement may still be observed because signal alteration happens in the ion source prior to detection. Using dilution or a smaller injection volume requires more sensitive instruments and introduces more error, in terms of accuracy and precision, for quantitative results. Additionally, optimal dilution factors depend on food matrices, instrument sensitivity, target pesticides, and LC conditions, so it is time-consuming to optimize the experimental conditions. Using internal standards might be too expensive to apply in multi-residue analysis. Matrix-matched calibration is commonly used for quantitation, but there are disadvantages associated with this approach. First, it is hard to collect blank matrix for each food commodity. Second, analytes in a matrix-matched environment are different from those in real samples, in which the analytes first interact with the matrix components and then are "modified" by sample preparation. Matrix-matched calibration standards would alleviate matrix effects on quantification only if sample matrices remained the same before and after the sample preparation, which is impossible to achieve. Therefore, this approach might only work well for simple matrices such as fresh produce, but not for more complex matrices, such as botanical samples. Third, it is laborious and time-consuming to prepare matrix-matched calibration standards for routine analysis, especially when samples of different commodities have to be analyzed on daily basis.

Obviously, the lack of well-suited approaches for circumventing matrix effects requires us to systematically investigate the problem so that, in theory, we will be able to describe and define the interactions between matrix components and analytes. In practice, we can quantitatively measure matrix effects and estimate the impact on quantitation and identification. At the present time, LC-MS/MS is known as the best instrument for target analysis and quantitation; however, it is limited by an incomplete understanding of matrix effects. This presents a significant challenge to researchers working to harness the sensitivity, selectivity, and specificity of LC-MS/MS to meet the growing need for better multi-residue analysis procedures.

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Dennis Claspell, Director of Marketing

How Did We Do?

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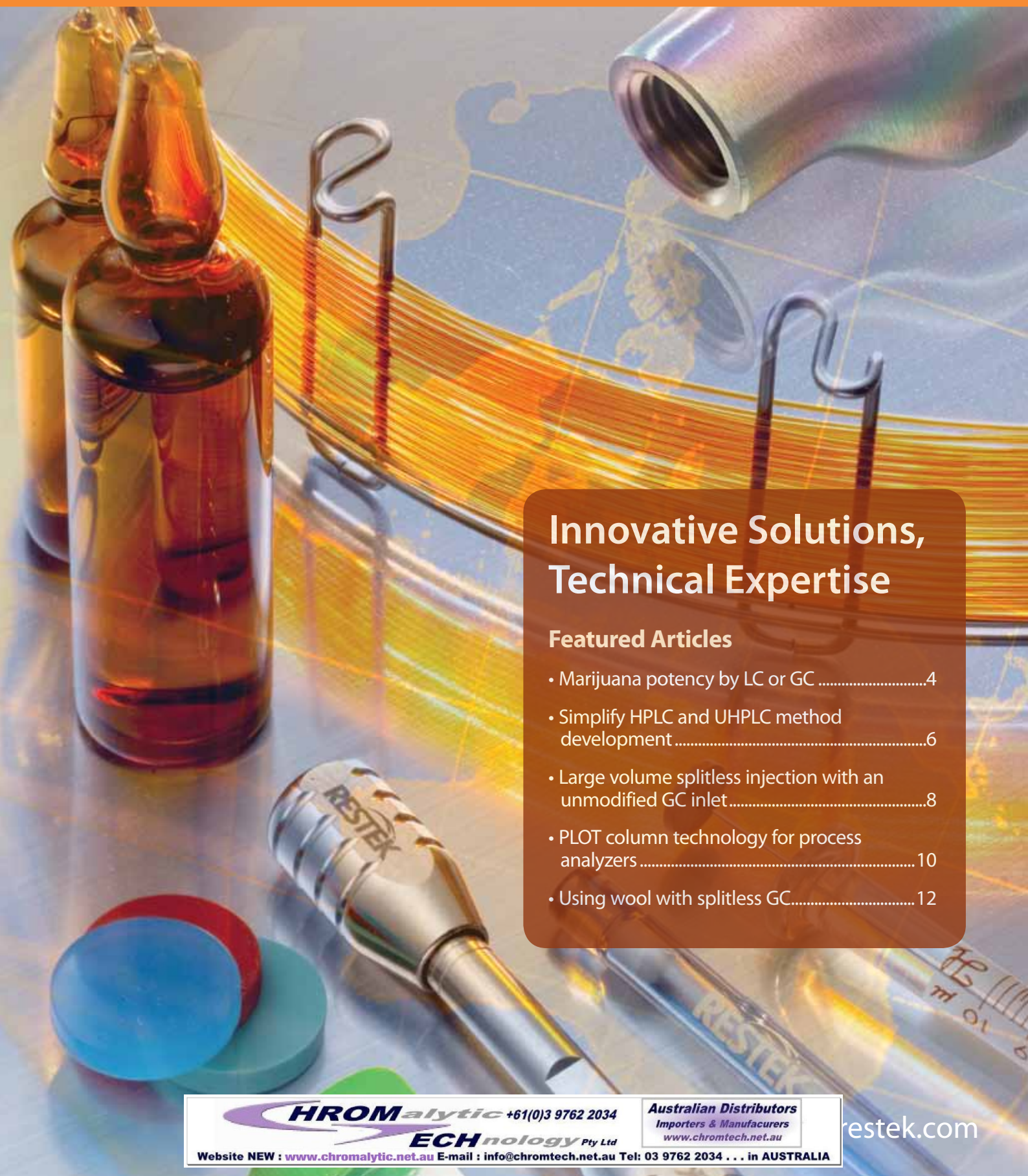

Innovative Solutions, Technical Expertise

Featured Articles

Restek Connections

In This Issue

Letter from the Bench

Welcome to the new look for your *Restek Advantage*!

When we sat down to plan this issue, one of our goals was to share more chromatography news and better connect with you, our reader. That's how our new Hot Topics and Restek Connections departments came to be.

Of course, as always, much of this *Advantage* highlights the application work of our Innovations Lab, where we're lucky to have seasoned veterans working alongside young, enthusiastic chemists to solve your toughest problems. Rick Lake and Ty Kahler show you how to get the most selectivity for your LC separations. Their work employs the hydrophobic subtraction model to define a highly selective and orthogonal set of 4 USLC™ columns.

You will also be interested in reading our article on marijuana potency testing, PLOT columns in process GC, wool in GC inlet liners, large volume splitless injection... We have something inside for every analyst.

Finally, we also set up a new email address: **advantage@restek.com** Use it to let us know what you think of your new *Restek Advantage*. I say "*your*" because we create this technical document with your needs and interests in mind. Your feedback will be invaluable for assembling future issues.

Cheers!

Jack Cochran
Director of New Business & Technology

You Have Opinions... And We Want Them.

We chemists are an opinionated bunch, so the odds are good that you have some thoughts about the *Restek Advantage*. Love it? Hate it? Want to see something different in the next issue? Maybe you have a response to one of our technical articles? Whatever you have to say, let's hear it! Email your comments to advantage@restek.com and you may even see them in an upcoming issue.

About Restek Corporation

A leading innovator of chromatography solutions for both LC and GC, Restek has been developing and manufacturing columns, reference standards, sample preparation materials, accessories, and more since 1985. We provide analysts around the world with products and services to monitor the quality of air, water, soil, food, pharmaceuticals, chemicals, and petroleum products. Our experts enjoy diverse areas of specialization in chemistry, chromatography, engineering, and related fields as well as close relationships with government agencies, international regulators, academia, and instrument manufacturers.

Hot Topics

Product Spotlight

Restek Introduces Secondary Columns for GCxGC

Restek now offers a full line of secondary columns with a wide range of polarities to help you accurately analyze highly complex samples using GCxGC. These new columns can be matched with any Restek Rxi® or Rtx® primary column to create the perfect orthogonal separation for your application—and our online column combination guide makes pairing simple. A 2 m length means greater convenience and reduced cost while 0.15, 0.18, and 0.25 mm ID formats accommodate varying sample capacities, speeds, and detectors. And, of course, because they're Restek columns, you know you're getting the high thermal stability and unrivaled inertness you've come to rely on. Our chemists have been performing comprehensive two-dimensional gas chromatography since its commercial inception, and now you can put our years of GCxGC experience to work in your lab, too.

www.restek.com/gcxgc

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Flip Seal™ inlet seals feature a patented design that lets you simply flip them and use them again instead of throwing them away, so you get twice the life for the same price. Soft Vespel® rings embedded in the top and bottom surfaces eliminate the need for a washer and require very little torque to make a reliable seal. Choose gold plating or Siltek® treatment to reduce breakdown and adsorption of active compounds for maximum transfer onto the GC column. For decreased costs and increased performance, you owe it to your data to try our reversible Flip Seal™ inlet seals today.

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Chromatography in the News

1,4-Dioxane in Your Bathwater

Next time you take a bath, you might just be enjoying a nice, long soak in 1,4-dioxane. Dioxane is a by-product of the ethoxylation process, which is employed most notably to create sodium myreth sulfate and sodium laureth sulfate for the manufacture of soaps and cosmetics. Unfortunately, 1,4-dioxane is also a possible human carcinogen and has also been classified by the World Health Organization's International Agency for Research on Cancer (IARC) as a Group 2B compound. Global concern has prompted companies to begin eliminating it from their products and has also led to regulatory changes. For example, in the U.S., the recently signed third Unregulated Contaminant Monitoring Regulation (UCMR 3) will require monitoring using newly promulgated methods. 1,4-dioxane will be analyzed according to U.S. EPA Method 522, which concentrates the sample using solid phase extraction (SPE) instead of the most common technique previously used for this compound: purge and trap. Restek offers dioxane reference standards specifically formulated for Method 522, and you can find them at **www.restek.com/epa522**

Questions From You

Our Technical Service specialists field an astounding variety of questions from our customers. Today's featured topic is the flowmeter.

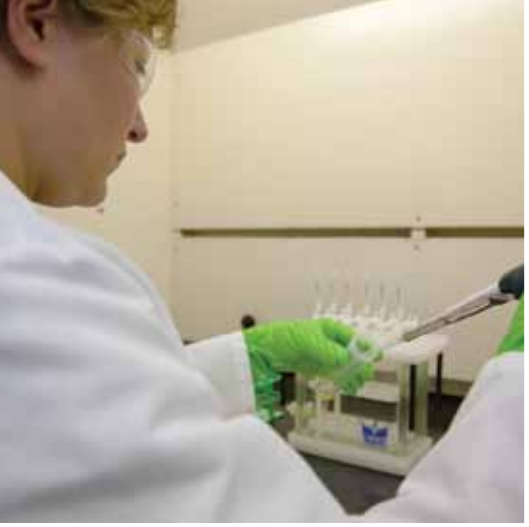
Q: Why do I see a difference in readings from different flowmeters?

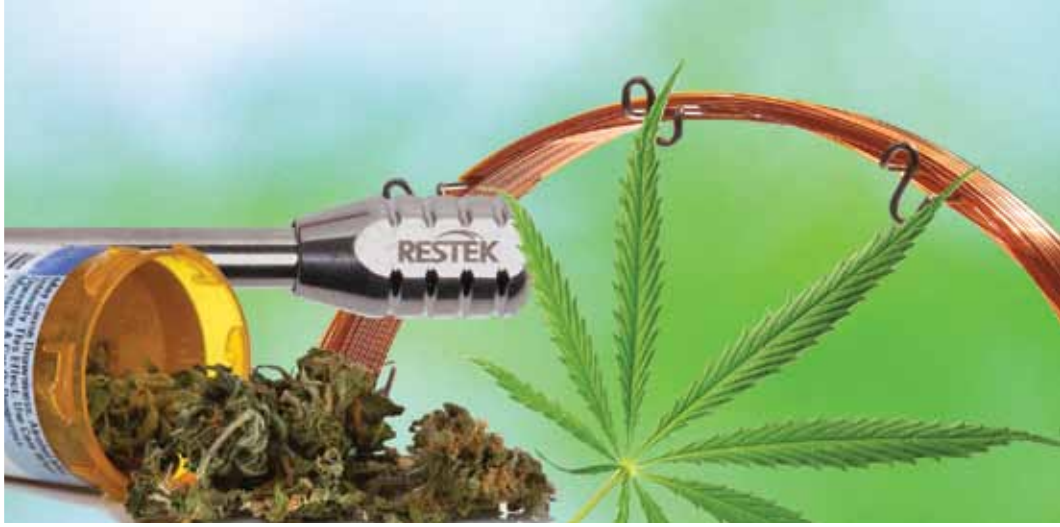
A: All flowmeters present some level of flow impedance, but the amount differs among meters. When any meter is connected to a flow source, the system is loaded which will usually result in a change of flow from the source. The amount of change in flow depends on the level of impedance. While each meter will display the correct current flow, they may have different readings because the actual flow changes based on the degree of impedance. For this reason, it is inappropriate to "check" the flow measurement of one volumetric flowmeter against that of another.

We just released a full FAQ on the ProFLOW 6000 flowmeter! Find answers to your questions at **www.restek.com/FAQFlow**

- **Brandon Tarr**
Product Development Engineer

Wrestling with a question of your own?
Call 1-814-353-1300, ext. 4, or email **support@restek.com** today!





Marijuana Potency Testing—Quick and Easy by GC or LC

By Amanda Rigdon and Jack Cochran

- Single extraction for both GC and LC.
- Fast results on Rxi®-5Sil MS GC or Ultra Aqueous C18 LC columns.
- Convenient standards for potency testing.

Although marijuana is illegal at the federal level in the United States, the use of medicinal marijuana is currently legal in many states. In some areas, it is widely used, and demand is rising for potency data for medicinal products purchased at dispensaries. Potency testing is more straightforward than impurity testing because the active compounds are present in much higher concentrations relative to matrix. Currently, GC is the most popular method for potency testing due to its ease of use and the availability of relatively inexpensive instrumentation. However, LC is also a viable technique for medical cannabis potency testing. As shown in this article, the same straightforward sample preparation technique can be used for cannabis potency testing by either GC or LC.

Simple Sample Prep

Cannabinoids were extracted from 7 different marijuana samples under the supervision of local law enforcement personnel. The extraction procedure consisted of weighing 0.2 g of sample into a 40 mL VOA vial, adding 40 mL of isopropyl alcohol, shaking for 5 minutes, and then allowing the sample to settle. The procedure was very quick and produced extracts that were compatible with both GC and LC analysis.

GC Analysis

The 3 compounds of interest for GC potency testing are Δ^9-tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD). While THC is primarily responsible for the hypnotic effects of marijuana, CBD acts to attenuate these effects. Since CBD has been shown to have medicinal properties, it is desired at higher concentrations in medical marijuana. Because the samples that were extracted were illicit samples seized by local law enforcement, the CBD levels were very low. In general, higher CBD levels are observed in medicinal marijuana strains. CBN is an indicator of sample breakdown due to age or poor storage conditions.

For GC potency testing, 1 µL of prepared extract was manually injected onto a 5890 GC equipped with a flame ionization detector and analyzed on a 15 m Rxi®-5Sil MS column (cat.# 13620). To ensure accurate and reproducible manual injections, a Merlin Microshot injector (cat.# 22229) was used. Figure 1 shows an overlay of a cannabinoid standard (cat.# 34014) that contains the 3 target analytes (blue trace) and a representative chromatogram of a marijuana sample (red trace). The use of a narrow-bore, thin-film analytical column resulted in sharp peaks, which improve sensitivity and allow a split injection to be used to reduce column contamination.

LC Analysis

LC potency testing requires the analysis of the 3 components discussed above, but also includes Δ^9- tetrahydrocannabolic acid (THCA). While THCA is not hallucinogenic, all THC in the marijuana plant exists as THCA, and only converts to THC upon heating (i.e., smoking, vaporizing, cooking, or injecting into a hot GC inlet). Since the sample extraction and LC analysis employ no heat, potency must be determined based on THCA when using LC, rather than with THC as is used in GC analysis.

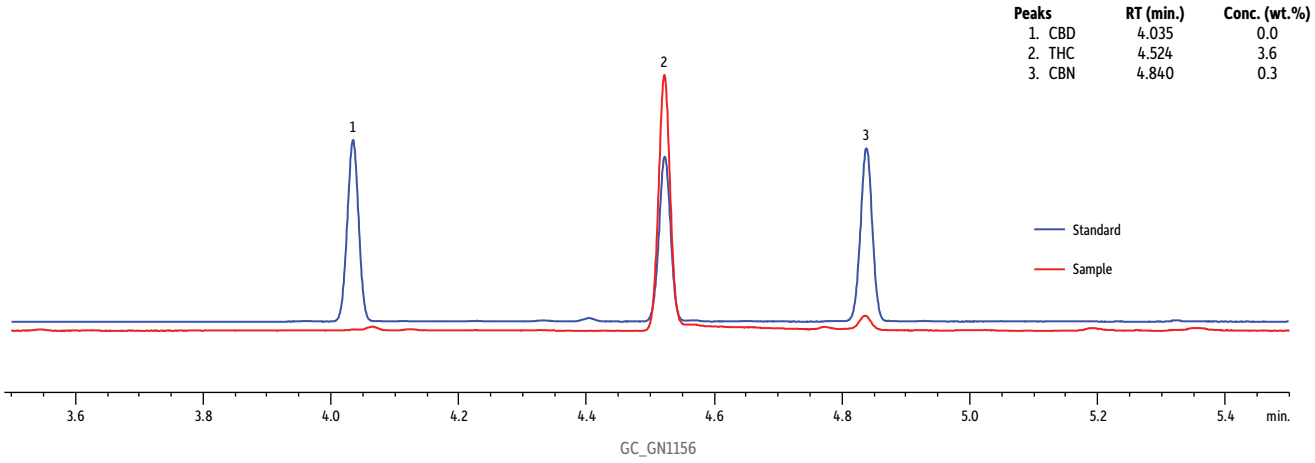
For LC potency testing, extracts were diluted 10x with isopropyl alcohol, and 10 µL of extract was injected onto a 3 µm Ultra Aqueous C18 column (cat.# 9178312). Figure 2 shows an overlay of the cannabinoid standard described above with the addition of THCA (blue trace) and a representative chromatogram of the same marijuana sample (red trace).

Summary

Both the GC and LC methods shown here for determining medical marijuana potency employ a straightforward and cost-effective extraction procedure and fast analysis times. This allows reliable potency analyses at a reasonable cost per sample.

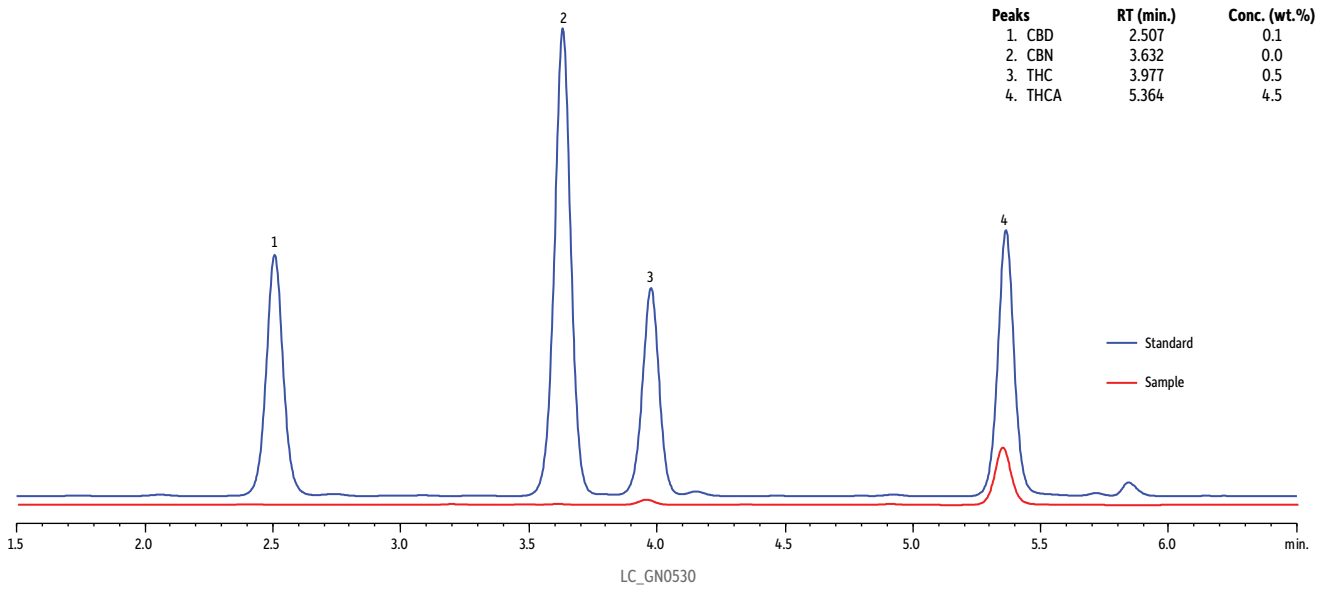
For further details, visit our technical blog at
www.restek.com/potpotency

Figure 1: Potency testing of marijuana using an Rxi®-5Sil MS GC column results in higher sensitivity for all target analytes.



Column: Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620); **Injection**: Inj. Vol.: 1 µL split (split ratio 20:1); Liner: Sky™ 4.0 mm ID single taper/gooseneck inlet liner w/wool (cat.# 23303.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp: 200 °C (hold 0 min.) to 300 °C at 15 °C/min. (hold 0 min.); **Carrier Gas**: H_2, constant pressure (7 psi, 48.3 kPa); Temp.: 200 °C; Dead Time: 0.6 min. @ 200 °C; **Detector**: FID @ 300 °C; Make-up Gas Flow Rate: 45 mL/min.; Make-up Gas Type: N_2; **Instrument**: HP5890 GC; **Notes**: Blue trace = cannabinoids standard (cat.# 34014) diluted to 100 µg/mL in isopropyl alcohol.; Red trace = extracted marijuana sample; Sample extraction: Weigh 0.2 g of sample into a 40 mL VOA vial, add 40 mL of isopropyl alcohol, shake for 5 minutes, and allow sample to settle.; Quantification: Potency values (weight%) were based on a 1-point standard curve using the standard show above.

Figure 2: Ultra Aqueous C18 columns easily separate THCA, which is used to determine marijuana potency when testing by LC.



Column: Ultra Aqueous C18 (cat.# 9178312); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 3 µm; Pore Size: 100 Å; Temp.: 30 °C; **Sample:** Inj. Vol.: 10 µL; **Mobile Phase:** A: Water + 10 mM potassium phosphate (pH = 2.5), B: Methanol; Flow: 0.4 mL/min.; Gradient (%B): 0 min. (80%), 1.0 min. (80%), 5.0 min. (95%), 6.0 min. (95%), 6.1 min. (80%), 8.0 min. (80%); **Detector**: UV/Vis @ 220, 4 nm; Cell Temp: 40 °C; **Instrument**: Shimadzu UFLCXR; **Notes**: Blue trace = cannabinoids standards (cat.#s 34014 and 34093) diluted to 100 µg/mL in isopropyl alcohol; Red trace = extracted marijuana sample; Sample extraction: Weigh 0.2 g of sample into a 40 mL VOA vial, add 40 mL of isopropyl alcohol, shake for 5 minutes, and allow sample to settle. Dilute extract 10x with isopropyl alcohol.; Quantification: Potency values (weight%) were based on a 1-point standard curve using the standard show above.

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; similar to 5% phenyl/95% dimethyl polysiloxane)

Description	temp. limits	cat.#
15m, 0.25mm ID, 0.25µm	-60 to 330/350°C	13620

similar **phases**

DB-5ms, VF-5ms, CP-Sil 8 Low-Bleed/MS, DB-5ms UI, Rtx-5Sil MS, ZB-5ms, Optima 5ms, AT-5ms, SLB-5ms, BPX-5

Ultra Aqueous C18 Columns (USP L1)

Description	cat.#
3µm Columns	
100mm, 2.1mm ID	9178312
3µm Columns	
100mm, 2.1mm ID (with Trident Inlet Fitting)	9178312-700

similar **phases**

AQUA C18, Aquasil C18, Hypersil Gold AQ, YMC ODS-Aq

Acknowledgment

Randy Hoffman, a Police Evidence Technician at The Pennsylvania State University (PSU), supplied the seized marijuana samples while overseeing their handling. Frank Dorman at PSU provided access to the samples and assisted with prep.

Simplify HPLC and UHPLC Method Development

With the Restek USLC™ Column Set

By Rick Lake and Ty Kahler

- Column selectivity has the most significant influence on chromatographic peak separation (i.e., resolution).
- Initially focusing on columns instead of mobile phases will drastically speed up method development.
- Restek's USLC™ column set boasts the widest range of selectivity available—using just 4 stationary phases!

Wasted effort. Lost time. Frustration. Making the wrong decisions can needlessly complicate and delay successful method development. By understanding selectivity's impact on resolution and focusing on column choice to create ***alternate*** selectivity, you can drastically speed up LC method development. Enter the new Restek Ultra Selective Liquid Chromatography™ (USLC™) columns.

Change Your Habits—and Your Columns—to Optimize Resolution

Resolution is the result of 3 cumulative terms: efficiency (N), retention capacity (k), and selectivity (α). How well and how quickly we resolve our analytes depends upon our ability to control these factors. Of the 3, selectivity affects resolution to the greatest degree (Equation 1). For that reason, any discussion about resolution in method development should focus on selectivity.

All too often, HPLC method developers use C18 columns and rely on adjusting mobile phases to alter selectivity and reach a desired separation. While it is true that mobile phase adjustments may alter selectivity, it is a laborious task that typically creates only marginal differences. In addition, some mobile phases are not practical with certain detection modes, including mass spectrometry (MS) and refractive index (RI). To save time and work, you should first focus on choosing the right stationary phases (i.e., columns). Columns pose fewer issues with MS and RI, change easily, and offer alternate and even orthogonal separations for maximum effect with each change.

Choosing columns can be incredibly difficult, but by characterizing stationary phase selectivity, we created new guidelines for easily making the right choice.

Equation 1: Selectivity is the driving parameter of resolution, as it affects peak separation to the greatest degree.

$$R = \tfrac{1}{4}\sqrt{N} \times (k/(k+1)) \times (\alpha-1)$$

Efficiency *Retention Factor* *Selectivity*

The Highest Range of Alternate Selectivity

Using the hydrophobic subtraction model (H-S model) [1], we quantified the selectivity of our stationary phases and determined which phases produce the greatest degree of dissimilarity compared to a C18 benchmark. We then matched these phases with specific solute types based on molecular interactions commonly encountered in reversed phase chromatography. By doing so, we were able to (1) find a small set of columns with the widest range of ***alternate*** selectivity available and (2) recommend columns based on the chemical properties of target analytes.

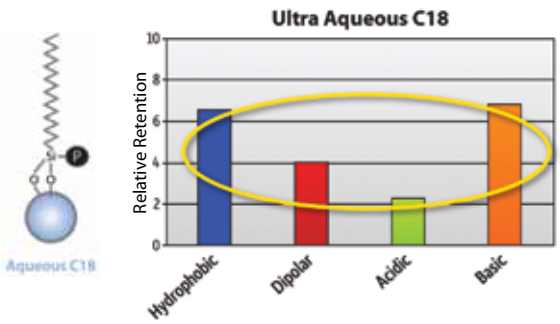
Figure 1 illustrates the retention profile of a C18 compared with those of the 4 Restek USLC™ columns. USLC™ phases are highly selective and exhibit significantly different retention profiles based on specific solute chemical properties, so you can match USLC™ columns to specific analytes and accelerate method development!

To confirm the orthogonality of the Restek USLC™ column set, we also quantified its selectivity (S) as described by Neue et al. [2] by looking at the degree of scatter along a regression line when compared to a conventional C18 (Figure 2). USLC™ phases produce the highest range of alternate selectivity available today—using only 4 columns.

Summary

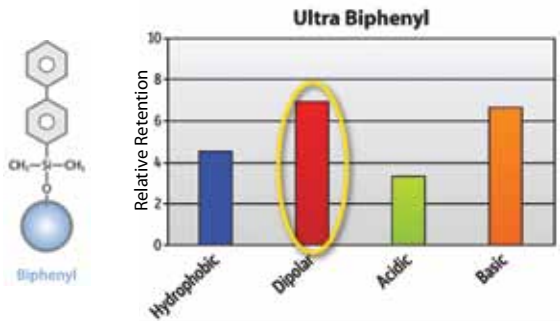
The Restek USLC™ column set has a profile that encompasses the widest range of reversed phase selectivity available today. Instead of manually altering mobile phases, operational parameters, or instrument settings—often with minimal effect on resolution—take advantage of the Restek USLC™ column set. These 4 orthogonal stationary phases and their defined retention profiles let you quickly determine the best column for almost any reversed phase situation.

Figure 1: Stationary phase selectivity can be characterized by looking for column types with varying retention profiles. When compared to a C18, the 4 Restek USLC™ phases offer diverse retention profiles—that is, a true range in selectivity.



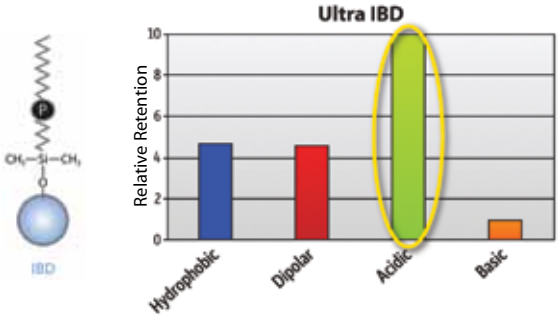
Restek USLC™ Phase: **Aqueous C18**

- **General purpose with a well-balanced retention profile.**
- Increased retention for acids and bases.
- Resistant to dewetting—compatible with 100% aqueous mobile phases.



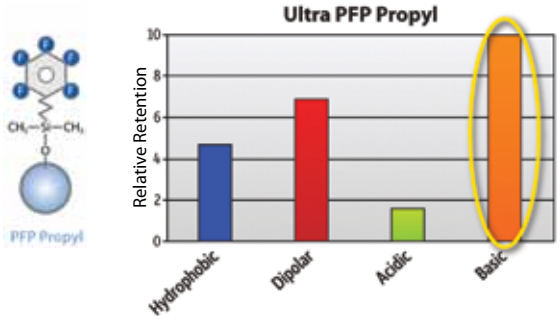
Restek USLC™ Phase: **Biphenyl**

- **Increased retention for dipolar, unsaturated, or conjugated solutes.**
- Increased retention for fused-ring solutes containing electron withdrawing ring substituents.
- Enhanced selectivity when used with methanolic mobile phase.



Restek USLC™ Phase: **IBD**

- **Increased retention for acids.**
- Moderate retention for hydrophobic and dipolar solutes.
- Resistant to dewetting—compatible with 100% aqueous mobile phases.
- Capable of multi-mode mechanisms.

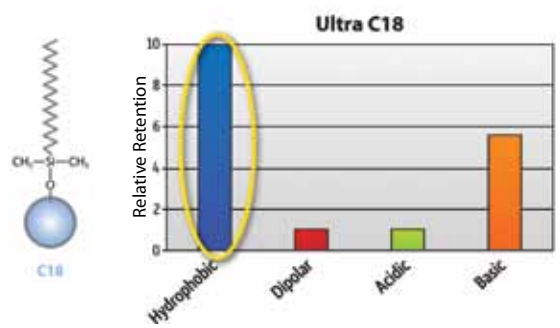


Restek USLC™ Phase: **PFP Propyl**

Properties:

- **Increased retention for protonated bases.**
- Increased retention for solutes containing dipolar moieties.
- Capable of multi-mode mechanisms.

C18 BENCHMARK

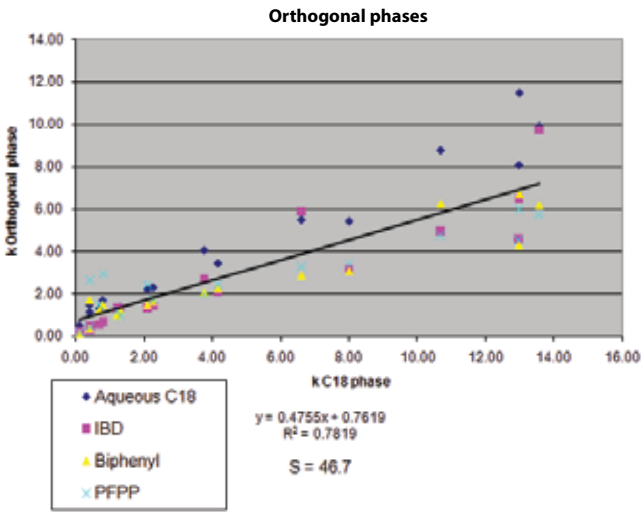


Restek Phase: **C18 Benchmark**

- General purpose.
- Strong hydrophobic retention.

All columns in Figures 1 and 2 were tested using the same silica support.

Figure 2: Restek has extended the selectivity (S) for a range of columns and defined a set—the 4 USLC™ phases—that is ideal for fast column selection and faster method development.



For a detailed analysis of USLC™ column selectivity data, visit **www.restek.com/USLCarticle**

References

[1] L.R. Snyder, J.W. Dolan, P.W. Carr, *The Hydrophobic-Subtraction Model of Reversed-Phase Column Selectivity,* J. Chromatogr. A 1060 (2004) 77.

[2] U.D. Neue, J.E. O'Gara, A. Mendez, *Selectivity in Reversed-Phase Separations Influence of the Stationary Phase,* J. Chromatogr. A 1127 (2006) 161.

Acknowledgements

The authors gratefully acknowledge the contributions of Dr. Lloyd Snyder from LC Resources and Dr. Frank Dorman from The Pennsylvania State University. The authors also wish to thank the contributing team of researchers Randy Romesberg, Bruce Albright, Mike Wittrig, Brian Jones, and Vernon Bartlett.

Large Volume Splitless Injection With an Unmodified GC Inlet

Lets You Skip Sample Concentration for Pesticides and BFRs in Drinking Water

By Michelle Misselwitz and Jack Cochran

- Eliminate time-consuming extract concentration without sacrificing sensitivity.
- Simplified approach uses standard injection port—no specialized equipment.
- Analyze at sub-ppb levels with faster, less labor-intensive procedure.

Using large volume splitless injection is advantageous when trying to analyze trace-level contaminants in clean matrices like drinking water because greater levels of target compounds are introduced onto the analytical column. A special injection port is generally required for large volume injection, which has limited its application. A concurrent solvent recondensation–large volume splitless injection (CSR-LVSI) technique described by Magni and Porzano [1,2] offered a more practical alternative, but involved some modification of a split/splitless injection port.

We have used CSR-LVSI successfully with a completely unmodified Agilent split/splitless GC inlet. The setup utilizes a pre-column (e.g., 5 m x 0.53 mm) press-fitted to the analytical column and a starting GC oven temperature below the boiling point of the solvent. A fast autosampler injection with liquid band formation into a liner containing glass wool is used to prevent backflash in the injection port. Here we investigated the applicability of this approach to analyzing pesticides and brominated flame retardants (BFRs) in drinking water according to U.S. EPA Method 527 [3].

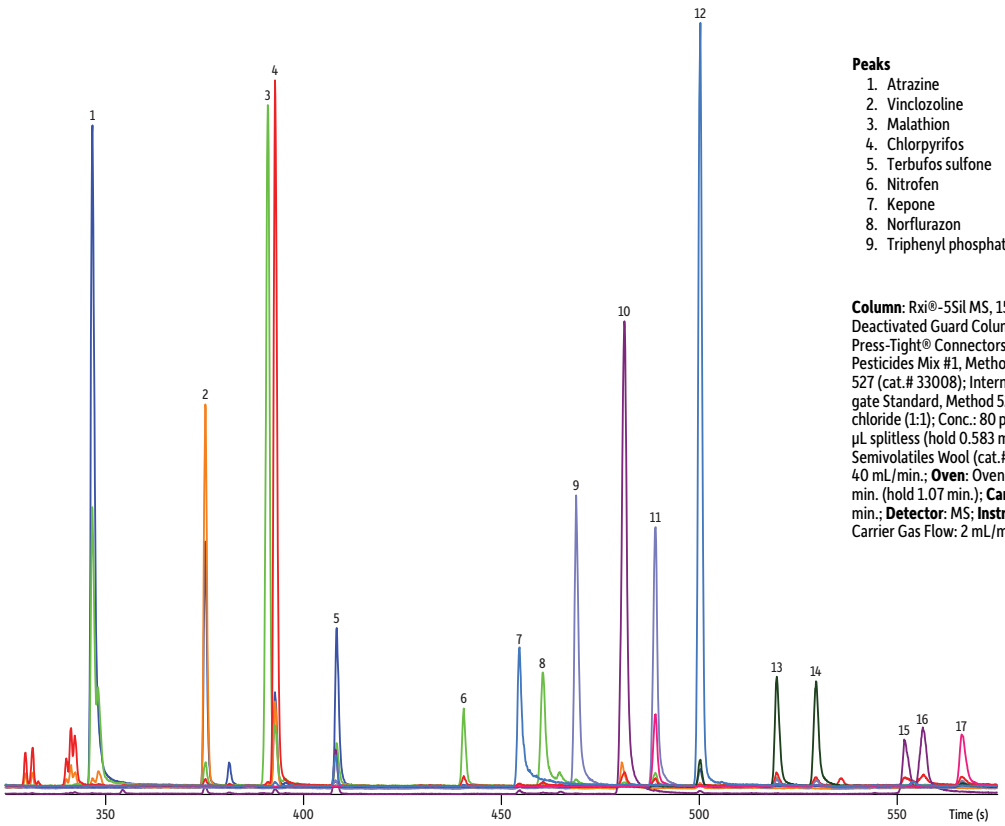
Table I: Calibration standards and concentration equivalents.

Level	Prepared Standard (pg/µL)	On-Column Amount Injected (pg/12.5 µL)	Equivalent Concentration in 1 L Samples (ug/L)
1	2	25	0.05
2	4	50	0.1
3	10	125	0.25
4	20	250	0.5
5	40	500	1
6	80	1,000	2

Table II: Average percent recoveries and relative standard deviations for 1 µg/L and 0.1 µg/L laboratory fortified blank samples analyzed using disk extraction with no extract concentration and CSR-LVSI GC-TOFMS (n = 3).

	1.0 µg/L % Recovery		0.1 µg/L % Recovery	
Compounds	AVG (n = 3)	%RSD	AVG (n = 3)	%RSD
Dimethoate	73	2.4	75	9.3
Atrazine	96	1.8	84	13
Propazine	93	3.3	92	8.5
Vinclozoline	97	4.0	97	8.0
Prometryne	179	3.0	113	7.9
Bromacil	78	2.2	66	3.1
Malathion	98	2.7	85	6.5
Thiobencarb	93	3.9	70	1.9
Chlorpyrifos	92	3.1	84	1.7
Parathion	94	0.7	92	4.6
Terbufos sulfone	88	2.8	105	11
Oxychlordane	75	8.5	74	10
Esbiol	88	2.7	79	6.5
Nitrofen	91	3.0	77	5.3
Kepone	102	18	56	32
Norflurazon	91	7.2	105	10
Hexazinone	87	0.8	68	2.1
Bifenthrin	100	3.0	81	3.2
BDE-47	96	4.4	87	15
Mirex	93	4.5	76	2.3
BDE-100	93	3.8	89	11
BDE-99	93	2.9	79	33
Perylene-D12	103	1.6	98	3.3
Fenvalerate	92	0.4	59	16
BB-153	88	3.4	45	14
Esfenvalerate	89	3.7	69	20
BDE-153	88	13	54	49

Figure 1: Extracted ion chromatogram of 80 pg/µL standard from 12.5 µL CSR-LVSI injections.



The typical procedure for preparing samples according to EPA Method 527 involves extracting a 1 L water sample, drying the extract, and concentrating it down to a final volume of 1 mL. To determine if using CSR-LVSI could eliminate the need for extract concentration, linearity and recovery were assessed. Water samples were fortified at 0.1 µg/L and 1 µg/L levels and then extracted using Resprep® resin SPE disks, dried with anhydrous sodium sulfate, and diluted to 25 mL with methylene chloride:ethyl acetate (1:1). This differs from the method, which calls for the samples to be concentrated to 1 mL after drying. In order to achieve the detection limits described in the method, a 12.5 µL injection volume was used.

Linear Responses for Challenging Compounds Using CSR-LVSI

Calibration curves were built using duplicate 12.5 µL injections of 2, 4, 10, 20, 40, and 80 pg/µL standards. All compounds exhibited good linearity down to 2 pg/µL, which is equivalent to 25 pg on-column and 0.05 µg/L in the original water sample (Table I). Results for Kepone (r = 0.995) are especially notable, as it can be problematic due to the formation of a hemiacetal that chromatographs poorly. Good chromatographic separations were obtained using a 15 m x 0.25 mm x 0.25 µm Rxi®-5Sil MS column, and the fast oven program resulted in an analysis time of less than 10 minutes (Figure 1).

Determine Sub-ppb Levels Without Extract Concentration

The average recovery for all compounds for the 1 µg/L (500 pg on-column) and 0.1 µg/L (50 pg on-column) spikes were quite good at 94% and 80%, respectively (Table II). Individual recoveries met EPA Method 527 criteria, except for the 0.1 µg/L value for hexabromobiphenyl 153 (BB-153) and the 1.0 µg/L value for prometryne. Recovery results demonstrated that employing CSR-LVSI and eliminating the concentration step can be an effective way to meet detection limits while reducing sample preparation time by more than an hour.

Summary

When the extract concentration step was eliminated, good linearity and recovery results were obtained while sample preparation time was significantly reduced. CSR-LVSI with an unmodified Agilent split/splitless GC inlet has been shown to be a technically viable approach that has the advantage of speeding up sample preparation without compromising sensitivity for pesticides and BFRs in drinking water.

For the complete version of this technical article, visit **www.restek.com/LVSI**

References

[1] P. Magni, T. Porzano, J. Sep. Sci. 26 (2003) 1491.
[2] Patent No: US 6,955,709 B2.
[3] U.S. Environmental Protection Agency, Method 527, Determination of Selected Pesticides and Flame Retardants in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS), April 2005.

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; similar to 5% phenyl/95% dimethyl polysiloxane)

Description	temp. limits	cat.#
15m, 0.25mm ID, 0.25µm	-60 to 330/350°C	13620

Resprep® Resin SPE Disks

Description	qty.	cat.#
Resprep Resin SPE Disks	20-pk.	26023

Extending the Power of Stabilized PLOT Column Technology to Process GC Analyzers

By Jaap de Zeeuw, Rick Morehead, and Tom Vezza

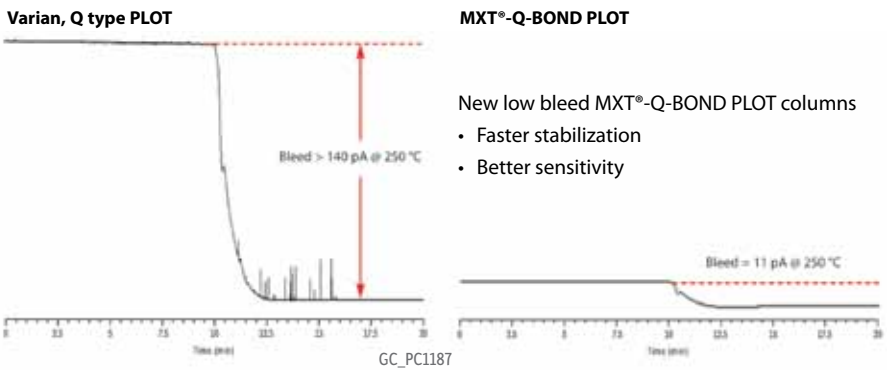
- New technology ensures consistent flows and predictable retention times.
- Rugged metal MXT® tubing stands up to process GC analyzer conditions.
- Available with all major adsorbents in 3.5" coils or on 7" 11-pin cages.

Porous layer open tubular (PLOT) columns are useful for analyzing volatiles in petrochemical product streams, as the specialized adsorbents provide good resolution and fast analysis times. However, conventional PLOT columns suffer from poor mechanical stability, limiting their use in process analyzers, which require robust columns for continual operation. Recently Restek developed new PLOT column bonding techniques that result in improved layer stability, consistent flow behavior, and more reproducible retention times. This technology, which was first developed for fused silica columns, has now been transferred to metal MXT® tubing, resulting in rugged columns that outperform typical metal PLOT columns and are ideal for process GC analyzers.

New Technology Improves Column Stability

Restek's PLOT columns are stabilized through a proprietary process that is based on concentric adsorption layers and improved particle bonding. New MXT® PLOT columns show greater thermal stability and much less phase bleed than the comparable competitor product (Figure 1). Lower bleed improves sensitivity and ensures faster stabilization times.

Figure 1: The bonding technology used in new MXT® PLOT columns increases thermal tolerance, resulting in lower bleed, faster stabilization times, and higher sensitivity.



Bleed comparison: Q type porous polymer columns were conditioned at 250 °C for equivalent periods and then tested to evaluate temperature stability. Split vent flow rate: 150 mL/min.; Oven: 250 °C (hold 10 min.) to 40 °C at 50 °C/min.; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

Figure 2: Conventional PLOT columns show continuous spiking resulting from particle generation. In contrast, the Restek column showed spikes during only the 2 initial analyses out of 240.

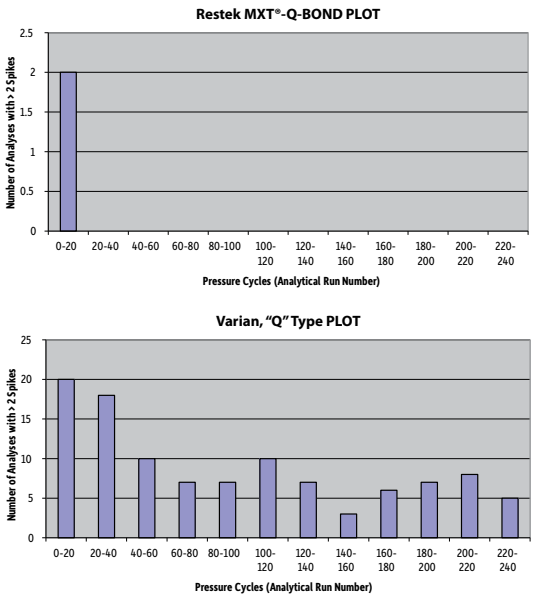
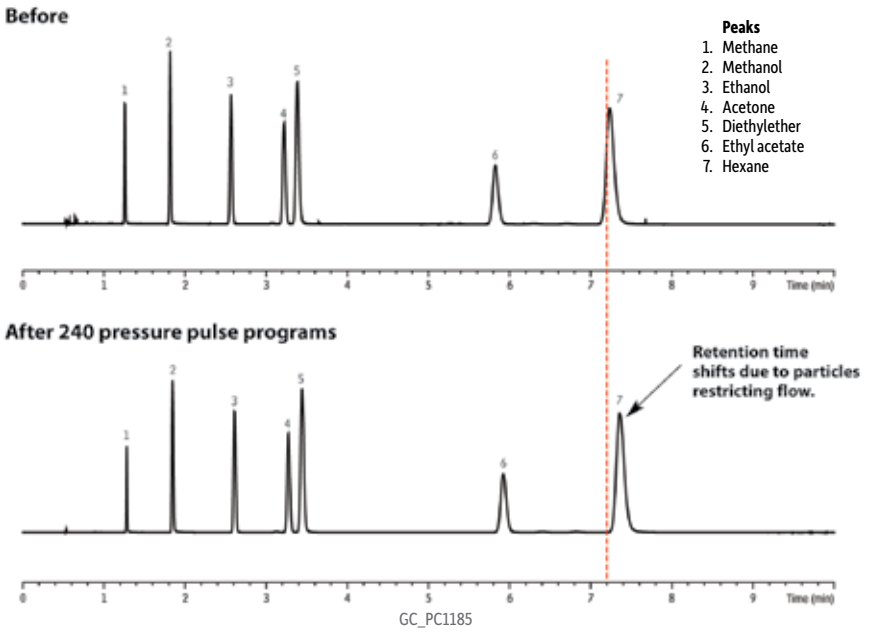
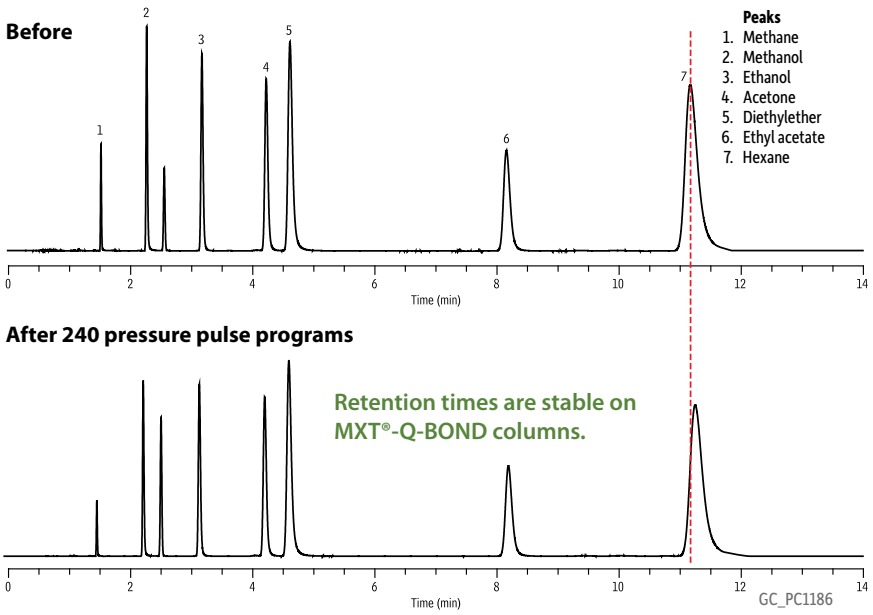


Figure 3: A conventional PLOT column releases particles following pressure pulsing, forming restrictions in the column that affect flow behavior and change retention time.



Isothermal testing before and after 240 pressure pulse cycles. Column: Varian Q type PLOT, 25 m x 0.53 mm ID; Sample: solvent mix; Injection: 1 µL split, 250 °C; Split vent flow rate: 150 mL/min.; Oven: 150 °C; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

Figure 4: MXT® PLOT columns are exceptionally stable; flow characteristics and retention times are highly consistent and not affected by pressure pulses.



Isothermal testing before and after 240 pressure pulse cycles. Column: MXT®-Q-BOND PLOT, 30 m x 0.53 mm ID x 20 µm (cat.# 79716); Sample: solvent mix; Injection: 1 µL split, 250 °C; Split vent flow rate: 150 mL/min.; Oven: 150 °C; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

Stable Flow Ensures Predictable Retention Times

To demonstrate the superior stability of MXT® PLOT columns, an MXT®-Q-BOND column and a competitor's Q type column were subjected to 240 pressure pulse cycles and the spiking observed in each analytical run was used as an indicator of particle generation, or phase instability. Results demonstrate that particle generation on the Varian column was significantly higher (Figure 2), resulting in restrictions in the column that caused a shift in retention time (Figure 3). In contrast, the MXT®-Q-BOND column showed little spiking. Greater phase stability resulted in consistent flow behavior and predictable retention times (Figure 4).

Key Phases Available for Optimized Separations

New metal MXT® columns are available for all major adsorbent types: porous polymer, molecular sieve, and alumina. Porous polymer MXT® columns, such as the MXT®-Q-BOND column, are highly inert and effective at separating both polar and nonpolar compounds. Volatiles are strongly retained, making these columns extremely useful for determining solvents. Molecular sieve columns provide efficient separation of argon and oxygen, as well as other permanent gases. Metal MXT® alumina columns are recommended for light hydrocarbon analysis, as alumina is one of the most selective adsorbents available and allows all C1-C5 isomers to be separated with the highest degree of resolution.

Summary

MXT® PLOT columns from Restek offer greater stability than conventional PLOT columns, making them a better choice for process monitoring. New bonding techniques produce columns with highly reproducible flow characteristics, improved layer stability, and excellent separation efficiencies. These robust columns produce exceptionally reproducible chromatography, providing the reliable performance needed for process GC analyzer applications.

For the complete version of this technical article, visit
www.restek.com/metalPLOT

MXT®-Q-BOND Columns

(Siltek®-treated stainless steel PLOT)

ID	df	temp. limits	3.5" coil 15-Meter	7" 11-pin cage 15-Meter	3.5" coil 30-Meter	7" 11-pin cage 30-Meter
0.25mm	8µm	to 280/300°C	79718-273	79718		
0.53mm	20µm	to 280/300°C			79716-273	79716

Other phases available, visit www.restek.com/metalPLOT for details.

Rethinking the Use of Wool With Splitless GC

By Scott Grossman

- An obstruction like wool is a must for efficient vaporization under split conditions.
- Wool is also necessary under splitless conditions to minimize sample loss and improve transfer onto column.
- With exceptionally inert Sky™ inlet liners, you can use wool with confidence.

When running a split injection with an autosampler, few would challenge that you need a liner with an obstacle like wool to achieve accurate, precise results. After all, when you combine a fast injection with a high split flow rate, your sample simply needs more time to vaporize or else it may be lost out the split vent. Wool stops the sample and gives it the time it needs to efficiently and completely vaporize, presenting a homogenous mixture to the column and split vent. Unlike in split injections, conventional wisdom has long held that you do not need wool under splitless conditions. However, a highly recommended paper by Bieri et al. argues that wool is just as important in splitless work. [1]

Should Splitless Mean Wool-Free?

Why do so many chromatographers believe that wool is not necessary to get accurate and representative sample transfer in a splitless run? The only flow out of the inlet (other than the septum purge) is through the column, so the thinking is that, since the flow will be so much slower than it is under split conditions, the sample will have ample time to vaporize and transfer onto the column without assistance. But, could autoinjecting the sample using a fast plunger speed pose a problem? And can't the sample still become trapped or be lost? The visualization and chromatographic experiments Bieri et al. outlined were very effective in supporting their claim that wool is a must for split ***and*** splitless runs alike. So, I decided to expand upon their work using common styles of splitless liners.

Putting Wool Through the Wringer

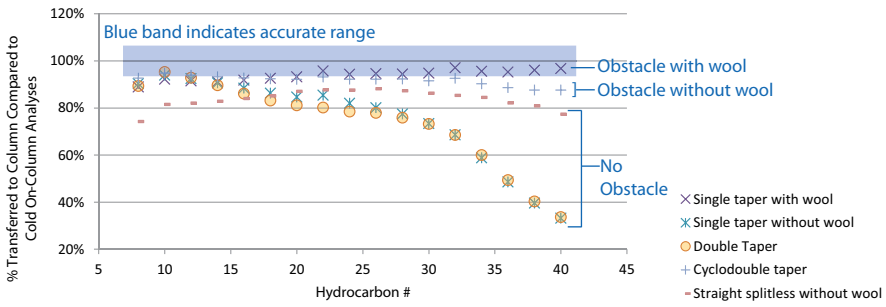
Since the integral question is whether you lose sample when performing splitless injections without wool, I opted to benchmark with cold on-column injections to force 100% of the sample onto the column. My sample was a 17-component mixture of straight-chain hydrocarbons spanning a molecular weight range from C8 to C40. In addition to cold on-column capability, my GC also had a split/splitless inlet, so I collected all response data using the same FID.

Figure 1 shows the data from a series of splitless analyses using the same sample but different liners. Results clearly illustrate that, for a wide molecular weight range, the use of wool—or to a lesser degree another obstacle like a cyclo double gooseneck—is necessary for accurate sample transfer and a reduction of molecular weight discrimination. You can also see that the only time the entire mass of analytes was transferred to the column under splitless conditions was when we employed a single gooseneck with wool. The liners with no obstruction had much less desirable results.

Use Wool With Confidence

Of course, there is a reason why one may prefer not to use wool: It is a common source of activity that can break down and trap sensitive analytes. In that case, how do you avoid counteracting wool's advantage in improving vaporization? The wool in a Sky™ inlet liner is made of fused quartz and is deactivated after packing, reducing the loss of sensitive analytes (Figure 2). By using Sky™ liners with exceptionally inert wool, you can help ensure efficient vaporization and improved transfer onto your column for more accurate results and lower detection limits. With Restek Sky™ inlet liners, you can use wool with confidence—and should under split ***and*** splitless conditions.

Figure 1: Only the liners with an obstruction were able to produce even 90% sample transfer with splitless injections—and only the liner with wool offered full accuracy.

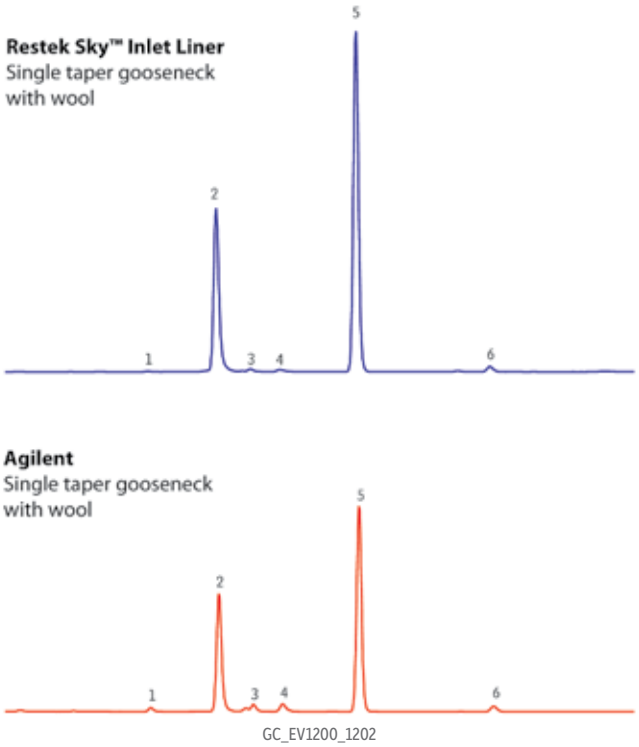


References

[1] Stefan Bieri, Philippe Christen, Maurus Biedermann, and Koni Grob, *Inability of Unpacked Gooseneck Liners to Stop the Sample Liquid After Injection With Band Formation (Fast Autosampler) Into Hot GC Injectors*, Anal. Chem. 76 (2004) 1696.

For a closer look at the form and function of GC inlet liners, view Scott's webinar at **www.restek.com/linerwebinar**

Figure 2: Endrin and DDT breakdown is significantly reduced with Sky™ liners, due to higher inertness and lower activity—even when using wool.



Inert Sky™ liners reduce analyte breakdown, giving you more accurate results.

% Breakdown

	Endrin	DDT
Restek	**4.8**	**1.3**
Agilent	12	5.2

Peaks

1. DDE*
2. Endrin
3. DDD*
4. Endrin aldehyde*
5. DDT
6. Endrin ketone*

*breakdown products

Column Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620); **Sample** endrin (50 ng/mL) and DDT (100 ng/mL) in hexane; **Injection** Inj. Vol.: 1 µL splitless (hold 0.75 min.); Liner: Comparison of Sky™ Single Taper Gooseneck Liner with Wool (cat.# 23303.5) and Agilent Single Taper Gooseneck Liner with Wool (cat.# 5062-3587); Inj. Temp.: 250 °C.; **Detector:** µ-ECD @ 300 °C.

Analysis of Brominated Flame Retardants by Liquid Chromatography Mass Spectrometry

By Dr. Chris Marvin, Environment Canada

***Dr. Chris Marvin** is a Research Scientist for Environment Canada, Burlington, Ontario. His research interests include new and emerging environmental contaminants, occurrence and fate of contaminants in the Great Lakes, and LC-MS methods development.*

A wide variety of brominated flame retardants (BFRs) are currently used in industry and commerce. Use of these compounds has increased exponentially in the past 50 years as a result of strict regulations regarding the flame retardancy of consumer products. Roughly 40% of all flame retardants on the market are brominated. Some of these compounds have the potential to be persistent, toxic, bioaccumulative, and are amenable to long range transport. In addition, the occurrence, distribution, and fate of many of these compounds in the environment remain largely unknown.

Polybrominated diphenyl ethers (PBDEs) remain the most widely studied of the BFRs, despite the penta- and octa-formulations being banned in Europe and voluntary cessation of production in North America. With the exception of the fully-substituted decabromodiphenyl ether (BDE-209), the PBDEs are easily determined by gas chromatography-mass spectrometry (GC-MS) and are now routinely measured in a wide range of environmental matrices. Due to its unique chemical and physical properties, including high molecular weight, poor solubility, and sensitivity to heat and light, accurate determination of BDE-209 remains a significant challenge. A host of other BFRs are not readily amenable to analysis by GC-MS and pose an analytical challenge as a result of their physical properties. Although their chemical structures appear quite simple, BFRs such as hexabromocyclododecane (HBCD), 1,2,5,6-tetrabromocyclooctane (TBCO) and tetrabromoethylcyclohexane (TBECH) thermally isomerize and partition poorly on GC stationary phases. HBCD is one of the most widely used BFRs with production globally in excess of 20,000 tons; HBCD is the primary flame retardant used in the extruded and expanded polystyrene foams used as thermal insulation in buildings, as well as in upholstery fabrics. Some laboratories continue to report HBCD concentrations as the sum of the three predominant isomers based on analysis by GC, i.e., the sum of α-, β- and γ-HBCD. These nonisomer specific analyses preclude thorough investigation of environmental pathways, and potential shifting of isomer profiles during manufacture or cycling in the environment. Differences in pathways of HBCD in the environment are evidenced by the predominance of γ-HBCD in the technical mixture and in sediment, while α-HBCD is dominant in

biota (typically >90%). In addition, an inherent property of aliphatic BFRs is that they exist as diastereomers. Therefore, the study of enantioselective accumulation of BFRs in food chains requires separation of the individual enantiomers.

The last decade has been a period of extraordinary progress in development of LC-MS technology. As a result, detection limits of some LC-MS methods are on a par with those of gas chromatography-high resolution mass spectrometry (GC-HRMS) methods. These technological advances allow the resolving power of contemporary LC stationary phases to be coupled with the sensitivity and specificity of state-of-the-art mass spectrometers. In addition, electrospray ionization (ESI), one of the most commonly used ionization mechanisms, is softer than electron ionization (EI) used in GC-MS. Robust LC-MS methods for analysis of BFRs, including HBCD and tetrabromobisphenol-A (TBBPA), are now routinely used in analytical laboratories. Most methods for analysis of BFRs are based on negative ion mass spectrometry. Despite these advances, significant analytical challenges remain in LC-MS methods development. LC-MS continues to be susceptible to matrix effects, and the technique still generally lacks the retention time reproducibility of GC-MS methods. The use of isotopically-labeled internal standards is effective in minimizing matrix effects, but investigations of new chemicals continue to be plagued by a paucity not only of labeled compounds, but authentic native standards.

Other challenges of LC-MS analysis of BFRs can include poor ionization efficiency and limited fragmentation. In the case of TBCO and TBECH, both ESI and atmospheric pressure chemical ionization (APCI) result in weak molecular ions or molecular ion adducts. Adequate detectability of the compounds can be achieved by monitoring the Br- ions in selected ion monitoring (SIM) mode; however, this approach negates the advantages of a triple quadrupole mass spectrometer, in that the power of tandem MS techniques cannot be exploited. Atmospheric pressure photoionization (APPI) is the latest ionization technique developed for LC-MS; in fact, the impetus behind development of APPI was the need to extend the range of compounds beyond those only amenable to ESI or APCI. Typical variations of the technique are based on vaporization of the liquid sample (similar to APCI), combination with a dopant, and subsequent ionization resulting from gas phase reactions initiated by photons from a krypton discharge lamp. APPI has shown great potential for analysis of compounds across a broad range of polarities, but particularly for nonpolar analytes. The method is also reportedly less susceptible to matrix effects than ESI and APCI.

Progress in LC-MS methods development continues as lessons learned from investigations of individual compounds are applied to subsequent generations of BFRs. A new challenge in the evolution of LC-MS methods for BFRs is the development of comprehensive methods for concurrent analysis of multiple compound classes. The primary challenge in development of comprehensive methods is identification of suitable LC stationary phases coupled with MS ionization techniques applicable to compounds exhibiting a broad range of chemical and physical characteristics. The LC stationary phase must provide adequate separation among compounds that can exhibit dramatically different retention behaviors; key factors include particle size, pore size, and stationary phase chemistry. In addition, even individual isomers within the same compound class can exhibit significantly different mass spectrometric response factors. A further convoluting factor is the limited solubility of BFRs in typical reversed phase (RP) HPLC mobile phases. Many BFR standards are marketed in nonpolar solvents such as toluene, necessitating a solvent exchange step prior to analysis. The same issue arises for BFRs isolated from environmental samples using conventional column cleanup methods, in that these techniques frequently culminate in the extracts being concentrated in nonpolar solvents amenable to analysis by GC.

The primary challenge in development of comprehensive methods is identification of suitable LC stationary phases coupled with MS ionization techniques applicable to compounds exhibiting a broad range of chemical and physical characteristics.

Ultimately, partnerships among experts in the field of analytical standards, separation science, and mass spectrometry will yield viable comprehensive methods for BFRs. In the past few years, suppliers of analytical standards and manufacturers of LC stationary phases and mass spectrometers have been astute in recognizing trends in analysis of compounds of potential environmental concern, and correspondingly have been proactive in developing technologies of great value to the toxics research and monitoring community.

Restek Has Added ISO Guide 34 and 17025 Accreditations

We Now Offer a Full Line of Certified Reference Materials!

Restek is proud to announce that our reference standard manufacturing and QA testing labs in Bellefonte, PA, have earned ISO Guide 34 and 17025 accreditations through A2LA. More than ever, you can rely on Restek for all of your reference standards, and now, you can also experience the advantages of our ISO accreditations:

- **Satisfy regulatory requirements** by sourcing CRMs from an accredited supplier.
- Benefit from the **exceptional product quality and customer service** needed to meet strict ISO 9001, Guide 34, and 17025 guidelines.
- Get the **same reliability and documentation with custom-formulated solutions** as you do with stock standards—both fall under Restek's accreditation.
- **Eliminate POs** by ordering primary- and secondary-source reference standards, GC and LC columns, sample prep supplies, and accessories from one vendor.

We invite you to visit **www.restek.com/iso** to learn more about our ISO quality credentials and view our certificates (including scopes of accreditation).

If you have any questions or would like more information, feel free to contact customer service at **814-353-1300, ext. 3**, or **csreps@restek.com**

Note: *If your lab must use certified reference materials (CRMs), please be sure to tell your Restek representative when ordering so we can help you meet your regulatory requirements as we transition our inventory.*

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Our expertise, experience, and enthusiasm is your Advantage.

RESTEK ADVANTAGE

Weeding Target Analytes Out of Complex Samples

Also in this issue

Restek Connections

In This Issue

Letter from the Bench

Welcome to the new look for your *Restek Advantage*!

When we sat down to plan this issue, one of our goals was to share more chromatography news and better connect with you, our reader. That's how our expanded Hot Topics and new Restek Connections departments came to be. My friend and colleague, George Fong, is retiring as head of the Florida Pesticide Residue Workshop after almost 50 years. Restek just introduced a new line of secondary columns for GCxGC. Get the latest scoop on these topics and more over the next 3 pages!

Of course, as always, much of this *Advantage* highlights the application work of our Innovations Lab, where we're lucky to have seasoned veterans working alongside young, enthusiastic chemists to solve your toughest problems. Looking to determine trace-level compounds in complex sample matrices like marijuana and urine? You'll be interested to read our articles on pesticide and synthetic cannabinoid analysis using both advanced GCxGC-TOFMS and LC-MS/MS platforms.

LC-MS/MS has revolutionized analytical chemistry, but it still relies on good chromatography. Rick Lake and Ty Kahler show you how to get the most selectivity for your LC separations. Their work employs the hydrophobic subtraction model to define a highly selective and orthogonal set of 4 USLC™ columns.

Chromatographic column selectivity has always been a Restek forte, and Jason Thomas proves it yet again using one Rtx®-CLPesticides column pair for 7 GC-ECD environmental methods. In these cases, chromatographic separation is mandatory for accurate, quantitative work, as the ECD is not a specific detector.

But that's not all: PLOT columns in process GC, wool in GC inlet liners, large volume splitless injection... We have something inside for every analyst.

Finally, we also set up a new email address: **advantage@restek.com** Use it to let us know what you think of your new *Restek Advantage*. I say "*your*" because we create this technical document with your needs and interests in mind. Your feedback will be invaluable for assembling future issues.

Cheers!

Jack Cochran
Director of New Business & Technology

You Have Opinions... And We Want Them.

We chemists are an opinionated bunch, so the odds are good that you have some thoughts about the *Restek Advantage*. Love it? Hate it? Want to see something different in the next issue? Maybe you have a response to one of our technical articles? Whatever you have to say, let's hear it! Email your comments to advantage@restek.com and you may even see them in an upcoming issue.

About Restek Corporation

A leading innovator of chromatography solutions for both LC and GC, Restek has been developing and manufacturing columns, reference standards, sample preparation materials, accessories, and more since 1985. We provide analysts around the world with products and services to monitor the quality of air, water, soil, food, pharmaceuticals, chemicals, and petroleum products. Our experts enjoy diverse areas of specialization in chemistry, chromatography, engineering, and related fields as well as close relationships with government agencies, international regulators, academia, and instrument manufacturers.

Sitting Down With a Chromatography Icon:
W. George Fong

By Jack Cochran

W. George Fong

Earlier this year, we received some sad news: George and Wilma Fong were retiring after almost 50 years at the helm of Florida Pesticide Residue Workshop (FPRW). The field of pesticide detection and analysis would not be what it is today without FPRW or George and Wilma Fong. They will be missed.

After cheering the Fongs when they accepted the inaugural FPRW service award—named in their honor—I was fortunate enough to catch up with George. Here's just a small peek at our discussion.

Jack: What made you decide to start FPRW?
George: I felt very isolated from technical information. I suggested... that a periodical meeting for all Chemical Residue Laboratory (CRL) chemists and inspectors to discuss analytical technology and regulatory matters was necessary.

The first intra-lab CRL meeting was held in Tallahassee during the holidays of 1964. The following meeting in 1965 was held at the Sanford field laboratory. The late Dr. Charles H. Van Middelem was invited to speak... Dr. Van Middelem presented to us the technical requirements of pesticide residue analysis. He suggested that CRL and Interregional Research Project (IR-4) could work closely and encouraged such meetings…

Jack: Has the meeting always been called the Florida Pesticide Residue Workshop?
George: There were no names for the first few meetings; they were like discussion gatherings. The 1966 workshop… had speakers from the FDA in addition to CRL chemists... We asked each attendee to speak or just to give a short talk about their laboratory work. We particularly encouraged attendees from government agencies to describe their programs. I believe the name [FPRW] was introduced a few years later.

Soon after, PCBs (polychlorinated biphenyls) became an issue. CRL was one of the first laboratories to analyze residues of PCBs and PCB congeners using the Pestilyzer. We shared our knowledge with other state laboratories…

Jack: How has FPRW impacted pesticide residue analysis over the years?
George: Its biggest impact has been in providing a way for us to share knowledge and network with colleagues… When a pesticide residue crisis arose, the agencies were no longer alone. They could find advice and assistance…

Only CRL personnel and a few chemists from the Florida Dept. of Ag. attended the first meetings.

For the entire interview, be sure to visit **www.restek.com/interview-fong**

Questions From You

Our Technical Service specialists field an astounding variety of questions from our customers. Today's featured topic is that staple of the workbench: the flowmeter.

Q: Why do I see a difference in readings from different flowmeters?

A: All flowmeters present some level of flow impedance, but the amount differs among meters. When any meter is connected to a flow source, the system is loaded which will usually result in a change of flow from the source. The amount of change in flow depends on the level of impedance. While each meter will display the correct current flow, they may have different readings because the actual flow changes based on the degree of impedance. For this reason, it is inappropriate to "check" the flow measurement of one volumetric flowmeter against that of another.

We just released a full FAQ on the ProFLOW 6000 flowmeter! Find answers to your questions at **www.restek.com/FAQFlow**

- **Brandon Tarr**
Product Development Engineer

Wrestling with a question of your own?
Call 1-800-356-1688, ext. 4, or email **support@restek.com** today!

ChromaBLOGraphy

Topical and Timely Insights
ChromaBLOGraphy is where Restek's renowned experts go to share their thoughts on current trends along with best practices and troubleshooting tips. Best of all, you have the opportunity to weigh in yourself.

Here's a look at some of our latest posts:

- *Effect of Source Temperature on 2,4-DNP Response at Low Concentrations*
- *Searching for the Holy Grail—LC Separations of Important PAHs and Their Interferences*
- *The Coalition Against Coelution (CAC) and GC Method Translation for PAHs*
- *Increasing the Life Time of your GC Columns*

Join the discussion at **blog.restek.com** today!

Hot Topics

Product Spotlight

Restek Introduces Secondary Columns for GCxGC

Restek now offers a full line of secondary columns with a wide range of polarities to help you accurately analyze highly complex samples using GCxGC. These new columns can be matched with any Restek Rxi® or Rtx® primary column to create the perfect orthogonal separation for your application—and our online column combination guide makes pairing simple. A 2 m length means greater convenience and reduced cost while 0.15, 0.18, and 0.25 mm ID formats accommodate varying sample capacities, speeds, and detectors. And, of course, because they're Restek columns, you know you're getting the high thermal stability and unrivaled inertness you've come to rely on. Our chemists have been performing comprehensive two-dimensional gas chromatography since its commercial inception, and now you can put our years of GCxGC experience to work in your lab, too.

www.restek.com/gcxgc

Turn to page 8 to see our secondary columns for GCxGC put to the test!

Have You Tried Our Reversible Inlet Seals?

Flip Seal™ inlet seals feature a patented design that lets you simply flip them and use them again instead of throwing them away, so you get twice the life for the same price. Soft Vespel® rings embedded in the top and bottom surfaces eliminate the need for a washer and require very little torque to make a reliable seal. Choose gold plating or Siltek® treatment to reduce breakdown and adsorption of active compounds for maximum transfer onto the GC column. For decreased costs and increased performance, you owe it to your data to try our reversible Flip Seal™ inlet seals today.

www.restek.com/flip

Chromatography in the News

1,4-Dioxane in Your Bathwater

Next time you take a bath, you might just be enjoying a nice, long soak in 1,4-dioxane. Dioxane is a by-product of the ethoxylation process, which is employed most notably to create sodium myreth sulfate and sodium laureth sulfate for the manufacture of soaps and cosmetics. Unfortunately, dioxane has also been classified as a Group 2B carcinogen, prompting companies to begin eliminating it from their products. Over 1 million people in the U.S. are exposed to low-ppb dioxane levels in their drinking water, and half of those exposures are above the health guidelines set by the EPA (3 ppb). The recently signed third Unregulated Contaminant Monitoring Regulation (UCMR 3) will require monitoring using newly promulgated methods. 1,4-dioxane will be analyzed according to EPA Method 522, which concentrates the sample using solid phase extraction (SPE) instead of the most common technique previously used for this compound: purge and trap. Thankfully, we have reference standards specifically formulated for Method 522, and you can find them at **www.restek.com/epa522**

The Tar Balls Keep Rolling In

Tar ball photo courtesy of Susan Forsyth

As you read this, tar balls from the Gulf of Mexico continue to wash up on the shores of the U.S. And while organizations like Woods Hole Oceanographic Institute (WHOI) have found that naturally occurring microbes are eating oil at a much faster pace than predicted, scientists still believe that this may only account for 10% of the total discharge. Samantha Joye, a marine scientist at the University of Georgia, recently took 250 core samples of the sea floor covering an area of 2,600 miles and found that many contained the oil fingerprint (MC252) from the Deepwater Horizon rig. The oil spill may be out of the headlines, but the need for reliable analysis is far from over. We have 17 blog entries and counting on the Gulf oil spill, and many more on petrochemical analysis in general. Stop by ChromaBLOGraphy today for the latest advice and tips!

Hydrofracking: Coming to a Town Near You

From Colorado to New York, we're in the midst of a new kind of gold rush as companies flock to shale sites like Devonian, Marcellus, and Utica to tap massive deposits of natural gas. Several regions have what are known by energy companies as "stacked plays"—areas where two or more gas shale regions overlap, resulting in huge potential output—and there's one in Pennsylvania, putting Restek right in the middle of a growing debate. To extract natural gas from shale, a process called hydraulic fracturing (hydrofracking) is used, and while it is very effective, it also has raised significant health, safety, and environmental concerns. As confirmed by the Dimock case, where 14 homes had their well water contaminated with methane, natural gas released by fracking can find its way into drinking water instead of storage tanks. That's why many states are expected to soon adopt a variation of Method RSK-175 for the analysis of natural gas in drinking water, and why you can expect many new posts about gas analysis on our blog!

Detecting Cancer Cola via HPLC

It looks like mom was right: too much soda really can be bad for you! But the biggest problem may not be obesity, diabetes, or tooth decay. It could be cancer.

There are 4 main ways to produce the caramel coloring that is added to many foods including colas, coffee, beer, whisky, and soy sauce. In particular, the process used to make Class IV caramel color reacts sugars with ammonia and generates 4-methylimidazole (4MI or 4-MEI) as a by-product. The Center for Science in the Public Interest (CSIP) is petitioning the FDA to ban the use of 4MI-containing colorings because there is some indication that it is harmful and possibly carcinogenic. In fact, 4MI has already been classified by California as a chemical known to cause cancer (OEHHA, 2011). And, researchers at the University of California at Davis recently found significant levels of 4MI in colas that far exceed what the state considers to be safe. All of this has set the stage for analytical testing.

Analysis of 4MI has traditionally been accomplished by GC-MS with derivatization or by reversed phase HPLC with ion pairing, but these options are neither simple nor easily reproducible. Now, a simpler, LC-MS-friendly HILIC analysis is available. Using an Ultra PFP Propyl column, you can analyze 4MI employing typical LC-MS mobile phases, water and methanol with formic acid, and isocratic conditions! Look at our work in detail at **www.restek.com/cola**

Event Recap

Tradeshows are an incredibly important way for us to meet with you face-to-face and share our latest breakthroughs. In fact, we have travelled to 24 tradeshows in 7 countries this year, and we have just as many planned for 2012! To catch us at a future event, consult **www.restek.com/events** And, in case you missed them, here's a look into 2 featured events we attended:

HPLC 2011 | June 19–23

This June, more than 1,300 analysts traveled to Hungary for what is one of the premier liquid chromatography conferences in the world. HPLC 2011 covered topics from biomarkers to industrial separations to Quality by Design (QbD).

We had the honor of meeting hundreds of terrific scientists and discussing their work. Over the course of the 5-day show, we also presented posters on LC phase selectivity, food safety, environmental analysis, and clinical forensics. To read through our presentations or contact the authors directly, visit **www.restek.com/hplc2011**

Be sure to watch for a special issue of *Journal of Chromatography A* that will contain selected papers from HPLC 2011, and don't forget to make plans for next June, when the conference returns stateside in Anaheim, CA. Finally, thank you to everyone in Budapest for a terrific show in a beautiful city. *Egészségedre!* (To your health!)

- Ty Kahler

FPRW 2011 | July 17–20

Steven Bradbury, the Director of the U.S. EPA's Office of Pesticide Programs (U.S. EPA OPP), opened the technical session of FPRW with an excellent talk on "Priorities, Challenges, and Vision" for his office. Steven is from the "old school" and did not use PowerPoint, but that did not make his wide-ranging talk any less interesting. He led with the National Children's Study, which will examine environmental effects, including pesticides in the diet, on the health of children. When he noted that a successful outcome depended upon analytical chemistry, he made an immediate connection with the audience.

It was obvious as Steven continued that U.S. EPA OPP has an ambitious and challenging agenda set for itself. Harmonizing maximum residue levels for commodities, studying honey bee colony collapse disorder, monitoring water quality and surveying wetlands (pyrethroids in sediments), mitigating risk of soil fumigation with pesticides (using impermeable tarps), developing methods for nanotechnology analysis, advancing metabolomics... The list goes on, and every item depends on rugged and sensitive analytical methods!

PS: Check out our FPRW posters at **www.restek.com/fprw**

- Jack Cochran

Quantifying Synthetic Cannabinoid Metabolites

Single Extraction LC-MS/MS Method for Both Hydroxylated and Carboxylated Metabolites

By Amanda Rigdon*, Paul Kennedy**, and Ty Kahler*
*Restek Corp., **Cayman Chemical

- Single SPE extraction replaces separate low and high pH liquid/liquid extractions.
- The Ultra Biphenyl LC column separates positional isomers that cannot be distinguished by MS/MS.
- Quantify both hydroxylated and carboxylated JWH-018 and JWH-073 metabolites in urine.

Recent increases in the use of herbal incense containing synthetic cannabinoids, such as JWH-018 and JWH-073, have resulted in greater demand for testing. In response, many laboratories are now developing methods to analyze human urine for these compounds. Research has shown that the parent molecules are extensively metabolized prior to excretion [1]; therefore, the more abundant metabolites are better targets for screening assays.

Major metabolites of JWH-018 and JWH-073 include mono- and di-hydroxylated, as well as carboxylated, compounds [1,2]. These groups are generally extracted separately due to differences in their pKa values. Both present chromatographic challenges: the hydroxylated analytes exist as multiple positional isomers that are indistinguishable by MS/MS detectors, and the carboxylated compounds are hydrophilic, making them difficult to retain using RP-HPLC. Here we show the analysis of authentic urine samples using a simplified extraction procedure and a chromatographic method that allows quantification of clinically relevant metabolites.

Simplified Extraction Speeds up Sample Prep

Previously published methods describe the use of a high pH liquid/liquid extraction for the analysis of synthetic cannabinoid metabolites [1]. While this is suitable for hydroxylated metabolites, carboxylated metabolites require a second liquid/liquid extraction at low pH for adequate recovery. In contrast, the SPE procedure used here recovers both mono-hydroxylated and carboxylated metabolites. This SPE extraction procedure allowed authentic samples to be prepared for analysis quickly using just a single procedure.

Analysis of Positional Isomers and Unknown Metabolites in Authentic Samples

Many JWH-018 and JWH-073 metabolites are positional isomers, meaning they have the same molecular weight, share several common fragments, and must be chromatographically resolved because they are indistinguishable by MS/MS detectors. The analytical method used here provides chromatographic separation of all major isomeric analytes (Figure 1) and was used to determine the clinically significant positional isomer metabolites in authentic samples (Figure 2).

Quantitative results for authentic samples are presented in Table I. All reported values met ion ratio criteria for the first qualifier MRM transition; however, most results for JWH-018 5-hydroxypentyl did not meet ion ratio criteria for the second qualifier. To determine if an interfering compound was coeluting, samples were re-analyzed using an isocratic method. Results revealed a coeluting peak with

Table I: Quantitative LC-MS/MS results for JWH metabolites in authentic urine samples.

Compounds	Sample 1 (ng/mL)	Sample 2 (ng/mL)	Sample 3 (ng/mL)	Sample 4 (ng/mL)	Sample 5 (ng/mL)	Sample 6 (ng/mL)
JWH-018 N-pentanoic acid	9.9	11.5	22.7	1.5	<1	44.3
JWH-018 5-hydroxypentyl + unknown metabolite	29.5*	14.7*	84.2*	5.4*	1.4*	48.9
JWH-073 4-hydroxybutyl	ND	ND	ND	ND	ND	ND
Unknown metabolite	14.2	35.2	21.6	1.70	<1	69.7
JWH-073 N-butanoic acid	13.7	1.2	9.3	1.3*	ND	1.4
JWH-018 4-hydroxyindole	ND	ND	ND	ND	ND	ND
JWH-018 5-hydroxyindole	ND	ND	<1	ND	ND	ND
JWH-018 6-hydroxyindole	<1	ND	1.1	ND	ND	ND
JWH-018 7-hydroxyindole	ND	ND	ND	ND	ND	ND
JWH-073 4-hydroxyindole	ND	ND	ND	ND	ND	ND
JWH-073 5-hydroxyindole	ND	ND	ND	ND	ND	ND
JWH-073 6-hydroxyindole	ND	ND	ND	ND	ND	ND
JWH-073 7-hydroxyindole	ND	ND	ND	ND	ND	ND

*Results did not meet ion ratio criteria (±20%) for the second qualifier MRM transition.
ND = no peak detected

the same transitions as JWH-018 5-hydroxypentyl. This peak was not present in any of the blank samples and, based on recent work by NMS Labs, is thought to be JWH-018 4-hydroxypentyl [3].

Although JWH-073 *n*-butanoic acid was present in several samples, no JWH-073 4-hydroxybutyl was found. However, a large peak with the same transitions as JWH-073 4-hydroxybutyl was detected at a slightly earlier retention time compared to the JWH-073 4-hydroxybutyl metabolite. Post-extraction spiking experiments confirmed that the observed peak was not due to JWH-073 4-hydroxybutyl. The unknown peak was not observed in any blank samples, suggesting that it is also an unknown metabolite of either JWH-018 or JWH-073. Comparison to an NMS Labs report indicates this peak is most likely JWH-073 3-hydroxybutyl [3].

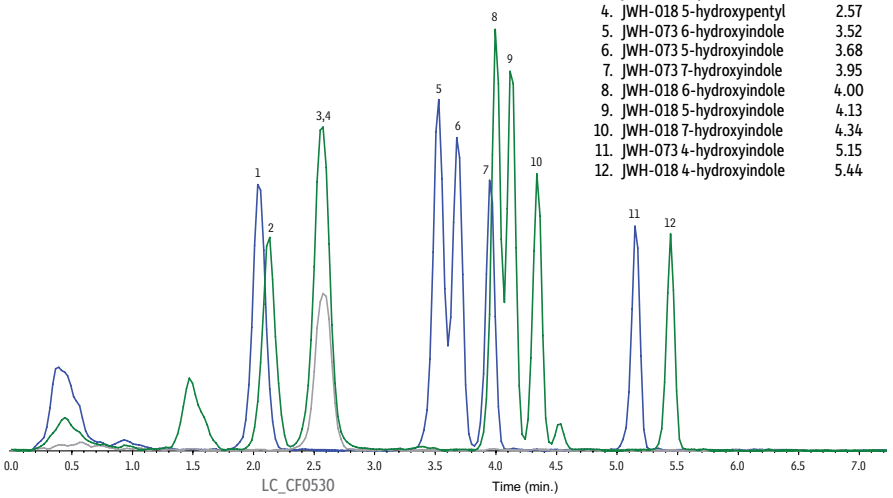
Summary

The extraction and chromatographic methods shown here perform well for the analysis of JWH-018 and JWH-073 metabolites in urine. The mid-range pH SPE extraction allows both mono-hydroxylated and carboxylated metabolites to be recovered from a single extraction. In addition, the Ultra Biphenyl column provides enough retention for the hydrophilic carboxylated metabolites, as well as the selectivity needed to separate positional isomers of the mono-hydroxylated metabolites.

For the complete version of this technical article, visit
www.restek.com/JWHmetabolites

Figure 1: LC-MS/MS chromatogram of a 1 ng/mL JWH metabolites calibration standard.

Peaks		RT (min.)
1.	JWH-073 4-hydroxybutyl	2.04
2.	JWH-073 N-butanoic acid	2.13
3.	JWH-018 N-pentanoic acid	2.59
4.	JWH-018 5-hydroxypentyl	2.57
5.	JWH-073 6-hydroxyindole	3.52
6.	JWH-073 5-hydroxyindole	3.68
7.	JWH-073 7-hydroxyindole	3.95
8.	JWH-018 6-hydroxyindole	4.00
9.	JWH-018 5-hydroxyindole	4.13
10.	JWH-018 7-hydroxyindole	4.34
11.	JWH-073 4-hydroxyindole	5.15
12.	JWH-018 4-hydroxyindole	5.44



Column: Ultra Biphenyl (cat.# 9109552); Dimensions: 50 mm x 2.1 mm ID; Particle Size: 5 µm; Pore Size: 100 Å; Temp.: 25 °C; **Sample:** Diluent: 50:50 mobile phase; Conc.: 1 ng/mL extracted spiked sample; Inj. Vol.: 10 µL; **Mobile Phase:** A: water + 0.05% acetic acid (pH approx. 3.4), B: acetonitrile + 0.05% acetic acid; **Flow:** 0.5mL/min.; Gradient (%B): 0 min. (45%), 2.00 min. (45%), 6.00 min. (85%), 6.10 min. (95%), 7.00 min. (95%), 7.10 min. (45%), 8.50 min. (stop); **Detector:** API 4000; Model #: API 4000; Ion Source: TurboIonSpray®; Ion Mode: ESI+; Ion Spray; Mode: MRM; **Instrument:** API LC MS-MS; For complete conditions and transitions, visit www.restek.com and enter LC_CF0530 in the search.

Sample was prepared according to the following method:

1) Spike 1 mL blank urine sample with analytes and internal standards.
2) *Hydrolyze sample*:
- Add 1 mL solution of beta-glucuronidase from keyhole limpet (Sigma-Aldrich cat.# G8132). Solution is prepared at a concentration of 5,000 Fishman units/mL in 100 mM ammonium acetate buffer (pH = 5.0).
- Incubate at 60 °C for 3 hours.

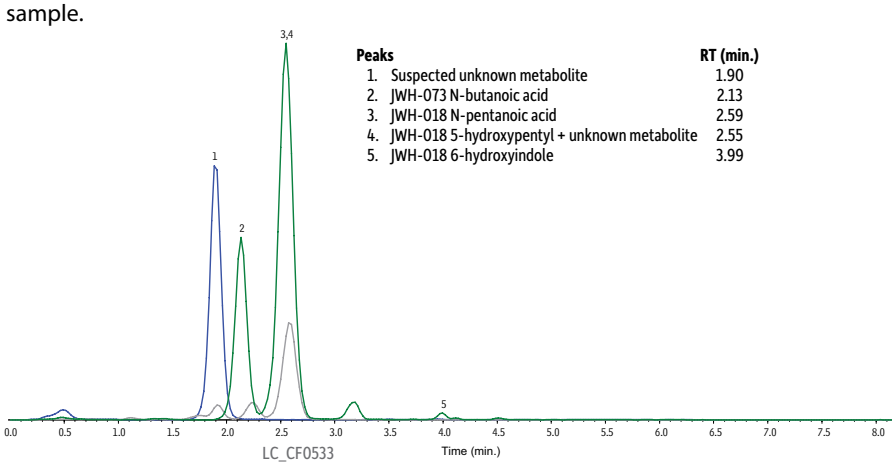
3) *Extract sample on 6 mL, 500 mg C18 high-load endcapped Resprep® SPE cartridge (cat.# 24052)*:
- Add 1 mL 5 mM ammonium acetate + 0.1% acetic acid (pH = 4.2) to sample.
- Condition cartridge with 3x 1 mL acetonitrile.
- Condition cartridge with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Apply sample and allow to pass through under gravity.
- Rinse with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Dry cartridge with vacuum for 10 minutes.
- Elute with 3 mL acetonitrile followed by 3 mL butyl chloride.

4) *Concentrate sample*:
- Evaporate sample to dryness under nitrogen at 40 °C.
- Reconstitute in 0.5 mL water + 0.05% acetic acid:acetonitrile + 0.05% acetic acid (50:50).

Acknowledgement: Special thanks to Cayman Chemical for reference standards

Figure 2: LC-MS/MS chromatogram of JWH metabolites found in an authentic urine sample.

Peaks		RT (min.)
1.	Suspected unknown metabolite	1.90
2.	JWH-073 N-butanoic acid	2.13
3.	JWH-018 N-pentanoic acid	2.59
4.	JWH-018 5-hydroxypentyl + unknown metabolite	2.55
5.	JWH-018 6-hydroxyindole	3.99



(See Figure 1 for instrument conditions and extraction procedure.)

Ultra Biphenyl Columns (USP L11)

Physical Characteristics:

particle size: 3µm or 5µm, spherical	endcap: fully endcapped
pore size: 100Å	pH range: 2.5 to 8
carbon load: 15%	temperature limit: 80°C

Description	cat.#
5µm Columns	
50mm, 2.1mm ID	9109552
50mm, 2.1mm ID (with Trident Inlet Fitting)	9109552-700

Resprep® SPE Cartridges

(Bonded Reversed Phases)

Hydrophobic (nonpolar) silica-based adsorbents, used to extract hydrophobic analytes from polar matrices, such as water (e.g., pesticides from water).

	6mL/500mg
C18 (high load, endcapped)	24052

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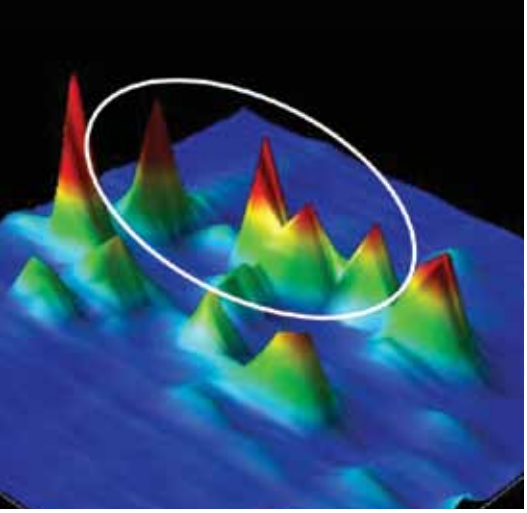
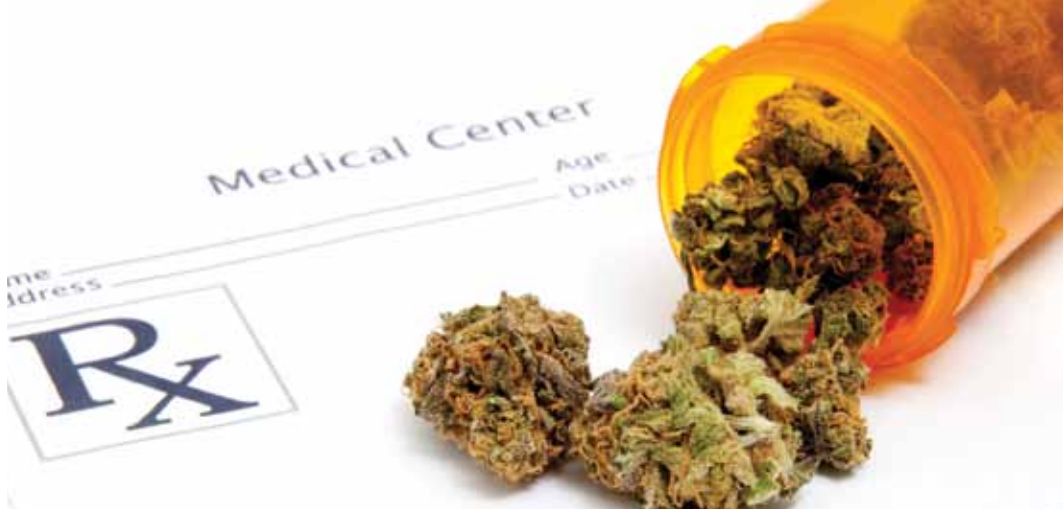




High Quality Analysis of Pesticides in Marijuana

Using QuEChERS, Cartridge SPE Cleanup, and GCxGC-TOFMS

By Jack Cochran, Julie Kowalski, Sharon Lupo, Michelle Misselwitz, and Amanda Rigdon

- Quickly and effectively extract medical marijuana samples for pesticide analysis.
- Cartridge SPE cleanups of dirty extracts improve GC inlet and column lifetimes.
- Selective GC columns increase accuracy of pesticide determinations for complex samples.

Over a dozen states in the U.S. have legalized medical marijuana because of therapeutic benefits for ailments such as cancer, multiple sclerosis, and ALS. Dosing methods include smoking or vaporizing and baked goods. Unlike other prescribed medicines regulated by U.S. FDA, marijuana is a Schedule 1 drug and is illegal on the federal level. As a result, medical marijuana patients have no safety assurances for their medication, which could contain harmful levels of pesticide residues. Currently, medical marijuana pesticide residue analysis methods are poorly defined and challenging to develop due to matrix complexity and a long list of potential target analytes.

In order to address matrix complexity, we combined a simple QuEChERS extraction approach with cartridge SPE (cSPE) cleanup, followed by GCxGC-TOFMS. Acceptable recoveries were obtained for most pesticides, and incurred pesticide residues were detected in some of the illicit marijuana samples used for method development.

QuEChERS Extraction Saves Time and Reduces Hazardous Solvent Use

Trace residue extraction procedures from dry materials like marijuana typically involve large amounts of solvent, long extraction times, and tedious concentration steps similar to the Soxhlet procedure or multiresidue methods from the Pesticide Analytical Manual. QuEChERS, with its simple 10 mL acetonitrile shake extraction and extract partitioning with salts and centrifugation, offers time savings, glassware use reduction, and lower solvent consumption.

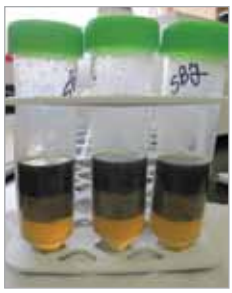
Water was added to finely ground, dry marijuana samples to increase QuEChERS extraction efficiency, especially for more polar pesticides. A vortex mixer was used to shake the solvent and sample for at least 30 minutes prior to extract partitioning. When finished, it was easy to transfer the supernatant from the QuEChERS extraction tube for subsequent cSPE cleanup prior to analysis with GC or LC (Figure 1).

Cartridge SPE Cleanup Improves GC Inlet Uptime

Injecting chlorophyll-laden extracts into a GC gives reduced recoveries for less volatile pesticides, and results in degradation of sensitive pesticides like DDT and Dicofol (Table I). SPE cleanup with a 500 mg graphitized carbon black/500 mg PSA cartridge removes chlorophyll and traps fatty acids that interfere with qualitative pesticide identification and bias quantification. cSPE has increased sorbent capacity over dispersive SPE for thorough cleanup of complex extracts.

Figure 1: A quick and easy QuEChERS extraction, combined with cSPE, effectively prepared extracts for pesticide residue analysis from highly complex marijuana samples.

A. Post-centrifugation QuEChERS extracts



B. QuEChERS extracts loaded on SPE cartridge

C. Final extract

Orthogonal GC Columns Greatly Increase Separation Power for More Accurate Pesticide Results

GCxGC is a powerful multidimensional approach that gives 2 independent separations in 1 instrumental analysis. An Rxi®-5Sil MS and Rtx®-200 column combination distributes pesticides broadly in both dimensions, providing a highly orthogonal GCxGC system. More important though is separating pesticides from potential isobaric matrix interferences, as seen in the surface plot for the insecticide cypermethrin (Figure 2). Cypermethrin gas chromatographs as 4 isomers, and all would have experienced qualitative interference and quantitative bias from peaks in the foreground of the surface plot had only 1-dimensional GC been used. With GCxGC-TOFMS, cypermethrin was unequivocally identified in a marijuana sample at a low ppm level (Figure 3).

Summary

QuEChERS and cSPE produced usable extracts from highly complex marijuana samples for high quality pesticide residue analysis. The multidimensional separation power of GCxGC-TOFMS was then used to correctly identify and quantify pesticides in these complex extracts.

Table I: Pesticide recoveries for a QuEChERS extract of marijuana give higher results when cSPE is used for cleanup. Dicofol and DDT are degraded in the inlet for the dirtier extract, yielding high DDD results.

Pesticide	Classification	With cSPE Cleanup (%)	Without cSPE Cleanup (%)
4,4´-DDD	Organochlorine	83	230
4,4´-DDT	Organochlorine	77	9
Bifenthrin	Pyrethroid	86	89
Dicofol	Organochlorine	84	ND
Azinphos methyl	Organophosphorus	79	53
trans-Permethrin	Organochlorine	68	17
Pyraclostrobin	Strobilurin	73	19
Fluvalinate	Pyrethroid	72	23
Difenoconazole	Triazole	67	21
Deltamethrin	Pyrethroid	68	20
Azoxystrobin	Strobilurin	72	27

ND = no peak detected

Figure 2: GCxGC-TOFMS and orthogonal Rxi®-5Sil MS and Rtx®-200 columns allow incurred cypermethrins in a marijuana extract to be separated from interferences (m/z 163 quantification ion).

Peaks	RT 1 (sec.)	RT 2 (sec.)
1. Cypermethrin 1	2292	1.50
2. Cypermethrin 2	2304	1.54
3. Cypermethrin 3	2310	1.53
4. Cypermethrin 4	2313	1.58

Column: Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623), Rtx®-200 1.3 m, 0.25 mm ID, 0.25 µm (cat.# 15124); **Sample:** Diluent: Toluene; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min.); Liner: Sky™ 4mm Single Taper w/Wool (cat.# 23303.1); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min.; **Oven:** Oven Temp: Rxi®-5Sil MS: 80 °C (hold 1 min.) to 310 °C at 5 °C/min., Rtx®-200: 85 °C (hold 1 min.) to 315 °C at 5 °C/min.; **Carrier Gas:** He, corrected constant flow (2 mL/min.); **Modulation**: Modulator Temp. Offset: 20 °C; Second Dimension Separation Time: 3 sec.; Hot Pulse Time: 0.9 sec.; Cool Time between Stages: 0.6 sec.; **Instrument**: LECO Pegasus 4D GCxGC-TOFMS; For complete conditions, visit www.restek.com and enter GC_FF1204 in the search.

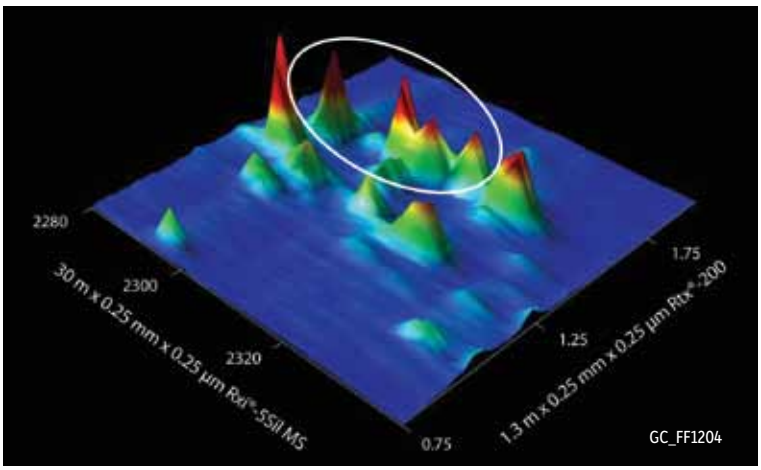
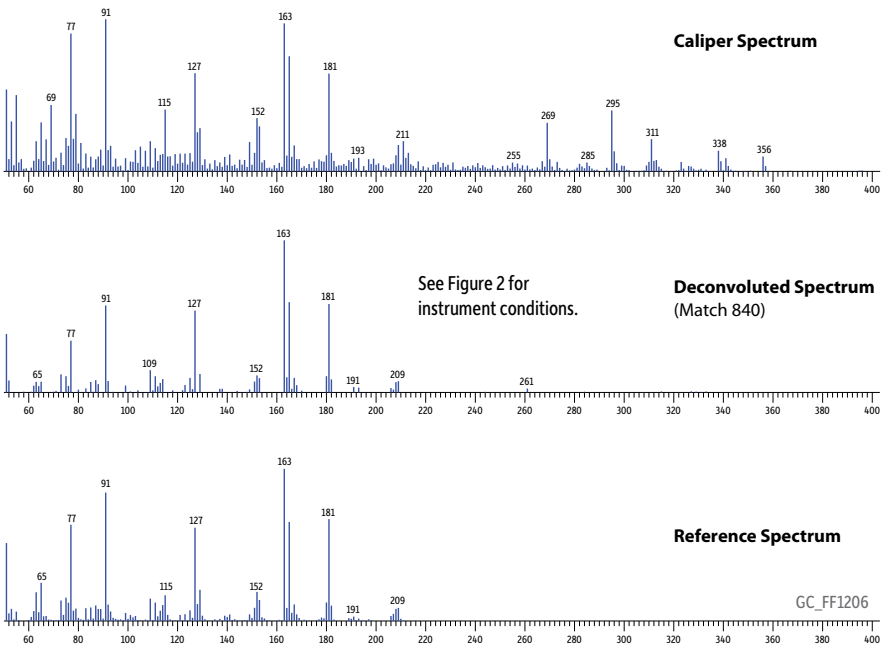


Figure 3: Positive mass spectral identification of incurred cypermethrin in illicit marijuana.



Acknowledgment
Randy Hoffman, a Police Evidence Technician at The Pennsylvania State University (PSU), supplied the seized marijuana samples while overseeing their handling. Frank Dorman at PSU assisted with QuEChERS extractions.

ChromaBLOGraphy

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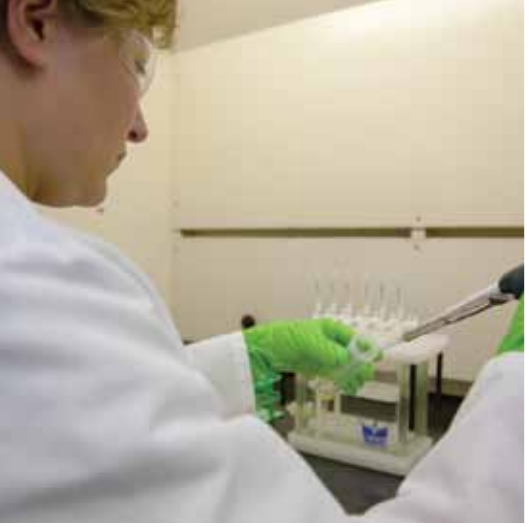
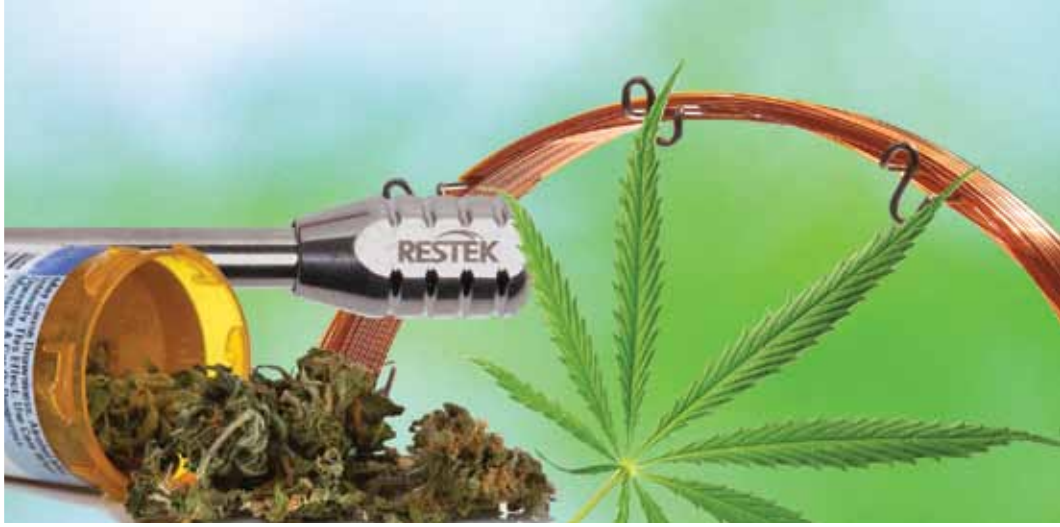




Marijuana Potency Testing—Quick and Easy by GC or LC

By Amanda Rigdon and Jack Cochran

- Single extraction for both GC and LC.
- Fast results on Rxi®-5Sil MS GC or Ultra Aqueous C18 LC columns.
- Convenient standards for potency testing.

Although marijuana is illegal at the federal level in the United States, the use of medicinal marijuana is currently legal in many states. In some areas, it is widely used, and demand is rising for potency data for medicinal products purchased at dispensaries. Potency testing is more straightforward than impurity testing because the active compounds are present in much higher concentrations relative to matrix. Currently, GC is the most popular method for potency testing due to its ease of use and the availability of relatively inexpensive instrumentation. However, LC is also a viable technique for medical cannabis potency testing. As shown in this article, the same straightforward sample preparation technique can be used for cannabis potency testing by either GC or LC.

Simple Sample Prep

Cannabinoids were extracted from 7 different marijuana samples under the supervision of local law enforcement personnel. The extraction procedure consisted of weighing 0.2 g of sample into a 40 mL VOA vial, adding 40 mL of isopropyl alcohol, shaking for 5 minutes, and then allowing the sample to settle. The procedure was very quick and produced extracts that were compatible with both GC and LC analysis.

GC Analysis

The 3 compounds of interest for GC potency testing are Δ^9-tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD). While THC is primarily responsible for the hypnotic effects of marijuana, CBD acts to attenuate these effects. Since CBD has been shown to have medicinal properties, it is desired at higher concentrations in medical marijuana. Because the samples that were extracted were illicit samples seized by local law enforcement, the CBD levels were very low. In general, higher CBD levels are observed in medicinal marijuana strains. CBN is an indicator of sample breakdown due to age or poor storage conditions.

For GC potency testing, 1 µL of prepared extract was manually injected onto a 5890 GC equipped with a flame ionization detector and analyzed on a 15 m Rxi®-5Sil MS column (cat.# 13620). To ensure accurate and reproducible manual injections, a Merlin Microshot injector (cat.# 22229) was used. Figure 1 shows an overlay of a cannabinoid standard (cat.# 34014) that contains the 3 target analytes (blue trace) and a representative chromatogram of a marijuana sample (red trace). The use of a narrow-bore, thin-film analytical column resulted in sharp peaks, which improve sensitivity and allow a split injection to be used to reduce column contamination.

LC Analysis

LC potency testing requires the analysis of the 3 components discussed above, but also includes Δ^9- tetrahydrocannabolic acid (THCA). While THCA is not hallucinogenic, all THC in the marijuana plant exists as THCA, and only converts to THC upon heating (i.e., smoking, vaporizing, cooking, or injecting into a hot GC inlet). Since the sample extraction and LC analysis employ no heat, potency must be determined based on THCA when using LC, rather than with THC as is used in GC analysis.

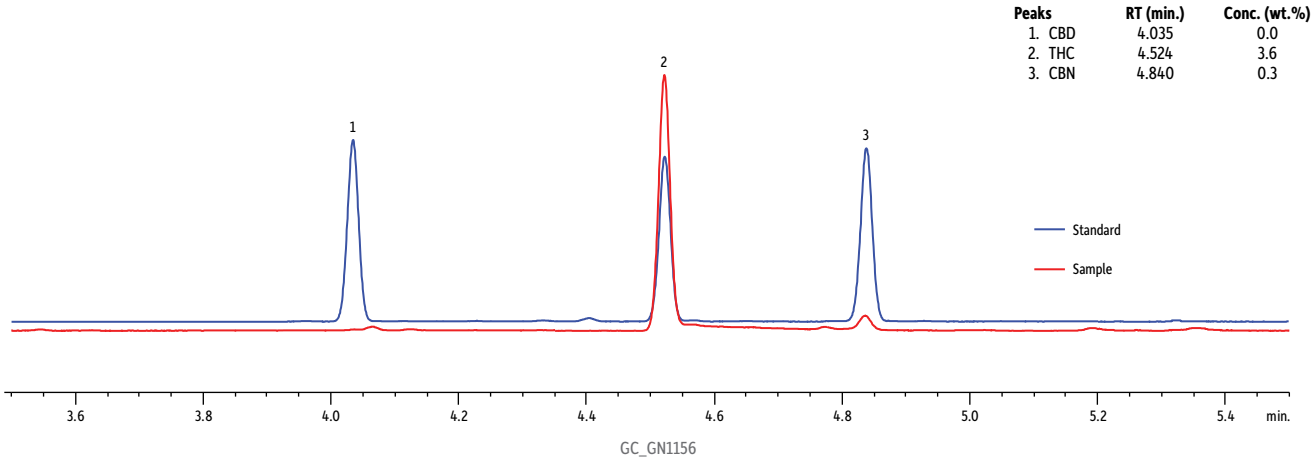
For LC potency testing, extracts were diluted 10x with isopropyl alcohol, and 10 µL of extract was injected onto a 3 µm Ultra Aqueous C18 column (cat.# 9178312). Figure 2 shows an overlay of the cannabinoid standard described above with the addition of THCA (blue trace) and a representative chromatogram of the same marijuana sample (red trace).

Summary

Both the GC and LC methods shown here for determining medical marijuana potency employ a straightforward and cost-effective extraction procedure and fast analysis times. This allows reliable potency analyses at a reasonable cost per sample.

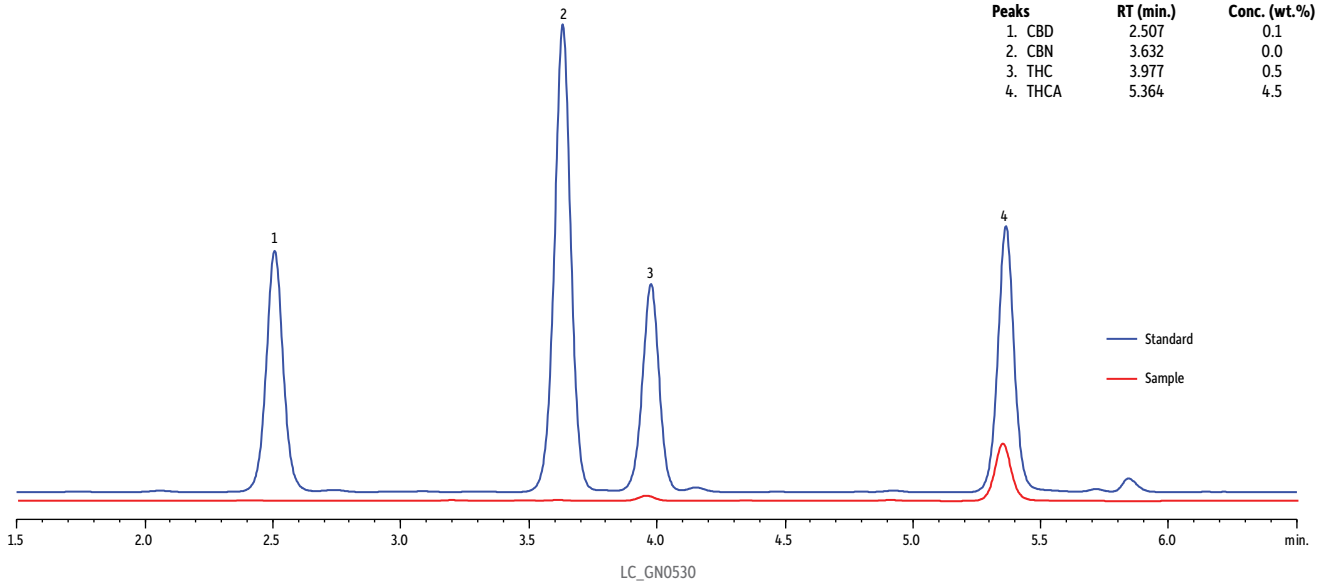
For further details, visit our technical blog at
www.restek.com/potpotency

Figure 1: Potency testing of marijuana using an Rxi®-5Sil MS GC column results in higher sensitivity for all target analytes.



Column: Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620); **Injection**: Inj. Vol.: 1 µL split (split ratio 20:1); Liner: Sky™ 4.0 mm ID single taper/gooseneck inlet liner w/wool (cat.# 23303.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp: 200 °C (hold 0 min.) to 300 °C at 15 °C/min. (hold 0 min.); **Carrier Gas**: H_2, constant pressure (7 psi, 48.3 kPa); Temp.: 200 °C; Dead Time: 0.6 min. @ 200 °C; **Detector**: FID @ 300 °C; Make-up Gas Flow Rate: 45 mL/min.; Make-up Gas Type: N_2; **Instrument**: HP5890 GC; **Notes**: Blue trace = cannabinoids standard (cat.# 34014) diluted to 100 µg/mL in isopropyl alcohol.; Red trace = extracted marijuana sample; Sample extraction: Weigh 0.2 g of sample into a 40 mL VOA vial, add 40 mL of isopropyl alcohol, shake for 5 minutes, and allow sample to settle.; Quantification: Potency values (weight%) were based on a 1-point standard curve using the standard show above.

Figure 2: Ultra Aqueous C18 columns easily separate THCA, which is used to determine marijuana potency when testing by LC.



Column: Ultra Aqueous C18 (cat.# 9178312); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 3 µm; Pore Size: 100 Å; Temp.: 30 °C; **Sample:** Inj. Vol.: 10 µL; **Mobile Phase:** A: Water + 10 mM potassium phosphate (pH = 2.5), B: Methanol; Flow: 0.4 mL/min.; Gradient (%B): 0 min. (80%), 1.0 min. (80%), 5.0 min. (95%), 6.0 min. (95%), 6.1 min. (80%), 8.0 min. (80%); **Detector**: UV/Vis @ 220, 4 nm; Cell Temp: 40 °C; **Instrument**: Shimadzu UFLCXR; **Notes**: Blue trace = cannabinoids standards (cat.#s 34014 and 34093) diluted to 100 µg/mL in isopropyl alcohol; Red trace = extracted marijuana sample; Sample extraction: Weigh 0.2 g of sample into a 40 mL VOA vial, add 40 mL of isopropyl alcohol, shake for 5 minutes, and allow sample to settle. Dilute extract 10x with isopropyl alcohol.; Quantification: Potency values (weight%) were based on a 1-point standard curve using the standard show above.

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; similar to 5% phenyl/95% dimethyl polysiloxane)

Description	temp. limits	cat.#
15m, 0.25mm ID, 0.25µm	-60 to 330/350°C	13620

similar **phases**

DB-5ms, VF-5ms, CP-Sil 8 Low-Bleed/MS, DB-5ms UI, Rtx-5Sil MS, ZB-5ms, Optima 5ms, AT-5ms, SLB-5ms, BPX-5

Ultra Aqueous C18 Columns (USP L1)

Description	cat.#
3µm Columns	
100mm, 2.1mm ID	9178312
3µm Columns	
100mm, 2.1mm ID (with Trident Inlet Fitting)	9178312-700

similar **phases**

AQUA C18, Aquasil C18, Hypersil Gold AQ, YMC ODS-Aq

Acknowledgment

Randy Hoffman, a Police Evidence Technician at The Pennsylvania State University (PSU), supplied the seized marijuana samples while overseeing their handling. Frank Dorman at PSU provided access to the samples and assisted with prep.

Simplify HPLC and UHPLC Method Development

With the Restek USLC™ Column Set

By Rick Lake and Ty Kahler

- Column selectivity has the most significant influence on chromatographic peak separation (i.e., resolution).
- Initially focusing on columns instead of mobile phases will drastically speed up method development.
- Restek's USLC™ column set boasts the widest range of selectivity available—using just 4 stationary phases!

Wasted effort. Lost time. Frustration. Making the wrong decisions can needlessly complicate and delay successful method development. By understanding selectivity's impact on resolution and focusing on column choice to create ***alternate*** selectivity, you can drastically speed up LC method development. Enter the new Restek Ultra Selective Liquid Chromatography™ (USLC™) columns.

Change Your Habits—and Your Columns—to Optimize Resolution

Resolution is the result of 3 cumulative terms: efficiency (N), retention capacity (k), and selectivity (α). How well and how quickly we resolve our analytes depends upon our ability to control these factors. Of the 3, selectivity affects resolution to the greatest degree (Equation 1). For that reason, any discussion about resolution in method development should focus on selectivity.

All too often, HPLC method developers use C18 columns and rely on adjusting mobile phases to alter selectivity and reach a desired separation. While it is true that mobile phase adjustments may alter selectivity, it is a laborious task that typically creates only marginal differences. In addition, some mobile phases are not practical with certain detection modes, including mass spectrometry (MS) and refractive index (RI). To save time and work, you should first focus on choosing the right stationary phases (i.e., columns). Columns pose fewer issues with MS and RI, change easily, and offer alternate and even orthogonal separations for maximum effect with each change.

Choosing columns can be incredibly difficult, but by characterizing stationary phase selectivity, we created new guidelines for easily making the right choice.

Equation 1: Selectivity is the driving parameter of resolution, as it affects peak separation to the greatest degree.

$$R = \tfrac{1}{4}\sqrt{N} \times (k/(k+1)) \times (\alpha-1)$$

Efficiency *Retention Factor* *Selectivity*

The Highest Range of Alternate Selectivity

Using the hydrophobic subtraction model (H-S model) [1], we quantified the selectivity of our stationary phases and determined which phases produce the greatest degree of dissimilarity compared to a C18 benchmark. We then matched these phases with specific solute types based on molecular interactions commonly encountered in reversed phase chromatography. By doing so, we were able to (1) find a small set of columns with the widest range of ***alternate*** selectivity available and (2) recommend columns based on the chemical properties of target analytes.

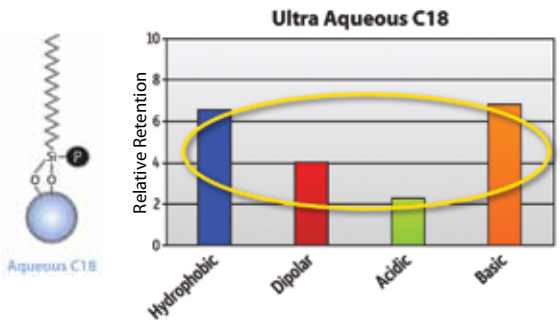
Figure 1 illustrates the retention profile of a C18 compared with those of the 4 Restek USLC™ columns. USLC™ phases are highly selective and exhibit significantly different retention profiles based on specific solute chemical properties, so you can match USLC™ columns to specific analytes and accelerate method development!

To confirm the orthogonality of the Restek USLC™ column set, we also quantified its selectivity (S) as described by Neue et al. [2] by looking at the degree of scatter along a regression line when compared to a conventional C18 (Figure 2). USLC™ phases produce the highest range of alternate selectivity available today—using only 4 columns.

Summary

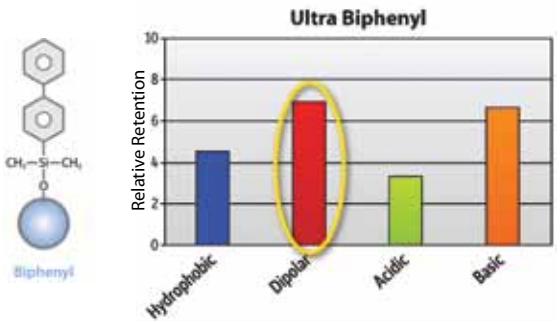
The Restek USLC™ column set has a profile that encompasses the widest range of reversed phase selectivity available today. Instead of manually altering mobile phases, operational parameters, or instrument settings—often with minimal effect on resolution—take advantage of the Restek USLC™ column set. These 4 orthogonal stationary phases and their defined retention profiles let you quickly determine the best column for almost any reversed phase situation.

Figure 1: Stationary phase selectivity can be characterized by looking for column types with varying retention profiles. When compared to a C18, the 4 Restek USLC™ phases offer diverse retention profiles—that is, a true range in selectivity.



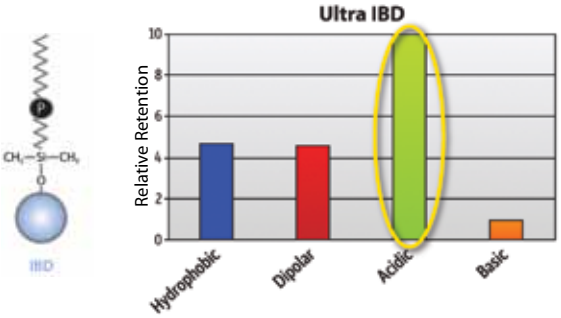
Restek USLC™ Phase: **Aqueous C18**

- **General purpose with a well-balanced retention profile.**
- Increased retention for acids and bases.
- Resistant to dewetting—compatible with 100% aqueous mobile phases.



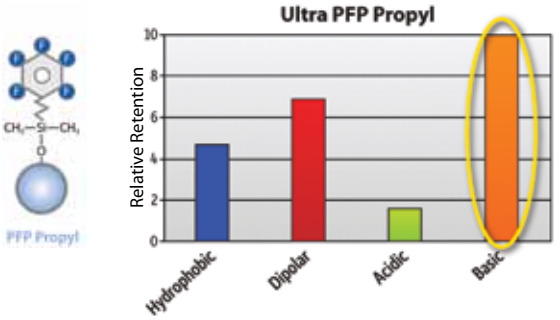
Restek USLC™ Phase: **Biphenyl**

- **Increased retention for dipolar, unsaturated, or conjugated solutes.**
- Increased retention for fused-ring solutes containing electron withdrawing ring substituents.
- Enhanced selectivity when used with methanolic mobile phase.



Restek USLC™ Phase: **IBD**

- **Increased retention for acids.**
- Moderate retention for hydrophobic and dipolar solutes.
- Resistant to dewetting—compatible with 100% aqueous mobile phases.
- Capable of multi-mode mechanisms.

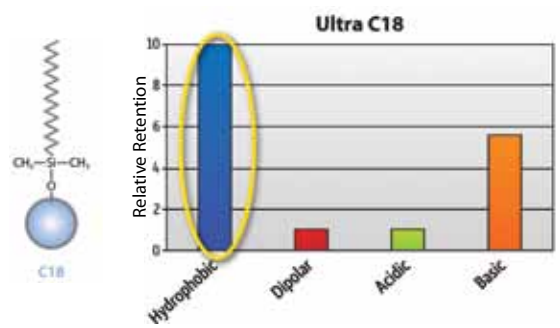


Restek USLC™ Phase: **PFP Propyl**

Properties:

- **Increased retention for protonated bases.**
- Increased retention for solutes containing dipolar moieties.
- Capable of multi-mode mechanisms.

C18 BENCHMARK

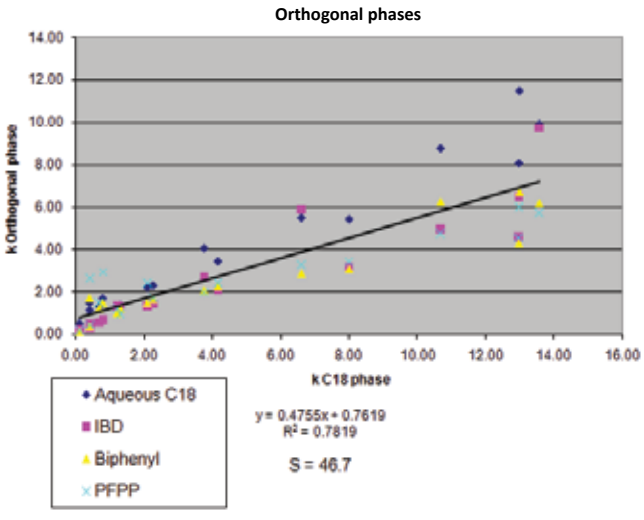


Restek Phase: **C18 Benchmark**

- General purpose.
- Strong hydrophobic retention.

All columns in Figures 1 and 2 were tested using the same silica support.

Figure 2: Restek has extended the selectivity (S) for a range of columns and defined a set—the 4 USLC™ phases—that is ideal for fast column selection and faster method development.



For a detailed analysis of USLC™ column selectivity data, visit **www.restek.com/USLCarticle**

References

[1] L.R. Snyder, J.W. Dolan, P.W. Carr, *The Hydrophobic-Subtraction Model of Reversed-Phase Column Selectivity,* J. Chromatogr. A 1060 (2004) 77.

[2] U.D. Neue, J.E. O'Gara, A. Mendez, *Selectivity in Reversed-Phase Separations Influence of the Stationary Phase,* J. Chromatogr. A 1127 (2006) 161.

Acknowledgements

The authors gratefully acknowledge the contributions of Dr. Lloyd Snyder from LC Resources and Dr. Frank Dorman from The Pennsylvania State University. The authors also wish to thank the contributing team of researchers Randy Romesberg, Bruce Albright, Mike Wittrig, Brian Jones, and Vernon Bartlett.

7 EPA Methods on 1 Column Pair

Analyze Pesticides, PCBs, Herbicides and More on a Single Rtx®-CLPesticides Column Set

By Jason Thomas

- Spend more time analyzing samples and less changing columns.
- Avoid downtime associated with dedicated instruments.
- Best performance of any column set offered specifically for multiple GC-ECD methods.

Although many new techniques, or previously underutilized ones, are coming into greater use in environmental labs to combat ever more complicated sample lists and difficult sample matrices, the electron capture detector (ECD) remains an important and powerful tool in determining the presence of many compounds of environmental concern. The ECD is a simple, inexpensive detector that provides excellent sensitivity for environmental compounds that are halogenated or contain other electron withdrawing functionalities. Because of this compound class selectivity, target environmental analytes can be detected without much interference from the sample matrix, an issue that can be problematic using less selective detectors.

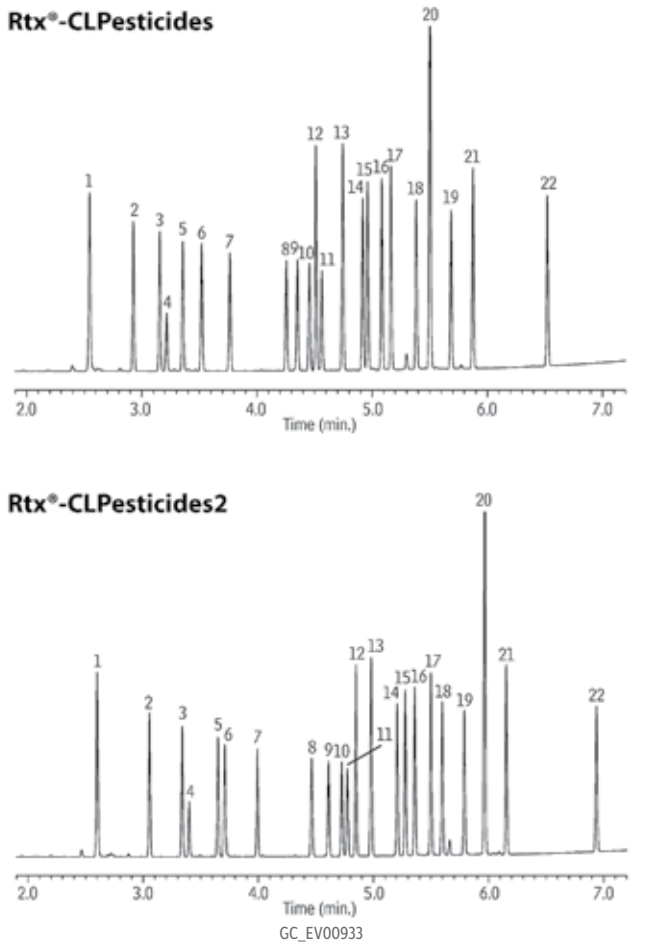
Numerous environmental contaminants are halogenated, and many tend to be quite toxic. Although some of these, like dioxins, are analyzed using HRMS for increased specificity, many EPA methods have been developed for pesticides, PCBs, DBPs, and other similar compounds using the ECD. These methods tend to use a column pair, where one column serves as a confirmation column in the event a target contaminant needs to be positively identified and quantified. One such pair, the Rtx®-CLPesticides

Table I: Rtx®-CLPesticides columns offer the best performance for multiple GC-ECD methods.

EPA Method	Column Pair	Analysis Time (min.)	Coelutions	Restek Advantage
8081B (Organochlorine pesticides)	Rtx-CLPesticides/ Rtx-CLPesticides2	7/7	0/0	• Increase sample throughput with 7 min. analyses.
	DB-35ms/DB-XLB	15/16	0/0	
	ZB-MR1/ZB-MR2	10/9	0/0	
8081B** (extended) (Organochlorine pesticides)	Rtx-CLPesticides/ Rtx-CLPesticides2	24/23	1/2	• Best balance of speed and selectivity. • All compounds are resolved between both columns.
	DB-35ms/DB-XLB	42/39	2/3	
	ZB-MR1/ZB-MR2	NDP/16	NDP/3	
8082A (Polychlorinated biphenyls [PCBs], Aroclors)	Rtx-CLPesticides/ Rtx-CLPesticides2	7/7	0/0	• Analyze PCBs 2x or 3x faster than on other ECD columns.
	DB-35ms/DB-XLB	14/16	0/0	
	ZB-MR1/ZB-MR2	24/21	0/0	
8151A (Chlorinated herbicides)	Rtx-CLPesticides/ Rtx-CLPesticides2	13/13	1/0	• Increase sample throughput with fastest run time.
	DB-35ms/DB-XLB	16/17	0/0	
	ZB-MR1/ZB-MR2	16/15	1/1	
504.1 (EDB, DBCP, TCP)	Rtx-CLPesticides/ Rtx-CLPesticides2	9/10	0/0	• Reliably separate analytes from trihalomethane interferences.
	DB-35ms/DB-XLB	NDP	NDP	
	ZB-MR1/ZB-MR2	NDP	NDP	
505 (Organohalide pesticides)	Rtx-CLPesticides/ Rtx-CLPesticides2	18/18	1/1	• Fast, reliable analysis.
	DB-35ms/DB-XLB	NDP	NDP	
	ZB-MR1/ZB-MR2	NDP	NDP	
508.1 (Chlorinated pesticides, herbicides, organohalides)	Rtx-CLPesticides/ Rtx-CLPesticides2	23/24	2/2	• All compounds resolved between both columns. • Best overall balance of speed and resolution.
	DB-35ms/DB-XL	22/24	2/4	
	ZB-MR1/ZB-MR2	18/NDP	2/NDP	
552.2 (Haloacetic acids, dalapon)	Rtx-CLPesticides/ Rtx-CLPesticides2	12/12	0/0	• No coelutions—get accurate results for compounds that coelute on other columns.
	DB-35ms/DB-XLB	8/9	2/1	
	ZB-MR1/ZB-MR2	NDP	NDP	

Comparison based on published competitor data.
NDP = no data published

Figure 1: Rtx®-CLPesticides columns provide a fast, 7 minute analysis time with no coelutions.



1. 2,4,5,6-Tetrachloro-*m*-xylene (SS)
2. α-BHC
3. γ-BHC
4. β-BHC
5. δ-BHC
6. Heptachlor
7. Aldrin
8. Heptachlor epoxide (isomer B)
9. *trans*-chlordane
10. *cis*-chlordane
11. Endosulfan I
12. 4,4´-DDE
13. Dieldrin
14. Endrin
15. 4,4´-DDD
16. Endosulfan II
17. 4,4´-DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl (SS)

Columns: Rtx®-CLPesticides 30 m, 0.32 mm ID, 0.32 µm (cat.# 11141) and Rtx®-CLPesticides2 30 m, 0.32 mm ID, 0.25 µm (cat.# 11324) using Rxi® Guard Column 5 m, 0.32 mm ID (cat.# 10039) with Deactivated Universal "Y" Press-Tight Connector (cat.# 20405-261); **Sample**: Organochlorine Pesticide Mix AB #2 (cat.# 32292), Pesticide Surrogate Mix, EPA 8080, 8081 (cat.# 32000); **Injection**: Inj. Vol.: 1 µL splitless (hold 0.3 min.), Liner: Gooseneck Splitless (4 mm) (cat.# 20799), Inj. Temp.: 250 °C; **Oven:** Oven Temp: 120 °C to 200 °C at 45 °C/min. to 230 °C at 15 °C/min. to 330 °C at 30 °C/min. (hold 2 min.); **Carrier Gas**: He; **Detector**: µ-ECD @ 330 °C; **Notes**: Instrument was operated in constant flow mode., Linear velocity: 60 cm/sec. @ 120 °C.

Table II: Sample throughput can be significantly improved by using Rtx®-CLPesticides and Rtx®-CLPesticides2 columns.

Vendor	Column Pair	Analysis Time	Coelutions	Runs/12 hr Shift*
Restek	Rtx-CLPesticides Rtx-CLPesticides2	7 7	0 0	**42**
Agilent	DB-35ms DB-XLB	15 16	0 0	27
Phenomenex	ZB-MR1 ZB-MR2	10 9	0 0	36

*Comparison based on published competitor data. Assuming a 5 minute cool-down and equilibration time and a 5 minute high temperature hold after the last compound elutes, samples run per 12 hour sequence are calculated as follows:

Restek: 5 min. + 5 min. + 7 min. = 17 min./sample; 720 min./17 min. = 42 samples
Agilent: 5 min. + 5 min. + 16 min. = 26 min./sample; 720 min./26 min. = 27 samples
Phenomonex: 5 min. + 5 min. + 10 min. = 20 min./sample; 720 min./20 min. = 36 samples

and Rtx®-CLPesticides2 column set, was originally developed for the organochlorine pesticides in EPA Method 8081. While popular among analysts for this method, the unique selectivity is also appropriate for many other common halogenated compounds, making them an excellent choice for many GC-ECD methods.

Optimal Performance for 7 ECD Methods

A key benefit of this column pair is that, since it works quite well for several common ECD methods, there is no need to dedicate one instrument strictly to an individual method or to change columns based on testing needs. In addition, compared to other column sets that are offered specifically for GC-ECD methods, the Rtx®-CLPesticides column set provides the best overall performance across all 7 commonly used EPA methods (Table I). Comparisons of analysis time and coelutions demonstrate that this column set is an ideal choice for chlorinated pesticides, PCBs, herbicides, haloacetic acids, and other halogenated compounds.

Cut Analysis Time in Half for Method 8081

The selectivity of the Rtx®-CLPesticides column set was originally tuned for the analysis of organochlorine pesticides by EPA Method 8081. This is one of the most common ECD methods used by environmental labs, and it provides an excellent example of the performance of the column pair. As shown in Figure 1, all compounds are fully resolved in just 7 minutes using standard 0.32 mm columns for analysis. This time savings translates to significantly higher sample throughput (Table II), which is an important consideration for most labs.

Summary

Instead of dedicating instruments to a single method or changing columns between methods, analysis of chlorinated pesticides, PCBs, herbicides, and other halogenated compounds can be done on a single column set. Rtx®-CLPesticides and Rtx®-CLPesticides2 columns outperform other column sets offered specifically for multiple GC-ECD methods and are recommended for labs interested in increasing operational efficiency.

For complete comparisons and chromatograms for all methods, visit **www.restek.com/CLP7**

Rtx®-CLPesticides Column (fused silica)
(proprietary Crossbond® phases)

Description	temp. limits	cat.#
30m, 0.32mm ID, 0.32µm	-60 to 320/340°C	11141

Rtx®-CLPesticides2 Column (fused silica)
(proprietary Crossbond® phases)

Description	temp. limits	cat.#
30m, 0.32mm ID, 0.25µm	-60 to 320/340°C	11324

Large Volume Splitless Injection With an Unmodified GC Inlet

Lets You Skip Sample Concentration for Pesticides and BFRs in Drinking Water

By Michelle Misselwitz and Jack Cochran

- Eliminate time-consuming extract concentration without sacrificing sensitivity.
- Simplified approach uses standard injection port—no specialized equipment.
- Analyze at sub-ppb levels with faster, less labor-intensive procedure.

Using large volume splitless injection is advantageous when trying to analyze trace-level contaminants in clean matrices like drinking water because greater levels of target compounds are introduced onto the analytical column. A special injection port is generally required for large volume injection, which has limited its application. A concurrent solvent recondensation–large volume splitless injection (CSR-LVSI) technique described by Magni and Porzano [1,2] offered a more practical alternative, but involved some modification of a split/splitless injection port.

We have used CSR-LVSI successfully with a completely unmodified Agilent split/splitless GC inlet. The setup utilizes a pre-column (e.g., 5 m x 0.53 mm) press-fitted to the analytical column and a starting GC oven temperature below the boiling point of the solvent. A fast autosampler injection with liquid band formation into a liner containing glass wool is used to prevent backflash in the injection port. Here we investigated the applicability of this approach to analyzing pesticides and brominated flame retardants (BFRs) in drinking water according to U.S. EPA Method 527 [3].

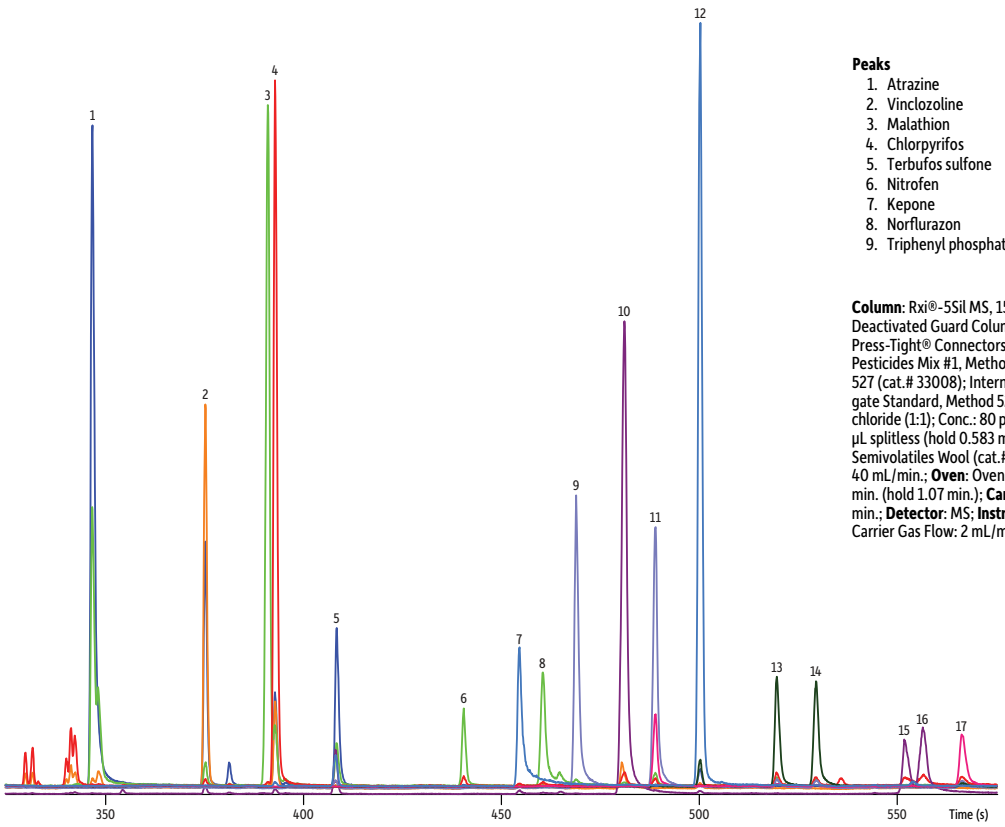
Table I: Calibration standards and concentration equivalents.

Level	Prepared Standard (pg/µL)	On-Column Amount Injected (pg/12.5 µL)	Equivalent Concentration in 1 L Samples (ug/L)
1	2	25	0.05
2	4	50	0.1
3	10	125	0.25
4	20	250	0.5
5	40	500	1
6	80	1,000	2

Table II: Average percent recoveries and relative standard deviations for 1 µg/L and 0.1 µg/L laboratory fortified blank samples analyzed using disk extraction with no extract concentration and CSR-LVSI GC-TOFMS (n = 3).

	1.0 µg/L % Recovery		0.1 µg/L % Recovery	
Compounds	AVG (n = 3)	%RSD	AVG (n = 3)	%RSD
Dimethoate	73	2.4	75	9.3
Atrazine	96	1.8	84	13
Propazine	93	3.3	92	8.5
Vinclozoline	97	4.0	97	8.0
Prometryne	179	3.0	113	7.9
Bromacil	78	2.2	66	3.1
Malathion	98	2.7	85	6.5
Thiobencarb	93	3.9	70	1.9
Chlorpyrifos	92	3.1	84	1.7
Parathion	94	0.7	92	4.6
Terbufos sulfone	88	2.8	105	11
Oxychlordane	75	8.5	74	10
Esbiol	88	2.7	79	6.5
Nitrofen	91	3.0	77	5.3
Kepone	102	18	56	32
Norflurazon	91	7.2	105	10
Hexazinone	87	0.8	68	2.1
Bifenthrin	100	3.0	81	3.2
BDE-47	96	4.4	87	15
Mirex	93	4.5	76	2.3
BDE-100	93	3.8	89	11
BDE-99	93	2.9	79	33
Perylene-D12	103	1.6	98	3.3
Fenvalerate	92	0.4	59	16
BB-153	88	3.4	45	14
Esfenvalerate	89	3.7	69	20
BDE-153	88	13	54	49

Figure 1: Extracted ion chromatogram of 80 pg/µL standard from 12.5 µL CSR-LVSI injections.



The typical procedure for preparing samples according to EPA Method 527 involves extracting a 1 L water sample, drying the extract, and concentrating it down to a final volume of 1 mL. To determine if using CSR-LVSI could eliminate the need for extract concentration, linearity and recovery were assessed. Water samples were fortified at 0.1 µg/L and 1 µg/L levels and then extracted using Resprep® resin SPE disks, dried with anhydrous sodium sulfate, and diluted to 25 mL with methylene chloride:ethyl acetate (1:1). This differs from the method, which calls for the samples to be concentrated to 1 mL after drying. In order to achieve the detection limits described in the method, a 12.5 µL injection volume was used.

Linear Responses for Challenging Compounds Using CSR-LVSI

Calibration curves were built using duplicate 12.5 µL injections of 2, 4, 10, 20, 40, and 80 pg/µL standards. All compounds exhibited good linearity down to 2 pg/µL, which is equivalent to 25 pg on-column and 0.05 µg/L in the original water sample (Table I). Results for Kepone (r = 0.995) are especially notable, as it can be problematic due to the formation of a hemiacetal that chromatographs poorly. Good chromatographic separations were obtained using a 15 m x 0.25 mm x 0.25 µm Rxi®-5Sil MS column, and the fast oven program resulted in an analysis time of less than 10 minutes (Figure 1).

Determine Sub-ppb Levels Without Extract Concentration

The average recovery for all compounds for the 1 µg/L (500 pg on-column) and 0.1 µg/L (50 pg on-column) spikes were quite good at 94% and 80%, respectively (Table II). Individual recoveries met EPA Method 527 criteria, except for the 0.1 µg/L value for hexabromobiphenyl 153 (BB-153) and the 1.0 µg/L value for prometryne. Recovery results demonstrated that employing CSR-LVSI and eliminating the concentration step can be an effective way to meet detection limits while reducing sample preparation time by more than an hour.

Summary

When the extract concentration step was eliminated, good linearity and recovery results were obtained while sample preparation time was significantly reduced. CSR-LVSI with an unmodified Agilent split/splitless GC inlet has been shown to be a technically viable approach that has the advantage of speeding up sample preparation without compromising sensitivity for pesticides and BFRs in drinking water.

For the complete version of this technical article, visit **www.restek.com/LVSI**

References

[1] P. Magni, T. Porzano, J. Sep. Sci. 26 (2003) 1491.
[2] Patent No: US 6,995,709 B2.
[3] U.S. Environmental Protection Agency, Method 527, Determination of Selected Pesticides and Flame Retardants in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS), April 2005.

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; similar to 5% phenyl/95% dimethyl polysiloxane)

Description	temp. limits	cat.#
15m, 0.25mm ID, 0.25µm	-60 to 330/350°C	13620

Resprep® Resin SPE Disks

Description	qty.	cat.#
Resprep Resin SPE Disks	20-pk.	26023

Extending the Power of Stabilized PLOT Column Technology to Process GC Analyzers

By Jaap de Zeeuw, Rick Morehead, and Tom Vezza

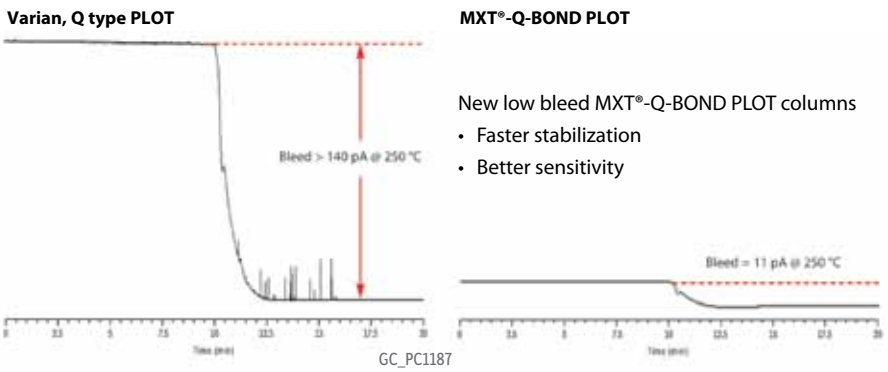
- New technology ensures consistent flows and predictable retention times.
- Rugged metal MXT® tubing stands up to process GC analyzer conditions.
- Available with all major adsorbents in 3.5" coils or on 7" 11-pin cages.

Porous layer open tubular (PLOT) columns are useful for analyzing volatiles in petrochemical product streams, as the specialized adsorbents provide good resolution and fast analysis times. However, conventional PLOT columns suffer from poor mechanical stability, limiting their use in process analyzers, which require robust columns for continual operation. Recently Restek developed new PLOT column bonding techniques that result in improved layer stability, consistent flow behavior, and more reproducible retention times. This technology, which was first developed for fused silica columns, has now been transferred to metal MXT® tubing, resulting in rugged columns that outperform typical metal PLOT columns and are ideal for process GC analyzers.

New Technology Improves Column Stability

Restek's PLOT columns are stabilized through a proprietary process that is based on concentric adsorption layers and improved particle bonding. New MXT® PLOT columns show greater thermal stability and much less phase bleed than the comparable competitor product (Figure 1). Lower bleed improves sensitivity and ensures faster stabilization times.

Figure 1: The bonding technology used in new MXT® PLOT columns increases thermal tolerance, resulting in lower bleed, faster stabilization times, and higher sensitivity.



Bleed comparison: Q type porous polymer columns were conditioned at 250 °C for equivalent periods and then tested to evaluate temperature stability. Split vent flow rate: 150 mL/min.; Oven: 250 °C (hold 10 min.) to 40 °C at 50 °C/min.; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

Figure 2: Conventional PLOT columns show continuous spiking resulting from particle generation. In contrast, the Restek column showed spikes during only the 2 initial analyses out of 240.

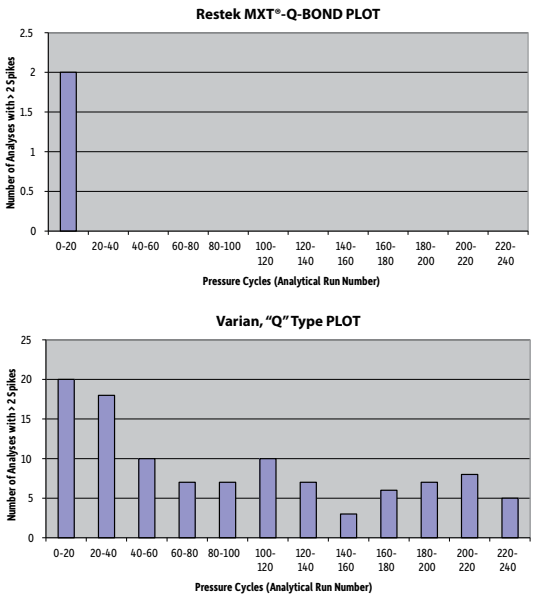
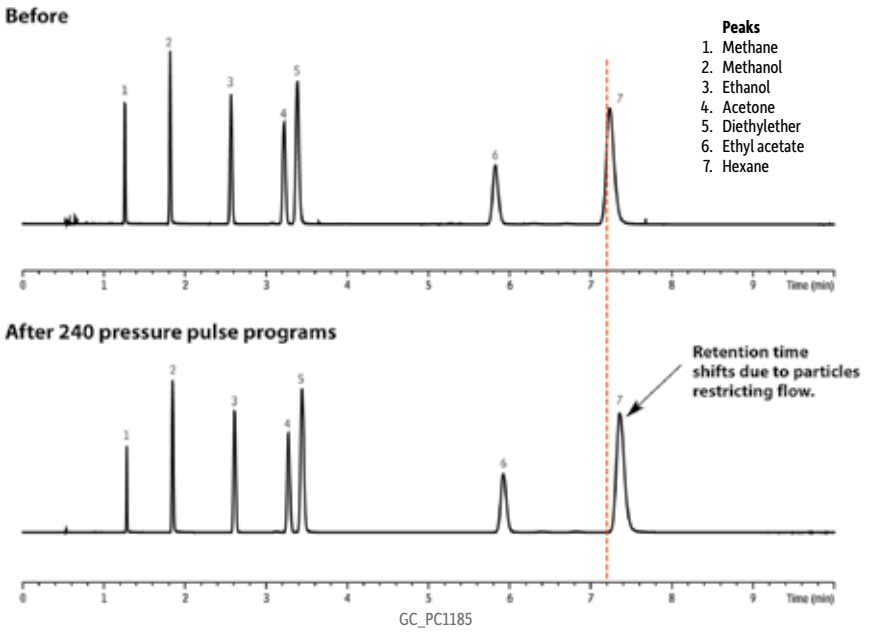
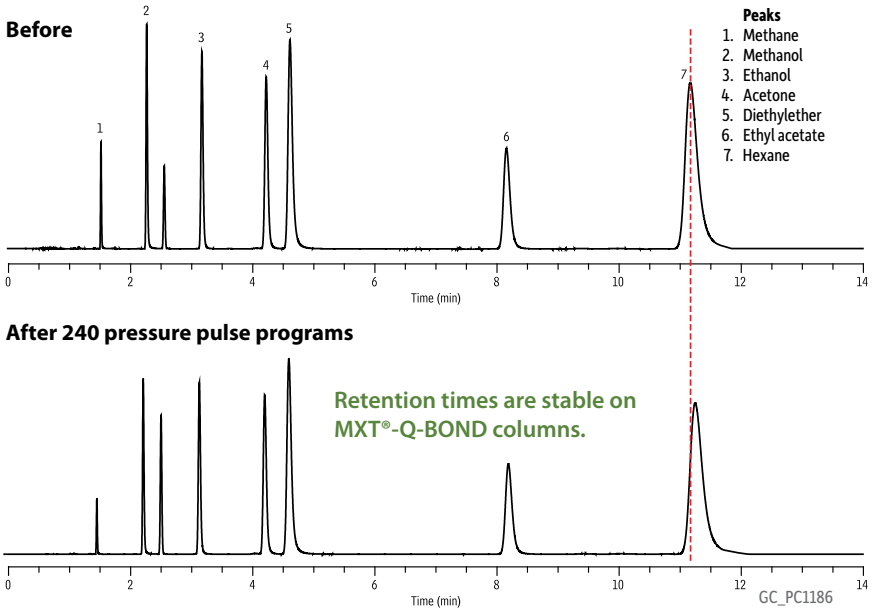


Figure 3: A conventional PLOT column releases particles following pressure pulsing, forming restrictions in the column that affect flow behavior and change retention time.



Isothermal testing before and after 240 pressure pulse cycles. Column: Varian Q type PLOT, 25 m x 0.53 mm ID; Sample: solvent mix; Injection: 1 µL split, 250 °C; Split vent flow rate: 150 mL/min.; Oven: 150 °C; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

Figure 4: MXT® PLOT columns are exceptionally stable; flow characteristics and retention times are highly consistent and not affected by pressure pulses.



Isothermal testing before and after 240 pressure pulse cycles. Column: MXT®-Q-BOND PLOT, 30 m x 0.53 mm ID x 20 µm (cat.# 79716); Sample: solvent mix; Injection: 1 µL split, 250 °C; Split vent flow rate: 150 mL/min.; Oven: 150 °C; Carrier gas: hydrogen, constant pressure (4 psi, 27.6 kPa); Detector: FID @ 250 °C.

Stable Flow Ensures Predictable Retention Times

To demonstrate the superior stability of MXT® PLOT columns, an MXT®-Q-BOND column and a competitor's Q type column were subjected to 240 pressure pulse cycles and the spiking observed in each analytical run was used as an indicator of particle generation, or phase instability. Results demonstrate that particle generation on the Varian column was significantly higher (Figure 2), resulting in restrictions in the column that caused a shift in retention time (Figure 3). In contrast, the MXT®-Q-BOND column showed little spiking. Greater phase stability resulted in consistent flow behavior and predictable retention times (Figure 4).

Key Phases Available for Optimized Separations

New metal MXT® columns are available for all major adsorbent types: porous polymer, molecular sieve, and alumina. Porous polymer MXT® columns, such as the MXT®-Q-BOND column, are highly inert and effective at separating both polar and nonpolar compounds. Volatiles are strongly retained, making these columns extremely useful for determining solvents. Molecular sieve columns provide efficient separation of argon and oxygen, as well as other permanent gases. Metal MXT® alumina columns are recommended for light hydrocarbon analysis, as alumina is one of the most selective adsorbents available and allows all C1-C5 isomers to be separated with the highest degree of resolution.

Summary

MXT® PLOT columns from Restek offer greater stability than conventional PLOT columns, making them a better choice for process monitoring. New bonding techniques produce columns with highly reproducible flow characteristics, improved layer stability, and excellent separation efficiencies. These robust columns produce exceptionally reproducible chromatography, providing the reliable performance needed for process GC analyzer applications.

For the complete version of this technical article, visit
www.restek.com/metalPLOT

MXT®-Q-BOND Columns

(Siltek®-treated stainless steel PLOT)

ID	df	temp. limits	3.5" coil 15-Meter	7" 11-pin cage 15-Meter	3.5" coil 30-Meter	7" 11-pin cage 30-Meter
0.25mm	8µm	to 280/300°C	79718-273	79718		
0.53mm	20µm	to 280/300°C			79716-273	79716

Other phases available, visit www.restek.com/metalPLOT for details.

Rethinking the Use of Wool With Splitless GC

By Scott Grossman

- An obstruction like wool is a must for efficient vaporization under split conditions.
- Wool is also necessary under splitless conditions to minimize sample loss and improve transfer onto column.
- With exceptionally inert Sky™ inlet liners, you can use wool with confidence.

When running a split injection with an autosampler, few would challenge that you need a liner with an obstacle like wool to achieve accurate, precise results. After all, when you combine a fast injection with a high split flow rate, your sample simply needs more time to vaporize or else it may be lost out the split vent. Wool stops the sample and gives it the time it needs to efficiently and completely vaporize, presenting a homogenous mixture to the column and split vent. Unlike in split injections, conventional wisdom has long held that you do not need wool under splitless conditions. However, a highly recommended paper by Bieri et al. argues that wool is just as important in splitless work. [1]

Should Splitless Mean Wool-Free?

Why do so many chromatographers believe that wool is not necessary to get accurate and representative sample transfer in a splitless run? The only flow out of the inlet (other than the septum purge) is through the column, so the thinking is that, since the flow will be so much slower than it is under split conditions, the sample will have ample time to vaporize and transfer onto the column without assistance. But, could autoinjecting the sample using a fast plunger speed pose a problem? And can't the sample still become trapped or be lost? The visualization and chromatographic experiments Bieri et al. outlined were very effective in supporting their claim that wool is a must for split ***and*** splitless runs alike. So, I decided to expand upon their work using common styles of splitless liners.

Putting Wool Through the Wringer

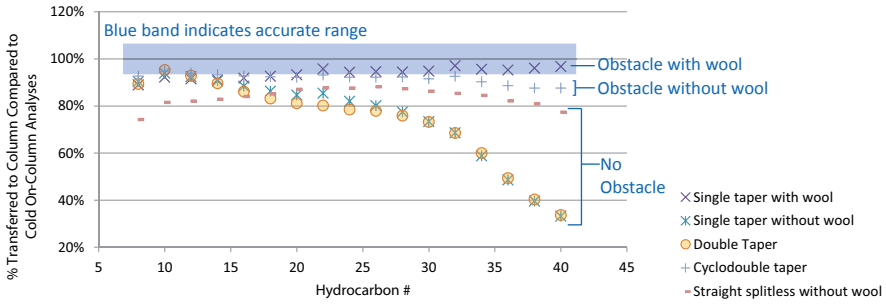
Since the integral question is whether you lose sample when performing splitless injections without wool, I opted to benchmark with cold on-column injections to force 100% of the sample onto the column. My sample was a 17-component mixture of straight-chain hydrocarbons spanning a molecular weight range from C8 to C40. In addition to cold on-column capability, my GC also had a split/splitless inlet, so I collected all response data using the same FID.

Figure 1 shows the data from a series of splitless analyses using the same sample but different liners. Results clearly illustrate that, for a wide molecular weight range, the use of wool—or to a lesser degree another obstacle like a cyclo double gooseneck—is necessary for accurate sample transfer and a reduction of molecular weight discrimination. You can also see that the only time the entire mass of analytes was transferred to the column under splitless conditions was when we employed a single gooseneck with wool. The liners with no obstruction had much less desirable results.

Use Wool With Confidence

Of course, there is a reason why one may prefer not to use wool: It is a common source of activity that can break down and trap sensitive analytes. In that case, how do you avoid counteracting wool's advantage in improving vaporization? The wool in a Sky™ inlet liner is made of fused quartz and is deactivated after packing, reducing the loss of sensitive analytes (Figure 2). By using Sky™ liners with exceptionally inert wool, you can help ensure efficient vaporization and improved transfer onto your column for more accurate results and lower detection limits. With Restek Sky™ inlet liners, you can use wool with confidence—and should under split ***and*** splitless conditions.

Figure 1: Only the liners with an obstruction were able to produce even 90% sample transfer with splitless injections—and only the liner with wool offered full accuracy.

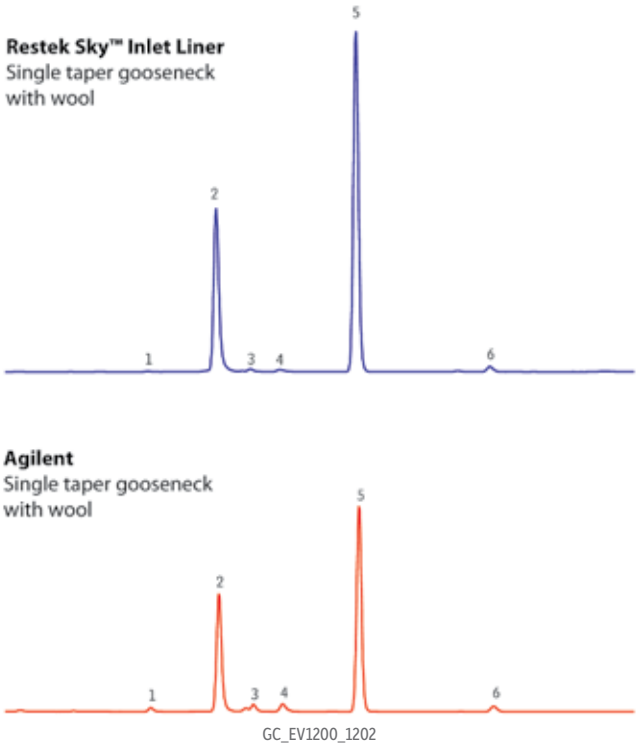


References

[1] Stefan Bieri, Philippe Christen, Maurus Biedermann, and Koni Grob, *Inability of Unpacked Gooseneck Liners to Stop the Sample Liquid After Injection With Band Formation (Fast Autosampler) Into Hot GC Injectors*, Anal. Chem. 76 (2004) 1696.

For a closer look at the form and function of GC inlet liners, view Scott's webinar at **www.restek.com/linerwebinar**

Figure 2: Endrin and DDT breakdown is significantly reduced with Sky™ liners, due to higher inertness and lower activity—even when using wool.



Inert Sky™ liners reduce analyte breakdown, giving you more accurate results.

% Breakdown

	Endrin	DDT
Restek	**4.8**	**1.3**
Agilent	12	5.2

Peaks
1. DDE*
2. Endrin
3. DDD*
4. Endrin aldehyde*
5. DDT
6. Endrin ketone*

*breakdown products

Column Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620); **Sample** endrin (50 ng/mL) and DDT (100 ng/mL) in hexane; **Injection** Inj. Vol.: 1 µL splitless (hold 0.75 min.); Liner: Comparison of Sky™ Single Taper Gooseneck Liner with Wool (cat.# 23303.5) and Agilent Single Taper Gooseneck Liner with Wool (cat.# 5062-3587); Inj. Temp.: 250 °C.; **Detector:** µ-ECD @ 300 °C.

Analysis of Brominated Flame Retardants by Liquid Chromatography Mass Spectrometry

By Dr. Chris Marvin, Environment Canada

***Dr. Chris Marvin** is a Research Scientist for Environment Canada, Burlington, Ontario. His research interests include new and emerging environmental contaminants, occurrence and fate of contaminants in the Great Lakes, and LC-MS methods development.*

A wide variety of brominated flame retardants (BFRs) are currently used in industry and commerce. Use of these compounds has increased exponentially in the past 50 years as a result of strict regulations regarding the flame retardancy of consumer products. Roughly 40% of all flame retardants on the market are brominated. Some of these compounds have the potential to be persistent, toxic, bioaccumulative, and are amenable to long range transport. In addition, the occurrence, distribution, and fate of many of these compounds in the environment remain largely unknown.

Polybrominated diphenyl ethers (PBDEs) remain the most widely studied of the BFRs, despite the penta- and octa-formulations being banned in Europe and voluntary cessation of production in North America. With the exception of the fully-substituted decabromodiphenyl ether (BDE-209), the PBDEs are easily determined by gas chromatography-mass spectrometry (GC-MS) and are now routinely measured in a wide range of environmental matrices. Due to its unique chemical and physical properties, including high molecular weight, poor solubility, and sensitivity to heat and light, accurate determination of BDE-209 remains a significant challenge. A host of other BFRs are not readily amenable to analysis by GC-MS and pose an analytical challenge as a result of their physical properties. Although their chemical structures appear quite simple, BFRs such as hexabromocyclododecane (HBCD), 1,2,5,6-tetrabromocyclooctane (TBCO) and tetrabromoethylcyclohexane (TBECH) thermally isomerize and partition poorly on GC stationary phases. HBCD is one of the most widely used BFRs with production globally in excess of 20,000 tons; HBCD is the primary flame retardant used in the extruded and expanded polystyrene foams used as thermal insulation in buildings, as well as in upholstery fabrics. Some laboratories continue to report HBCD concentrations as the sum of the three predominant isomers based on analysis by GC, i.e., the sum of α-, β- and γ-HBCD. These nonisomer specific analyses preclude thorough investigation of environmental pathways, and potential shifting of isomer profiles during manufacture or cycling in the environment. Differences in pathways of HBCD in the environment are evidenced by the predominance of γ-HBCD in the technical mixture and in sediment, while α-HBCD is dominant in

biota (typically >90%). In addition, an inherent property of aliphatic BFRs is that they exist as diastereomers. Therefore, the study of enantioselective accumulation of BFRs in food chains requires separation of the individual enantiomers.

The last decade has been a period of extraordinary progress in development of LC-MS technology. As a result, detection limits of some LC-MS methods are on a par with those of gas chromatography-high resolution mass spectrometry (GC-HRMS) methods. These technological advances allow the resolving power of contemporary LC stationary phases to be coupled with the sensitivity and specificity of state-of-the-art mass spectrometers. In addition, electrospray ionization (ESI), one of the most commonly used ionization mechanisms, is softer than electron ionization (EI) used in GC-MS. Robust LC-MS methods for analysis of BFRs, including HBCD and tetrabromobisphenol-A (TBBPA), are now routinely used in analytical laboratories. Most methods for analysis of BFRs are based on negative ion mass spectrometry. Despite these advances, significant analytical challenges remain in LC-MS methods development. LC-MS continues to be susceptible to matrix effects, and the technique still generally lacks the retention time reproducibility of GC-MS methods. The use of isotopically-labeled internal standards is effective in minimizing matrix effects, but investigations of new chemicals continue to be plagued by a paucity not only of labeled compounds, but authentic native standards.

Other challenges of LC-MS analysis of BFRs can include poor ionization efficiency and limited fragmentation. In the case of TBCO and TBECH, both ESI and atmospheric pressure chemical ionization (APCI) result in weak molecular ions or molecular ion adducts. Adequate detectability of the compounds can be achieved by monitoring the Br- ions in selected ion monitoring (SIM) mode; however, this approach negates the advantages of a triple quadrupole mass spectrometer, in that the power of tandem MS techniques cannot be exploited. Atmospheric pressure photoionization (APPI) is the latest ionization technique developed for LC-MS; in fact, the impetus behind development of APPI was the need to extend the range of compounds beyond those only amenable to ESI or APCI. Typical variations of the technique are based on vaporization of the liquid sample (similar to APCI), combination with a dopant, and subsequent ionization resulting from gas phase reactions initiated by photons from a krypton discharge lamp. APPI has shown great potential for analysis of compounds across a broad range of polarities, but particularly for nonpolar analytes. The method is also reportedly less susceptible to matrix effects than ESI and APCI.

Progress in LC-MS methods development continues as lessons learned from investigations of individual compounds are applied to subsequent generations of BFRs. A new challenge in the evolution of LC-MS methods for BFRs is the development of comprehensive methods for concurrent analysis of multiple compound classes. The primary challenge in development of comprehensive methods is identification of suitable LC stationary phases coupled with MS ionization techniques applicable to compounds exhibiting a broad range of chemical and physical characteristics. The LC stationary phase must provide adequate separation among compounds that can exhibit dramatically different retention behaviors; key factors include particle size, pore size, and stationary phase chemistry. In addition, even individual isomers within the same compound class can exhibit significantly different mass spectrometric response factors. A further convoluting factor is the limited solubility of BFRs in typical reversed phase (RP) HPLC mobile phases. Many BFR standards are marketed in nonpolar solvents such as toluene, necessitating a solvent exchange step prior to analysis. The same issue arises for BFRs isolated from environmental samples using conventional column cleanup methods, in that these techniques frequently culminate in the extracts being concentrated in nonpolar solvents amenable to analysis by GC.

The primary challenge in development of comprehensive methods is identification of suitable LC stationary phases coupled with MS ionization techniques applicable to compounds exhibiting a broad range of chemical and physical characteristics.

Ultimately, partnerships among experts in the field of analytical standards, separation science, and mass spectrometry will yield viable comprehensive methods for BFRs. In the past few years, suppliers of analytical standards and manufacturers of LC stationary phases and mass spectrometers have been astute in recognizing trends in analysis of compounds of potential environmental concern, and correspondingly have been proactive in developing technologies of great value to the toxics research and monitoring community.

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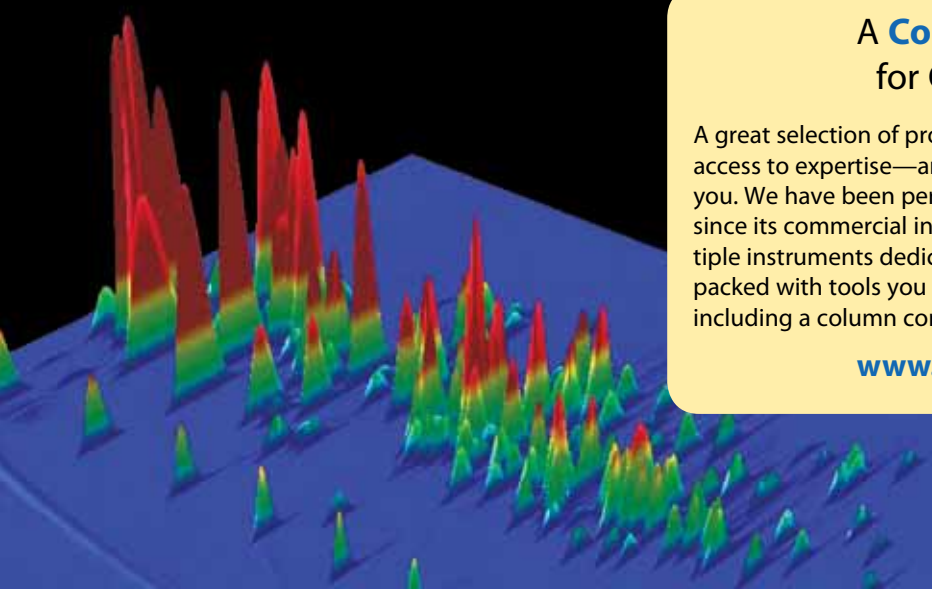

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Rxi®-17Sil MS Columns

Offering Unique Selectivity for PAHs

- **Separate PAHs** that cannot be distinguished by mass spectrometry.
- **Increase accuracy for PAHs** of regulatory and health concern.
- **Rxi® technology** assures reliable trace level results.

For more information on PAH analyses using **Rxi-17Sil MS** columns, see the **Food Safety** feature articles on pages 2 & 3.

Also in this issue

www.restek.com

New! Rxi®-17Sil MS Column

Separate PAHs that Cannot be Distinguished by Mass Spectrometry

- Unique phase chemistry provides better resolution than other "17" type columns.
- Optimized selectivity separates a wide range of key PAHs.
- Rxi® technology assures accurate, reliable trace level analyses.

Rxi® GCColumns

Polycyclic aromatic hydrocarbon (PAH) analysis is a growing area of environmental and food safety testing, due to the ubiquitous presence and reported genotoxicity and carcinogenicity of some compounds in this class. As target lists expand and health concerns drive detection levels lower, reporting requirements are more difficult to meet and column selectivity becomes an important factor in achieving accurate results.

New Rxi®-17Sil MS columns are optimized for PAHs and are the best choice for accurate, trace level detection. Rxi®-17Sil MS columns differ in phase chemistry from conventional 17 type (50% diphenyl) columns, and the resulting selectivity provides better resolution of critical PAHs (Figure 1). Not all 50% phenyl columns are equivalent—Rxi®-17Sil MS columns let you quantify isobaric PAHs that cannot be determined by mass spectrometry.

Unique Selectivity Means More Accurate PAH Data

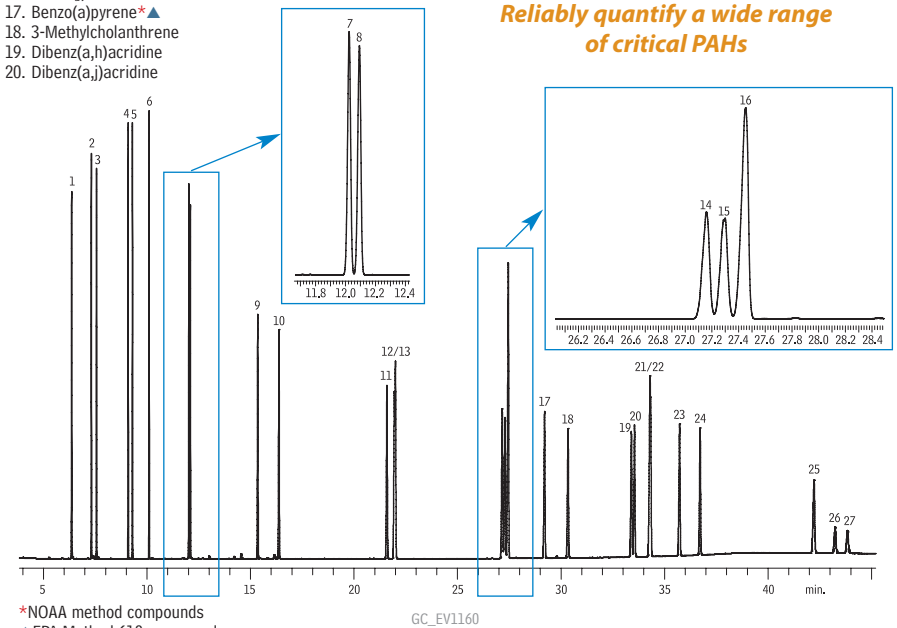
Little differences mean a lot. At first glance, PAH separations on the new Rxi®-17Sil MS and typical "17" type columns appear to be similar, but the difference in selectivity becomes clear when looking at critical separations (Figure 2). Isobaric compounds phenanthrene and anthracene have essentially indistinguishable mass spectra and must be chromatographically resolved. The Rxi®-17Sil MS column provides baseline resolution of these critical com-

Figure 1 Rxi®-17Sil MS columns ensure excellent resolution of PAHs of regulatory or health concern.

1. Naphthalene*▲
2. 2-Methylnaphthalene
3. 1-Methylnaphthalene
4. Acenaphthylene▲
5. Acenaphthene▲
6. Fluorene*▲
7. Phenanthrene*▲
8. Anthracene*▲
9. Fluoranthene*▲
10. Pyrene*▲
11. Benz(a)anthracene*▲
12. Chrysene*▲
13. Triphenylene
14. Benzo(b)fluoranthene*▲
15. Benzo(k)fluoranthene*▲
16. Benzo(j)fluoranthene
17. Benzo(a)pyrene*▲
18. 3-Methylcholanthrene
19. Dibenz(a,h)acridine
20. Dibenz(a,j)acridine
21. Indeno(1,2,3-cd)pyrene*▲
22. Dibenz(a,h)anthracene*▲
23. Benzo(ghi)perylene▲
24. 7H-Dibenzo(c,g)carbazole
25. Dibenzo(a,e)pyrene
26. Dibenzo(a,i)pyrene
27. Dibenzo(a,h)pyrene

Column: Rxi®-17Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 14123)
Sample: SV Calibration Mix #5 / 610 PAH Mix (cat.# 31011); EPA Method 8310 PAH Mixture (cat.# 31841);
Diluent: dichloromethane; Conc.: 10 ppm
Injection: Inj. Vol.: 0.5 µL splitless (hold 1.75 min.)
Liner: Auto SYS XL PSS Split/Splitless w/Wool (cat.# 21718)
Inj. Temp.: 320 °C; Purge Flow: 75 mL/min.
Oven: Oven Temp: 65 °C (hold 0.5 min.) to 220 °C at 15 °C/min. to 330 °C at 4 °C/min. (hold 15 min.)
Carrier Gas: He, constant flow; Flow Rate: 2.0 mL/min.
Detector:FID @ 320 °C
Instrument:PE Clarus 600 GC
Acknowledgement: Instrument provided by PerkinElmer

Reliably quantify a wide range of critical PAHs



*NOAA method compounds
▲EPA Method 610 compounds

pounds, which are only partially separated with a typical 17 type column. Similarly, benzofluoranthenes b, k, and j are isobaric compounds that must be reported separately, and the Rxi®-17Sil MS column reliably resolves all 3 isomers, even in a 15 m length. The unique selectivity of the Rxi®-17Sil MS column gives you more resolving power and better accuracy for challenging PAHs.

For more information on new Rxi®-17Sil MS columns, visit **www.restek.com/adv010**

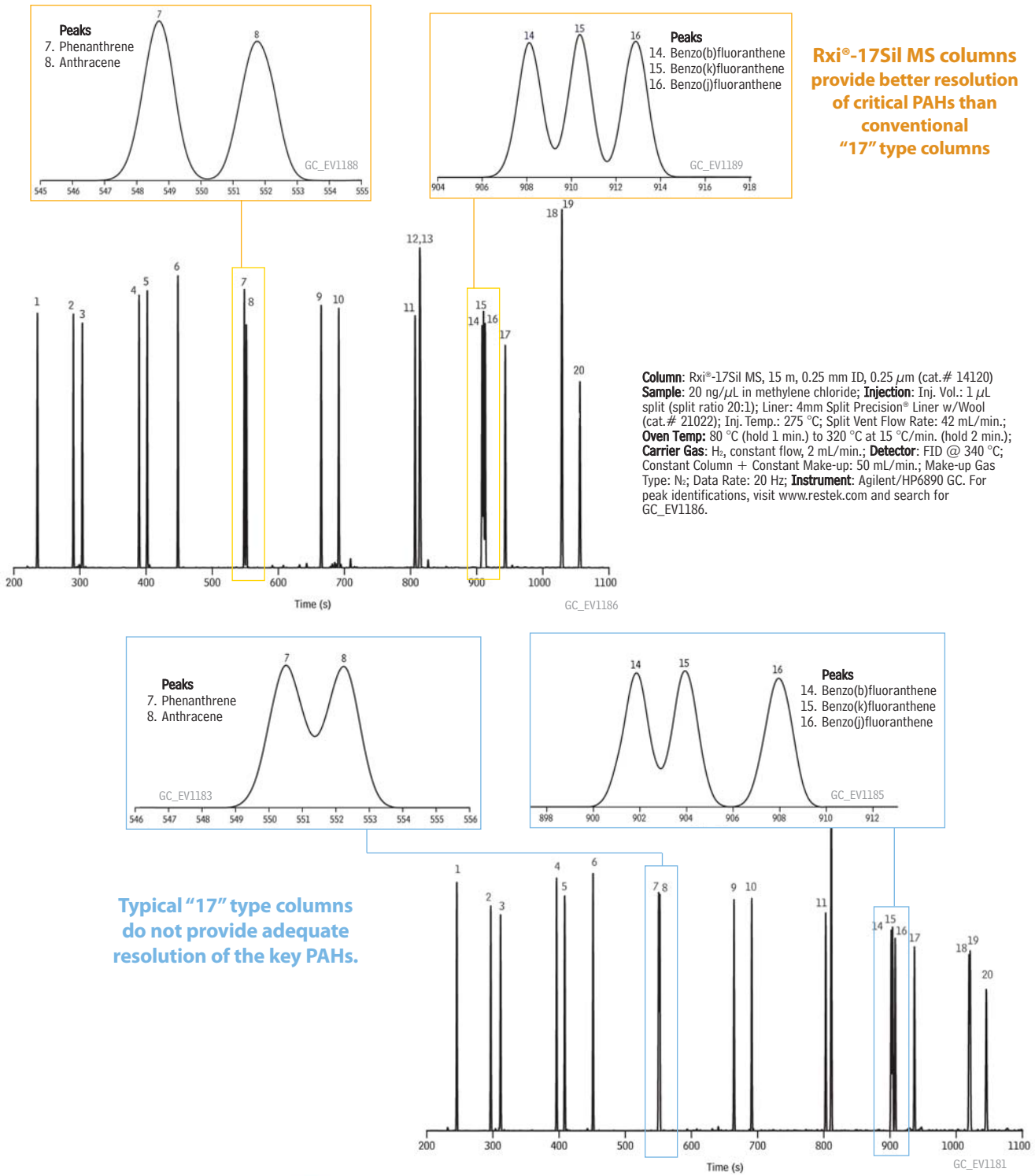
Rxi®-17Sil MS Columns (fused silica)

(mid polarity Crossbond® silarylene phase; equivalent to 50% phenyl methyl polysiloxane)

ID	df (µm)	temp. limits	length	qty.	cat. #
0.18mm	0.18µm	40 to 340/360°C	20m	ea.	14102
0.18mm	0.36µm	40 to 340/360°C	20m	ea.	14111
0.25mm	0.25µm	40 to 340/360°C	15m	ea.	14120
0.25mm	0.25µm	40 to 340/360°C	30m	ea.	14123
0.25mm	0.25µm	40 to 340/360°C	60m	ea.	14126
0.32mm	0.25µm	40 to 340/360°C	15m	ea.	14121
0.32mm	0.25µm	40 to 340/360°C	30m	ea.	14124

*Maximum temperatures listed are for 15- and 30-meter lengths. Longer lengths may have a slightly reduced maximum temperature.

Figure 2 Rxi®-17Sil MS columns let you accurately report PAHs that cannot be distinguished by MS.



Novel Approach for PAHs in Seafood:

Reduce Sample Prep from Days to Hours Using QuEChERS and GCxGC

By Jack Cochran, Director of New Business and Technology

- Prepare samples in hours vs. days, using QuEChERS instead of the NOAA method.
- GCxGC analysis minimizes matrix interference, for accurate trace-level results.
- Selectivity of Rxi®-17Sil MS column assures separation of benzofluoranthenes.

Consumer safety concern in the wake of the Deepwater Horizon oil spill has increased demand for rapid, accurate test methods for polycyclic aromatic hydrocarbons (PAHs) in seafood. The FDA has issued a protocol to reopen closed fishing waters that includes chemical testing of seafood for PAHs, but the NOAA sample preparation method that was proposed is extremely tedious and time-consuming, requires expensive pressurized fluid extraction and gel permeation chromatography equipment, and uses large volumes of environmentally-unfriendly methylene chloride. Alternative methods are being explored, and initial results for a novel approach that combines a rapid QuEChERS extraction with the accuracy of GCxGC-TOFMS are presented here.

QuEChERS Saves Time and Money

While QuEChERS was originally developed to simplify extraction and cleanup of pesticide residues in fruits and vegetables, it is rapidly expanding to other applications due to its speed, simplicity, and cost-effectiveness, so it was natural to consider it as a replacement for the NOAA method. For this work, samples of freeze-dried mussel tissue containing NIST certified levels of PAHs were prepared in less than 2 hours using a simple procedure that was quicker, easier, and more cost-effective than the NOAA method (Figure 1).

GCxGC with Rxi®-17Sil MS and Rxi®-1ms Columns Ensures Unbiased Separation of Key PAHs

Mussel samples were too complex for traditional GC/MS analysis, so GCxGC was employed. The key to maximizing separations between peaks with this technique is to choose columns that differ significantly in phase chemistry. An Rxi®-17Sil MS column was chosen for the first separation, as it is optimized for PAH separations (see article on p. 2), and a standard Rxi®-1ms column was used for the second dimension to separate interfering fatty acids and sterols from the PAHs of interest.

Figure 1 QuEChERS extraction and cleanup procedure.

Note: Dried sample was used here; for fresh samples, start at Step 3.

1. Weigh 1.0 g of NIST SRM 2974a tissue into a 50 mL FEP centrifuge tube and add 10 mL organic-free water. Shake 1 min. to wet sample.
2. Aggressively vortex the sample for 15 min., then allow sample to settle for 30 min.
3. Add 10 mL acetonitrile and 20 µL of 25 ng/µL Semivolatiles Internal Standard Mix (diluted from cat.# 31206).
4. Shake sample by hand for 1 min., then vortex for 15 min.
5. Add 1 packet of Q110 EN 15662 QuEChERS extraction salts (cat.# 26236) and shake hard.
6. Aggressively vortex the sample for 15 min., then centrifuge at 3,000 g for 5 min.
7. Transfer 1mL supernatant extract to a 10 mL FEP tube with 150 mg $MgSO_4$ and 50 mg PSA, and 50 mg C18 and shake 1 min. to remove some fatty acids and lipids.
8. Centrifuge at 3,000 g for 5 min.
9. Withdraw extract for GCxGC-TOFMS analysis of PAHs.

Example time savings:

Estimated processing time for 30 fresh samples

QuEChERS: 10 hours
NOAA: 3-5 days

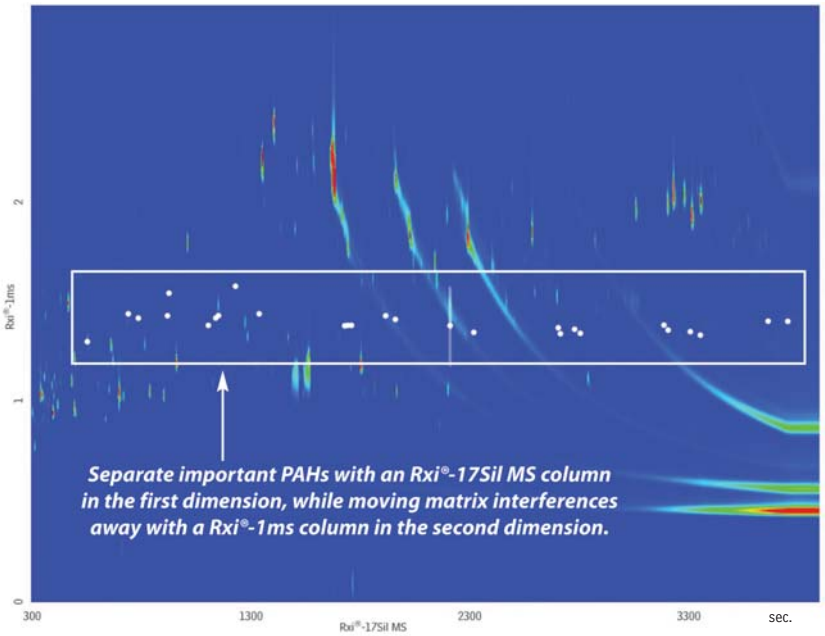
Results in Table I demonstrate that good recoveries were obtained under these preliminary conditions, especially considering that hydrophobic compounds were being extracted in hydrophilic QuEChERS solvent. High recoveries were noted for some compounds (e.g. fluoranthene), but were not due to isobaric interference as evidenced by high efficiency separation of PAHs from matrix (Figure 2) and by good agreement between sample and reference spectra (Figure 3).

Conclusion

Combining QuEChERS extraction with GCxGC-TOFMS, using Rxi®-17Sil MS and Rxi®-1ms columns shows great promise for analyzing PAHs in seafood. Labs interested in alternatives to the NOAA method should consider procedures based on this approach.

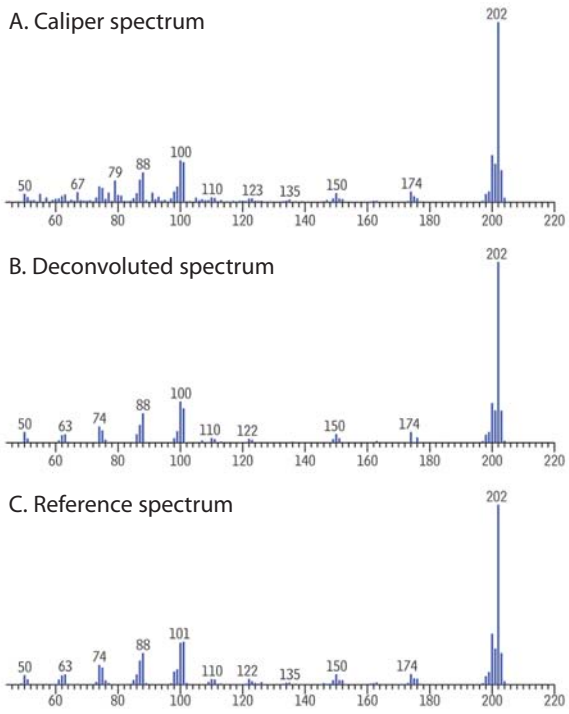
Visit our technical blog at **www.restek.com/adv011** for more details.

Figure 2 GCxGC-TOFMS contour plot of PAHs in mussels (QuEChERS extraction).



Column: Rxi®-17Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 14123); Rxi®-1ms 1.2 m, 0.15 mm ID, 0.15 µm (cat.# custom);
Sample: NIST SRM 2974a freeze-dried; mussel tissue with incurred residues; Diluent: Acetonitrile.
For complete conditions visit www.restek.com and search for GC_FF1197.

Figure 3 Good agreement between sample and reference spectra show target PAHs were separated from isobaric interferences.



Column:
Rxi®-17Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 14123);
Rxi®-1ms 1.2 m, 0.15 mm ID, 0.15 µm (cat.# custom);
Sample: NIST SRM 2974a freeze-dried mussel tissue with incurred residues; Diluent: Acetonitrile.

For complete conditions visit www.restek.com and search for GC_FF1198.

Table I Preliminary conditions gave good recoveries for most PAHs (n = 3).

PAH	Q Mass	NIST µg/kg	Average µg/kg by GCxGC	RSD%
Naphthalene-D8	136	ISTD	ISTD	ISTD
Naphthalene	128	9.68	63	5
2-Methylnaphthalene	142	8.1	8.6	15
1-Methylnaphthalene	142	5.8	5.4	8
Biphenyl	154	NA	4.2	1
2,6-Dimethylnaphthalene	156	NA	9.1	3
Acenaphthylene	152	NA	1.7	18
Acenaphthene-D10	162	ISTD	ISTD	ISTD
Acenaphthene	154	NA	3.3	22
2,3,5-Trimethylnaphthalene	170	NA	4.0	13
Fluorene	166	NA	8.8	8
Phenanthrene-D10	188	ISTD	ISTD	ISTD
Phenanthrene	178	74.4	113	5
Anthracene	178	2.46	8.1	8
1-Methylphenanthrene	192	17.6	29	12
Fluoranthene	202	287	376	5
Pyrene	202	186	229	4
Benzo(a)anthracene	228	31.1	39	9
Chrysene-D12	240	ISTD	ISTD	ISTD
Chrysene	228	123.6	199	5
Benzo(b)fluoranthene	252	41.5	53	0
Benzo(k)fluoranthene	252	18.95	22	12
Benzo(j)fluoranthene	252	21.4	18	3
Benzo(a)pyrene	252	9.73	12	5
Perylene-D12	264	ISTD	ISTD	ISTD
Perylene	252	6.80	5.0	3
Indeno(1,2,3-cd)pyrene	276	14.9	13	1
Benzo(ghi)perylene	276	23.7	20	10

ISTD = internal standard
NA = not analyzed by NIST

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26235

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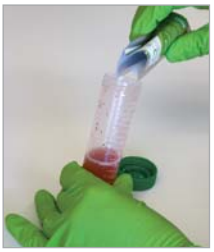

Description	Material	Methods	qty.	cat.#
Q110 Kit	4g $MgSO_4$, 1g NaCl, 1g TSCD, 0.5g DHS with 50mL Centrifuge Tube	European EN 15662	50 packets & 50 tubes	26235
Q110 Packets	4g $MgSO_4$, 1g NaCl, 1g TSCD, 0.5g DHS	European EN 15662	50 packets	26236
Q150 Kit	6g $MgSO_4$, 1.5g NaOAc with 50mL Centrifuge Tube	AOAC 2007.01	50 packets & 50 tubes	26237
Q150 Packets	6g $MgSO_4$, 1.5g NaOAc	AOAC 2007.01	50 packets	26238
Empty 50mL Centrifuge Tube, Polypropylene			50-pk.	26239
Empty 50mL Centrifuge Tube, Teflon FEP			2-pk.	23997

TSCD—trisodium citrate dihydrate
DHS—disodium hydrogen citrate sesquihydrate
NaOAc—sodium acetate

NEW! Q-sep™

Dimensions: 9"h x 14.5"w x 17"d
(22.9 cm x 36.8 cm x 43.2 cm)

26249

Q-sep™ 3000 Centrifuge

for QuEChERS

- Meets requirements of AOAC and European QuEChERS methodology.
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Priced to fit your laboratory's budget, the Q-sep™ 3000 Centrifuge is the first centrifuge specifically designed for QuEChERS methodology. This compact, quiet, yet powerful, unit spins at the 3,000 g force required by the European method.

Centrifuge includes 50 mL tube carriers (6), 50 mL conical tube inserts (6), 4-place 15 mL tube carriers (6), and 2 mL tube adaptors (24).

26232

26233

26234

Description	qty.	cat.#
Q-sep 3000 Centrifuge, 110V	ea.	26230
Q-sep 3000 Centrifuge, 220V	ea.	26231
Replacement Accessories		
50mL Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26232
50mL Conical Tube Insert for Q-sep 3000 Centrifuge	6-pk.	26249
4-Place Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26233
2mL Tube Adaptors for Q-sep 3000 Centrifuge	4-pk.	26234

Flip Seal Dual Vespel® Ring Inlet Seals

A **reversible** Dual Vespel® Ring Inlet Seal that lasts twice as long, for the same great price!

Our new Flip Seal greatly improves injection port performance while saving you time and money. This reversible inlet seal allows twice as many uses as other inlet seals, at the same cost. By using our patented Dual Vespel® Ring technology, the Flip Seal features two soft Vespel® rings, one on the top and one on the bottom, which eliminate the need for a washer. Our new reversible design allows you to flip the inlet seal and use it twice as many times.

1/16" Adaptor Fitting

Feature	Benefit
Reversible design.	Allows twice as many uses as other seals, at the same price.
Vespel® ring embedded in bottom surface.	Eliminates need for a washer.
Vespel® ring embedded in top surface.	Very little torque required to make seal—reduces operator variability.
Lower leak rate compared to OEM metal inlet seals.	Less detector noise.
Prevents oxygen from permeating the carrier gas.	Increases column lifetime.
Gold or Siltek Treated seals.	Reduces breakdown and adsorption of compounds, maximizing component transfer to GC column.
Kit includes 1/16-inch split/splitless adaptor fitting.	Works with standard OEM capillary ferrules.

0.8mm ID Flip Seal Dual Vespel Ring Inlet Seal	2-pk.	10-pk.
Gold-Plated	23407	23409
Siltek Treated	23408	23410
1.2mm ID Flip Seal Dual Vespel Ring Inlet Seal	**2-pk.**	**10-pk.**
Gold-Plated	23411	23413
Siltek Treated	23412	23414

Flip Seal Dual Vespel Ring Inlet Seal Kit	qty.	cat.#
Includes: gold-plated 0.8mm ID inlet seal, reducing nut adaptor, 1/16" SS nut	kit	23406

Fully Resolve Critical PAHs with an Optimized HPLC Column

Although most HPLC methods recommend a C18 column for the analysis of polycyclic aromatic hydrocarbons, resolution of isobaric compounds, such as the benzofluoranthenes, can be quite poor. Restek offers 2 HPLC columns that have been optimized specifically for PAHs and offer greater selectivity for these key compounds. Critical PAHs that cannot be distinguished by mass spectrometry can be reliably separated using either Pinnacle® II PAH or Pinnacle® DB PAH columns. Pinnacle® II PAH columns are available in standard formats, while the Pinnacle® DB PAH columns are offered on 1.9µm silica. Labs analyzing PAHs by either HPLC or UHPLC will benefit from the reliable separations obtained using Restek PAH columns.

Pinnacle® DB PAH UHPLC Columns

Physical Characteristics:

particle size: 1.9µm
pore size: 140Å
endcap: yes
pH range: 2.5 to 8
temperature limit: 80°C

1.9µm Columns	cat. #
50mm, 2.1mm ID	9470252
100mm, 2.1mm ID	9470212

Pinnacle® II PAH Columns

Physical Characteristics:

particle size: 4µm, spherical
pore size: 110Å
endcap: fully endcapped
pH range: 2.5 to 8
temperature limit: 80°C

4µm Columns	cat. #
50mm, 2.1mm ID	9219452
150mm, 2.1mm ID	9219462
50mm, 3.2mm ID	9219453
150mm, 3.2mm ID	9219463
50mm, 4.6mm ID	9219455
150mm, 4.6mm ID	9219465

ordering note

For guard cartridges for these columns, visit our website at **www.restek.com**.

Visit **www.restek.com/chromatograms** for PAHs and other HPLC applications.

Analyze 40% More Samples per Shift

Using Split Injection for Semivolatiles

By Michelle Misselwitz, Innovations Chemist, and Jack Cochran, Director of New Business and Technology

- Faster oven cycle increases sample throughput.
- Better precision at trace levels, compared to splitless injection.
- Reliably meet or exceed method requirements for sensitivity and linearity.

Semivolatiles are typically analyzed using splitless injection, but this approach results in slow analysis times and injection-to-injection variability. Combined, these factors reduce the number of samples that can be analyzed before quality control criteria are no longer met. This article demonstrates the advantages of split injection in terms of sample throughput, sensitivity, and linearity for EPA Method 8270D.

Increase Sample Throughput with Faster Oven Cycles

Split injection produces narrower injection bands and uses higher initial oven temperatures than splitless injection. Two oven programs starting at 80 °C were compared to a typical splitless program, and the faster oven cycle times used with split injection allowed up to 10 more samples to be analyzed per shift (Table I). The fastest program resulted in reduced separation of dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene (Figure 1), but these compounds were fully resolved using the alternate split conditions. The 80 °C oven start temperature could not be used with splitless injection, as it resulted in extremely broad peaks that could not be integrated.

Split Injection Results in More Reliable Sensitivity and Excellent Linearity

In addition to increasing sample throughput, split injection provided good sensitivity and better injection-to-injection repeatability at 0.5 ng on-column than splitless injection. Minimum response factor criteria were easily met and lower relative standard deviations (% RSD) for base/neutral and acid extractable compounds were achieved at the lowest calibration level (Table II). Calibration curves (5-160 ng/µL) were also assessed and, even with the 10:1 split, response factors met the method criterion of <20% RSD, except for 2,4-dinitrophenol (Table III). In this case, calibration was established based on the correlation (r = 0.9997). Better repeatability at low levels makes it easier to meet method criteria and allows more injections to be made before maintenance is required.

Table I Split injection significantly increases sample throughput compared to splitless injection.

	Split (Fast Cycle)	Split (Faster Cycle)	Splitless
Total run time (min.)	21	18.5	25.5
Sample analysis (min.)	18	15	20
Oven cooling (min.)	3	3.5	5.5
Sample throughput* (Samples/shift)	30	34	24
% Increase in sample throughput (vs. splitless)	25%	42%	--

* 12-hr. shift = 10.5 hr. sample analysis period + 1.5 hr. quality control/method performance analysis period. Sample throughput calculation based on number of samples that can be analyzed in 10.5 hours.

Table II Using split injection results in greater repeatability at 0.5 ng on-column, allowing more samples to be analyzed before maintenance is required.

		Split (10:1)		Splitless	
	8270D Min. RF	RF	%RSD	RF	%RSD
Pyridine	--	1.534	2	1.038	9
Phenol	0.800	1.861	0.7	1.857	5
1,4-Dichlorobenzene-d4	ISTD	ISTD	ISTD	ISTD	ISTD
N-Nitroso-di-*n*-propylamine	0.500	1.053	2	1.266	3
2,4-Dichlorophenol	0.200	0.317	2	0.325	3
Naphthalene-d8	ISTD	ISTD	ISTD	ISTD	ISTD
Naphthalene	0.700	1.249	0.5	1.238	2
Hexachlorocyclopentadiene	0.050	0.407	1	0.414	5
2-Nitroaniline	0.010	0.395	3	0.514	3
Acenaphthylene	0.900	2.188	0.9	2.139	1
Acenaphthene-d10	ISTD	ISTD	ISTD	ISTD	ISTD
2,4-Dinitrophenol	0.010	0.113	8	0.127	13
4-Nitrophenol	0.010	0.256	6	0.296	5
4,6-Dinitro-2-methylphenol	0.010	0.175	6	0.110	9
N-Nitrosodiphenylamine	0.010	0.712	1	0.694	1
Pentachlorophenol	0.050	0.115	3	0.098	5
Phenanthrene-d10	ISTD	ISTD	ISTD	ISTD	ISTD
Phenanthrene	0.700	1.252	0.7	1.259	2
Perylene-d12	ISTD	ISTD	ISTD	ISTD	ISTD
Benzo(ghi)perylene	0.500	0.940	4	0.252	26
		Avg. %RSD	3	Avg. %RSD	6

Comparison based on faster cycle split conditions shown in Figure 1; 0.5 ng on-column (n = 5).
ISTD = internal standard

Conclusion

Sample throughput for semivolatiles analysis can be significantly increased by employing split injection with a higher initial oven temperature and faster cycle time. Compared to splitless injection, analysis times are faster and repeatability is improved, allowing more samples to be run per shift.

For the complete technical details, visit **www.restek.com/adv012**

Table III Split injection provides excellent linearity across the typical calibration range of 5–160 ng/µL.

	Avg. RF	Avg. %RSD
Pyridine	1.533	0.9
Phenol	1.787	2
N-Nitroso-di-*n*-propylamine	0.991	2
2,4-Dichlorophenol	0.272	3
Naphthalene	0.998	5
Hexachlorocyclopentadiene	0.383	6
2-Nitroaniline	0.414	6
Acenaphthylene	1.824	3
2,4-Dinitrophenol	0.157	26
4-Nitrophenol	0.264	8
4,6-Dinitro-2-methylphenol	0.123	19
N-Nitrosodiphenylamine	0.608	3
Pentachlorophenol	0.127	16
Phenanthrene	1.082	5
Benzo(ghi)perylene	0.942	5

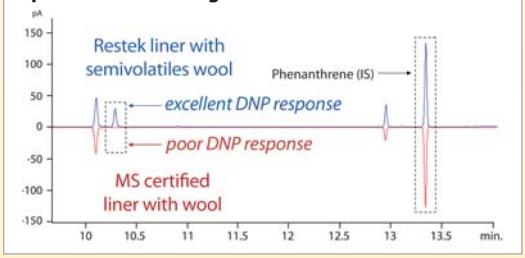
Data acquired using faster cycle split conditions shown in Figure 1; 6-point calibration curve (5, 20, 40, 80, 120, and 160 µg/mL), n = 3 at each level.

TECH TIP!

Increase Accuracy with an Inert Sample Path

Semivolatiles Wool from Restek improves precision and accuracy, while protecting your column from contamination. This new wool is more inert than a competitor's MS Certified Wool and gives you more reliable trace level results. For the complete comparison, visit **www.restek.com/adv017**

Response of 10 ng of 2,4-dinitrophenol relative to phenanthrene using a flame ionization detector.



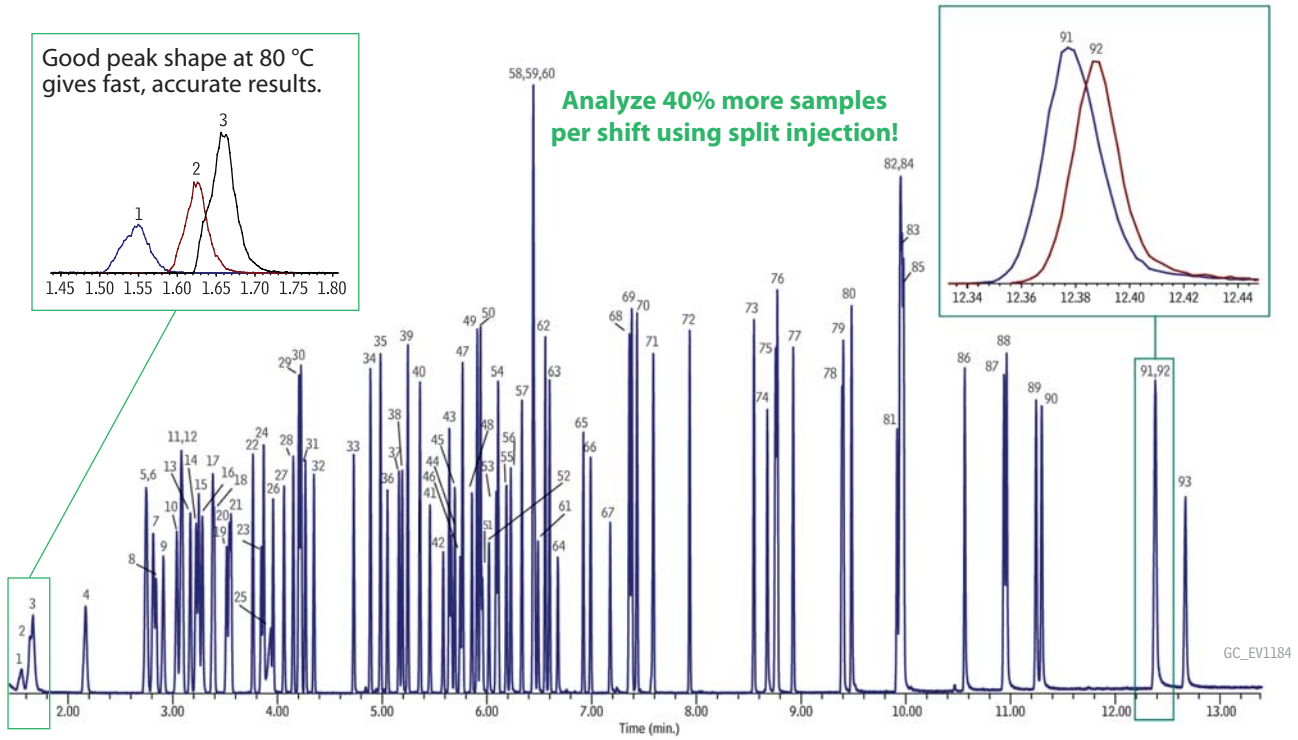
To order new Semivolatiles Wool in prepacked liners, add the corresponding suffix number to the liner catalog number.

Visit **www.restek.com/liners** for a full product listing.

qty.	Deactivated Liner with Semivolatiles Wool
each	-231.1
5-pk.	-231.5
25-pk.	-231.25

For column information, see page 7 or visit **www.restek.com**

Figure 1 Analyzing semivolatiles in less than 13 min. is possible with split injection, due to faster temperature programming.



Column: Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623) **Sample:** 8270 MegaMix® (cat.# 31850) Benzoic acid (cat.# 31879); 8270 Benzidines Mix (cat.# 31852); Acid Surrogate Mix (4/89 SOW) (cat.# 31025); 1,4-dioxane (cat.# 31853); Revised B/N Surrogate Mix (cat.# 31887); SV Internal Standard Mix (cat.# 31206); Diluent: Methylene chloride; Conc.: 40 µg/mL (4 ng on-column); **Injection:** Inj. Vol.: 1.0 µL split (split ratio 10:1); Liner: 4mm Split Precision® Liner w/ Semivolatiles Wool (cat.# 21023-231.5); Inj. Temp.: 270 °C; Split Vent Flow Rate: 60 mL/min.; Oven Temp: 80 °C (hold 1 min.) to 320 °C at 25 °C/min. to 330 °C at 5 °C/min. (hold 2 min.); **Carrier Gas**: He, constant flow; Flow Rate: 1.2 mL/min.; **Detector**: MS; Mode: Scan; Transfer Line Temp.: 280 °C; Analyzer Type: Quadrupole; Source Temp.: 250 °C; Quad Temp.: 150 °C; Tune Type: DFTPP; Ionization Mode: EI; Scan Range: 35-400 amu; **Instrument**: Agilent 7890A GC & 5975C MSD. For peak list, enter chromatogram GC_EV1184 in search box on www.restek.com

Avoid Resampling Soil Vapors

Confirm Tracer Gas in the Field Using a Leak Detector

By Irene DeGraff, Air Monitoring Product Marketing Manager, Russell Pellegrino, Director of Technical Services*, and Kelli Steindl, GC Accessories Product Marketing Manager
* Centek Laboratories, LLC

- Confirm system integrity before sample collection.
- Minimize resampling by detecting leaks prior to sampling.
- Eliminate costly and time-consuming lab analysis of tracer gas.

Vapor intrusion occurs when pollutants from contaminated soil or ground water migrate into buildings and ambient air. Adverse health effects can result when vapors occur in high concentrations, or if toxic volatile organics are present. These compounds are monitored using a variety of sampling procedures, including soil vapor, sub-slab, indoor, and ambient air testing. Sample collection for volatile organic compounds (VOCs) typically is performed with an air canister and passive sampling kit according to EPA Method TO-15 or a similar method.

Costly Detection in Lab Doesn't Prevent Resampling

The primary challenge in vapor intrusion monitoring is distinguishing vapor intrusion from other sources of exposure. In order to establish that VOCs are from soil vapor, rather than from the surrounding environment, sampling systems (ports) must be tested with tracer compounds, such as helium, and shown to be properly sealed. Sample collection system integrity can be demonstrated by including the tracer gas in the list of target analytes reported by the laboratory; however, if high levels are found the sample is rejected and costly resampling may result.

Using a Leak Detector in the Field Saves Time and Money

Detection of tracer gas in the field is a cost-effective alternative to lab analysis that assures the integrity of the sampling system before sampling occurs. The Restek Leak Detector provides good screening of helium tracer gas at concentrations of 10%, the level at which sample port resealing is required. In addition, this unit is just a fraction of the cost of other field portable devices, such as photionization detectors, which may be too sensitive for screening purposes.

Real-time detection of helium tracer gas in the field using a Restek Leak Detector as shown in Figure 1 is a simple, inexpensive way to minimize resampling by establishing system integrity prior to sample collection. Centek Laboratories pioneered this technique and contributed to its inclusion in the New York State Department of Health method[1].

References
1. New York State Department of Health, October 2006, Guidance for Evaluating Soil Vapor Intrusion in the State of New York, http://www.nyhealth.gov/environmental/investigations/soil_gas/svi_guidance/docs/svi_main.pdf (accessed August 27, 2010).

Prevent costly resampling—use a Restek Electronic Leak Detector to ensure sample collection system integrity prior to sampling.

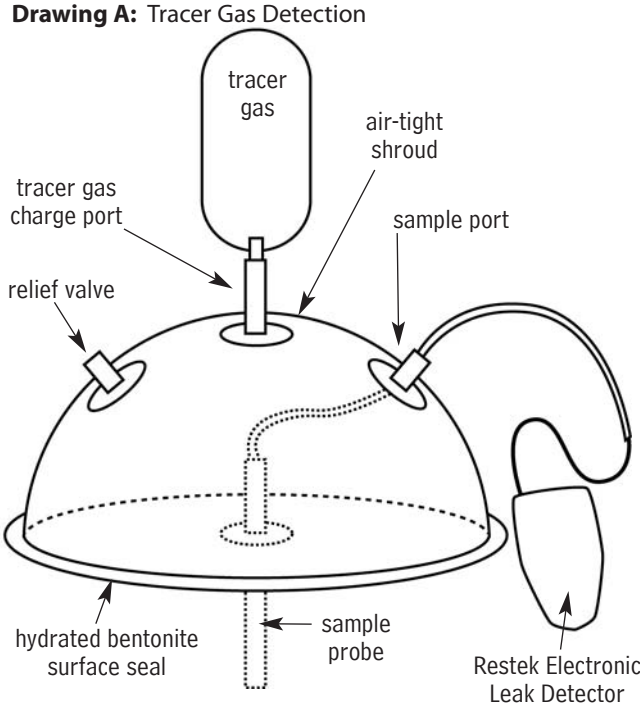
Use a Restek Leak Detector to verify the sample port is sealed (Drawing A).

1. Prepare sampling port by installing sample probe and shroud as described in NY DOH method.
2. Turn on Restek Electronic Leak Detector and allow it to equilibrate for a few seconds prior to use.
3. Insert leak detector probe tip into the open end of the tubing connected to the sealed sample port.
4. Wait 10 seconds and inject a charge of helium into the open space of the shroud.
5. Wait several minutes. An alarm will sound if helium is detected at >10%, indicating a leak in the sample port.

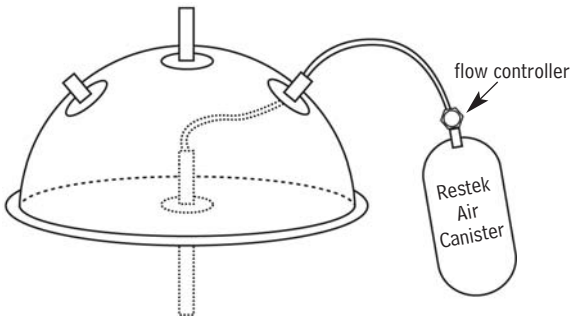
Photos courtesy of Centek Laboratories, LLC

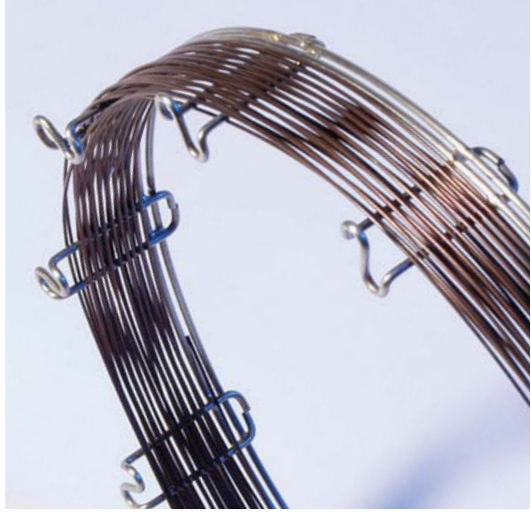
Collect sample using any Restek air canister or mini-can (Drawing B).

Drawing A: Tracer Gas Detection



Drawing B: Sample Collection





New D3606 Column Set

Outperforms TCEP Columns for Benzene Analysis

By Barry Burger, Petroleum Chemist and Jan Pijpelink, Petrochemical Market Development Manager

- Complete resolution of ethanol and benzene allows tighter process control.
- Fully conditioned column set—ready to use out of the box.
- Each column set is tested for method applicability and includes chromatogram.

Demand for finished gasolines containing ethanol continues to increase, as these fuels reduce greenhouse gas emissions and can help control air pollution. Ethanol is a cost-effective additive, but its presence significantly complicates the analysis of benzene, a regulated carcinogen which is added to increase octane levels. A new D3606 column set developed by Restek separates benzene and ethanol completely and more reliably than the 1,2,3-tris(2-cyanoethoxy) propane (TCEP) column listed in ASTM Method D3606.

Independent Testing Shows New D3606 Column Set Outperforms TCEP

It is widely recognized that TCEP columns often fail to adequately separate ethanol and benzene (Figure 1). In contrast, the new D3606 column set from Restek reliably produces resolution values greater than 3.00 for these compounds, allowing easy integration and more accurate quantification of benzene than is typically obtained on TCEP columns (Figure 2). Independent analysis of finished gasoline by beta testers has also produced excellent results (Figure 3). Linearity was assessed and correlations of 0.99999 and 1.00000 were obtained for benzene and toluene calibration curves respectively. Beta testers also reported that repeatability was excellent and that overall reliability exceeded typical TCEP column performance.

Reliable Performance Guaranteed

In addition to inadequate resolution of ethanol and benzene, TCEP columns often show poor thermal stability (max 135 °C). This results in short column lifetimes, making TCEP columns a relatively expensive choice in terms of cost-per-injection and the downtime required for frequent column changes. In comparison, Restek's D3606 column set is stable to 165 °C and exhibits very low bleed, allowing accurate integration and quantification. Reliable performance is assured, as all D3606 column sets are individually tested for method applicability.

Conclusion

Both in-house data and results from independent testers demonstrate that the Restek D3606 column set substantially outperforms TCEP columns and provides more accurate and reliable data for quantifying benzene in finished gasolines.

For the complete version of this condensed article, visit **www.restek.com/adv013**

did you **know**?

The D3606 column set developed by Restek provides accurate, reliable results for benzene and toluene in finished gasoline and will be added to the appendix of ASTM Method D3606. Compared to TCEP columns, this new column set provides better separation of benzene and ethanol.

D3606 Application Column Set

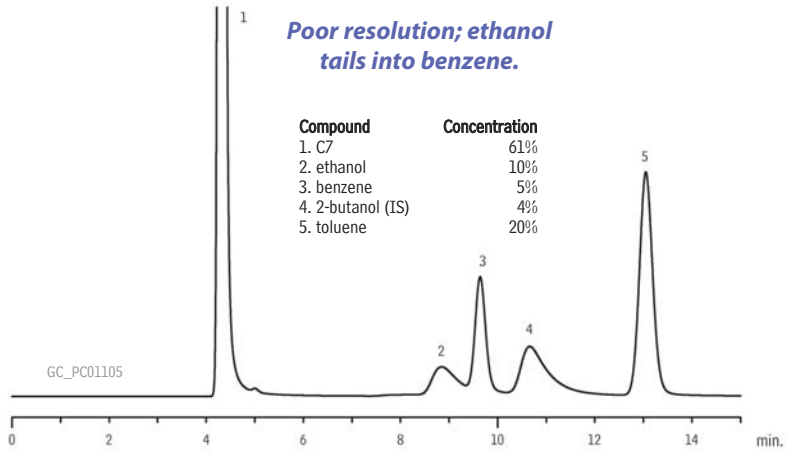
(2 column set)**:
Column 1: 6' (1.8m), 1/8" OD, 2.0mm ID, nonpolar Rtx-1
Column 2: 16' (4.9m), 1/8" OD, 2.0mm ID, proprietary packing material

Description	cat.#*
D3606 Application Column (2 column set)**	83606-

*Please add column configuration suffix number from our catalog to cat.# when ordering—see our catalog or website.
**The column set is designed to accommodate both valve injection and/or syringe injection. Column 1 is configured with a 2" inlet void to facilitate on-column injection. The inlet is identified on both column 1 and column 2. Note: The inlet of column 2 is identified for proper orientation for connection to the valve.

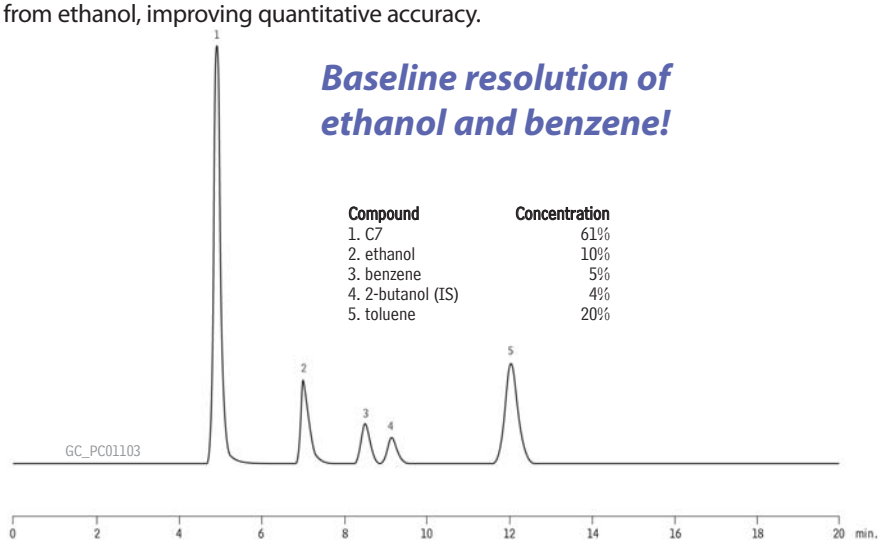
For a complete list of D3606 reference standards, visit **www.restek.com/petro**

Figure 1 TCEP columns often fail to adequately resolve benzene from ethanol, resulting in poor quantitative results



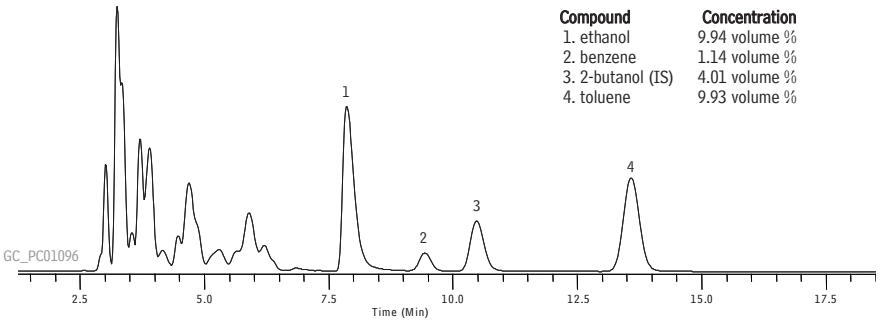
Column: D3606 TCEP Column Set-Up (Column 1: 5', 1/8" OD, 10% OV 101 on Chromosorb PAW 80/100, Column 2: 5', 1/8" OD, 20% TCEP on Chromosorb PAW 80/100, and Column 3: 15', 1/8" OD, 15% Carbowax 1540 on Chromosorb W); **Instrument:** Agilent 6890; **Sample:** mixed standard prepared in C7; **Inj.:** 1 µL, direct; **Inj. temp.:** 200 °C; **Carrier gas:** helium, constant flow; **Flow rate:** 26 mL/min.; **Oven temp.:** 135 °C, isothermal; **Det.:** TCD @ 200 °C

Figure 2 Restek's new D3606 column set accurately and reliably separates benzene from ethanol, improving quantitative accuracy.



Column: D3606 Application Column (2 column set, cat.# 83606) (Column 1: nonpolar Rtx®-1, 6' (1.8 m), 1/8" OD, 2.0 mm ID and Column 2: proprietary packing material, 16' (4.9 m), 1/8" OD, 2.0 mm ID); **Instrument:** Wasson 3606/5599 combo 6890N GC; **Sample:** mixed standard prepared in C7; **Inj.:** 1 µL, direct; **Inj. temp.:** 200 °C; **Backflush time:** 3 min.; **Carrier gas:** helium, constant flow; **Flow rate:** 25 mL/min.; **Oven temp.:** 135 °C, isothermal ; **Det.:** TCD @ 200 °C

Figure 3 Ethanol and benzene are reliably resolved in commercial gasoline by beta testers using the D3606 column set.



Column: D3606 Application Column (2 column set, cat.# 83606-800) (Column 1: 6' (1.8 m), 1/8" OD, 2.0 mm ID, nonpolar Rtx®-1 and Column 2: 16' (4.9 m), 1/8" OD, 2.0 mm ID, proprietary packing material); **Sample:** gasoline; **Inj**.: 1 µL; **Backflush:** 3.0 min.; **Carrier gas**: helium; **Flow rate**: 20.4 mL/min.; **Oven temp**.: 135 °C; **Det**.: TCD; Courtesy of Joaquin Lubkowitz, Separation Systems, Gulf Breeze, Florida

TECH TIP!

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Using Restek gas filters is an easy way to increase GC column lifetime. Our triple gas filters protect your column by removing hydrocarbons, oxygen, and moisture.

Restek Super-Clean Gas Filter Kit

- High-purity output ensures 99.9999% pure gas (at max. flow of 2L/min.).
- "Quick connect" fittings for easy, leak-tight cartridge changes.
- Glass inside to prevent diffusion; polycarbonate housing outside for safety.
- All traps measure 10 5/8" x 1 3/4" (27 x 4.4 cm).
- Each base plate unit measures 4" x 4" x 1 7/8" (10.2 x 10.2 x 4.8 cm).

Description	qty.	cat.#
Carrier Gas Cleaning Kit Includes: mounting base plate, 1/8" inlet/outlet fittings, and oxygen/moisture/hydrocarbon Triple Gas Filter	kit	22019

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Develop Methods Quickly and Easily Using Ultra Selective Chromatography

Ultra Selective Liquid Chromatography™

What is Ultra Selective Liquid Chromatography™? USLC™ is the directed application of selectivity—the most influential factor affecting resolution—to optimize separations and improve method performance. Restek has extensively studied reversed phase selectivity to provide practicing chromatographers with the most effective and widest range of USLC™ stationary phase chemistries available.

RESTEK USLC™
Ultra Selective Liquid Chromatography™

Selectivity Drives Separations

By understanding and controlling selectivity through USLC™, chromatographers have the best opportunity for fast, effective analyte resolution.

One of the most significant challenges in method development is finding the proper stationary and mobile phase chemistry for a particular separation. As sample complexity increases, achieving adequate resolution between matrix components and target analytes becomes more difficult. Despite recent advancements in column format, such as sub-2 micron packings and pellicular particles, resolution can still be difficult to obtain because, while these formats can increase chromatographic efficiency and analysis speed, they do not significantly influence resolution. Selectivity, as shown in Equation 1, is the single most powerful factor affecting resolution, and it is largely dependent upon stationary phase composition.

Real Diversity in Phase Chemistry

Restek columns offer the widest range of selectivities available on a single column line. More choices mean optimized separations and more robust methods.

Equation 1: Selectivity drives resolution—USLC™ considers column selectivity during method development, resulting in fast, effective separations.

$$R = 1/4\sqrt{N} \times (k'/k'+1) \times (\alpha-1/\alpha)$$

Efficiency *Retention capacity* *Selectivity*

While numerous bonded phases are available for reversed phase chromatography, many are similar and offer only moderate changes in retention (e.g. C8 and C18), rather than significant differences in selectivity. Method development is less laborious and time-consuming when using a full range of column selectivities, including orthogonal phase chemistries like polar embedded, phenyl, and fluorophenyl columns. Restek has led the development of unique USLC™ phases across these phase classes in order to provide chromatographers with a more effective range of column selectivities and innovative column chemistries for method development. The phases shown in Figure 1 provide the widest range of reversed phase selectivity available on any column line, and can be used to guide the least understood and most practically significant part of method development—proper column selection.

Evaluating and Extending Selectivity

Restek leads the industry in USLC™ phase diversity because optimal differences in selectivity are built in during the research and development of our bonded phases.

The diversity in selectivity provided by USLC™ columns can be demonstrated empirically using the hydrophobic-subtraction (HS) model [1]. This model is a novel procedure for characterizing selectivity that uses test probes to define the solute and stationary phase interactions in reversed phase separations. Restek is leading the commercial application of this model by implementing it in the research and development of USLC™ bonded phases. To evaluate phase selectivity using the hydrophobic-subtraction model, the retention characteristics of the solute probes are compared across different phases on the same silica base. In this approach, the range of selectivity is indicated by the degree of scatter along the regression line; high correlations indicate similarity and low correlations represent changes in selectivity across phases (Figure 2). The difference in selectivity across columns can then be quantified based on the correlation by calculating the selectivity (S) statistic for the comparison [2].

Figure 1: Restek columns offer the widest range of unique and effective column chemistries to aid the chromatographer in fast and easy method development.

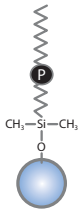
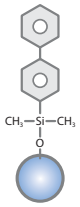
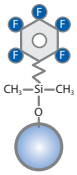
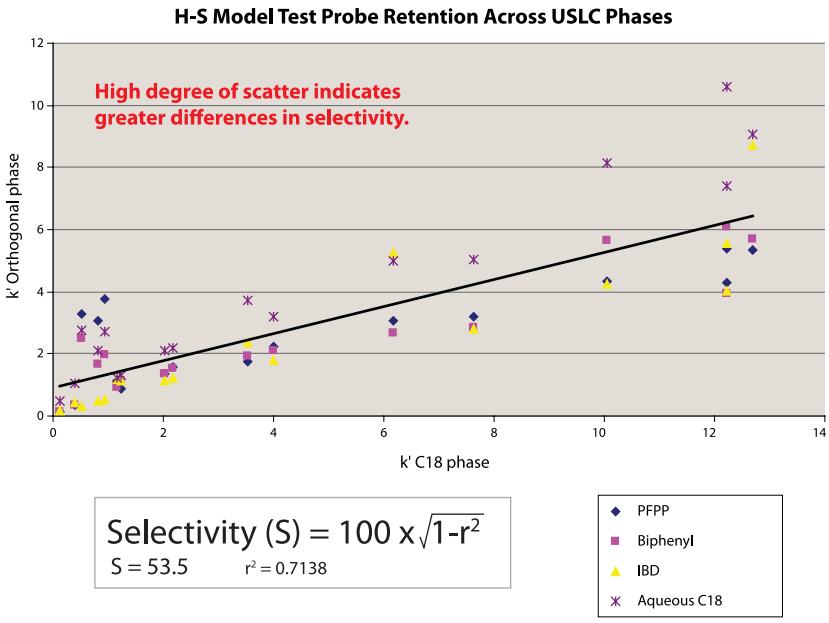
Restek phase (column class)	**Aqueous C18** (alkyl)	**IBD** (polar embedded)	**Biphenyl** (phenyl)	**PFP Propyl** (fluorophenyl)
				
Ligand type	Proprietary polar modified and functionally bonded C18	Proprietary polar functional embedded alkyl	Unique Biphenyl	Proprietary end-capped pentafluorophenyl propyl
Characteristics and uses	• C18 phase for balanced retention of multiple solute types. • Compatible with up to 100% aqueous mobile phases.	• Enhanced retention of polar acids. • Moderate retention of both acidic and basic solutes.	• Increased retention of aromatic, unsaturated, conjugated solutes, or solutes containing an electron withdrawing ring substituent. • Enhanced retention and selectivity when used with methanolic mobile phases.	• Increased retention of protonated bases and solutes containing aromatic moieties.

Figure 2 Restek has extended the selectivity range for reversed phase separations as illustrated by the hydrophobic-subtraction model and corresponding selectivity (S) value.



USLC™ Columns: Selectivity Choices Optimize Separations

Restek USLC™ columns, available in both HPLC and UHPLC formats, offer the widest range of selectivities available and are an integral part of successful method development. Ideal for column switching systems, these columns provide the orthogonal separations needed to create optimal resolution and robust methods. Combining USLC™ phases with a suitable column format gives practicing chromatographers the most powerful tool available for successful method development.

References

1. L.R. Snyder, J.W. Dolan, P.W. Carr, J. Chromatogr. A 1060 (2004) 77.
2. U.D. Neue, J.E O'Gara, A. Mendez, J. Chromatogr. A 1127 (2006) 161.

We're here to help!

To discuss the right selectivity for your separation or to find a comparable column, **contact us at support@restek.com or 800-356-1688.**

Rugged Rxi®-5Sil MS Columns Stand up to Derivatization Reagents, Reducing Downtime and Replacement Costs

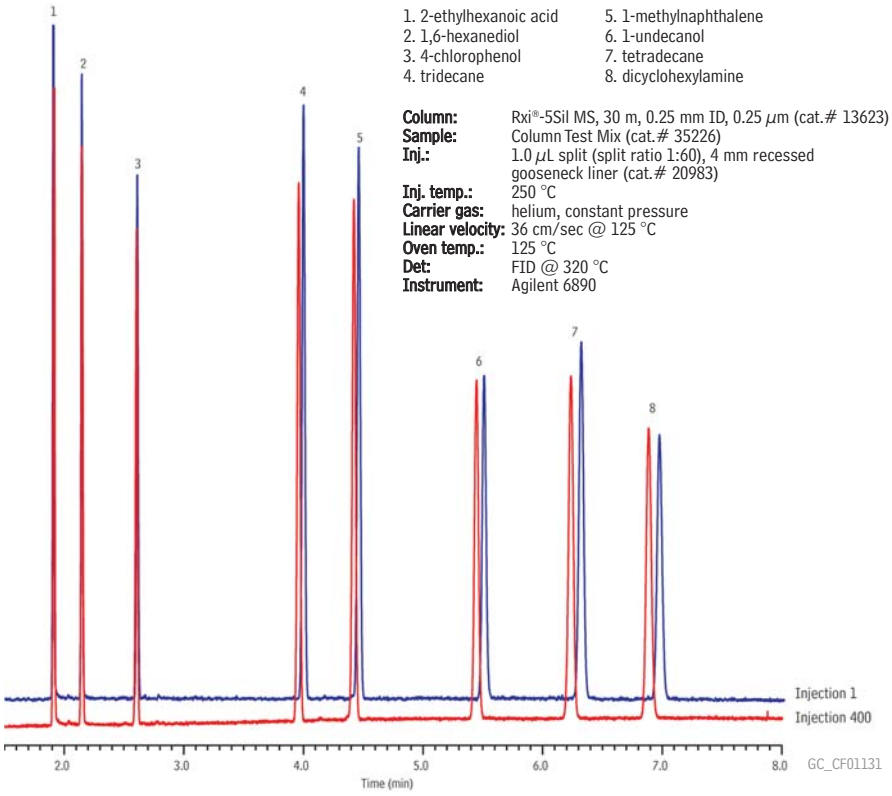
By Amanda Rigdon, Clinical/Forensic Innovations Chemist and Gary Stidsen, GC Columns Product Marketing Manager

- Save costs with long column lifetime.
- Reduce downtime from column trimming and replacement.
- Improve peak shape for active compounds.

When performing GC/MS analysis of drugs, many chemists choose to derivatize samples prior to analysis. Derivatization not only increases the volatility of some drug compounds, but it also reduces activity, resulting in improved peak shape and more accurate quantification. An additional advantage is that derivatized compounds have a higher molecular weight, thus producing more reliable mass spectra than underivatized compounds. Despite these benefits, derivatization reagents are often harsh and can damage analytical columns, leading to high bleed, significant reduction in retention times, and increased tailing for active compounds. Often, this damage is concentrated near the head of the column, so trimming a short length can improve results. However, trimming is a finite solution as repeated clipping ultimately results in decreased efficiency and shorter column lifetimes. Choosing a more rugged column, such as the Rxi®-5Sil MS column, is a better alternative. The Rxi®-5Sil MS column is extremely stable and holds up to harsh treatment, including repeated exposure to derivatization reagents.

The analysis of amphetamine illustrates the ruggedness of the arylene-based Rxi®-5Sil MS polymer. Amphetamine is typically derivatized, because the underivatized form is an active basic compound that produces only a few low molecular weight ions for monitoring. In contrast, upon derivatization, activity decreases, resulting in dramatically improved peak shape and more accurate quantitation. Additionally, several higher molecular weight ions are produced, which can be monitored for definitive identification.

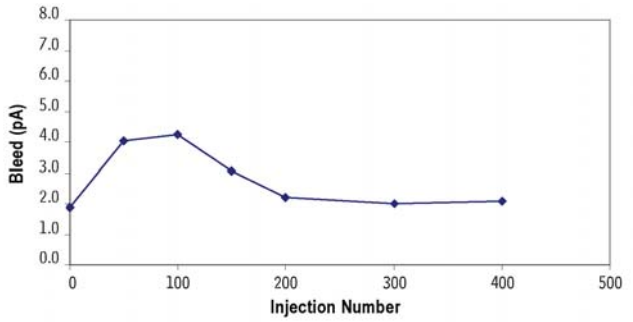
Figure 1 Rugged Rxi®-5Sil MS columns produce consistent retention times, even after 400 injections of derivatization reagent.



Phase Stability Extends Column Lifetime

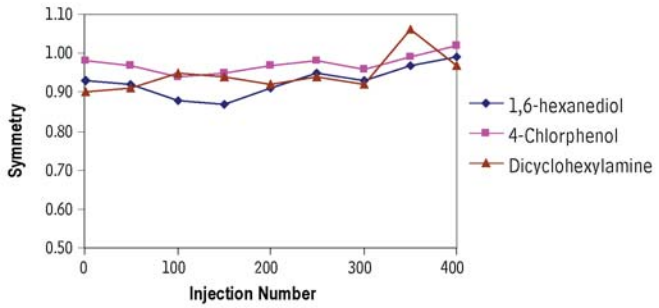
In order to demonstrate the ruggedness of the Rxi®-5Sil MS column, 400 injections of heptafluorobutyric acid anhydride (HFBA) in butyl chloride were performed. HFBA is a very harsh derivatization reagent, and the concentration of reagent in the solvent was equivalent to that of a derivatized sample. Throughout the course of 400 injections, bleed, retention, and peak shape for active compounds were monitored by periodically injecting a column test mix containing active compounds (1,6-hexanediol, 4-chlorophenol, and dicyclohexylamine). Chromatographic results were remarkably consistent, even after 400 injections (Figure 1). Column bleed was monitored over the course of the experiment and remained below 5 pA (Figure 2). The consistency of retention time data and low bleed levels demonstrate phase stability, which results in longer column lifetimes and reduced maintenance and replacement costs.

Figure 2 Low column bleed results in long column lifetimes, saving labs replacement costs.



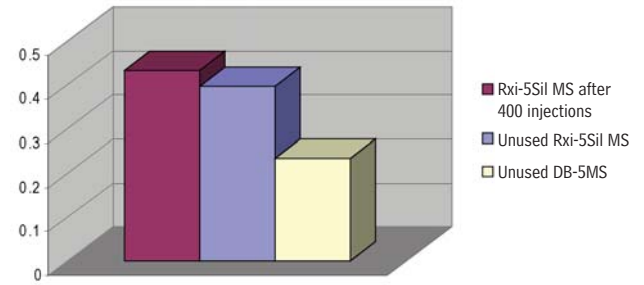
Column bleed over 400 injections of HFBA derivatization reagent.

Figure 3 Active probes show consistent, symmetric peak shape, demonstrating the inertness needed for accurate quantification.



Test probe symmetry over 400 injections of HFBA derivatization reagent.
Symmetry values <1 indicate peak tailing and values >1 indicate fronting.

Figure 4 Peak symmetry for underivatized amphetamine is significantly better on an Rxi®-5Sil MS than on a competitor column, even after 400 injections of HFBA derivatization reagent.



Symmetry values <1 indicate peak tailing and values >1 indicate fronting.

Symmetric Peaks for More Accurate Results

Peak shape was also monitored to ensure column inertness was stable over time—an important factor in maintaining accuracy. Peaks for the active test probes were symmetric even after 400 injections, allowing easy identification and consistent integration (Figure 3). In a second experiment to complement the test probe results, underivatized amphetamine was injected onto a new Rxi®-5Sil MS column, an Rxi®-5Sil MS column after 400 injections of derivatization reagent, and a new competitor column of equivalent phase chemistry. Even though underivatized amphetamine is highly active, peak symmetry on the Rxi®-5Sil MS column was consistent and unaffected by exposure of the column to derivatization reagent. Additionally, peak shape on both the exposed and unexposed Rxi®-5Sil MS column was better than that on the new competitor column (Figure 4).

Conclusion

The rugged arylene phase of the Rxi®-5Sil MS column results in highly stable performance, even under the most demanding of analytical conditions, and its exceptional inertness ensures good peak shape for reproducible quantitation. The stability of the Rxi®-5Sil MS column results in longer column lifetimes, reducing both downtime and replacement costs.

For an online version of this article, visit **www.restek.com/adv018**

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	qty.	cat. #
0.10mm	0.10µm	-60 to 330/350°C	10m	ea.	43601
0.18mm	0.18µm	-60 to 330/350°C	20m	ea.	43602
0.18mm	0.36µm	-60 to 330/350°C	20m	ea.	43604
0.25mm	0.10µm	-60 to 330/350°C	15m	ea.	13605
0.25mm	0.10µm	-60 to 330/350°C	30m	ea.	13608
0.25mm	0.25µm	-60 to 330/350°C	15m	ea.	13620
0.25mm	0.25µm	-60 to 330/350°C	15m	ea.	13620-127
0.25mm	0.25µm	-60 to 330/350°C	30m	ea.	13623
0.25mm	0.25µm	-60 to 330/350°C	30m	6-pk.	13623-600
0.25mm	0.25µm	-60 to 330/350°C	30m	ea.	13623-124
0.25mm	0.25µm	-60 to 330/350°C	30m	ea.	13623-127
0.25mm	0.25µm	-60 to 330/350°C	60m	ea.	13626
0.25mm	0.50µm	-60 to 330/350°C	15m	ea.	13635
0.25mm	0.50µm	-60 to 330/350°C	15m	ea.	13635-124
0.25mm	0.50µm	-60 to 330/350°C	30m	ea.	13638
0.25mm	0.50µm	-60 to 330/350°C	30m	ea.	13638-124
0.25mm	0.50µm	-60 to 330/350°C	30m	ea.	13638-127
0.25mm	1.00µm	-60 to 325/350°C	15m	ea.	13650
0.25mm	1.00µm	-60 to 325/350°C	30m	ea.	13653
0.25mm	1.00µm	-60 to 330/350°C	60m	ea.	13697
0.32mm	0.25µm	-60 to 330/350°C	15m	ea.	13621
0.32mm	0.25µm	-60 to 330/350°C	30m	ea.	13624
0.32mm	0.50µm	-60 to 330/350°C	30m	ea.	13639
0.32mm	0.50µm	-60 to 330/350°C	30m	ea.	13639-125
0.32mm	1.00µm	-60 to 325/350°C	30m	ea.	13654
0.32mm	1.00µm	-60 to 325/350°C	30m	ea.	13654-125
0.53mm	1.50µm	-60 to 310/330°C	30m	ea.	13670

Rxi® GC Columns

Analytical Chemistry Shapes Response to the Deepwater Horizon Oil Spill

By Ed Overton, Analytical Specialists, Inc.

***Ed Overton** developed the microFAST GC and is the founder of Analytical Specialists, Inc. (ASI). He currently is the principal investigator on a grant to provide NOAA's Office of Response and Restoration with chemical hazard assessments for oil and hazardous chemical spills within US jurisdiction. Prior to retiring in May 2009, Ed held the Claiborne Chair in Environmental Toxicology and Air Quality, an endowed professorship at Louisianna State University.*

The Deepwater Horizon oil spill was an unprecedented event in the annals of US petroleum exploration and development. Much has been made about the comparison to the tragic 1989 Exxon® Valdez spill. Both the current spill and the Alaskan incident are examples of spills that should not have happened and, when all details are known, could have been avoided with more attention to best operational practices and standard safe operating procedures. However, both did occur and we are now faced with trying to mitigate the effects of another major oil spill.

The Exxon® Valdez incident involved the loss of 11,000,000 gallons of oil fairly quickly into the cold waters of Prince William Sound from a floating vessel in close proximity to land. The oil quickly impacted the western islands and shoreline of the sound, and most of the response effort after the first few weeks involved activities to clean these rocky beaches. In the Deepwater Horizon spill, oil was entering the environment at a slower pace, approximately 2,300,000 gallons per day. However, the input was from a leak 1 mile below the Gulf's surface, 50 miles from the closest land, and considerably farther from most shorelines. Unfortunately, the shoreline types most vulnerable to damage from oil spills are marshy, grassy shorelines, like the mostly marshy Louisiana shoreline which represents the open water-land interface for some 40% of our nation's wetlands. Additional coastal marshes were in harm's way along the coasts of Mississippi, Alabama, and Florida.

The Deepwater Horizon spill was a slow moving spill that continued for 87 days and dumped over 200,000,000 gallons of a very volatile, light sweet crude oil into the waters of the Gulf of Mexico. Fresh oil reached the surface each day, and it appears that the oil was mixing with water as it ascended from the depths, stripping mostly aromatic compounds from small droplets. Surface oil formed a water-in-oil emulsion, a mousse, that floated in the water at the surface. Most of the volatile components readily evaporat-

ed. Some percentage of the oil entering the Gulf from the wellhead was both naturally and chemically dispersed at depth, and this very dilute dispersed oil resides and is being degraded in deep water. All of these factors presented scientific and engineering challenges when figuring out how to most effectively mitigate this horrible event.

Human cleanup options consisted mostly of using Corexit® 9500 to disperse the oil on the surface and at the wellhead. *In situ* burning and skimming tactics were also used. Dispersing surface oil in offshore waters certainly speeds up biodegradation, but it also spreads oil within the top 10-20 meters of the water column where marine animal exposure occurs. Dispersing surface oil most certainly mitigated the potential impacts of floating oil on marshy coastlines and sandy beaches along the northern Gulf coastline. It also fueled a massive natural offshore biological treatment process that, as we are now seeing, is rapidly degrading residual spilled oil and allowing the Gulf's environment to recuperate from this massive assault.

Tens of thousands of scientists, engineers, and response personnel worked tirelessly 24/7 to mitigate this spill. Analytical chemists, using techniques like GC/MS, UV fluorescence, and HPLC, played a critical role in guiding response efforts, following the environmental impacts, and ensuring the safety of the seafood harvested from the Gulf region. Analytical chemists are essential in responding to massive environmental disasters, like oil spills, and in monitoring environmental damage and ecological recovery. Thank God for analytical chemists, their impressive technologies, and all the supply companies that support high quality chemical analysis.

Bringing Back the Bluebirds

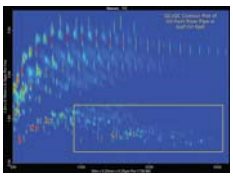
Restek chemist Mike Wittrig, a life-long bird enthusiast, has been supplying local bluebirds and tree swallows with nesting habitat for the past 9 years. What started as a backyard hobby quickly expanded to local parks, and also to the Restek campus where he works with the facility maintenance team to locate nesting boxes in prime locations. "Over the years I've learned the importance of both nest box location and spacing in improving reproductive success rates by limiting competition from sparrows," says Mike. Local initiatives like Mike's project have helped bring Eastern Bluebird populations back to healthy levels, following a critical midcentury decline due to habitat destruction and nesting competition.

HOT TOPICS in Chromatography

ChromaBLOGraphy www.restek.com/blog

GCxGC-TOFMS of Riser Pipe Oil from BP Gulf Oil Spill

posted by Jack Cochran



I recently analyzed an oil sample collected by an ROV from the riser pipe at the BP Gulf oil spill site using GCxGC-TOFMS, a powerful multidimensional technique capable of characterizing complex samples that defy one-dimensional GC-MS. The column setup was a 30 m x 0.25 mm x 0.25 µm Rxi-17Sil MS in the first dimension with a 1.2 m x 0.15 mm x 0.15 µm Rxi-1ms in the second dimension. This arrangement puts the highly aromatic compounds (e.g. PAHs) at the bottom of the contour plot while the aliphatics are retained by the Rxi-1ms in the second dimension, eluting away from the aromatics. Given that PAHs are considered the "toxic" compounds in crude oil, this is an efficient arrangement for their interference-free determination. Having a full mass range TOFMS allows spectral fingerprinting of the resolved components, including PAHs. Read full blog or post comments at **www.restek.com/blog**

See page 4 for a GCxGC analysis of PAHs in mussels!

New NJ-EPH Method

The New Jersey Department of Environmental Protection began phasing in a new analytical method for extractable petroleum hydrocarbons on Sept. 1, 2010. **Restek has all the standards, GC columns, SPE tubes needed for this new method**—contact us for assistance getting set up.

Flip Seal Doubles Lifetime

New reversible Flip Seals from Restek last twice as long as other inlet seals. Simply use—flip—then use again.

- Same easy sealing as Dual Vespel® Ring Inlet Seals—no extra washer needed.
- Lower leak rate than OEM metal seals.
- Maximum transfer of analytes to column.

Patented.

See page 7 for details.

FPRW QuEChERS Session

The 47th Florida Pesticide Residue Workshop took place July 18-21 in St. Pete Beach, Florida. As usual, this meeting featured an excellent technical program, which included sessions on the Gulf oil spill, multiclass/multiresidue analyses, veterinary drug residues, global chemical contaminant conflicts/resolutions, and US government residue programs.

In addition to the formal presentations, there was a lively evening discussion on developing a unified QuEChERS method that harmonizes the two current official methods (AOAC and EN 15662), which differ slightly in their approach. No consensus was reached, but attendees enjoyed a vigorous debate moderated by QuEChERS inventors Steve Lehotay (USDA) and Michelangelo Anastassiades (CVUA-Stuttgart). Much discussion centered on efficiencies for just a few pesticides, which is ironic, in a way, considering the effectiveness of QuEChERS for hundreds of pesticides!

Visit **www.restek.com/quechers** for a complete selection of QuEChERS products, technical applications, and resources.

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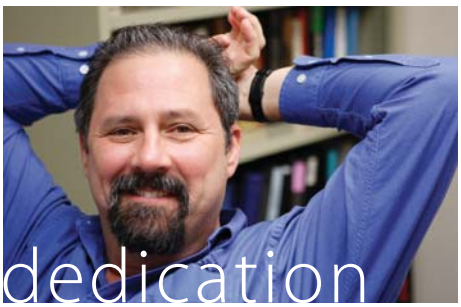
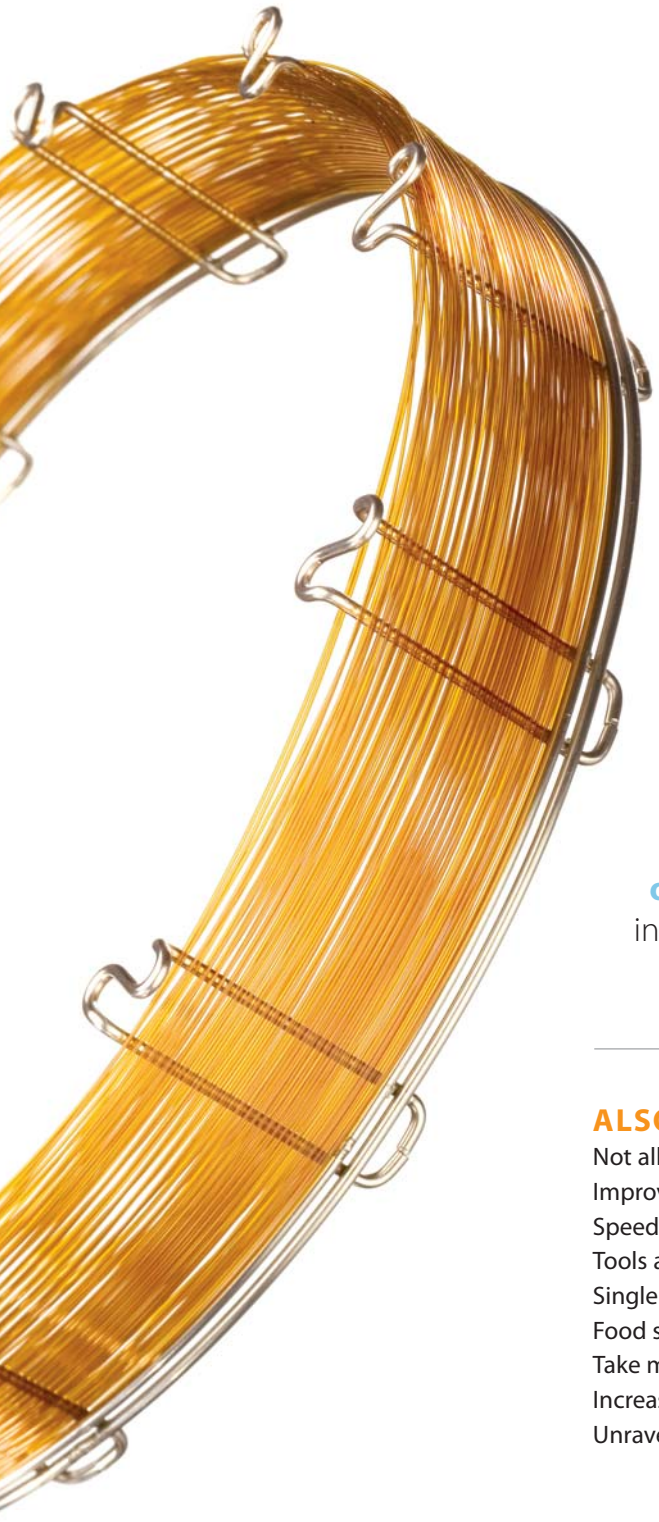


Jack Cochran, Director of New Business and Technology

2010.1

the RESTEK ADVANTAGE



Not All "624s" Are Equivalent

Improve Volatiles Analyses with New **Rxi®-624Sil MS** Columns:

- **Lower detection limits for active compounds**—See why Rxi®-624Sil MS columns improve sensitivity, accuracy, and MS performance.

p.2

- **Best-in-class G43**—Increase system suitability pass rates for USP <467> with the most selective G43 available.

p.4

- **Optimized method for volatile organics**—Minimize downtime by syncing instrument cycles with your purge and trap.

p.6

ALSO IN THIS ISSUE

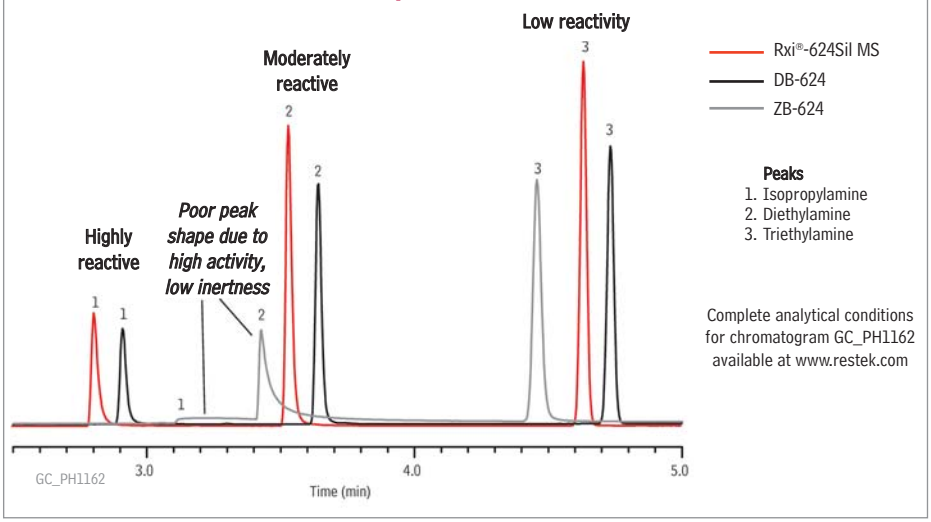
Not all "624s" are Equivalent Introducing Rxi®-624Sil MS Columns

New Rxi®-624Sil MS Columns Give Better Peak Shape, Improving Sensitivity

Whether you are developing methods for residual solvents, analyzing environmental VOCs, or running other applications for volatile organics, you can improve data quality with Rxi®-624Sil MS columns.

These new columns are more inert than other 624 type columns, resulting in higher response, better peak symmetry, and easier integration of active compounds (Figure 1). Since active analytes can be quantified at lower levels compared to similar products (Figure 2), Rxi®-624Sil MS columns are the best choice when increased sensitivity is desired.

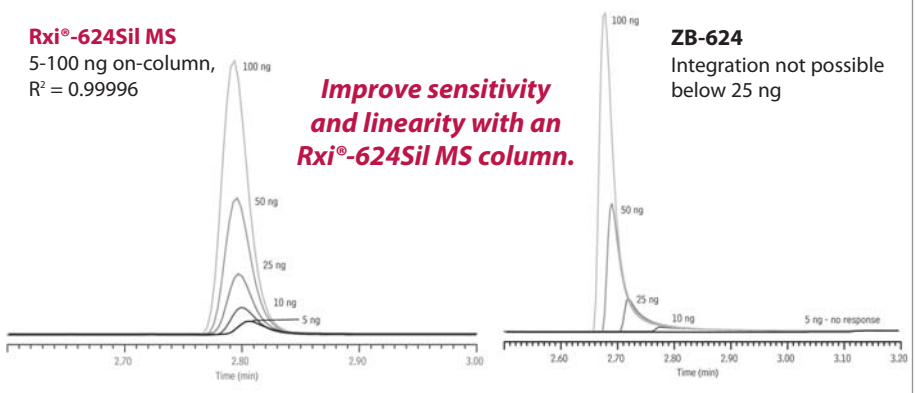
Figure 1 Highly inert Rxi®-624Sil MS columns provide better peak shape and simplify integration for active compounds at low levels (5 ng on-column).



get more

For more information on Rxi®624-Sil MS columns, download PHFL1245 at www.restek.com

Figure 2 Active compounds like isopropylamine can be more accurately integrated on an Rxi®-624Sil MS column, lowering levels of quantification (LOQs) and increasing accuracy.



Lowest Bleed 624 Available—Assured GC/MS Compatibility

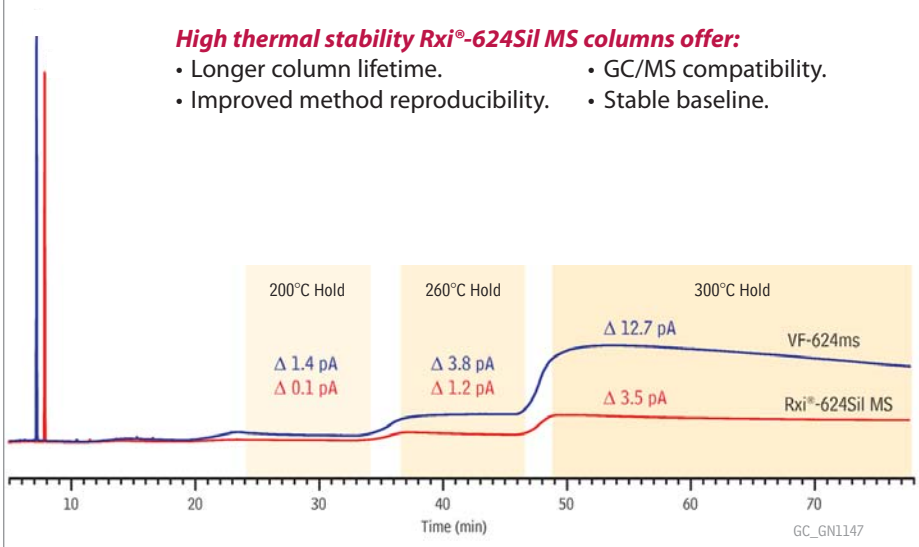
In addition to providing higher inertness and more accurate quantification of active compounds, Rxi®-624Sil MS columns offer greater thermal stability, resulting in lower bleed than any column in its class (Table I, Figure 3). While other 624 columns generate too much bleed to be useful for mass spec work, the Rxi®-624Sil MS column is fully compatible with mass spectrometry. Other benefits related to thermal stability include stable baselines, longer column lifetime, and improved method reproducibility.

Table I The Rxi®-624Sil MS column has the highest thermal stability of any 624 column.

Column	Manufacturer	Maximum Programmable Temperature
Rxi-624Sil MS	Restek	320 °C
VF-624ms	Varian	300 °C
DB-624	Agilent J&W	260 °C
ZB-624	Phenomenex	260 °C

Data obtained from company website or literature for a 30 m x 0.25 mm x 1.4 µm df column.

Figure 3 The Rxi®-624Sil MS column has the lowest bleed of any column in its class and provides true GC/MS capability.



Column: 30 m, 0.25 mm ID, 1.4 µm (Columns are of equivalent dimensions and were tested after equivalent conditioning)
Sample: Fluorobenzene in methanol, 10 ng on-column; **Detector:** FID @ 250 °C. Complete analytical conditions for chromatogram GC_GN1147 available at www.restek.com

Make your next Volatiles Column an Rxi®-624Sil MS Column

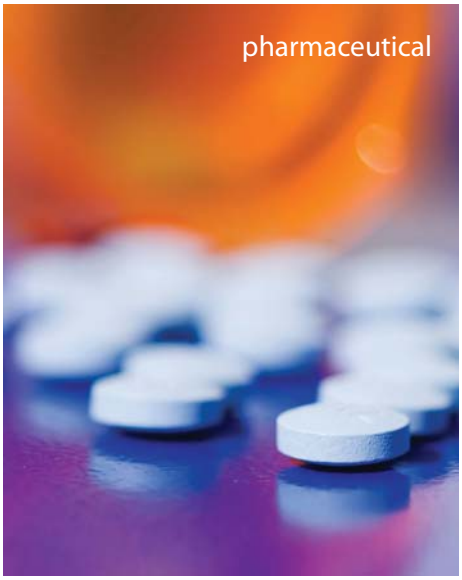
You can get more accurate low level results for volatile organics with a mass spec compatible Rxi®-624Sil MS column. See our articles in this issue for pharmaceutical (p. 4) and environmental (p. 6) applications, or contact us to discuss your own method needs.

Rxi®-624Sil MS Columns (fused silica)

(mid polarity Crossbond® silarylene phase; equivalent to 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	1.00	-20 to 300/320°C	20-Meter	13865
0.25mm	1.40	-20 to 300/320°C	30-Meter	13868
0.32mm	1.80	-20 to 300/320°C	30-Meter	13870
0.32mm	1.80	-20 to 300/320°C	60-Meter	13872
0.53mm	3.00	-20 to 280/300°C	30-Meter	13871

Visit **www.restek.com/rxi** for detailed comparisons and to learn how exceptional Rxi® inertness, bleed, and reproducibility can improve your data.

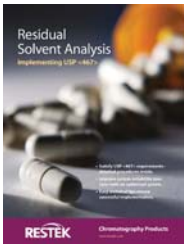


Improve Pass Rates for Residual Solvents by USP <467> With New Rxi®-624Sil MS GC Columns

By Rick Lake, Pharmaceutical Market Development Manager and Amanda Rigdon, Innovations Chemist

- Greatest resolution of acetonitrile and dichloromethane of any G43 column.
- Stable baseline for improved sensitivity of carbon tetrachloride.
- Exceptional column-to-column reproducibility.

It has been over a year since the revised USP <467> Residual Solvents general chapter became effective. Since then, many labs have experienced difficulty implementing this more expansive procedure. Most of the problems encountered relate to the selectivity and sensitivity needed to meet system suitability requirements in Procedure A. Finding an instrument setup that adequately resolves acetonitrile and dichloromethane in the Class 2 Mixture A standard, while also maintaining carbon tetrachloride sensitivity in the Class 1 solution, can prove difficult. Since increasing system suitability pass rates directly increases lab productivity, Restek has developed a new G43 capillary column that better meets USP <467> requirements.



get more

For more information on USP <467> analysis, download PHFL1018A at www.restek.com

Rxi®-624Sil MS Columns (fused silica)
(mid polarity Crossbond® silarylene phase; equivalent to 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length*	cat. #
0.18mm	1.00	-20 to 300/320°C	20	13865
0.25mm	1.40	-20 to 300/320°C	30	13868
0.32mm	1.80	-20 to 300/320°C	30	13870
0.32mm	1.80	-20 to 300/320°C	60	13872
0.53mm	3.00	-20 to 280/300°C	30	13871

*Length in meters.

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USP-equivalent residual solvent Class 1 & 2 mixes & singles.
Visit **www.restek.com/usp467** for a complete selection

Greater Resolution Improves Pass Rates

The Class 2 Mixture A solution contains the most difficult selectivity requirement of the method: the resolution between acetonitrile and dichloromethane must be greater than 1. This is often difficult to achieve on conventional G43 columns, which only give marginal selectivity for this pair. Poor selectivity can result in lower overall pass rates, and, thus, decreased sample throughput. In contrast, the Rxi®-624Sil MS column incorporates a distinctive bonding chemistry that results in resolution values consistently greater than 3 (Figure 1). The greater resolution routinely achieved on the Rxi®-624Sil MS column results in more consistent system suitability pass rates, and thus greater lab productivity.

Higher Inertness Gives Increased Sensitivity

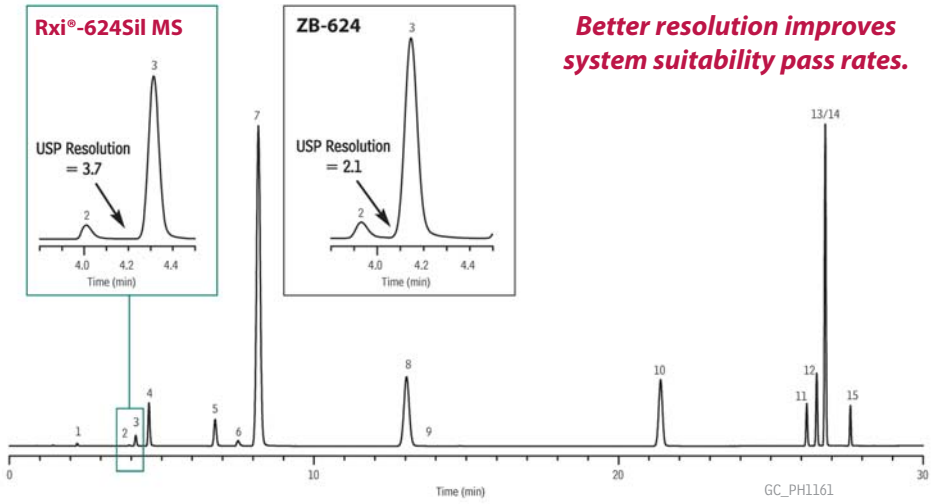
Rxi®-624Sil MS columns are manufactured using proprietary Rxi® technology, which produces extremely inert and stable columns. The high thermal stability of the Rxi®-624Sil MS column produces a very stable baseline, which leads to accurate and consistent integration. For example, carbon tetrachloride in the Class 1 system suitability solution, the most difficult sensitivity requirement in the method, can be easily and consistently integrated, reliably providing the necessary sensitivity. Another significant advantage of the Rxi®-624Sil MS column for the Class 1 solution is the complete resolution of benzene and 1,2-dichloroethane (Figure 2). Complete resolution of these analytes is often not achieved on other columns, making the Rxi®-624Sil MS column particularly beneficial for testing programs using USP <467>.

Conclusion

Not all G43 columns are equivalent for residual solvent testing, and the new Rxi®-624Sil MS column offers best-in-class performance advantages for all aspects of USP <467> system suitability testing. These columns reliably produce improved resolution and sensitivity, increasing system suitability pass rates and ensuring more productive laboratory time.

For the complete version of this condensed article, visit **www.restek.com/adv001**

Figure 1 Rxi®-624Sil MS columns reliably resolve Class 2 Mix A compounds.

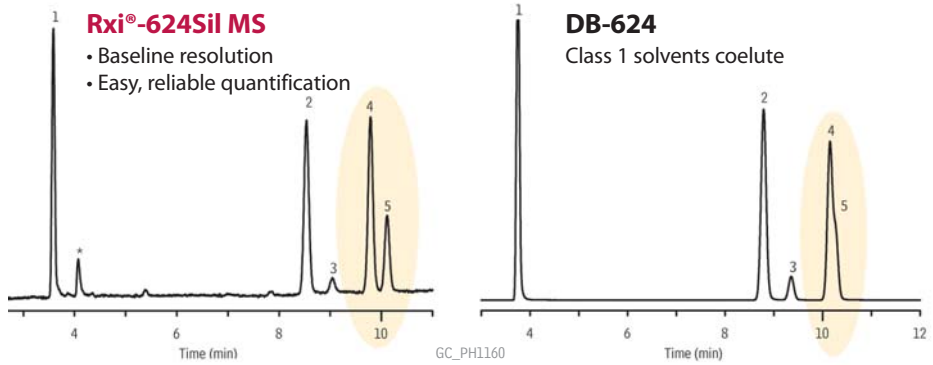


Better resolution improves system suitability pass rates.

Peaks	RT (min.)	Conc. (µg/mL)
1. Methanol	2.281	25.00
2. Acetonitrile	4.009	3.42
3. Dichloromethane	4.313	5.00
4. *trans*-1,2-Dichloroethene	4.798	7.83
5. *cis*-1,2-Dichloroethene	7.028	7.83
6. Tetrahydrofuran	7.706	5.75
7. Cyclohexane	8.708	32.33
8. Methylcyclohexane	14.099	9.83
9. 1,4-Dioxane	15.054	3.17
10. Toluene	22.018	7.42
11. Chlorobenzene	26.570	3.00
12. Ethylbenzene	26.837	3.07
13. *m*-Xylene	27.147	10.85
14. *p*-Xylene	27.147	2.53
15. *o*-Xylene	27.927	1.63

Column: Rxi®-624Sil MS, 30 m, 0.32 mm ID, 1.80 µm (cat.# 13870)
Sample: Residual Solvents Class 2 - Mix A (cat.# 36271)
Complete analytical conditions for chromatogram GC_PH1161 available at www.restek.com

Figure 2 The Rxi®-624Sil MS column provides complete resolution of the USP <467> Class 1 solution components—a result not often achieved on other G43 columns.



Peaks	RT (min.)	Conc. (µg/mL)
1. 1,1-Dichloroethene	3.586	0.07
2. 1,1,1-Trichloroethane	8.536	0.08
3. Carbon tetrachloride	9.042	0.03
4. Benzene	9.787	0.02
5. 1,2-Dichloroethane	10.112	0.04
* DMSO interference		

Column: Rxi®-624Sil MS, 30 m, 0.32 mm ID, 1.80 µm (cat.# 13870)
Sample: Residual Solvents - Class 1 (cat.# 36279)
Complete analytical conditions for chromatogram GC_PH1160 available at www.restek.com

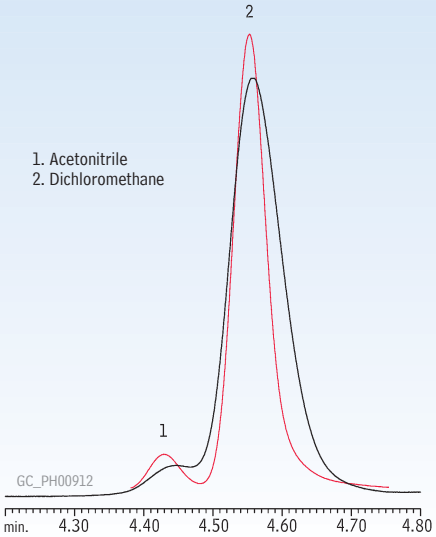
Tim Herring, Technical Service Specialist

TECH TIP!

When running USP <467> by headspace, using a **smaller bore liner** (1 mm) **can improve system suitability pass rates.** Larger bore liners (4 mm) are used with direct liquid injection because the sample is vaporized in the injection port and the liner must be able to accommodate the solvent expansion volume. In contrast, in headspace analysis, the sample is vaporized in a vial instead of the injection port, so a large volume liner is not needed, and in fact it can be deleterious. In headspace methods, using a smaller bore liner reduces band broadening by increasing linear velocity, allowing faster sample transfer and improving resolution.

See p. 9 for select 1mm liners.

Resolution passes USP <467> criteria when using a 1mm liner (red line), but fails if a 4mm liner is used (black line).



Are Your Volatiles Methods Slowing You Down? Minimize Downtime with an Rxi®-624Sil MS and Our Synchronized GC Conditions

By Michelle Misselwitz, Innovations Chemist, Gary Stidsen, Product Manager, and Chris English, Innovations Manager

- Optimized analysis allows for 36 runs per 12-hour shift, increasing instrument productivity.
- Rxi®-624Sil MS column selectivity and inertness resolve critical pairs.
- High temperature stability reduces bleed profile, resulting in lower detection limits.

Maximizing sample throughput while maintaining adequate resolution can be a delicate balancing act when analyzing volatile organic compounds (VOCs). Conditions optimized for resolution can result in long analysis times, but using faster run times can result in problematic coelutions. Often, "624" type columns are chosen for their selectivity, but thermal stability is usually poor, resulting in phase bleed that decreases detector sensitivity. New Rxi®-624Sil MS columns offer reliable resolution of VOCs and also provide lower bleed and greater inertness than other 624 columns. Labs interested in optimizing sample throughput and resolution can adopt the synchronized conditions established here on Rxi®-624Sil MS columns to maximize productivity and assure accurate, reliable results.

Want lower detection limits for active compounds?

See page 2 to learn why Rxi®-624Sil MS columns improve sensitivity, accuracy, and MS performance for active analytes.

Reduce Downtime and Resolve Critical Pairs

In order to minimize downtime between injections while ensuring good resolution, we established parameters that synchronized the purge and trap and instrument cycles while maintaining desired separations. Several critical pairs were chosen for computational modeling using Pro *ez*GC software. The initial temperature program determined by the software provided the best resolution, but resulted in an analysis time of 19 minutes. Since the purge and trap cycle time was 16.5 minutes, we tested other conditions to see if adequate resolution could be maintained using a faster instrument cycle. The program shown in Figure 1 reduced instrument downtime by better synchronizing injection and analysis, and also provided excellent resolution. Using a highly inert, low bleed Rxi®-624Sil MS column under the conditions established here, optimizes sample throughput while assuring good resolution of volatile organic compounds.

For the complete version of this condensed article, visit **www.restek.com/adv002**

Rxi®-624Sil MS Columns (fused silica)
(mid polarity Crossbond® silarylene phase; equivalent to 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID(mm)	df (µm)	temp. limits	length*	cat. #
0.18	1.00	-20 to 300/320°C	20	13865
0.25	1.40	-20 to 300/320°C	30	13868
0.32	1.80	-20 to 300/320°C	30	13870

*Length in meters

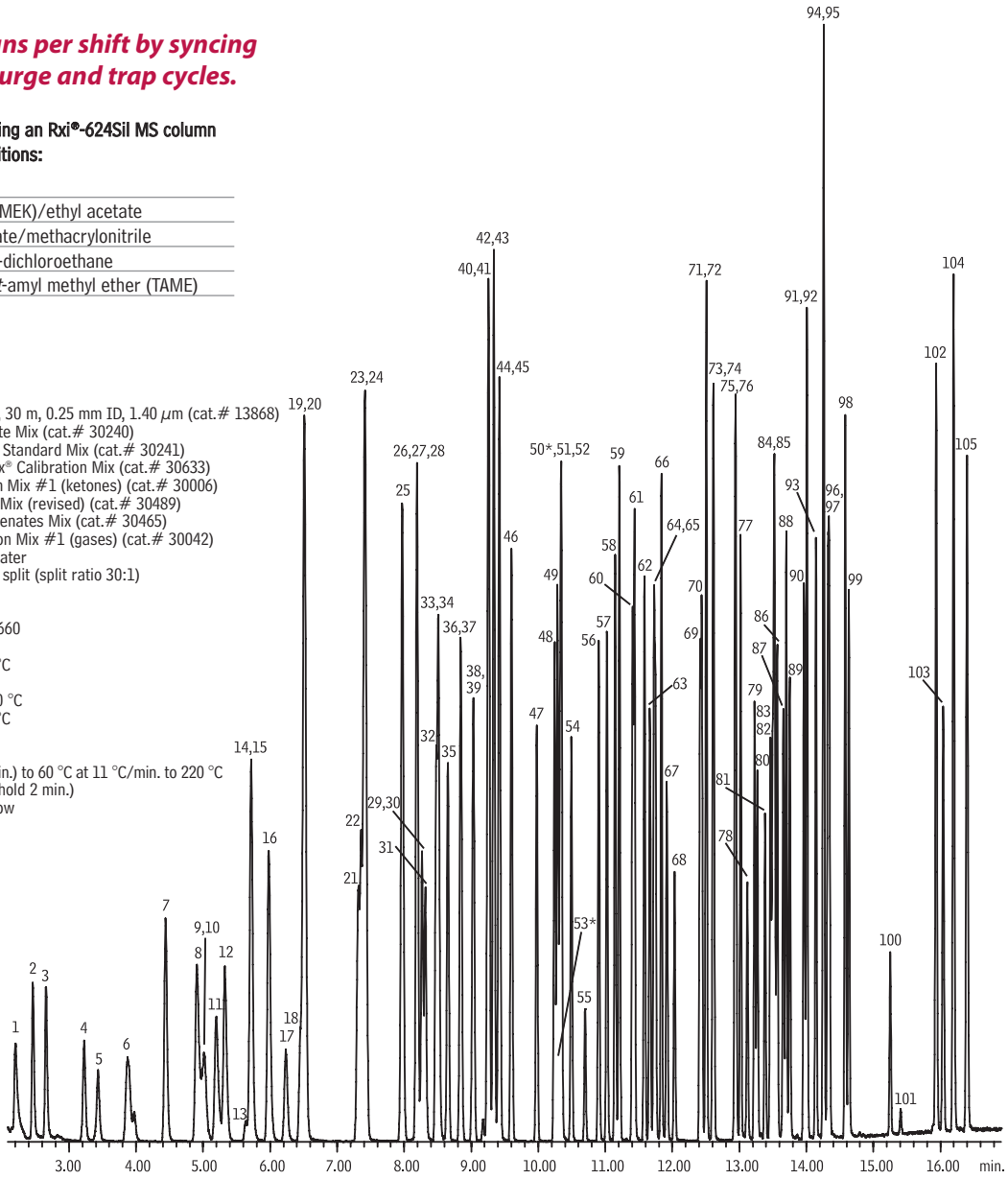
Figure 1 Using an Rxi®-624Sil MS column under optimized conditions assures good resolution with minimal downtime.

Analyze up to 36 runs per shift by syncing instrument and purge and trap cycles.

Critical pairs resolved using an Rxi®-624Sil MS column under synchronized conditions:

Peak #s	Compounds
26/29	2-butanone (MEK)/ethyl acetate
31/32	methyl acrylate/methacrylonitrile
41/42	benzene/1,2-dichloroethane
41/45	benzene/*tert*-amyl methyl ether (TAME)

Column: Rxi®-624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868)
Sample: 8260A Surrogate Mix (cat.# 30240)
8260A Internal Standard Mix (cat.# 30241)
8260B MegaMix® Calibration Mix (cat.# 30633)
VOA Calibration Mix #1 (ketones) (cat.# 30006)
8260B Acetate Mix (revised) (cat.# 30489)
California Oxygenates Mix (cat.# 30465)
502.2 Calibration Mix #1 (gases) (cat.# 30042)
Conc.: 25 ppb in RO water
Injection purge and trap split (split ratio 30:1)
Inj. Temp.: 225 °C
Purge and Trap
Instrument: OI Analytical 4660
Trap Type: 10 Trap
Purge: 11 min. @ 20 °C
Desorb Preheat Temp.: 180 °C
Desorb: 0.5 min. @ 190 °C
Bake: 5 min. @ 210 °C
Interface Connection: injection port
Oven
Oven Temp: 35 °C (hold 5 min.) to 60 °C at 11 °C/min. to 220 °C at 20 °C/min. (hold 2 min.)
Carrier Gas: He, constant flow
Flow Rate: 1.0 mL/min.
Detector: MS
Mode: Scan
Transfer Line Temp.: 230 °C
Analyzer Type: Quadrupole
Source Temp.: 230 °C
Quad Temp.: 150 °C
Electron Energy: 70 eV
Solvent Delay Time: 1.5 min.
Tune Type: BFB
Ionization Mode: EI
Scan Range: 36-260 amu
Instrument:
Agilent 7890A GC & 5975C MSD
Notes
Other Purge and Trap Conditions:
Sample Inlet: 40°C
Sample: 40°C
Water Management:
Purge 110°C, Desorb 0°C, Bake, 240°C



GC_EV1169

Peaks	RT (min.)
1. Dichlorodifluoromethane (CFC-12)	2.198
2. Chloromethane	2.459
3. Vinyl chloride	2.659
4. Bromomethane	3.226
5. Chloroethane	3.434
6. Trichlorofluoromethane (CFC-11)	3.876
7. Diethyl ether (ethyl ether)	4.440
8. 1,1-Dichloroethene	4.909
9. 1,1,2-Trichlorotrifluoroethane (CFC-113)	4.998
10. Acetone	5.029
11. Iodomethane	5.195
12. Carbon disulfide	5.323
13. Acetonitrile	5.637
14. Allyl chloride	5.715
15. Methyl acetate	5.723
16. Methylene chloride	5.981
17. *tert*-Butyl alcohol	6.234
18. Acrylonitrile	6.451
19. Methyl *tert*-butyl ether (MTBE)	6.509
20. *trans*-1,2-Dichloroethene	6.512
21. 1,1-Dichloroethane	7.315
22. Vinyl acetate	7.359
23. Diisopropyl ether (DIPE)	7.407
24. Chloroprene	7.429
25. Ethyl *tert*-butyl ether (ETBE)	7.970
26. 2-Butanone (MEK)	8.193
27. *cis*-1,2-Dichloroethene	8.193
28. 2,2-Dichloropropane	8.193
29. Ethyl acetate	8.265
30. Propionitrile	8.276
31. Methyl acrylate	8.318
32. Methacrylonitrile	8.476
33. Bromochloromethane	8.507
34. Tetrahydrofuran	8.521
35. Chloroform	8.651
36. 1,1,1-Trichloroethane	8.843
37. Dibromofluoromethane	8.848
38. Carbon tetrachloride	9.026
39. 1,1-Dichloropropene	9.037
40. 1,2-Dichloroethane-d4	9.246
41. Benzene	9.262
42. 1,2-Dichloroethane	9.334
43. Isopropyl acetate	9.340
44. Isobutyl alcohol	9.421
45. *tert*-Amyl methyl ether (TAME)	9.421
46. Fluorobenzene	9.598
47. Trichloroethene	9.976
48. 1,2-Dichloropropane	10.243
49. Methyl methacrylate	10.290
50. 1,4-Dioxane (ND)	10.299*
51. Dibromomethane	10.326
52. Propyl acetate	10.346
53. 2-Chloroethanol (ND)	10.368*
54. Bromodichloromethane	10.496
55. 2-Nitropropane	10.698
56. *cis*-1,3-Dichloropropene	10.904
57. 4-Methyl-2-pentanone (MIBK)	11.026
58. Toluene-D8	11.148
59. Toluene	11.210
60. *trans*-1,3-Dichloropropene	11.407
61. Ethyl methacrylate	11.435
62. 1,1,2-Trichloroethane	11.585
63. Tetrachloroethene	11.662
64. 1,3-Dichloropropane	11.729
65. 2-Hexanone	11.749
66. Butyl acetate	11.837
67. Dibromochloromethane	11.921
68. 1,2-Dibromoethane (EDB)	12.035
69. Chlorobenzene-d5	12.412
70. Chlorobenzene	12.440
71. Ethylbenzene	12.507
72. 1,1,1,2-Tetrachloroethane	12.507
73. *m*-Xylene	12.612
74. *p*-Xylene	12.612
75. *o*-Xylene	12.935
76. Styrene	12.949
77. *n*-Amyl acetate	13.018
78. Bromoform	13.118
79. Isopropylbenzene (cumene)	13.226
80. *cis*-1,4-Dichloro-2-butene	13.268
81. 4-Bromofluorobenzene	13.385
82. 1,1,2,2-Tetrachloroethane	13.456
83. *trans*-1,4-Dichloro-2-butene	13.496
84. Bromobenzene	13.515
85. 1,2,3-Trichloropropane	13.526
86. *n*-Propylbenzene	13.565
87. 2-Chlorotoluene	13.657
88. 1,3,5-Trimethylbenzene	13.699
89. 4-Chlorotoluene	13.751
90. *tert*-Butylbenzene	13.965
91. Pentachloroethane	14.007
92. 1,2,4-Trimethylbenzene	14.010
93. *sec*-Butylbenzene	14.140
94. 4-Isopropyltoluene (*p*-cymene)	14.254
95. 1,3-Dichlorobenzene	14.263
96. 1,4-Dichlorobenzene-D4	14.321
97. 1,4-Dichlorobenzene	14.340
98. *n*-Butylbenzene	14.579
99. 1,2-Dichlorobenzene	14.635
100. 1,2-Dibromo-3-chloropropane (DBCP)	15.252
101. Nitrobenzene	15.407
102. 1,2,4-Trichlorobenzene	15.935
103. Hexachloro-1,3-butadiene	16.040
104. Naphthalene	16.196
105. 1,2,3-Trichlorobenzene	16.396

* ND = not detected; retention time determined by wet needle injection

Perfect Complements

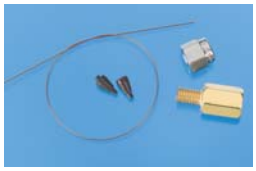
To Your New Rxi®-624Sil MS GC Column

EZ No-Vent® GC Column-Mass Spectrometer Connector

for Agilent GCs

- Change GC/MS columns in minutes without venting.
- Easy to install and maintain—no special tools or plumbing required.
- Deactivated transfer line keeps analytes focused.
- High-temperature polyimide ferrules eliminate leaks.
- Lower cost than other "no-vent" fittings.

Change columns in minutes—without venting!



Kit installs easily, without special tools or plumbing.

Available for Agilent GCs with 5971/5972, 5973, or 5975 GC/MS.
For other instrument specific EZ No-Vent® connectors, visit ***www.restek.com/eznovent***

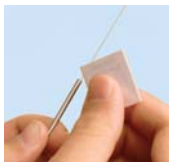
Description	qty.	cat.#
EZ No-Vent Connector Kit includes: EZ No-Vent Connector, two 0.4mm ID adaptor ferrules for capillary column, two 0.4mm ID ferrules for transfer line, 100µm deactivated transfer line (3 ft.), column plug, column nut	kit	21323
Replacement ferrules for connecting capillary column to EZ No-Vent Connector: 0.4mm ID (Polyimide)	2-pk.	21015
0.5mm ID (Polyimide)	2-pk.	21016
Replacement ferrules for connecting transfer line to EZ No-Vent Connector: 0.4mm ID	2-pk.	21043
Replacement 100µm deactivated transfer line	3 ft.	21018
Replacement EZ No-Vent Column Nut	5-pk.	21900
Replacement EZ No-Vent Plug	2-pk.	21915
Open-End Wrenches, 1/4" x 5/16"	2-pk.	20110

Capillary Installation Gauge

for Agilent 5973/5975 MS

Easily seat ferrules for consistent installations in Agilent 5973 MS.

Install the nut and ferrule onto the column, then insert the column through the installation tool, exposing several centimeters at the exit end, then tighten the nut on to the gauge.



Score and remove the exposed end of the column, then loosen the nut.

Ready to go!

Description	qty.	cat.#
Capillary Installation Gauge for Agilent 5973/5975 MS	ea.	21894

Capillary gauges for other instrument manufacturers are available. Visit us online.

GC/MS Cleaning Kit

The Restek GC/MS Cleaning Kit (cat.#s 27194, 27195) includes:

- lint free nylon gloves (small-2pair)
- lint free nylon gloves (large-2 pair)
- lint free cotton cloth, 9 x 9 (10-pk.)
- micro mesh 4 x 6 sheet (4-pk.)
- aluminum oxide (75 gram jar)
- cotton tip applicators
- tweezers, large
- tweezers, small
- septum puller
- Dremel® tool, battery-operated (optional, 27194)
- tool kit bag

Poor sensitivity, loss of sensitivity at high masses, or high multiplier gain during an auto tune are all indicators that your mass spectrometer source may need to be cleaned. Restek has assembled all of the necessary components for cleaning and polishing your ion source.

Description	qty.	cat.#
Mass Spec Cleaning Kit with Dremel Tool	kit	27194
Mass Spec Cleaning Kit without Dremel Tool	kit	27195
Mass Spec Cleaning Kit Replacement Parts Kit (includes cloths, micro mesh sheets, small and large gloves)	kit	27196

FREE Base Plate with Purchase of 2 Triple Filters!

Get Super-Clean!

Super-Clean Gas Filter and Base Plate Kit

- 99.9999% pure gas.
- Quick connect fittings for easy, leak-tight filter changes.

Offer valid through Dec. 31, 2010. Cannot be combined with other offers or discounts.

Description	qty.	cat.#
Restek Exclusive Triple Filter Kit	kit	22859-409

Dual Vespel® Ring Cross-Disk Inlet Seals

for Agilent GCs

- Ideal for high-flow split applications.
- Washerless, leak-tight seals.

restek **innovation**!

No washer required! Vespel® ring seal on top and bottom surfaces.

	2-pk.	10-pk.
Gold-Plated	22083	22084
Siltek Treated	22085	22086
Stainless Steel	22087	22088

get **more** For discussion about the benefits of using a dual Vespel® ring inlet seal, visit http://www.restek.com/adv009

ETP Electron Multipliers for Mass Spec

- Air stable.
- 2-year shelf life guarantee.
- Discrete dynode design results in extended operating life.

Other ETP Electron Multipliers are available upon request.
Call us if you do not see your instrument listed.

Description	qty.	cat.#
Electron Multipliers for Agilent GC/MS and LC/MS		
For Agilent 5970 GC/MS	ea.	23072
For Agilent 5971, 5972, GC GC/MS	ea.	23073
For Agilent 5973 & 5975 GC/MS (includes mount for initial installation)*†	ea.	23074
For Agilent 5973 & 5975 GC/MS and LC/MSD (Replacement Multiplier)*†	ea.	23075
For Agilent LC/MSD (includes mount for initial installation)*†	ea.	23076
Electron Multiplier for Applied Biosystems (Sciex)		
For API 300, 3000 & 4000 Applied Biosystems	ea.	23077
Electron Multiplier for Thermo Finnigan GC/MS		
For Thermo TRACE DSQ, DSQII, and Polaris-Q GC/MS	ea.	23081

*First time installation requires a mount which includes the mechanical housing. After initial installation, only the replacement electron multiplier is required.
†This unit is designed for use in the 5975, 5973 GC and the LC/MSD.

Restek Electronic Leak Detector

Protect your data and analytical column!

High temperature methods are extremely sensitive to carrier gas impurities such as water and oxygen. Make sure you have clean carrier gas and frequently check connections and injection system fittings for leaks using Restek's Electronic Leak Detector.

Leak Detector with Universal Charger Set (US, UK, European, Australian)
Cat.# 22839

www.restek.com/leakdetector

Narrow Bore Inlet Liners

Restek offers inlet liners, including narrow bore liners, for all major instrument manufacturers. Visit us at www.restek.com/liners

Split Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#
1mm Split**		
1.0mm x 6.3mm x 78.5mm	ea.	20972
1.0mm x 6.3mm x 78.5mm	5-pk.	20973

Zero Dilution Liners for PerkinElmer Auto SYS™ and Clarus GCs

ID* x OD & Length	qty.	cat.#
Zero Dilution Inner Liner		
1.0mm x 2.0mm x 73mm	ea.	22990
1.0mm x 2.0mm x 73mm	5-pk.	22991

Split Liners for Shimadzu GCs

ID* x OD & Length	qty.	cat.#
1mm Split		
1.0mm x 5.0mm x 95mm	ea.	20976
1.0mm x 5.0mm x 95mm	5-pk.	20977
1.0mm x 5.0mm x 95mm	25-pk.	20978

Split Liners for Varian 1075/1077 GCs

ID* x OD & Length	qty.	cat.#
1mm Split		
1.0mm x 6.3mm x 72mm	ea.	20970
1.0mm x 6.3mm x 72mm	5-pk.	20971

*Nominal ID at syringe needle expulsion point.
**Use this liner for increased sensitivity.

free poster

Get your liner anatomy wall chart!
Request GNWC1014 at www.restek.com

Aqueous C18 LC Columns—More Versatile than a C18 for Vitamins and Organic Acids in Dietary Supplements

Ty W. Kahler, Innovations Chemist and Rick Lake, Pharmaceutical Market Development Manager

- Simplify method development for polar compounds.
- Higher retention and selectivity compared to a C18.
- Compatible with 100% aqueous mobile phases.

Conventional alkyl (C18) columns are frequently used for initial method development, but often are not the best choice. C18 columns have poor retention for polar compounds and do not perform well with aqueous mobile phases. In contrast, Aqueous C18 columns are a more versatile choice, due to much higher polar retention and compatibility with 100% aqueous mobile phases. In this article, we demonstrate the utility of Aqueous C18 columns across a range of analytes relevant to dietary supplement testing.

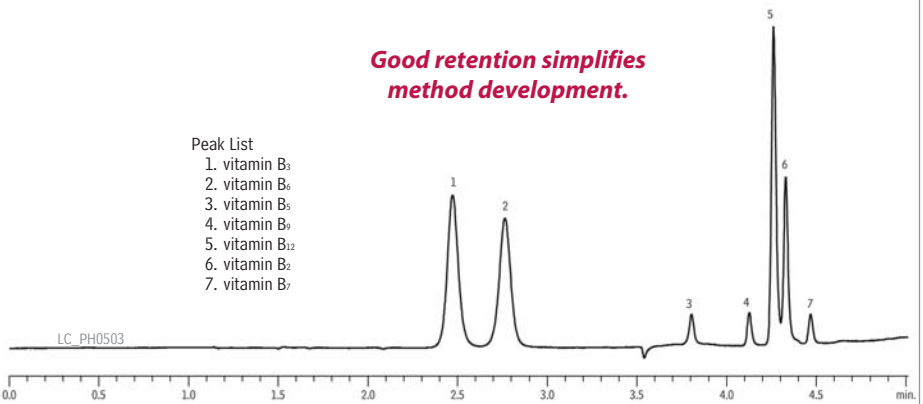
Ideal for Multi-Vitamin Analyses—Easily Retains Water-Soluble Vitamins

Many consumers are augmenting their diets with multi-vitamins. These supplements usually contain multiple water-soluble vitamins in a variety of chemical forms and concentrations. While water-soluble vitamins can be analyzed by HPLC, obtaining adequate retention of hydrophilic analytes is often problematic. As shown in Figure 1, the Ultra II® Aqueous C18 column provides excellent retention and completely resolves a test mix of B vitamins.

Need to test for pesticides too?

See p.12 for an LC/MS/MS analysis of 280 pesticides on an Ultra Aqueous C18 column.

Figure 1 A selective separation of seven B vitamins using a simple buffer:acetonitrile gradient on an Ultra II® Aqueous C18 column.



Inj.: 1µL; **Conc.:** 100µg/mL each component; **Sample diluent:** water:methanol (70:30); **Column:** Ultra II® Aqueous C18; **Cat.#:** 9608565; **Dimensions:** 150mm x 4.6mm; **Particle size:** 5µm; **Mobile phase:** A. 10mM potassium phosphate (pH 2.5), B. acetonitrile; **Gradient (%B):** 0 min. (2%), 1.5 min. (2%), 5 min. (75%); **Flow:** 1.5mL/min.; **Det.:** UV @ 210nm
Complete analytical conditions for chromatogram LC_PH0503 available at www.restek.com

Better for Organic Acids—More Retentive, Selective and Stable than a C18
Aqueous C18 columns are also an excellent choice for analyzing organic acids. For example, in Figure 2, an Ultra II® Aqueous C18 column provides greater retention and selectivity for organic acids than a conventional C18. The unique bonding chemistry of an Aqueous C18 column improves the retention of polar compounds and allows 100% aqueous mobile phases to be used, making it an excellent choice when developing methods for dietary supplement testing.

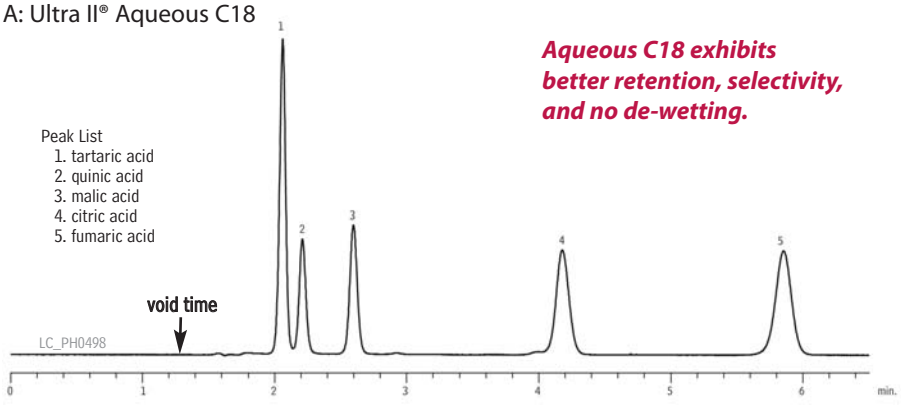
For the complete version of this condensed article, visit **www.restek.com/adv003**

Learning LINKS

Why use a reversed phase column specifically designed for highly aqueous mobile phases?
Learn why at www.restek.com/adv008

Figure 2 Ultra II® Aqueous C18 columns outperform conventional C18 columns for the analysis of organic acids in a 100% aqueous mobile phase.

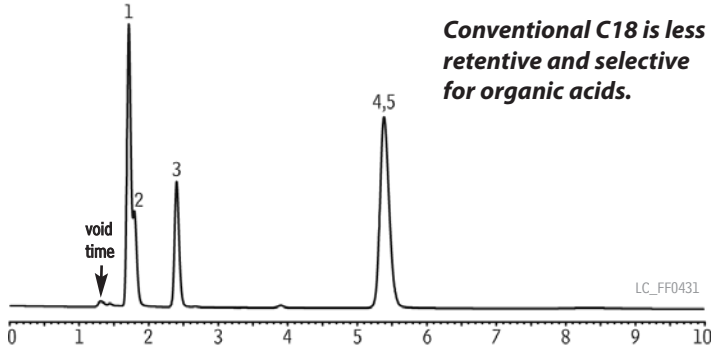
A: Ultra II® Aqueous C18



Aqueous C18 exhibits better retention, selectivity, and no de-wetting.

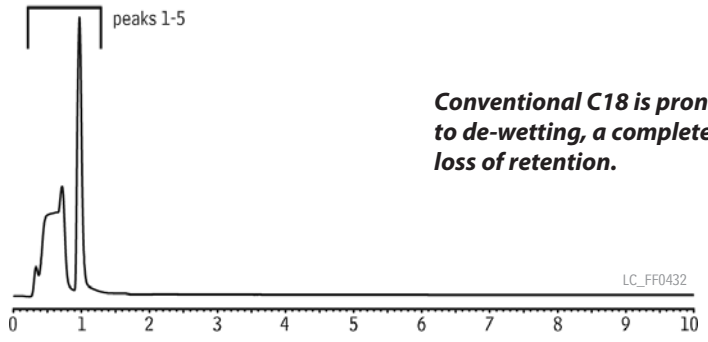
Sample: Inj.: 5µL; **Conc.:** 10µg/mL fumaric acid, 2,000µg/mL each other acids; **Sample diluent:** water; **Column:** Ultra II® Aqueous C18; **Cat.#:** 9608565; **Dimensions:** 150mm x 4.6mm; **Particle size:** 5µm; **Mobile phase:** 100% 20mM potassium phosphate (pH 2.5); **Det.:** UV @ 226nm Complete analytical conditions for chromatogram LC_PH0498 available at www.restek.com

B: Conventional C18, initial analysis



Conventional C18 is less retentive and selective for organic acids.

C: Conventional C18, post pressure release



Conventional C18 is prone to de-wetting, a complete loss of retention.

Conditions used in A, B, and C were identical. De-wetting in Figure 1C was caused for illustrative purposes by releasing column pressure.

Ultra II® Aqueous C18 Columns (USP L1)

Physical Characteristics:

particle size: 2.2µm, 3µm or 5µm, spherical
pore size: 100Å
carbon load: 15%
endcap: no
pH range: 2.5 to 7.5
temperature limit: 80°C

Chromatographic Properties:

Highly retentive and selective for reversed phase separations of polar analytes. Highly base-deactivated. Compatible with highly aqueous (up to 100%) mobile phases.

Length	3.2mm ID cat.#	4.6mm ID cat.#
3µm Columns		
30mm	9608333	9608335
50mm	9608353	9608355
100mm	9608313	9608315
150mm	9608363	9608365
5µm Columns		
30mm	9608533	9608535
50mm	9608553	9608555
100mm	9608513	9608515
150mm	9608563	9608565
200mm	9608523	9608525
250mm	9608573	9608575

More dimensions available online.

Fruit Juice Organic Acid Standard

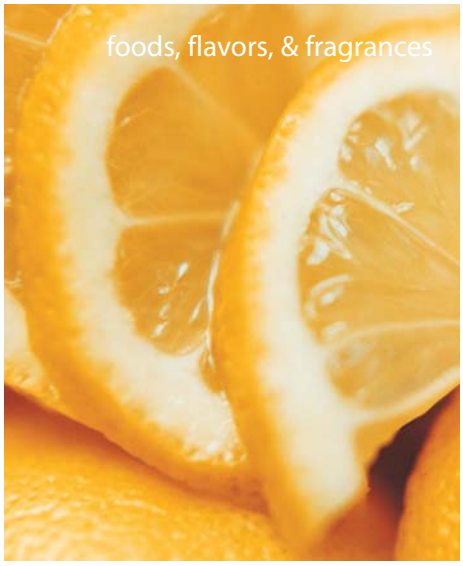
(5 components)

citric acid	2,000µg/ml	quinic acid	2,000
fumaric acid	10*	tartaric acid	2,000
malic acid	2,000		

In water, 1mL/ampul
cat. # 35080 (ea.)

In water, 5mL/ampul
cat. # 35081 (ea.)

*Fumaric acid is a trace impurity in malic acid, as well as an added component of the mix. The amount of fumaric acid in malic acid will not affect the stated concentration of malic acid, but can represent a significant and variable deviation from the low concentration of fumaric acid stated to be in the mix. All other components of the mix are at the specified concentration. Quantity discounts not available.



Comprehensive Pesticide Residue Analysis by LC/MS/MS Using an Ultra Aqueous C18 Column

By Becky Wittrig, Ph.D., AB Sciex, and André Schreiber, Ph.D., Applied Biosystems/MDS Analytical Technologies

- Easily resolve and quantify more than 280 pesticide species.
- Use LC/MS/MS to reliably monitor difficult polar and/or thermally unstable species.
- Aqueous C18 phase offers optimal selectivity and retention.

Food safety is a topic of great interest globally. With recent contamination issues in a wide range of commodities, ensuring the quality of our food supply is becoming increasingly important. Pesticide residue content is one area of concern. While pesticides have typically been monitored by gas chromatography, polar and/or thermally unstable pesticides are difficult or impossible to monitor using this approach. Thus, traditional HPLC techniques are used for select pesticide classes, such as the carbamate and phenylurea pesticides.

With recent advances in LC/MS/MS instrumentation, this technique is quickly gaining acceptance for pesticide residue testing. LC/MS/MS can be used to simultaneously monitor hundreds of potential contaminants—including those difficult to detect by GC. Using both LC/MS/MS and GC approaches allows for a faster, more complete picture of pesticide residues. MS/MS technology also permits identification of the target pesticides through the selection of specific MRM transitions for each compound. For example, aldicarb, a carbamate pesticide, uses two MRM transitions of 208.2→89.1amu and 208.2→116.1amu.

While the MS/MS detector allows for specific, sensitive detection of the pesticide species, the LC separation is still important to ensure the highest quality data. Conventional C18 stationary phases are typically used for pesticide monitoring, but the selectivity and retention is poor for more polar species. In contrast, Ultra Aqueous C18 columns are ideal for multi-pesticide residue monitoring methods. In Figure 1, the analysis of more than 280 pesticides using the 3µm Ultra Aqueous C18 is shown. Optimized stationary phase selectivity allows for an even distribution of the compounds throughout the retention time window (see www.restek.com/adv004 for peak lists and retention times). As well, retention of more polar pesticides is greatly improved, as demonstrated in Figure 1C. The Ultra Aqueous C18 column, in a 100 x 2.1mm, 3µm configuration is the column of choice for LC/MS/MS pesticide monitoring methods.

Using LC/MS/MS technology and Aqueous C18 columns, in combination with gas chromatography, results in the most comprehensive monitoring of pesticide residues. Labs interested in more complete multi-residue analysis of pesticides in food matrices, including difficult polar or thermally unstable compounds, should consider adding LC/MS/MS and Aqueous C18 columns to routine testing procedures.

Acknowledgements
The authors wish to thank the US FDA for their collaboration and recognize the participation of multiple FDA labs in this work.

For the complete version of this condensed article, visit **www.restek.com/adv004**

Ultra Aqueous C18 Columns (USP L1)

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 100Å
carbon load: 15%
endcap: no
pH range: 2.5 to 7.5
temperature limit: 80°C

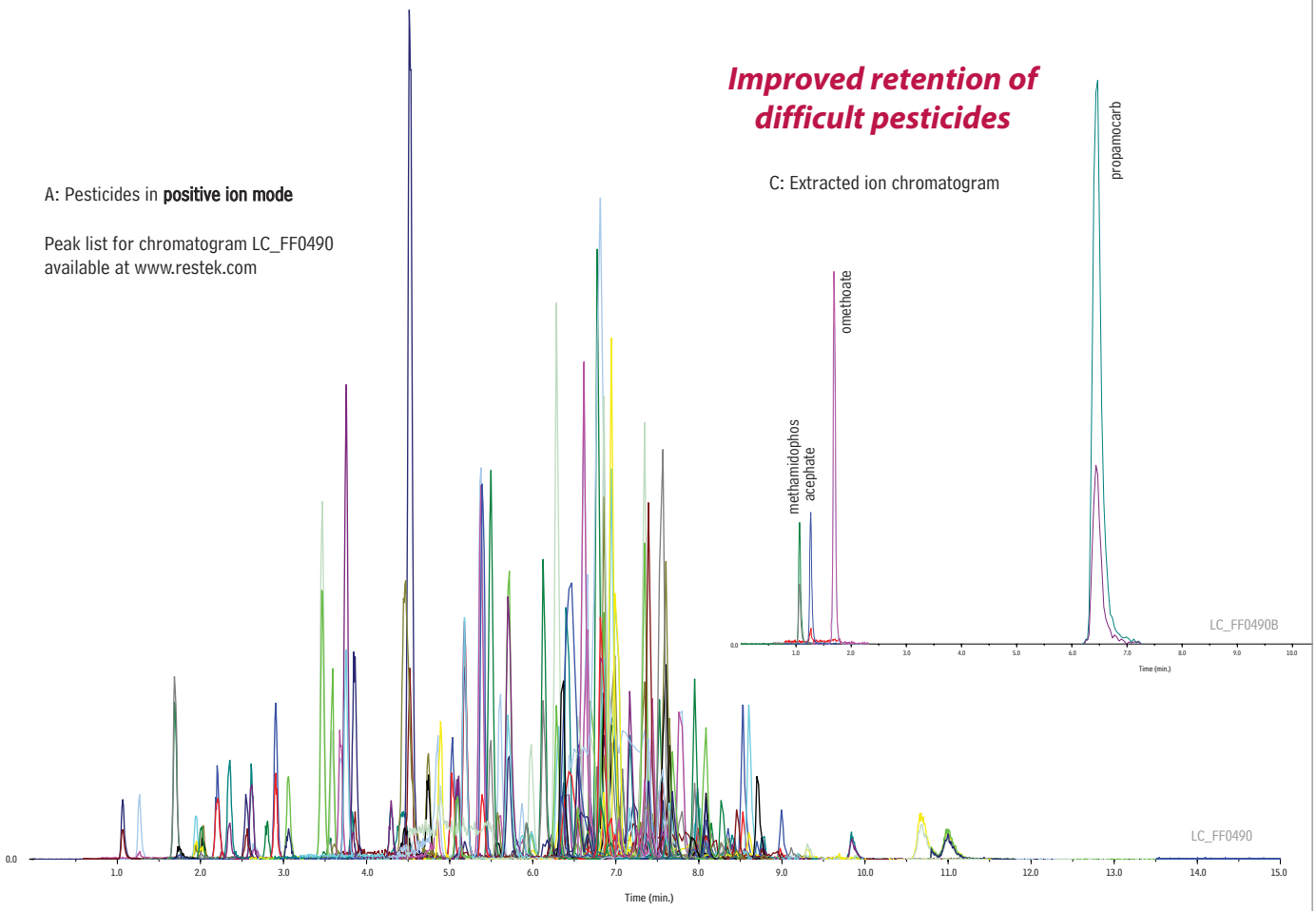
Chromatographic Properties:

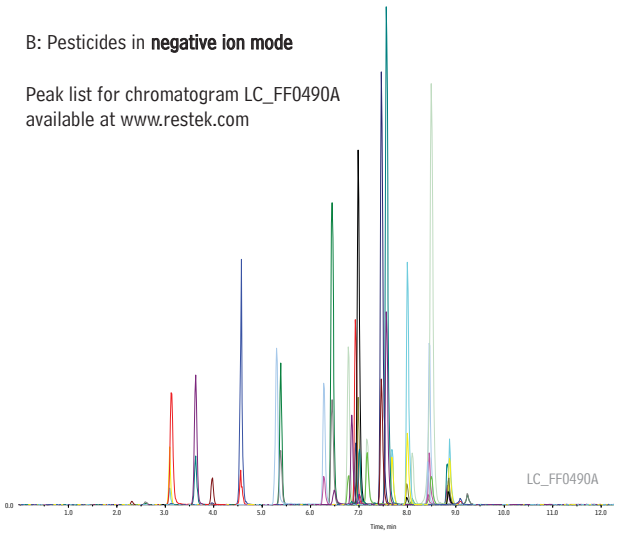
Highly retentive and selective for reversed phase separations of polar analytes. Highly base-deactivated. Compatible with highly aqueous (up to 100%) mobile phases.

Length	1.0mm ID cat.#	2.1mm ID cat.#
3µm Columns		
30mm	9178331	9178332
50mm	9178351	9178352
100mm	9178311	9178312
5µm Columns		
30mm	9178531	9178532
50mm	9178551	9178552
100mm	9178511	9178512
150mm	9178561	9178562
200mm	9178521	9178522
250mm	9178571	9178572

More dimensions available online.

Figure 1 Easily monitor over 280 pesticides by LC/MS/MS.





Sample: multicomponent pesticide standard; **Inj.:** 10µL; **Conc.:** 1ppb each pesticide; **Sample diluent:** acetonitrile; **Column:** Ultra Aqueous C18; **Cat. #:** 9178312; **Dimensions:** 100mm x 2.1mm; **Particle size:** 3µm; **Pore size:** 100Å; **Instrument:** Shimadzu Prominence® UFLCXR; **Mobile phase:** 10 mM NH_4OAc in water, B: 10 mM NH_4OAc in methanol; **Gradient (%B):** 0 min. (20%), 8.0 min. (90%), 12.0 min. (100%), 14.8 min. (100%), 14.9 min. (20%); **Flow:** 500µL/min; **Temp.:** 35°C; **Detector:** Applied Biosystems 4000 QTRAP® LC/MS/MS system; **Ion Source:** TurboIonSpray®, A & C: ESI+, B: ESI-; **IonSpray Voltage:** 5kV (ESI+), -4.2kV (ESI-); **Gas 1:** 50psi; **Gas 2:** 60psi; **Source Temp.:** 600°C.

LC/MS/MS Analysis of Diuretics in Urine: Biphenyl Column Takes Matrix Out of the Equation

By Amanda Rigdon, Clinical/Forensic Innovations Chemist, Takeo Sakuma, AB Sciex, and Becky Wittrig, Ph.D., AB Sciex

- Ultra II® Biphenyl columns separate compounds that coelute on phenyl hexyl columns.
- Improve quantitation through resolution of diuretics from isobaric matrix interferences.
- Fast analysis time supports high sample throughput.

Diuretics can mask the presence of performance enhancing drugs since they act to dilute the urine. Because of this, the use of diuretics has been banned by the World Anti-Doping Agency (WADA) and diuretic compounds are included in drug testing of athletes. Most common diuretics are highly functionalized compounds, making them hydrophilic and difficult to retain using C18 columns. Phenyl columns are a good alternative, as they generally have better hydrophilic retention; however, not all phenyl columns are retentive enough to ensure adequate resolution. While chromatographic resolution is not always required for LC/MS/MS analyses, it is necessary when isobaric interferences are present, such as when testing for diuretics in urine.

Better Retention Reduces Matrix Interference

Ultra II® Biphenyl columns can retain hydrophilic compounds longer than other phenyl-based stationary phases, due to the unique selectivity of the Biphenyl ligand for highly-functionalized aromatic compounds. As shown in Figure 2, using an Ultra II® Biphenyl column ensures complete separation of the diuretic amiloride from matrix peaks ($k' = 5$). In contrast, matrix interference occurs on a Gemini® C6-Phenyl (phenyl hexyl) column ($k' = 0.6$), preventing accurate quantitation. During this experiment, 10 diuretics from 4 classes were analyzed and excellent retention and resolution were obtained for all compounds in just 8 minutes, including re-equilibration time, on an Ultra II® Biphenyl column (see full chromatogram and conditions at www.restek.com/adv005).

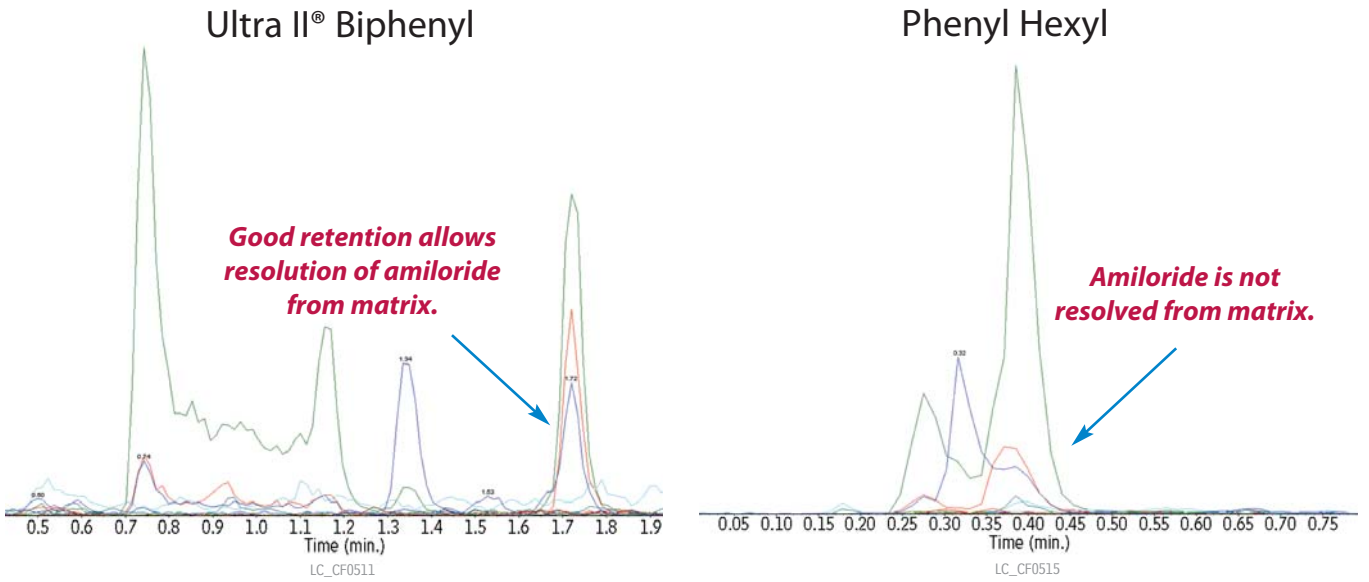
get more

For more information on biphenyl columns, download GNFL1277 at www.restek.com

Despite the power of modern LC/MS/MS instrumentation, isobaric matrix interferences often complicate analyses involving biological samples and using a column that produces adequate retention is critical for accurate quantitation. An Ultra II® Biphenyl column, in combination with LC/MS/MS, provides fast, reliable results when analyzing diuretics in urine.

For the complete version of this condensed article, visit **www.restek.com/adv005**

Figure 1 Higher retention on Ultra II® Biphenyl columns allows quantitation of diuretics that coelute with matrix on other phenyl phases.



Column: Ultra II® Biphenyl (cat.# 9609352); **Dimensions:** 50 mm x 2.1 mm ID; **Particle Size:** 3 µm; **Pore Size:** 100 Å; **Sample conc.:** 50 ng/mL diuretics in urine, diluted 10x in mobile phase; Complete analytical conditions for chromatogram LC_CF0511 available at www.restek.com

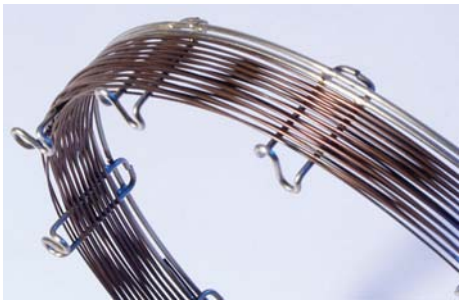
Column: Gemini® C6-Phenyl (phenyl hexyl); **Dimensions:** 50 mm x 2.0 mm ID; **Particle Size:** 3 µm; **Pore Size:** 110 Å; **Sample conc.:** 50 ng/mL diuretics in urine, diluted 10x in mobile phase; Complete analytical conditions for chromatogram LC_CF0515 available at www.restek.com

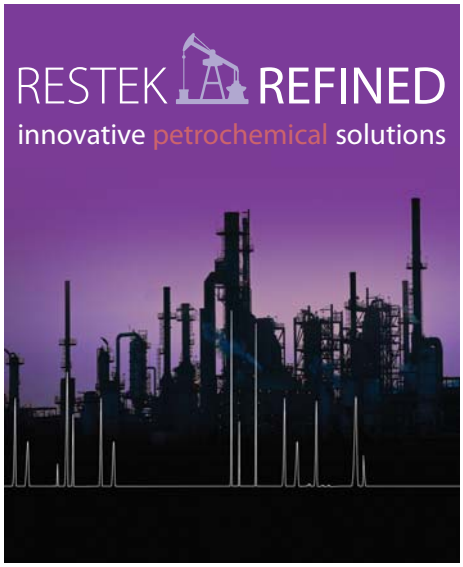
Ultra II® Biphenyl Columns (USP L11)

Physical Characteristics:

particle size: 2.2µm, 3µm or 5µm, spherical
pore size: 100Å
carbon load: 15%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

Length	1.0mm ID cat.#	2.1mm ID cat.#
1.9µm Columns		
30mm	–	9609232
50mm	–	9609252
100mm	–	9609212
2.2µm Columns		
30mm	–	9609832
50mm	–	9609852
100mm	–	9609812
3µm Columns		
30mm	9609331	9609332
50mm	9609351	9609352
100mm	9609311	9609312
150mm	9609361	9609362
5µm Columns		
30mm	9609531	9609532
50mm	9609551	9609552
100mm	9609511	9609512
150mm	9609561	9609562
200mm	9609521	9609522
250mm	9609571	9609572





Increase Productivity: Get More Runs from Your SimDist Setup Using Next Generation MXT®-1HT Columns

By Jan Pijpelink, Petrochemical Market Development Manager and Barry Burger, Petrochemical Innovations Chemist

- Stable up to 450°C—lowest bleed for longest column lifetime.
- Reliably meet all ASTM D6352 and D7500 specifications.
- 100% dimethyl polysiloxane phase allows easy comparisons to historical data.

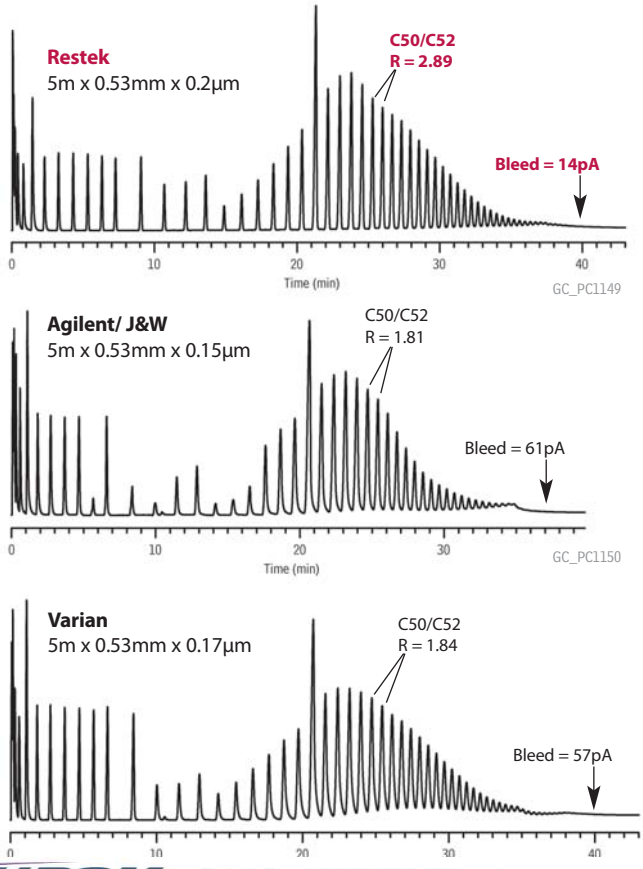
Accurate boiling point determination for medium and heavy fractions using GC simulated distillation requires columns and phase polymers that are robust enough to withstand high temperatures without significant degradation. Metal columns are a better alternative than fused silica, and the new MXT®-1HT SimDist columns are the lowest bleed, highest efficiency column available.

When compared to columns from other manufacturers, MXT®-1HT SimDist columns meet all D6352 method criteria and easily outperform competitors (Figure 1). In addition, field testing under accelerated conditions further demonstrates column robustness, even at 430°C (Figure 2). The exceptionally low bleed and high efficiency characteristics of the new MXT®-1HT SimDist columns translate directly into assured method performance, more analyses per calibration, and longer column lifetimes.

get more

For more information on petro solutions, download PCFL1195A at www.restek.com

Figure 1 Low bleed, high efficiency MXT®-1HT SimDist columns outperform competitors (ASTM D6352 conditions).



Lower bleed means:

- Longer column lifetime.
- More stable calibrations.
- Accurate boiling point determinations.

Restek advantage:

Longer column lifetime and more accurate data!

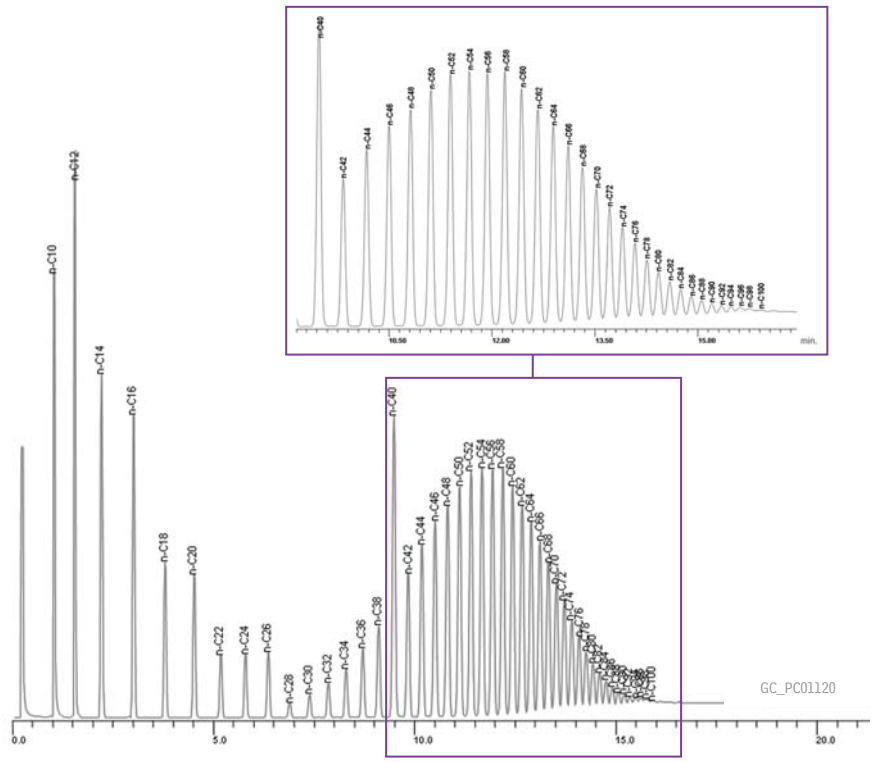
Higher efficiency means:

- Greater resolution; analyze more samples before method criteria are reached.
- Assured method performance.

Restek advantage:

Run more samples within method specifications!

Figure 2 Robust MXT®-1HT SimDist columns meet all ASTM D6352 requirements, even under accelerated conditions.



Column: MXT®-1HT Sim Dist, 5m, 0.53mm ID, 0.20μm (cat.# 70115); **Sample:** C10-C100, 1% in carbon disulfide; **Inj.:** 0.2μL near on-column (PTV); **Inj. temp.:** 40°C to 430°C @ 100°C/min.; **Carrier gas:** helium, constant flow; **Flow rate:** 20mL/min.; **Oven temp.:** 40°C to 430°C @ 25°C/min.; **Det.:** FID @ 430°C; Chromatograms courtesy of Joaquin Lubkowitz, Separation Systems, Gulf Breeze, FL.

Table I Recommended SimDist columns (100% PDMS) for use in ASTM SimDist methods.

ASTM Method	Range	Recommended Column
D2887	C5-C44	5/10m x 0.53mm, df = 0.88 – 2.65μm
D7213 (2887-ext)	C5-C60	5m x 0.53mm, df = 0.15 – 1.2μm
D3710	Gasoline up to FBP 260°C (C14)	10m x 0.53mm, df = 2.65μm
D5307	Crude up to FBP 538°C (C42)	5m x 0.53mm, df = 0.2μm
D6352/ D7500	C10-C90/ C7-C110	5m x 0.53mm, df = 0.1 – 0.2μm
D7169	C5-C100	5m x 0.53mm, df = 0.2 μm

FBP=final boiling point

MXT®-1HT Sim Dist Column
(Siltek® treated stainless steel)
(nonpolar phases)

ID	df (μm)	temp. limits	length*	cat. #
0.53mm	0.10	-60 to 430/450°C	5	70112
0.53mm	0.20	-60 to 430/450°C	5	70115
0.53mm	0.21	-60 to 430/450°C	10	70118
0.53mm	0.88	-60 to 400/430°C	5	70131
0.53mm	1.0	-60 to 380/400°C	10	70130
0.53mm	1.2	-60 to 380/400°C	10	70119
0.53mm	2.65	-60 to 360/400°C	10	70132
0.53mm	5.0	-60 to 360/400°C	10	70133

*Length in meters

Al Carusone, Technical Service Specialist

TECH TIP!

Oxygen and moisture will dramatically reduce siloxane phase stability, especially at temperatures over 400°C. To ensure maximum column lifetime, follow these guidelines for proper instrument set-up.

Use gas filters to remove oxygen and moisture from the carrier gas.

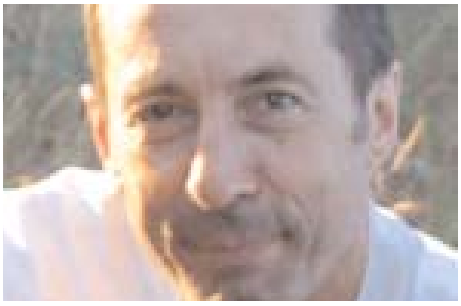
See the triple filter special offer on page 8.

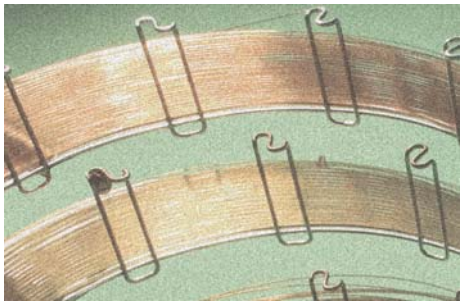
When installing a column, prevent leaks by using a proper cutting device (such as a scoring wafer or MXT® tubing scorer) to ensure the column is not crushed. (cat. # 20523)

Use graphite ferrules for column installation; Vespel®/graphite ferrules may leak, due to expansion and contraction at high temperatures (>400°C).

Check the system for leaks using an electronic leak detector. (cat. # 22839)

Visit **www.restek.com/petro** for a complete list of petroleum standards and accessories.





Unraveling Scent Signals to Protect African Wild Dogs

Peter Apps, Ph.D, Botswana Predator Conservation Trust

Innovators in Chromatography

A continuing series of guest editorials contributed by collaborators and internationally recognized leaders in chromatography.

***Peter Apps** runs the BPCT Paul G. Allen Family Foundation Wildlife Chemistry Laboratory in Maun, northern Botswana. He is a zoologist with a long career in chromatography, a rare combination that led him back to his zoological roots to set up the laboratory in July 2008.*

Although chromatography's versatility leads to its application to a host of diverse problems, helping to protect endangered African wild dogs from conflicts with people is perhaps not one that you would expect. With a grant from the Paul G. Allen Family Foundation, the Botswana Predator Conservation Trust (BPCT) has established a GC/MS laboratory to identify the chemical signals that African wild dogs use to mark their territory boundaries. The ultimate aim is to use artificial scent marks as "BioBoundaries" to limit movements by wild dogs into areas where they come into conflict with people and their livestock.

The BPCT BioBoundary project is led by Dr. John "Tico McNutt," who has been studying wild dogs since 1989, on the fringe of the Moremi Game Reserve and the Okavango Delta in northern Botswana. The GC/MS laboratory is located in the village of Maun, just 65 km from the BPCT study area, so that it can keep in close contact with field operations.

African wild dogs *(Lycaon pictus)* are intensely social predators. They live in packs of up to 27 adults and yearlings, in which usually only one pair breeds but everyone cares diligently for the pups. Numbering less than 6,000, they are one of Africa's most endangered carnivores, and their habitats are increasingly threatened by the expansion of human activities. Because wild dog packs have huge territories, only the very largest of protected wildlife areas can sustain viable populations. In Africa, wildlife areas with free-ranging carnivores are often separated from people and their livestock by only a line on a map or fences that are easily penetrated. Predators in livestock areas threaten peoples' livelihoods and the dogs' usual fate is to be shot, snared, or poisoned. The aim of the BPCT BioBoundaries project is to deploy artificial territorial scent marks, formulated with chemicals identified in natural wild dog marks, along protected area boundaries to create "virtual" neighboring packs that will deter dogs from crossing into areas where they are at risk. The stakes are high—population models predict that wild dogs will be extinct in the wild in 50 years, unless new ways are found to protect them.

Wild dogs, like nearly all mammals, live in a world dominated by odors. Airborne chemical signals, known as semiochemicals, play critical roles in their sexual and social behavior. The pack's dominant pair assiduously overmark each others feces and urine, and these double marks stake out the pack's territory.

Chemically, mammal scents are bafflingly complex, with the active messenger compounds at trace levels among hundreds of other components. Quantities of active compounds range down to picograms and concentrations of 10^{-18} molar. Nonetheless, mammal chemical signals are within range of gas chromatography and mass spectrometry, as long as the technology is used to its full potential. Maximum resolution and reproducibility

along with minimum contamination, discrimination, and limits of detection are required so that biological differences are not obscured by analytical artifacts and variability.

Sample preparation is both the most critical step and the Achilles heel. To preserve the integrity of the signal I have to sample what the dogs do: the volatiles in the air around a scent mark. Solid phase microextraction (SPME) and adsorption/thermal desorption looked promising, but yielded too many peaks from contaminants and too few from wild dogs. A simpler system was required to reduce contamination, variability, and analytical artifacts. Direct thermal desorption from urine-marked soil and cryotrapping with sample flow paths of glass and fused silica has provided the cleanest chromatograms so far. In nature, the scent marks are still active on hot, dry sand; therefore, samples can be dried prior to desorption to prevent icing of the cryotrap and then desorbed at 60°C.

The complexity of most mammal odors puts them well inside the Giddings zone, where at least 20% of chromatographic peaks overlap; not surprisingly, a dog mark chromatogram is so complex it has no clean baseline. Overlapping peaks cannot be properly quantified or identified and most failures to find an MS library match are due to coelutions that produce a mixed mass spectrum—only a minority of those without matches are new and, therefore, exciting compounds. To get cleanly resolved peaks, I will be using two-dimensional GC to transfer incompletely separated peaks from one column to another column with complementary selectivity.

Identifying everything in scent mark odor is unnecessary and impractical; the spotlight needs to fall on the few compounds that send the message. The critical challenge then is to differentiate the biologically relevant signal from the chemical noise, and this is where close links between the laboratory and the field operations play an absolutely critical role. Only dominant dogs produce territorial marks, so the signaling compounds will be present in their marks, but absent from subordinates' marks. The marks withstand 65K temperature differences in the soil substrate between midwinter midnights and summer afternoons. The marks last for at least six weeks and their emissions of territorial semiochemicals should be stable for at least as long. Without a detailed behavioral and social context for each sample it would be impossible to recognize the semiochemicals among the forest of extraneous peaks.

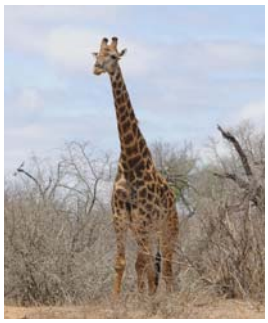
The wild dog boundary semiochemicals have to stand out against a background of the millions of natural chemicals that permeate the environment, and so I expect them not to be common constituents of mammal scent marks, feces or urine, or volatiles from plants or soil. Library searches of integer resolution mass spectra will eliminate compounds that are known to come from these sources.

Now that the sampling and separation conditions are worked out, in the months to come I will be running scent mark samples from several dogs in different packs searching for a peak, or a pattern of peaks that is present only in the marks of dominant animals, that stays the same with time and temperature, and that is not part of the environmental background. When I find it (or them) the next challenge will be to identify the compound(s). That will be a story for another time.

For more information on the BPCT BioBoundaries project and African wild dog research, visit www.bpctrust.org or www.wildentrust.org.

Travels in South Africa

Jack Cochran, Restek's Director of New Business and Technology, recently took these pictures on a photo safari while visiting South Africa to give seminars and collaborate on research projects. Jack was invited by ChromSA, the Chromatography Division of the South African Chemical Institute, to teach a course called, "Improving Your Gas Chromatographic Analyses." Following this and other speaking engagements at universities across the country, Jack spent several weeks working at the National Metrology Institute of South Africa on QuEChERS, GCxGC/TOFMS, PCB and dioxin analyses, on-column injection techniques, and various other gas chromatography projects at the invitation of Jayne de Vos.



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2009.01

Global Restek Advantage

Successfully Implement the Revised USP <467> Method

The USP general chapter <467> Residual Solvents is a widely used compendial method for identifying and quantifying residual solvents when no information is available on what solvents are likely to be present. In an attempt to harmonize with the ICH guidelines, the USP has proposed a more comprehensive method in the current USP 30/NF 25. This revision significantly increases the number of residual solvents to be routinely tested and includes three distinct procedures.[1]

Continued on page 2.

Also Inside:

New Restek Electronic Leak Detector

Prepare Samples in Half the Time Using a Fraction of the Solvent

Increase Retention of Hydrophilic Compounds Using Biphenyl Columns

Contents

Successfully Implement the Revised USP <467> Method

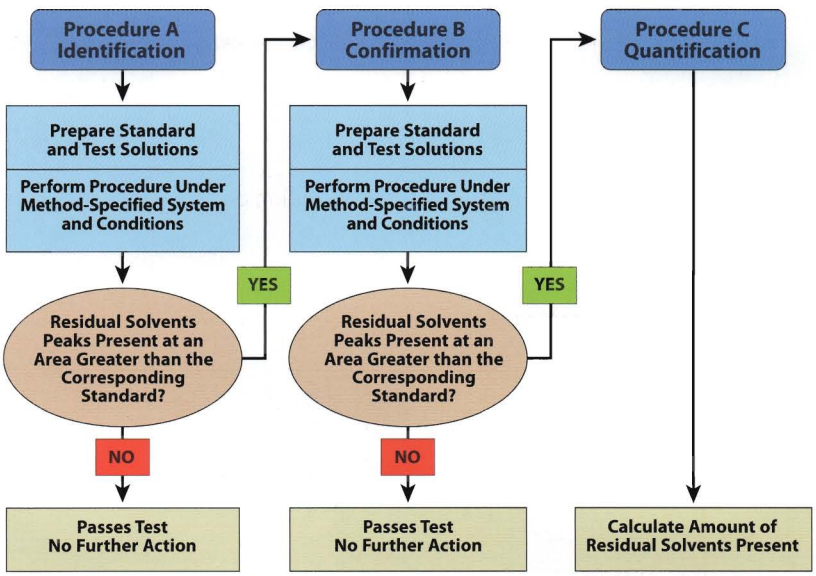
Continued from page 1.

Overview of Method

The revised USP <467> method consists of a static headspace extraction coupled with a gas chromatographic separation and flame ionization detection. In this guide we demonstrate the USP <467> application using two different types of headspace autosamplers. Procedure A was performed using a pressured loop autosampler and transfer line. Procedure B was performed using a heated syringe injection. Either system can be used to meet method requirements.

USP <467> is divided into two separate sections based upon sample solubility: water-soluble and water-insoluble articles. The methodology for both types of articles is similar, but the diluent used in both standard and sample preparations differs based upon the solubility of the test article. The test method consists of three procedures (A, B, and C), that are designed to identify, confirm, and then quantify residual solvents in drug substances and products (Figure 1).

Figure 1 Analytical flow chart for residual solvent testing under the revised USP <467> method.



[1] This number of analytes to be tested represents the sum of Class 1 and 2 residual solvents that can be effectively assayed using HS/GC. The actual number of analytes may be more if xylenes, ethyl benzene and *cis/trans* 1,2 dichloroethylene are differentiated, or if circumstances require the quantification of specific Class 3 residual solvents.

Analytical Reference Materials

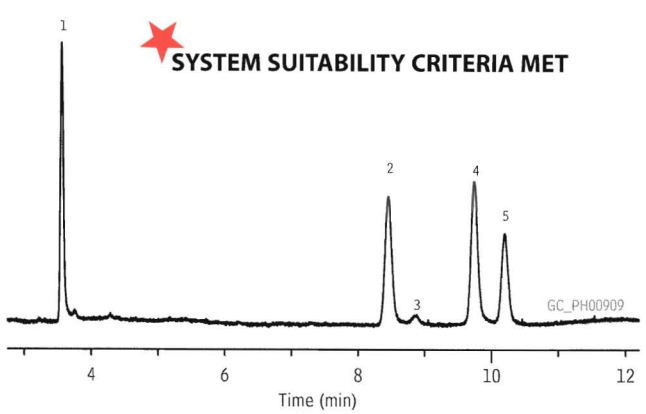
The ICH guideline classifies residual solvents by class according to toxicity. Class 1 compounds are carcinogenic and pose a risk to both the consumer and the environment. The use of these solvents must be avoided or tightly controlled. Class 2 compounds are nongenotoxic animal carcinogens and their concentration should be limited. Both Class 1 and 2 compounds require chromatographic determination and are separated into 3 test mixes: Class 1 Mixture, Class 2 Mixture A, and Class 2 Mixture B. Class 3 compounds have low toxic potential. Concentration levels of up to 0.5% are acceptable and, therefore, they can be assayed by nonspecific techniques, such as weight loss on drying. Class 2 Mixture C is not used in the second supplement of USP 30/NF 25, but contains solvents that are not readily detectable by headspace analysis. These solvents should be assayed by other appropriately validated procedures.

Procedure A - Identification

Procedure A is the first step in the identification process and is performed on a G43 column to determine if any residual solvents are present in the sample at detectable levels. First, Class 1 standard and system suitability solutions and Class 2 Mix A standard solutions are assayed under the method-specified operating conditions to establish system suitability. All peaks in the Class 1 system suitability solution must have a signal-to-noise ratio not less than 3, the Class 1 standard solution must have a 1,1,1-trichloroethane response greater than 5, and the resolution of acetonitrile and dichloromethane must be not less than 1 in the Class 2 Mixture A solution. When system suitability has been achieved, the test solutions are assayed along with the Class 1 and Class 2 Mixtures A and B standard solutions. If a peak is determined in the sample that matches a retention time and has a greater response than that of a corresponding reference material, then Procedure B is performed for verification of the analyte. In the second supplement of USP 30/NF 25, an exemption is made for 1,1,1-trichloroethane, where a response greater than 150 times the peak response denotes an amount above the percent daily exposure limit. Figures 2 through 4 (pages 3–4) illustrate the analysis of Class 1, Class 2 Mixture A, and Class 2 Mixture B residual solvent mixes by Procedure A. The resolution between acetonitrile and dichloromethane was easily achieved using an Rtx®-1301 column.

Continued on page 4.

Figure 2 USP residual solvent Class 1 standard solution on an Rtx®-1301 column (G43).

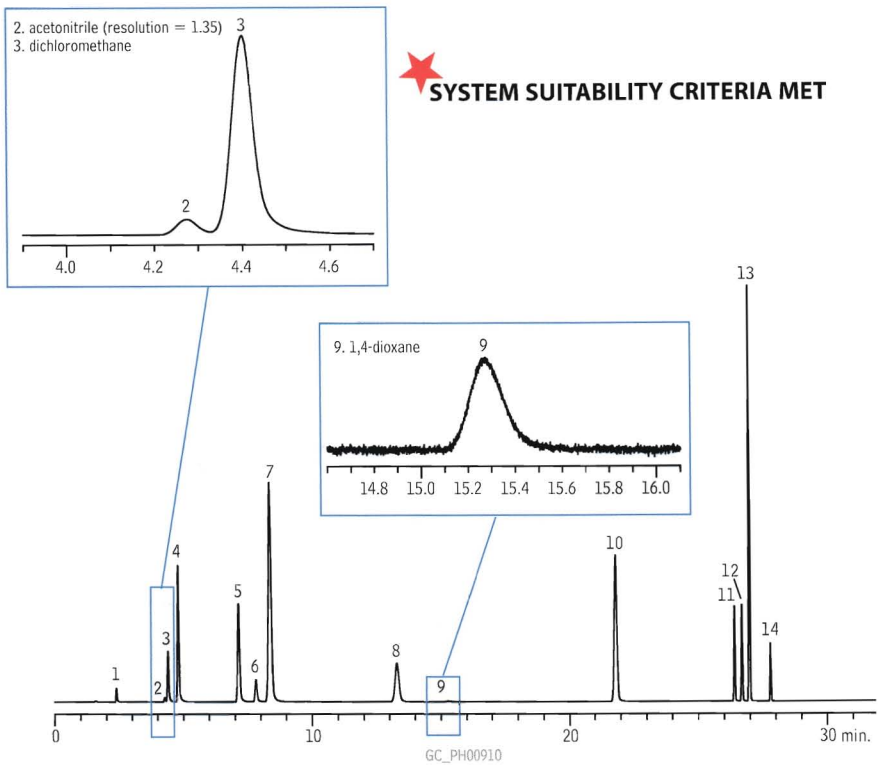


Column:	Rtx®-1301, 30m, 0.32mm ID, 1.8µm (cat.# 16092)	Headspace Conditions	
Sample:	USP <467> Class 1 standard solution (cat.# 36279) in 20mL headspace vial	Instrument:	Tekmar HT3
		Transfer line temp.:	105°C
Inj.:	headspace injection (split ratio 1:5), 1mm split liner, Siltek® deactivated (cat.# 20972-214.1)	Valve oven temp.:	105°C
		Sample temp.:	80°C
Inj. temp.:	140°C	Sample equil. time:	45 min.
Carrier gas:	helium, constant flow	Vial pressure:	10psi
Flow rate:	2.16mL/min., 35.3cm/sec.	Pressurize time:	0.5 min.
Oven temp.:	40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)	Loop fill pressure:	5psi
		Loop fill time:	2.00 min.
Det.:	FID @ 240°C	Inject time:	1.00 min.

Successfully Implement the Revised USP <467> Method

Continued from page 3.

Figure 3 USP residual solvent Class 2 Mixture A standard solution on an Rtx®-1301 column (G43).



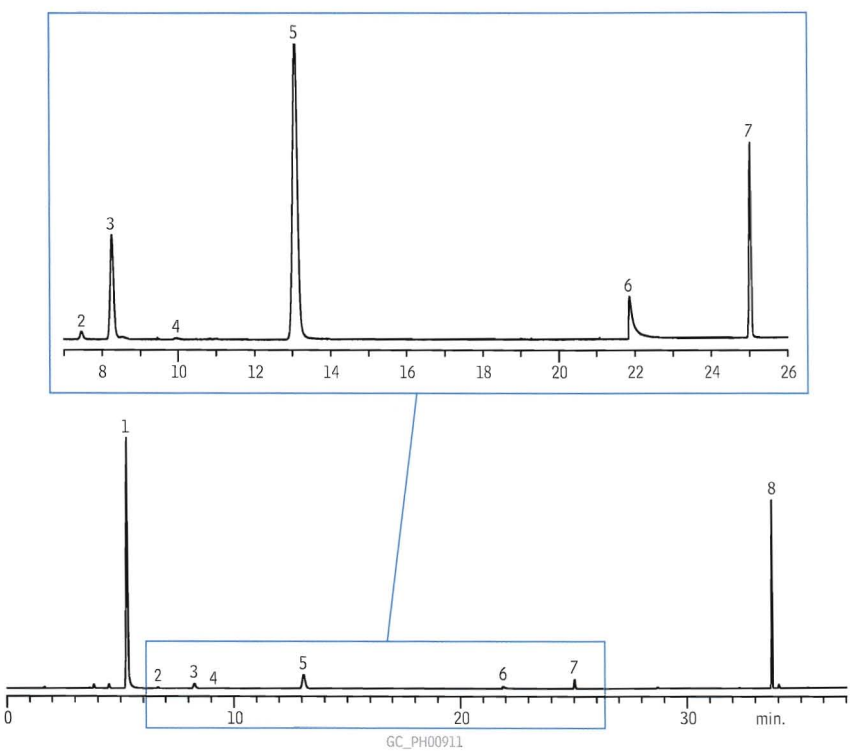
1. methanol
2. acetonitrile (resolution=1.35)
3. dichloromethane
4. *trans*-1,2-dichloroethene
5. *cis*-1,2-dichloroethene
6. tetrahydrofuran
7. cyclohexane
8. methylcyclohexane
9. 1,4-dioxane
10. toluene
11. chlorobenzene
12. ethyl benzene
13. *m*-xylene/*p*-xylene
14. *o*-xylene

Column:	Rtx®-624, 30m, 0.32 ID, 1.8µm (cat.# 10970)
Sample:	USP <467> Class 2 Mixture A standard solution (cat.# 36271) in 20mL headspace vial
Inj.:	headspace injection (split ratio 1:5), 1mm split liner, Siltek® deactivated (cat.# 20972-214.1)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.16mL/min., 35.3cm/sec.
Oven temp.:	40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)
Det.:	FID @ 240°C

Headspace Conditions

Instrument:	Tekmar HT3
Transfer line temp.:	105°C
Valve oven temp.:	105°C
Sample temp.:	80°C
Sample equil. time:	45 min.
Vial pressure:	10psi
Pressurize time:	0.5 min.
Loop fill pressure:	5psi
Loop fill time:	2.00 min.
Inject time:	1.00 min.

Figure 4 USP residual solvent Class 2 Mixture B standard solution on an Rtx®-1301 column (G43).



1. hexane
2. nitromethane
3. chloroform
4. 1,2-dimethoxyethane
5. trichloroethylene
6. pyridine
7. 2-hexanone
8. tetralin

Column:	Rtx®-624, 30m, 0.32mm ID, 1.8µm (cat.# 10970)
Sample:	USP <467> Class 2 Mixture B standard solution (cat.# 36280) in 20mL headspace vial
Inj.:	headspace injection (split ratio 1:5), 1mm split liner Siltek® deactivated (cat.# 20972-214.1)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.16mL/min., 35.3cm/sec.
Oven temp.:	40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)
Det.:	FID @ 240°C

Headspace Conditions

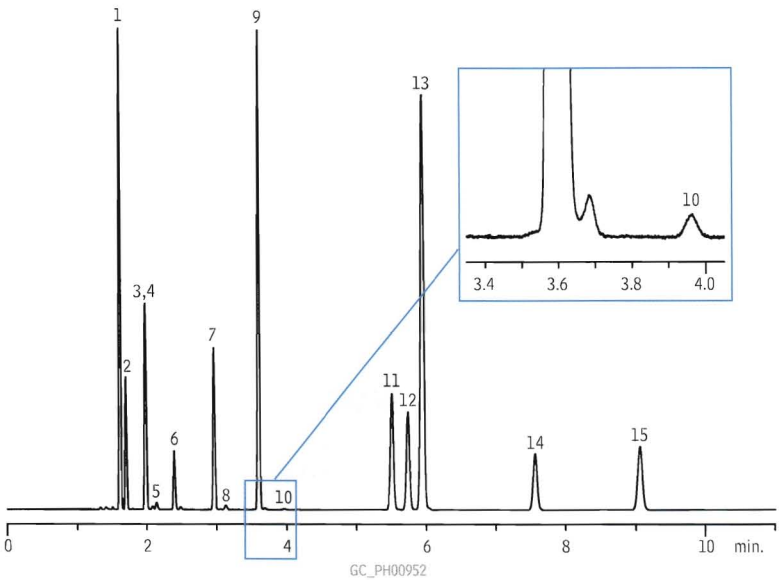
Instrument:	Tekmar HT3
Transfer line temp.:	105°C
Valve oven temp.:	105°C
Sample temp.:	80°C
Sample equil. time:	45 min.
Vial pressure:	10psi
Pressurize time:	0.5 min.
Loop fill pressure:	5psi
Loop fill time:	2.00 min.
Inject time:	1.00 min.

Successfully Implement the Revised USP <467> Method

Continued from page 5.

Figure 6 USP residual solvent Class 2 Mixture A standard solution on a Stabilwax® column (G16).

SYSTEM SUITABILITY CRITERIA MET—
RESOLUTION BETWEEN PEAKS 7 & 8 > 1.0



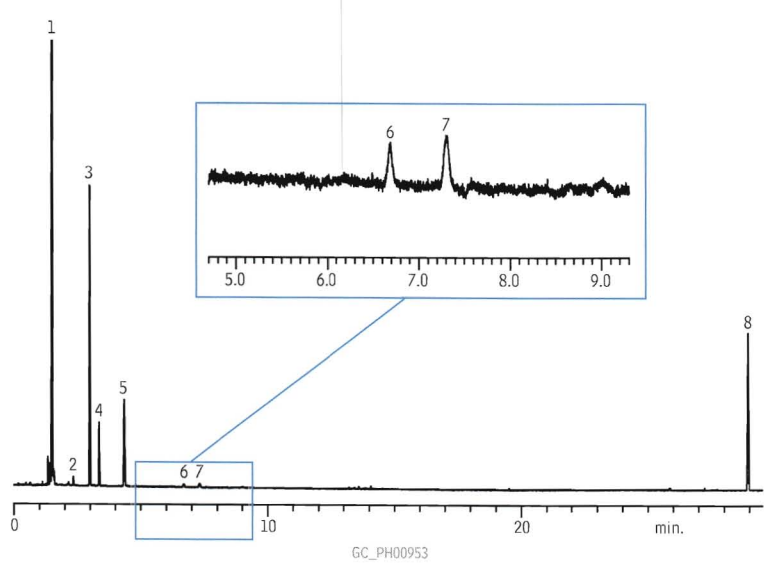
1. cyclohexane
2. methylcyclohexane
3. *trans*-1,2-dichloroethene
4. tetrahydrofuran
5. methanol
6. dichloromethane
7. *cis*-1,2-dichloroethene
8. acetonitrile
9. toluene
10. 1,4-dioxane
11. ethyl benzene
12. *p*-xylene
13. *m*-xylene
14. *o*-xylene
15. chlorobenzene

Column:	Stabilwax®, 30m, 0.32mm ID, 0.25µm (cat.# 10624)
Sample:	USP Stock Standard Residual Solvents Class 2 Mix A (cat.# 36271) in 20mL headspace vial (cat.# 24685), water diluent
Inj.:	headspace injection (split ratio 1:5), 2mm splitless liner IP deactivated (cat.# 20712)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.15mL/min., 35.2cm/sec.
Oven temp.:	50°C for 20 min. to 165°C @ 6°C/min. (hold for 20 min.)
Det.:	FID @ 250°C

Headspace Conditions

Instrument:	Overbrook Scientific HT200H
Syringe temp.:	100°C
Sample temp.:	80°C
Sample equil. time.:	45 min.
Injection vol.:	1.0mL
Injection speed:	setting 8
Injection dwell:	5 sec.

Figure 7 USP residual solvent Class 2 Mixture B standard solution on a Stabilwax® column (G16).



1. hexane
2. 1,2-dimethoxyethane
3. trichloroethylene
4. chloroform
5. 2-hexanone
6. nitromethane
7. pyridine
8. tetralin

Column:	Stabilwax®, 30m, 0.32mm ID, 0.25µm (cat.# 10624)
Sample:	USP Stock Standard Residual Solvents Class 2 Mix B (cat.# 36272) in 20mL headspace vial (cat.# 24685), water diluent
Inj.:	headspace injection (split ratio 1:5), 2mm splitless liner IP deactivated (cat.# 20712)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.15mL/min., 35.2cm/sec.
Oven temp.:	50°C for 20 min. to 165°C @ 6°C/min. (hold for 20 min.)
Det.:	FID @ 250°C

Headspace Conditions

Instrument:	Overbrook Scientific HT200H
Syringe temp.:	100°C
Sample temp.:	80°C
Sample equil. time.:	45 min.
Injection vol.:	1.0mL
Injection speed:	setting 8
Injection dwell:	5 sec.

Procedure B - Confirmation

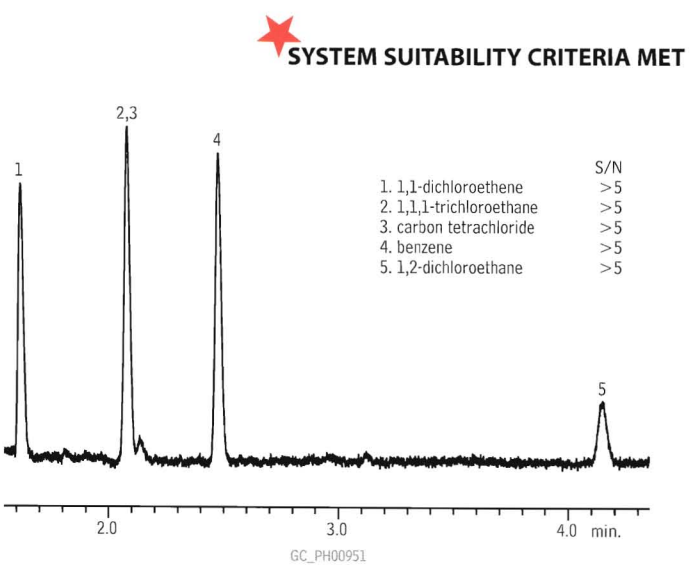
Once a residual solvent is identified and found to be above the percent daily exposure limit, Procedure B is performed to confirm analyte identity. A G16 capillary column is used here as a confirmation column, because it yields an alternate selectivity compared to a G43 column. The same standard and system suitability preparations are used in Procedures A and B. The system suitability requirements differ here in that the Class 1 standard solution must have a benzene response greater than 5 and the resolution of acetonitrile and cis-dichloroethene must not be less than 1 in the Class 2 Mixture A solution, a change from the original version. If the analyte identified in Procedure A again matches the retention time and exceeds the peak response of the reference materials (with the same exception to 1,1,1-trichloroethane), the analyst must quantify the analyte using Procedure C. Figures 5 through 7 (pages 5–6) illustrate the analysis of Class 1, Class 2 Mixture A, and Class 2 Mixture B residual solvent mixes on a Stabilwax® column. Again, the system suitability requirements were easily met.

Procedure C – Quantification

Once a residual solvent has been identified and verified, Procedure C is used to quantify the analyte by analyzing the sample against compound-specific reference materials. Individual standards are prepared by diluting the analyte in solution to a concentration of 1/20 of the concentration limit given under concentration limit Table 1 or 2 of the method. Following the procedure and instrument conditions in either Procedure A or B (whichever provides the most definitive results), a quantifiable result is produced. For water-insoluble articles, the same procedure is followed, except dimethylformamide or dimethylsulfoxide is used as the diluent.

Continued on page 6.

Figure 5 USP residual solvent Class 1 standard solution on a Stabilwax® column (G16).



Column:	Stabilwax®, 30m, 0.32mm ID, 0.25µm (cat.# 10624)	Headspace Conditions	
Sample:	USP Stock Mixture USP<467> Residual Solvents Class 1 Mix (cat.# 36279) in 20mL headspace vial (cat.# 24685), water diluent	Instrument:	Overbrook Scientific HT200H
		Syringe temp.:	100°C
		Sample temp.:	80°C
		Sample equil. time.:	45 min.
		Injection vol.:	1.0mL
Inj.:	headspace injection (split ratio 1:5), 2mm splitless liner IP deactivated (cat.# 20712)	Injection speed:	setting 8
		Injection dwell:	5 sec.
Inj. temp.:	140°C		
Carrier gas:	helium, constant flow		
Flow rate:	2.15mL/min., 35.2cm/sec.		
Oven temp.:	50°C for 20 min. to 165°C @ 6°C/min. (hold for 20 min.)		
Det.:	FID @ 250°C		

Product Listing

Capillary Column—Procedure A

Rtx®-1301 (G43) Columns (fused silica)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.80	-20 to 240°C	30-Meter	16092
0.53mm	3.00	-20 to 240°C	30-Meter	16085

Capillary Column—Procedure B

Stabilwax® Columns (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	40 to 250°C	30-Meter	10624
0.53mm	0.25	40 to 250°C	30-Meter	10625

Protect Your Data and Analytical Column with a

Restek Electronic Leak Detector

- Optimized sample flow path.
- A sleek, new ergonomic, hand-held design.
- Rugged side grips for added durability.
- Handy probe storage for cleanliness.
- Longer lasting battery, up to 6 hours of continuous use.
- Automatic shut-off capabilities.
- A convenient carrying and storage case.
- A universal charger set (US, European, UK and Australian plugs included).

Detect small leaks before they become a big problem.

Did you ever have a small leak turn into a costly repair? Protect your data and analytical column by using a Restek Leak Detector. Backed by a 1 year warranty, the new Restek Leak Detector will again set an industry standard for performance and affordability in hand-held Leak Detectors.

Table I Leak Detector Facts

Detectable gases:	helium, nitrogen, argon, carbon dioxide, hydrogen
Battery:	rechargeable Ni-MH internal battery pack (6 hours normal operation)
Operating Temp. Range:	32°-120°F (0°-48°C)
Humidity Range:	0-97%
Warranty:	one year
Certifications:	CE, Japan
Compliance:	WEEE, RoHS

Table II Limits of Detection

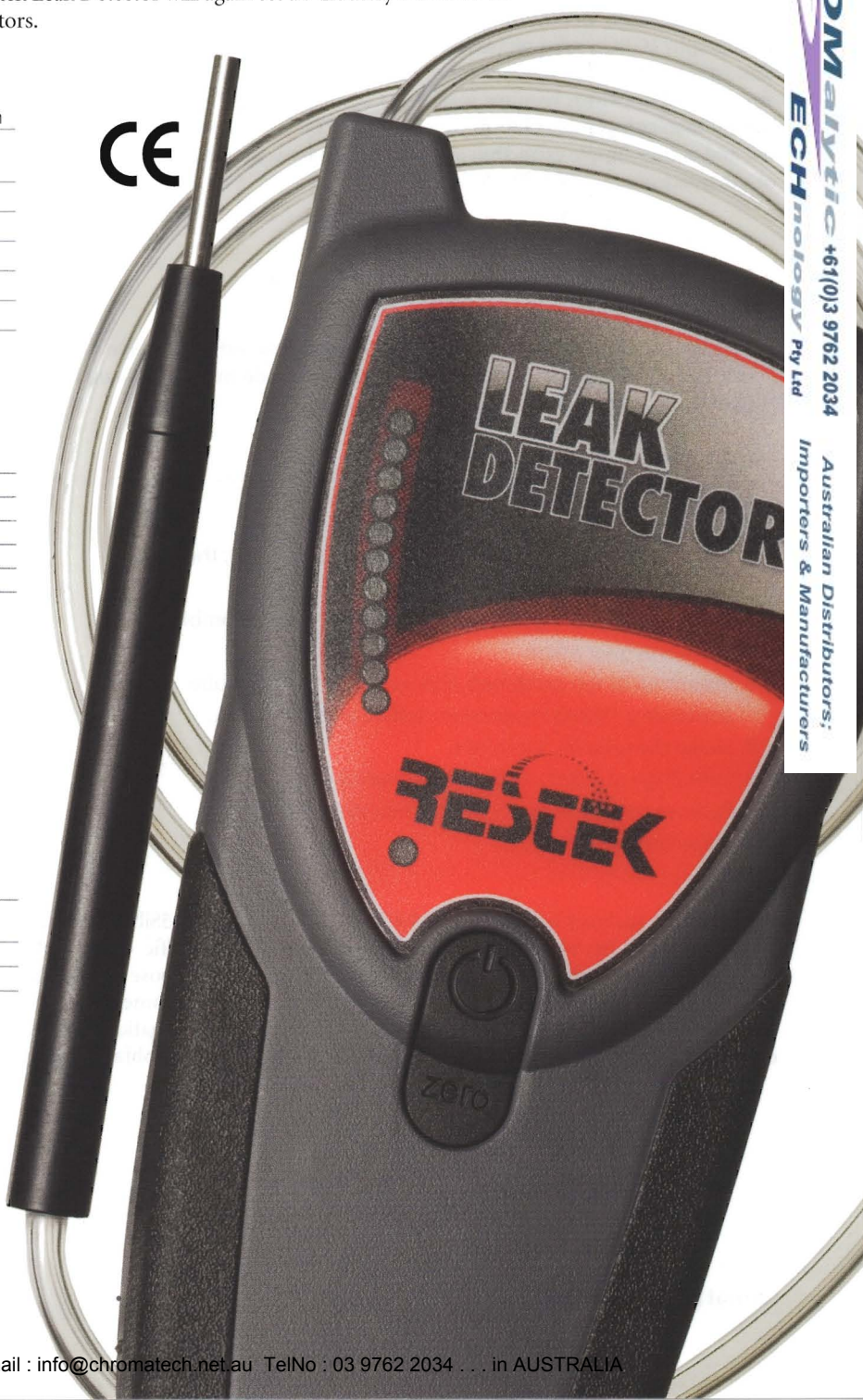
Gas	Minimum Detectable Leak Rate (atm cc/sec.)	Indicating LED Light Color
Helium	1.0×10^{-5}	Red
Hydrogen*	1.0×10^{-5}	Red
Nitrogen	1.4×10^{-3}	Yellow
Argon	1.0×10^{-4}	Yellow
Carbon Dioxide	1.0×10^{-4}	Yellow

Carrying/storage case included with purchase of unit.

Description	qty.	cat.#
Leak Detector with Universal Charger Set (US, UK, European, Australian)	ea.	22839
Soft-Side Carry/Storage Case	ea.	22657
Small Probe Adaptor	ea.	22658

Avoid using liquid leak detectors on a capillary system! Liquids can be drawn into the system.

† Caution: The Restek Electronic Leak Detector is NOT designed for determining leaks in a combustible environment. A combustible gas detector should be used for determining combustible gas leaks under any condition. The Restek Electronic Leak Detector may be used for determining trace amounts of hydrogen in a GC environment only.



Global RESTEK Advantage

Simplify and Speed Up Sample Preparation With Resprep dSPE tubes!

Here we show the extraction and clean-up of pesticide residues from olive oil samples—twice as fast as GPC, with only a fraction of the solvent required for conventional SPE.

Olive oil is considered a healthy fat source and is a staple in many recommended diets. However, concerns about potentially negative health effects associated with pesticide residues have increased consumer interest in testing. While organophosporus pesticides are currently used in olive orchards to control pests, organochlorine pesticides are still tested for as persistent organic pollutants (residues), even though they are no longer in commercial use. There are several existing methods for measuring pesticide residues in olive oil, all of which involve sample extraction and clean-up.[1] The common goal of these methods is to remove lipids that are harmful to the analytical system.[2] Efficient sample clean-up procedures are critical to maximizing sample throughput and minimizing labor and material costs. Here we demonstrate the efficiency of a dSPE clean-up procedure, as well as the capabilities of both method-specific and general purpose analytical columns.

Simple Procedure Uses Half the Time and Minimal Solvent

Sample extraction and clean-up can be accomplished with gel permeation chromatography (GPC), solid phase extraction (SPE), or dispersive solid phase extraction (dSPE) methods. However the dSPE method shown here is much less expensive than GPC (which requires specialized equipment) and uses substantially less solvent than comparable GPC or SPE methods (Table I).[3] The method is simple to use and allows sample extraction and clean-up to be accomplished in half the time of other techniques (Table II).

Extraction and dSPE Clean-up for Pesticide Residues in Olive Oil

Test sample: A 1.5mL sample of commercially obtained virgin olive oil was spiked with a standard organochlorine pesticide mix. The spiked sample was processed as follows.

1. Dilute with 1.5mL hexane.
2. Add 6mL of acetonitrile (ACN).
3. Mix for 30 minutes on a shaker.
4. Allow layers to separate (approximately 20 minutes), then collect the top (ACN) layer.
5. Repeat the liquid-liquid extraction (steps 2-4) and combine both ACN extract layers.
6. Place 1mL of the combined ACN extract in a 1.5mL tube containing 150mg magnesium sulfate and 50mg PSA.
7. Shake the tube for 2 minutes.
8. Centrifuge at 3,000 U/min. for approximately 5 minutes.
9. Remove the top layer and inject directly into the gas chromatograph system.

Extracts were analyzed using both Rtx®-CLPesticides2 and Rxi®-5Sil MS columns (Figure 1). The Rtx®-CLPesticides2 column is a method specific column that resolves all compounds. The Rxi®-5Sil MS column is a general purpose column that has one coelution that can easily be extracted by a mass spectrometer detector (MSD). Only α-BHC was not detected, a subject of further investigation, however either column can be used effectively. Recoveries of 70%-80% were obtained, levels

Table I Resprep dSPE method uses 42% and 89% less solvent than SPE and GPC methods respectively.

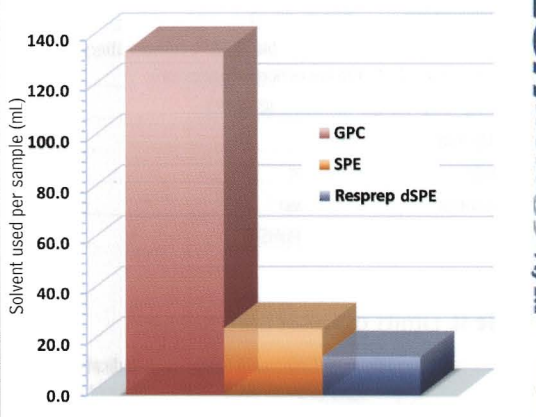


Table II Cut extraction/clean-up time by 50% using Resprep dSPE method.

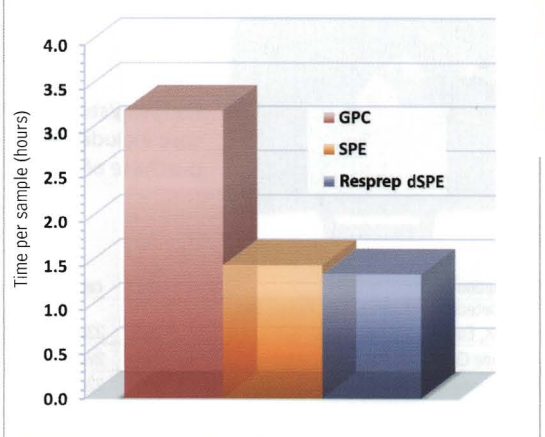
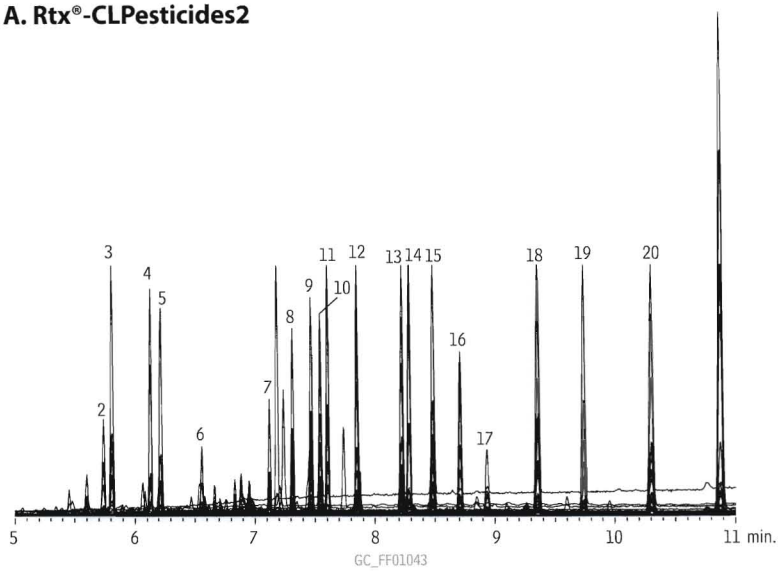
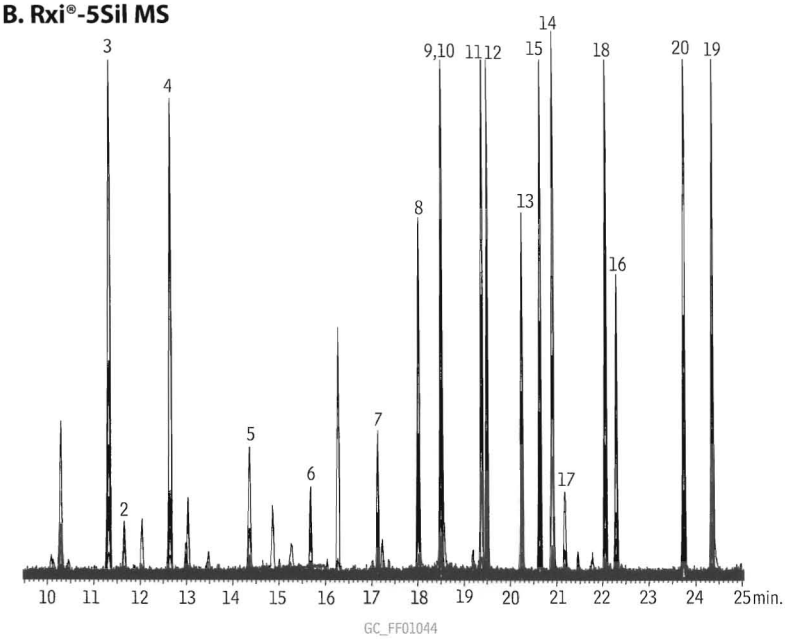


Figure 1 Chlorinated pesticide residues in olive oil are easily separated on either Rtx®-CLPesticides 2 or Rxi®-5Sil MS columns.

A. Rtx®-CLPesticides2



B. Rxi®-5Sil MS



Column:	Rtx®-CLPesticides2, 30m, 0.25mm ID, 0.20µm (cat.# 11323)
Sample:	10µg/mL Organochlorine Pesticide Mix AB # 3 (cat.# 32415) in olive oil
Inj.:	1µL, splitless (hold 0.5 min.), 3.5mm single gooseneck liner (cat.# 20962) packed with wool
Inj. temp.:	225°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	140°C (hold 0.5 min.) to 268°C @ 20°C/min. to 290°C @ 3°C/min. to 330°C (hold 5 min.) @ 20°C/min.
Det:	MS
Transfer line temp.:	320°C
Ionization:	EI
Mode:	SIM

Compound	Quant. ion	Q1	Q3
*1. α-BHC	219	181	109
2. γ-BHC	219	181	109
3. β-BHC	219	181	109
4. δ-BHC	219	181	109
5. heptachlor	272	237	100
6. aldrin	263	293	220
7. heptachlor epoxide	263	237	81
8. γ-chlordane	272	237	65
9. α-chlordane	272	237	65
10. endosulfan I	195	207	241
11. 4,4'-DDE	246	318	176
12. dieldrin	79	263	277
13. endrin	263	281	81
14. 4,4'-DDD	235	165	199
15. endosulfan II	195	207	-
16. 4,4'-DDT	235	165	199
17. endrin aldehyde	67	250	345
18. endosulfan sulfate	272	229	239
19. methoxychlor	227	274	-
20. endrin ketone	67	317	281
* not present			

comparable to conventional SPE—without the necessity of vacuum manifolds or high pressure systems. The GPC method attained recoveries of > 95%. However this method requires large amounts of solvent and takes over twice as long as other methods.

The dSPE method shown here is an efficient, cost-effective way to clean up chlorinated pesticide residues in olive oil. With good recoveries and minimal matrix interference, it is an easy way to reduce solvent usage, compared to conventional SPE, and is more cost-effective than GPC.

References
1. C. Lentza-Rizos, E.J. Avramides, Rev. Environ. Contam. Toxicol. 141 (1995) 111.
2. S. Cunha, S. Lehotay, K. Mastovska, J. Sep. Sci. 30 (2007) 620.
3. M. Crawford, M. Halvorson, J. Stevens, The Examination and Automation of GPC, SPE and QuEChERS Utilized in Extracting Pesticides from Olive Oil. HPLC 2008 poster presentation.

Product Listing

dSPE Tube for Clean-Up of Pesticide Residue Samples

Description	Material	Methods	qty.	cat#
2mL Micro-Centrifuge Tubes for dSPE				
Resprep Q250	150mg $MgSO_4$, 50mg PSA	AOAC 2007.1	100-pk.	26124

PSA—primary and secondary amine exchange material.

Organochlorine Pesticide Mix AB # 3

(20 components)

aldrin	dieldrin
α-BHC	endosulfan I
β-BHC	endosulfan II
δ-BHC	endosulfan sulfate
γ-BHC (lindane)	endrin
α-chlordane	endrin aldehyde
γ-chlordane	endrin ketone
4,4'-DDD	heptachlor
4,4'-DDE	heptachlor epoxide (isomer B)
4,4'-DDT	methoxychlor

2,000µg/mL each in hexane:toluene (1:1), 1mL/ampul
cat. # 32415 (ea.)

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.20	-60 to 320/340°C	30-Meter	11323

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623

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Beyond C18—Increase Retention of Hydrophilic Compounds Using Biphenyl Columns

*Searching for a better way to retain hydrophilic aromatic drug compounds? Biphenyl phases, such as the **Pinnacle™ DB Biphenyl** column, provide greater retention than alkyl phases. Use a Biphenyl column to separate difficult-to-retain polar aromatics from unretained matrix contaminants.*

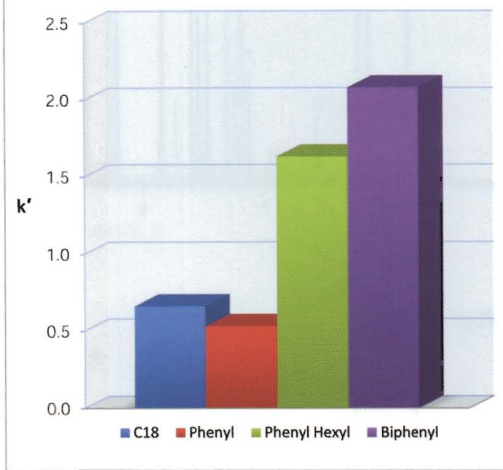
Many drug classes include compounds with aromatic ring structures, some of which also contain a sulfone or sulfoxide group. Both sulfur groups have dipole moments, adding a hydrophilic character to compounds containing these functional groups. The analysis of hydrophilic compounds on a traditional alkyl column (e.g., C18) can be problematic, since alkyl columns depend on hydrophobic (dispersive) interactions for retention. Since the sulfone and sulfoxide groups contain π bonds, the Biphenyl column's affinity toward compounds containing these bonds makes it a logical choice when increased retention of compounds containing these groups is desired.

To explore the selectivity of the biphenyl phase towards sulfur-containing aromatic compounds, phenyl sulfone, a simple probe, was analyzed on alkyl (C18), phenyl, phenyl hexyl, and Biphenyl columns to determine the relative retention of each phase, as measured by capacity factor (k'). In order to ensure separation of analytes from unretained contaminants, a minimum k' value of 2 is recommended for most analyses, however in cases where there is little to no matrix interference, a k' of 1 may be acceptable. The data in Figure 1 show that phenyl sulfone is retained to a much greater degree on the Pinnacle™ DB Biphenyl column, than on the other phases tested (k' = 2.08). This is due to the unique retention mechanism of the biphenyl stationary phase, which can interact with both the hydrophobic aromatic ring and the hydrophilic sulfone group through π-π interactions. Although the phenyl stationary phase also allows for the use of π-π interactions, the biphenyl phase has a larger electron cloud and is significantly more retentive.

To further test the retention of the Biphenyl column, a second set of probes, consisting of compounds in the NSAID family, was analyzed. Tenoxicam, which contains a sulfone group, and sulfinpyrazone, which contains a sulfoxide group, were analyzed along with a void marker (uracil). Although these compounds are more complex than the probe used in the first experiment, the same pattern of retention was observed (Figure 2). The Pinnacle™ DB Biphenyl column exhibited the greatest retention for tenoxicam. With k' values of 0.33 on the C18 and 0.49 on the phenyl columns, tenoxicam shows almost no retention on these stationary phases. The phenyl hexyl phase performed slightly better with a k' value of 1.52 for tenoxicam. However, when tenoxicam was analyzed on the Biphenyl column under the same conditions, the k' value increased to 2.22, a value much more likely to provide adequate resolution from matrix components. Sulfinpyrazone, a less polar compound, also followed the same pattern of retention (Table I).

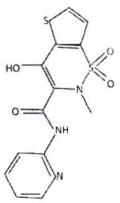
The improved retention for hydrophilic aromatics shown here is due to the unique π-π interaction retention mechanism of the Biphenyl phase. This mechanism is particularly useful for analysis of sulfone- and sulfoxide-containing drug compounds, which are not easily retained on alkyl or phenyl phases. The Biphenyl phase provides greater retention than alkyl and phenyl phases and is ideal for separating difficult-to-retain polar aromatics from unretained matrix contaminants.

Figure 1 The Biphenyl phase is more retentive for phenyl sulfone than other alkyl and phenyl phases.

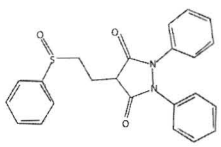


Biphenyl columns are much more effective than alkyl, phenyl, or phenyl hexyl phases when increased retention of hydrophilic aromatics is desired.

Figure 2 Only the Biphenyl phase retains both test probes to k' > 2, the level recommended to ensure separation from unretained matrix contaminants.



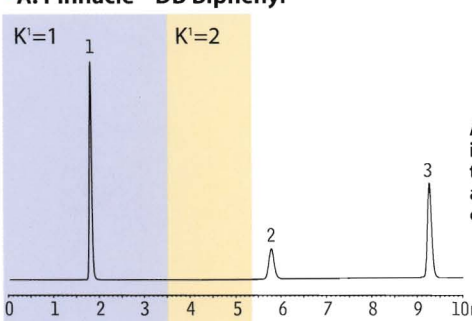
2. tenoxicam



3. sulfinpyrazone

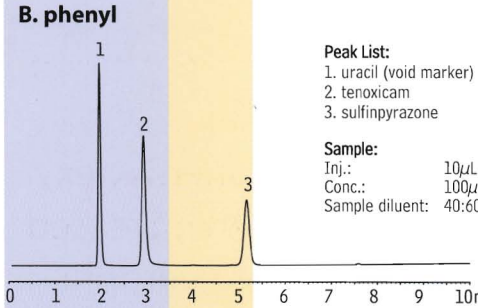
More retention with < ½ the carbon load, compared to phenyl hexyl columns

A. Pinnacle™ DB Biphenyl



A minimum k' value of 2 is generally recommended to fully separate target analytes from matrix contaminants.

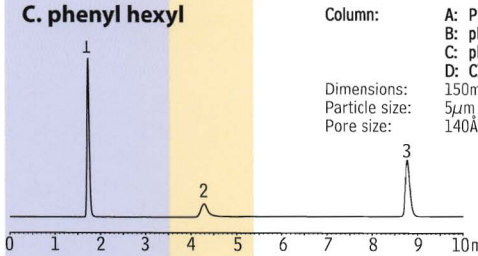
B. phenyl



Peak List:
1. uracil (void marker)
2. tenoxicam
3. sulfinpyrazone

Sample:
Inj.: 10µL
Conc.: 100µg/mL each component
Sample diluent: 40:60 water:0.1% formic acid:methanol

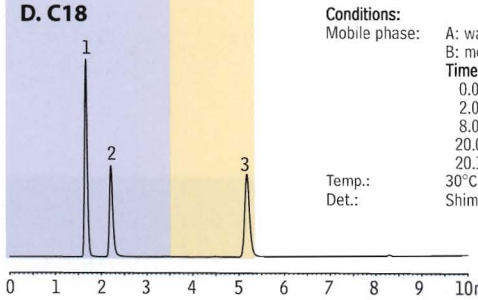
C. phenyl hexyl



Column: A: Pinnacle™ DB Biphenyl (cat.# 9409565)
B: phenyl
C: phenyl hexyl
D: C18

Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 140Å

D. C18



Conditions:
Mobile phase: A: water w/ 0.1% formic acid
B: methanol

Time (min.)	Flow (mL/min.)	%B
0.0	1.0	60
2.0	1.0	60
8.0	1.0	90
20.0	1.0	90
20.1	1.0	60

Temp.: 30°C
Det.: Shimadzu PDA (SPD-M20A) @ 254nm

Table I Biphenyl columns show improved retention of sulfone- and sulfoxide-containing aromatic drugs.

K' Value	Biphenyl	Phenyl hexyl	Phenyl	C18
Tenoxicam	2.23	1.39	0.637	0.235
Sulfinpyrazone	4.18	3.90	1.88	1.89

Product Listing

Pinnacle™ DB Biphenyl Columns (USP L11)

particle size: 1.9µm, 3µm or 5µm, spherical
pore size: 140Å
carbon load: 8%
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

3µm Column, 1.0mm	cat. #
30mm	9409331
50mm	9409351
100mm	9409311
150mm	9409361
3µm Column, 2.1mm	**cat. #**
30mm	9409332
50mm	9409352
100mm	9409312
150mm	9409362
3µm Column, 3.2mm	**cat. #**
30mm	9409333
50mm	9409353
100mm	9409313
150mm	9409363
3µm Column, 4.6mm	**cat. #**
30mm	9409335
50mm	9409355
100mm	9409315
150mm	9409365
5µm Column, 1.0mm	**cat. #**
30mm	9409531
50mm	9409551
100mm	9409511
150mm	9409561
200mm	9409521
250mm	9409571
5µm Column, 2.1mm	**cat. #**
30mm	9409532
50mm	9409552
100mm	9409512
150mm	9409562
200mm	9409522
250mm	9409572
5µm Column, 3.2mm	**cat. #**
30mm	9409533
50mm	9409553
100mm	9409513
150mm	9409563
200mm	9409523
250mm	9409573
5µm Column, 4.6mm	**cat. #**
30mm	9409535
50mm	9409555
100mm	9409515
150mm	9409565
200mm	9409525
250mm	9409575

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Global RESTEK Advantage

vol. 082

Increase Sample Throughput for Complex Drinking Water Pesticides

Using Rtx®-CLPesticides and Rtx®-CLPesticides2 Capillary Columns

- Optimized conditions cut analysis time in half, for higher sample throughput.
- Unique selectivity fully resolves complex compound list.
- Meets all method QA requirements, reducing rework.

With the advent of modern agriculture, and its vast selection of chemical pest control measures, the farming community has made significant increases in productivity and efficiency. Crop yield per acre is at an all time high, due in part to the role of pesticides and herbicides in mitigating the devastating effects of many plant and insect pests.[1] However, the use of these chemicals can have drawbacks, including surface and ground water contamination. EPA Methods, such as 508.1, are used to monitor pesticides and herbicides in drinking and ground water. The optimized dual column method shown here satisfies all method requirements in half the analysis time, significantly improving sample throughput.

Continued on page 2.

Increase Sample Throughput for Complex Drinking Water Pesticides

Continued from page 1.

EPA Method 508.1 includes many of the components as Method 505, a similar GC/ECD method, but also contains several others, expanding the list to 38 compounds. This method calls for solid phase extraction and extract concentration, followed by analysis using a GC/ECD system. In order to increase sample throughput, an optimized method was developed using a dual column configuration with the Rtx®-CLPesticides/Rtx®-CLPesticides2 column pair. These columns, used under the conditions shown, offer a unique selectivity that allows the target analytes to be resolved in approximately half the analysis time of the original method (Figure 1). There was one coelution on the primary column, but these compounds were separated on the second column. Both columns easily passed the comprehensive system performance criteria adapted from 508.1 (Table I).[2]

In conclusion, due to the complexity of the compound list in Method 508.1, a very high degree of selectivity is required of the capillary column in order to achieve adequate resolution of all target analytes in a reasonable time. The optimized dual column method shown here offers a significantly faster analysis time, while maintaining excellent resolution of challenging drinking water pesticides and herbicides.

References

1. http://www.usda.gov/nass/pubs/trackrec/track00a.htm#principal
2. US EPA Method 508.1, James W Eichelberger Rev 1.0 1994.

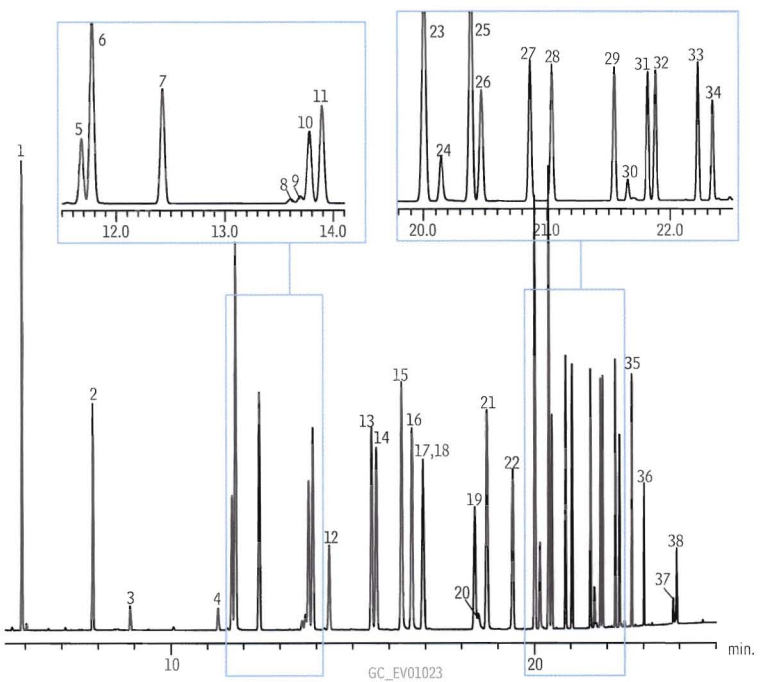
Conditions for Figure 1

Column:	Rtx®-CLPesticides2, 30m, 0.32mm ID, 0.25µm (cat.# 11324) and Rtx®-CLPesticides, 30m, 0.32mm ID, 0.32µm (cat.# 11141) with 5m x 0.32mm ID Rxi® deactivated guard tubing (cat.# 10039), connected using Universal "Y" Press-Tight® Connector (cat.# 20405-261)
Sample:	50ng/mL 508.1 Calibration Mix #1 (cat.# 32094), 100ng/mL 508.1 Calibration Mix #2 (cat.# 32095), 100ng/mL 508.1 Calibration Mix #3 (cat.# 32096), 50ng/mL 508.1 Internal Standard (cat.# 32091), 250ng/mL 508.1 Surrogate (cat.# 32092), 500ng/mL Atrazine (cat.# 32208), 500ng/mL Simazine (cat.# 32236) in ethyl acetate
Inj.:	2µL splitless (hold 0.75 min.), 4mm cyclo double gooseneck liner (cat.# 20896)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Linear velocity:	26cm/sec. @ 80°C
Oven temp.:	80°C (hold 0.5 min.) to 155°C (hold 1 min.) @ 19°C/min. to 210°C @ 4°C/min. to 310°C (hold 0.5 min.) @ 25°C/min.
Detector temp.:	ECD @ 325°C

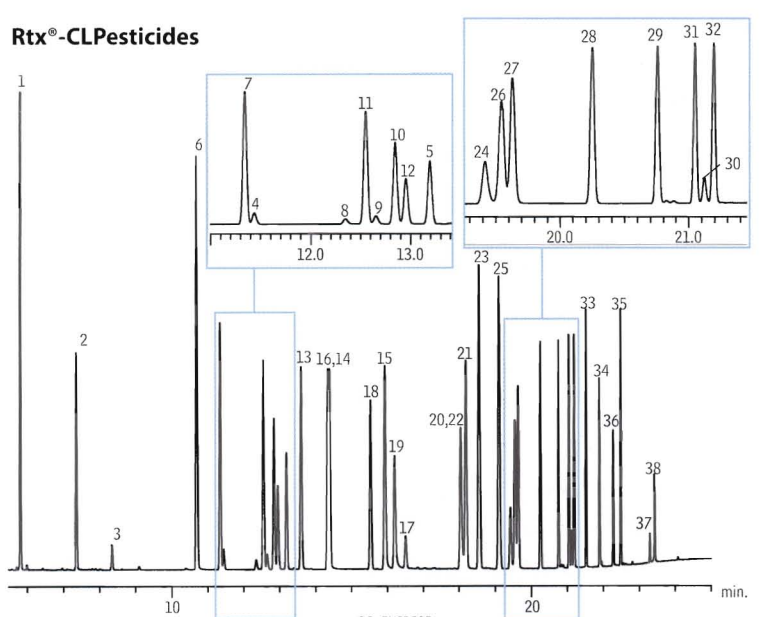
Figure 1 Resolve all critical pairs using Rtx®-CLPesticides and Rtx®-CLPesticides2 columns.

1. hexachlorocyclopentadiene
2. etridiazole
3. chlorneb
4. propachlor
5. trifluralin
6. hexachlorobenzene
7. α-BHC
8. simazine
9. atrazine
10. pentachloronitrobenzene (IS)
11. γ-BHC
12. β-BHC
13. δ-BHC
14. heptachlor
15. chlorothalonil
16. metribuzin
17. alachlor
18. aldrin
19. 4,4'-dibromobiphenyl (SS)
20. metachlor
21. DCPA
22. heptachlor epoxide
23. γ-chlordane
24. cyanazine
25. α-chlordane
26. endosulfan I
27. 4,4'-DDE
28. dieldrin
29. endrin
30. chlorobenzilate
31. 4,4'-DDD
32. endosulfan II
33. 4,4'-DDT
34. endrin aldehyde
35. endosulfan sulfate
36. methoxychlor
37. *cis*-permethrin
38. *trans*-permethrin

Rtx®-CLPesticides2



Rtx®-CLPesticides



Satisfy all method requirements in half the time!

Table I Rtx®-CLPesticides and Rtx®-CLPesticides2 columns easily pass EPA Method 508.1 performance criteria.

Test/Requirement	Analyte	Concentration (ppb)	Rtx®-CLPesticides2	Rtx®-CLPesticides
Inertness (breakdown <20%)	endrin	50	0.9%	1.4%
Inertness (breakdown <20%)	4,4'-DDE	100	1.0%	1.1%
Sensitivity (S/N>3)	chlorpyrifos	2	12.0	6.2
Chromatographic performance (0.8<PGF<1.15)	DCPA	50	1.03	1.06
Column performance (resolution>0.50)	chlorothalonil	50	9.9	26.8
Column performance (resolution>0.50)	gamma-BHC	40	9.9	26.8

Rxi® Guard/Retention Gap Columns (fused silica)

Nominal ID	Nominal OD	5-Meter	5-Meter/6-pk.	10-Meter	10-Meter/6-pk.
0.25mm	0.37 ± 0.04mm	10029	10029-600	10059	10059-600
0.32mm	0.45 ± 0.04mm	10039	10039-600	10064	10064-600
0.53mm	0.69 ± 0.05mm	10054	10054-600	10073	10073-600

Universal "Y" Press-Tight® Connectors

Description	ea.	3-pk.
Universal "Y" Press-Tight Connector	20405	20406
Deactivated Universal "Y" Press-Tight Connector	20405-261	20406-261
Siltek Treated Universal "Y" Press-Tight Connector	20485	20486

Rtx®-CLPesticides Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.32	-60 to 320/340°C	30-Meter	11141

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	-60 to 320/340°C	30-Meter	11324

508.1 Calibration Mix #1 (17 components)

aldrin
α-BHC
β-BHC
δ-BHC
γ-BHC (lindane)
4,4'-DDD
4,4'-DDE
4,4'-DDT
dieldrin
endosulfan I
endosulfan II
endosulfan sulfate
endrin
endrin aldehyde
heptachlor
heptachlor epoxide (isomer B)
methoxychlor

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32094

508.1 Calibration Mix #2 (11 components)

chlorobenzilate
α-chlordane
γ-chlordane
chlorneb
DCPA (Dacthal®)
etridiazole
hexachlorobenzene
cis-permethrin*
trans-permethrin*
propachlor
trifluralin

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32095

*1000µg/mL total permethrin. Exact content of each isomer listed on certificate of analysis.

508.1 Calibration Mix #3 (8 components)

alachlor
atrazine
chlorthalonil
cyanazine
hexachlorocyclopentadiene
metolachlor
metribuzin
simazine

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32096

508.1 Internal Standard

pentachloronitrobenzene
100µg/mL in ethyl acetate, 1mL/ampul
cat. # 32091

508.1 Surrogate

4,4'-dibromobiphenyl
500µg/mL in ethyl acetate, 1mL/ampul
cat. # 32092

Atrazine

1,000µg/mL in acetone, 1mL/ampul
cat. # 32208

Simazine

1,000µg/mL in acetone, 1mL/ampul
cat. # 32236

Splitless Liners for Agilent

ID* x OD & Length	qty.	cat.#
Cyclo Double Gooseneck (4mm)		
4.0mm x 6.5mm x 78.5mm	5-pk.	20896

*Nominal ID at syringe needle expulsion point.

Resprep™-C18 SPE Disks

Description	qty.	cat.#
Resprep™-C18 47mm SPE Disks	20-pk.	24004

Fast, Simple Sample Cleanup

Using QuEChERS SPE Tubes

- Achieve a four-fold increase in sample throughput.
- Significantly reduce material costs.
- Convenient, ready to use centrifuge tubes with ultra pure, pre-weighed adsorbent mixtures.

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe, the QuEChERS ("catchers") method for extracting pesticides from food is based on researcl by the US Department of Agriculture.[1] In addition to using less solvent and materials versus conventional SPE methods, QuEChERS employ a novel and much quicker dispersive solid phase extraction cleanup (dSPE). QuEChERS methods, including an AOAC Official Method[2] anc modifications to the methods, have been posted on the Internet.[3] These methods have several basic steps in common:

Step 1: Sample preparation and extraction– Commodities are uniformly comminuted. Acetonitrile solvent is added for a shake extraction. Salts, acids and buffers may be added to enhance extraction efficiency and protect sensitive analytes. Surrogate standards can be added to monitor extraction efficiencies.

Step 2: Extract cleanup – A subsample of solvent extract is cleaned up using dSPE, a key improvement incorporated in the QuEChERS technique. Small polypropylene centrifuge tubes are prefilled with precise weights of $MgSO_4$ and SPE adsorbents to remove excess water and unwanted contaminants from the extracted samples. After agitation and centrifugation, the cleaned extracts are ready for analysis.

Step 3: Sample analysis – Samples may be pH adjusted to protect sensitive pesticides and/or solvent-exchanged to improve analysis by either GC/MS or LC/MS. Internal standards can be added.

QuEChERS methods are convenient, rugged methods that simplify extract cleanup, reduce material costs, and improve sample throughput. Here we demonstrate the effectiveness of QuEChERS sample cleanup using a multiresidue analysis of pesticides on strawberries.

Experimental

Strawberry extracts were prepared, spiked, and dSPE treated according to Table I. Analytical conditions are presented in Table II.

One microliter splitless injections of the extracts were performed by a Shimadzu AOC-20i autosampler using "mid" injection speed into a Shimadzu QP-2010 Plus GC-MS system operated under the conditions in Table II.

Table I Modified mini-multiresidue QuEChERS for pesticides from strawberries.

Sample preparation and extraction	
Sample:	10g of strawberries were homogenized and placed in a 50mL PTFE centrifuge tube
Solvent:	10mL of acetonitrile were added to homogenate Shake for 1 minute, until uniform
Salts:	4.0g MgSO4 (powder or granular) 1.1.0g NaCl 1.0g trisodium citrate dihydrate 0.5g disodium hydrogencitrate sesquihydrate Salts were added and vigorously shaken for 1 minute. Sample was centrifuged and the supernatant removed for cleanup. Pesticides standards (200ng/mL) were spiked in at this point.
Sample extract cleanup	
QuEChERS tubes:	1mL of supernatant from the previous step was placed into several 2mL polypropylene centrifuge tubes, each containing one of the following adsorbent mixes: A. 50mg PSA + 150mg $MgSO_4$ (cat.# 26124) B. 50mg PSA + 150mg $MgSO_4$ + 50mg C18 (cat.# 26125) C. 50mg PSA + 150mg $MgSO_4$ + 50mg GCB (cat.# 26123)
Cleanup:	Samples were shaken with the adsorbents for 30 seconds (carbon for 2 minutes), then centrifuged to produce a clear supernatant for GC/MS analysis.
Internal standard:	Pentachloronitrobenzene in a formic acid solution, pH 5.

PSA—primary and secondary amine exchange material.
GCB—graphitized carbon black

Table II Instrument conditions.

Column:	Rtx®-CLPesticides2 20m, 0.18mm ID, 0.14µm (cat.# 42302)
Sample:	custom pesticide mix 200µg/mL each pesticide, internal standards: 8140-8141 ISTD, 1000µg/mL (cat.# 32279), 508.1 ISTD 100µg/mL (cat.# 32091), triphenylphosphate 1000µg/mL (cat.# 32281)
Inj.:	1.0µL splitless (hold 1 min.)
Inj. temp.:	250°C
Carrier gas:	helium
Flow rate:	constant linear velocity @ 40cm/sec
Oven temp.:	40°C (hold 1 min.) to 320°C @ 12°C/min.
Det:	Shimadzu GCMS-QP2010 Plus
Transfer line temp.:	300°C
Ionization:	Electron ionization
Mode:	Selected ion monitoring

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.14	-60 to 310/330°C	20-Meter	42302

Results and Discussion

Primary and secondary amine exchange material (PSA) is the base sorbent used for dSPE cleanup of QuEChERS fruit and vegetable extracts because it removes many organic acids and sugars that might act as instrumental interferences.

A pesticide-spiked strawberry extract (200ng/mL) subjected to dSPE with PSA was used to generate one-point calibration curves. Spiked strawberry extracts subjected to additional dSPE sorbents were analyzed and the results versus PSA dSPE are shown as percent recoveries in Table III. C18 is suggested for use when samples might contain fats; not an issue for a strawberry extract, but it was important to verify that gross losses of more hydrophobic pesticides (e.g. Endrin and DDT) would not occur. GCB is used to remove pigments, and when treated, the pink/red strawberry extract became clear. However, GCB can also have a negative effect on certain pesticides, especially those that can assume a planar shape like chlorothalonil and thiabendazole.

Restek dSPE products in a variety of standard sizes and formats make QuEChERS even simpler. The centrifuge tube format, available in 2mL and 15mL sizes, contains magnesium sulfate (to partition water from organic solvent) and a choice of SPE sorbents, including PSA (to remove sugars and fatty acids), C18 (to remove nonpolar interferences such as fats), and GCB (to remove pigments and sterols). Custom products also are available by request. If you are frustrated by the time and cost involved with your current approach to pesticide sample cleanup, we suggest you try this simple and economical new method.

References

1. Michelangelo Anastassiades, Steven J. Lehotay, Darinka Štajnbaher, Frank J. Schenck. "Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and Dispersive Solid-Phase Extraction for the Determination of Pesticide Residues in Produce." *J. AOAC International*, 2003, vol. 86(22), pp.412-431.
2. AOAC Official Method 2007.01, "Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate."
3. http://www.quechers.com/

References not available from Restek

Table III Pesticide percent recoveries in strawberry extracts treated with C18 or GCB dSPE, relative to PSA only.

Rt (min.)	pesticide	CAS Number	action/Use	classification	C18*	GCB**
9.50	Dichlorvos	62-73-7	Insecticide	Organophosphorus	111	116
9.67	Methamidophos	10265-92-6	Insecticide	Organophosphorus	105	107
11.75	Mevinphos	7786-34-7	Insecticide	Organophosphorus	112	130
12.02	*o*-Phenylphenol	90-43-7	Fungicide	Unclassified	106	97
12.14	Acephate	30560-19-1	Insecticide	Organophosphorus	128	147
13.89	Omethoate	1113-02-6	Insecticide	Organophosphorus	120	119
14.74	Diazinon	333-41-5	Insecticide	Organophosphorus	108	127
14.98	Dimethoate	60-51-5	Insecticide	Organophosphorus	124	151
15.69	Chlorothalonil	1897-45-6	Fungicide	Organochlorine	125	13
15.86	Vinclozolin	50471-44-8	Fungicide	Organochlorine	102	98
16.21	Metalaxyl	57837-19-1	Fungicide	Organonitrogen	105	117
16.28	Carbaryl	63-25-2	Insecticide	Carbamate	114	111
16.60	Malathion	121-75-5	Insecticide	Organophosphorus	124	160
16.67	Dichlofluanid	1085-98-9	Fungicide	Organohalogen	122	103
17.51	Thiabendazole	148-79-8	Fungicide	Organonitrogen	88	14
17.70	Captan	133-06-2	Fungicide	Organochlorine	88	91
17.76	Folpet	133-07-3	Fungicide	Organochlorine	108	63
18.23	Imazalil	35554-44-0	Fungicide	Organonitrogen	115	95
18.39	Endrin	72-20-8	Insecticide	Organochlorine	104	101
18.62	Myclobutanil	88671-89-0	Fungicide	Organonitrogen	119	114
19.07	4,4-DDT	50-29-3	Insecticide	Organochlorine	102	95
19.22	Fenhexamid	126833-17-8	Fungicide	Organochlorine	118	77
19.40	Propargite 1	2312-35-8	Acaricide	Organosulfur	110	95
19.43	Propargite 2	2312-35-8	Acaricide	Organosulfur	121	114
19.75	Bifenthrin	82657-04-3	Insecticide	Pyrethroid	106	81
20.04	Dicofol	115-32-2	Acaricide	Organochlorine	98	54
20.05	Iprodione	36734-19-7	Fungicide	Organonitrogen	118	90
20.21	Fenpropathrin	39515-41-8	Insecticide	Pyrethroid	113	96
21.32	*cis*-Permethrin	52645-53-1	Insecticide	Pyrethroid	106	65
21.47	*trans*-Permethrin	51877-74-8	Insecticide	Pyrethroid	109	71
23.74	Deltamethrin	52918-63-5	Insecticide	Pyrethroid	97	52

*50mg PSA, 50mg C18, **50mg PSA, 50mg GCB

$$\% \text{ recovery} = \frac{\text{RRF C18 or GCB}}{\text{RRF PSA}} \times 100$$

QuEChERS SPE Tubes

AOAC Method 2007.1	Benefits/Uses	qty.	cat#
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate and 50mg PSA	Cleanup of agricultural produce extracts, 1mL sample volume.	100-pk.	26124
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate, 50mg PSA, and 50mg Graphitized Carbon	Cleanup of 1mL sample extract with residual pigments and sterols.	100-pk.	26123
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate, 50mg PSA, and 50mg C18	Cleanup of 1mL sample extract with residual fat.	100-pk.	26125
15mL QuEChERS SPE Centrifuge Tube Contains 900mg Magnesium Sulfate, 300mg PSA, and 150mg Graphitized Carbon	Cleanup of 6mL sample extract with residual pigments and sterols.	50-pk.	26126

PSA—primary and secondary amine exchange material.

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Easy Transfer of HPLC Methods to UHPLC

Using Fully Scalable Pinnacle™ DB Columns

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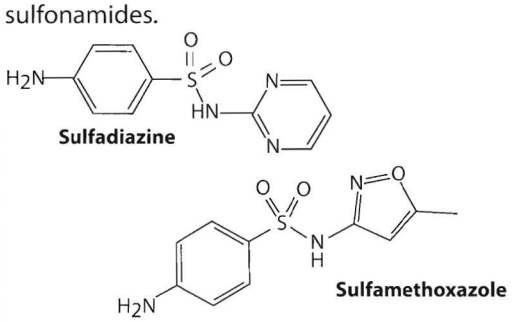
- Pinnacle™ DB columns are 100% Restek manufactured–from base silica to final packed column.
- Restek offers the widest selection of stationary phases for UHPLC—more choices mean better selectivity for your analytes.

Ultra High Pressure Liquid Chromatography (UHPLC) is a rapidly growing technique that produces significantly faster analysis times compared to conventional HPLC. While transferring HPLC methods to UHPLC can increase sample throughput, comparable method parameters must be used to maintain equivalent separations. Here we review which column properties and operating conditions should remain consistent and which need to be optimized in order to maintain selectivity.

In this example, we will perform a scale-down method transfer for sulfonamides (Figure 1). For optimal selectivity and faster analysis times, we used a Pinnacle™ DB Biphenyl stationary phase for this application (Figure 2). When performing a scale-down procedure, column pore size, carbon load, and support material must remain the same. Changes to other parameters can be made using a few simple calculations. Let's go through them sequentially.

Figure 1 Chemical structures for example sulfonamides.



Adjusting Column Size

The first calculation determines the appropriate column length. Keeping the same column length while decreasing the particle size increases the number of theoretical plates. Therefore, column length can be shortened without losing resolution. By adjusting the column length properly, using Equation 1, we can maintain the same separation.

Adjusting Injection Volume

Once we have determined the proper column length, we can calculate injection volume. Decreasing the column internal diameter and length decreases the overall column volume and sample capacity. Therefore, we must alter the injection volume as described in Equation 2. Note that since overall column volume has decreased, it is important to match the sample solvent to the starting mobile phase composition. Mismatched sample solvents can cause irreproducible retention times, efficiencies, and even changes in selectivity.

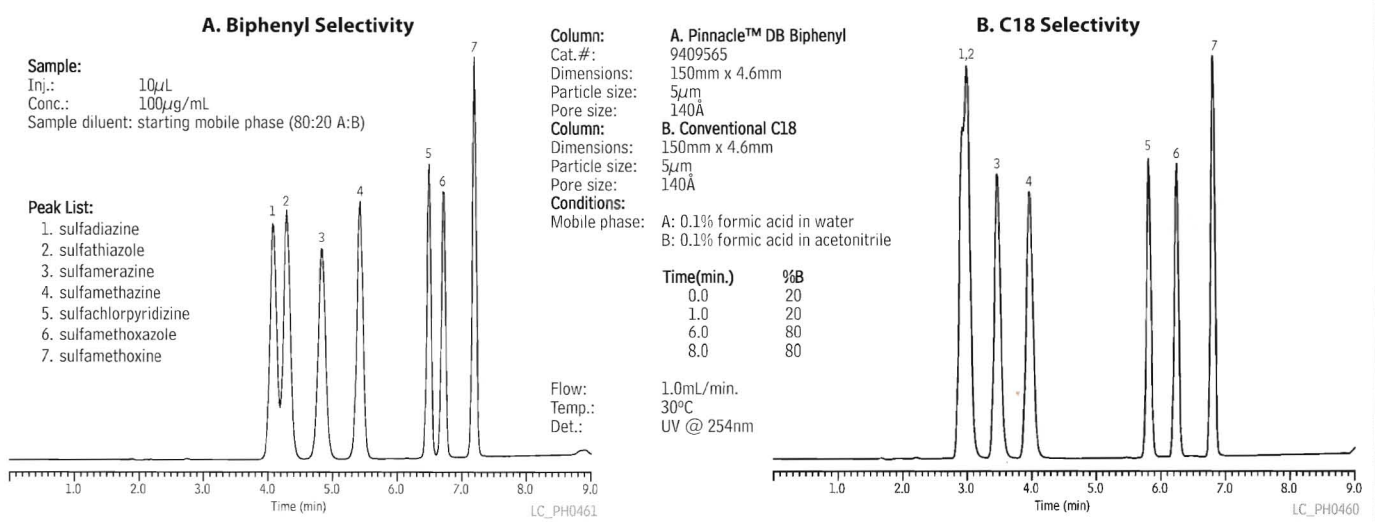
Adjusting Flow rate

Next, flow rate must be adjusted to maintain comparable linear velocity through a column with smaller internal diameter. To maintain the same linear velocity (which is important in maintaining efficiencies), flow rates must be decreased. Also, since smaller particle sizes give rise to higher optimal linear velocities, isocratic flow rates should be calculated with particle size taken into account. In this example, a gradient elution was used and, therefore, particle size was not included in the equation. Equation 3 can be used to estimate the adjusted flow rate needed for equivalent chromatography. Also, note that since <2µm particle sizes are less affected by flow rate, faster flow rates can be used in isocratic systems without detrimental effects on peak efficiency.

Adjusting Time Program

After determining the proper column length, injection volume, and flow rate, we can calculate the time needed for gradient or step elutions. As an analytical method is scaled down, the time program also needs to be scaled down to keep the phase interactions the same. Time can be adjusted using Equation 4.

Figure 2 A 1.9µm Pinnacle™ DB Biphenyl column is more selective for sulfonamides than a conventional C18 column.



Equation 1 Adjusted column length can easily be calculated when scaling from HPLC to UHPLC.

$$L_{c^2} = \frac{L_{c^1} \cdot dp_2}{dp_1}$$

Example:

$$L_{c^2} = \frac{150\text{mm} \cdot 1.9\mu\text{m}}{5\mu\text{m}}$$

$$L_{c^2} = 57\text{mm}$$

L_c = Column Length
dp = Particle Size

Equation 2 Changing column dimensions requires an adjusted injection volume.

$$V_{I^2} = V_{I^1} \cdot \left(\frac{d_{c^2}^2 \cdot L_{c^2}}{d_{c^1}^2 \cdot L_{c^1}}\right)$$

Example:

$$V_{I^2} = 10\mu\text{L} \cdot \left(\frac{2.1\text{mm}^2 \cdot 50\text{mm}}{4.6\text{mm}^2 \cdot 150\text{mm}}\right)$$

$$V_{I^2} = 0.69\mu\text{L}$$

V_I = Injection Volume
L_c = Column Length
d_c = Column Diameter

Equation 3 Changing column internal diameter requires using an adjusted flow rate.

$$F_{c^2} = \left(\frac{d_{c^2}}{d_{c^1}}\right)^2 \cdot F_{c^1}$$

Example:

$$F_{c^2} = \left(\frac{2.1\text{mm}}{4.6\text{mm}}\right)^2 \cdot 1.0\text{ml/min.}$$

$$F_{c^2} = 0.208\text{ ml/min.}$$

F_C = Column Flow
d_c = Column Diameter

Equation 4 When scaling down a gradient method, the time program needs to be adjusted.

$$t_{g^2} = t_{g^1} \cdot \left(\frac{F_{c^1}}{F_{c^2}}\right) \cdot \left(\frac{d_{c^2}^2}{d_{c^1}^2}\right) \cdot \left(\frac{L_{c^2}}{L_{c^1}}\right)$$

Example:

$$t_{g^2} = 5\text{ min.} \cdot \left(\frac{1.0\text{mL/min.}}{0.2\text{mL/min.}}\right) \cdot \left(\frac{2.1\text{mm}^2}{4.6\text{mm}^2}\right) \cdot \left(\frac{50\text{mm}}{150\text{mm}}\right)$$

$$t_{g^2} = 1.7\text{ min.}$$

t_g = Gradient Time
F = Column Flow
L_c = Column Length
d_c = Column Diameter

Conclusion

After determining the equivalent conditions for scaling-down the analysis of sulfonamides, we can see that the separations are equivalent, while the analysis time was greatly reduced (Figure 3). By following the procedure described here to ensure that the columns are equivalent, scaling analytical procedures from HPLC to UHPLC can easily be accomplished using Pinnacle™ DB columns.

Pinnacle™ DB Biphenyl Columns (USP L11)

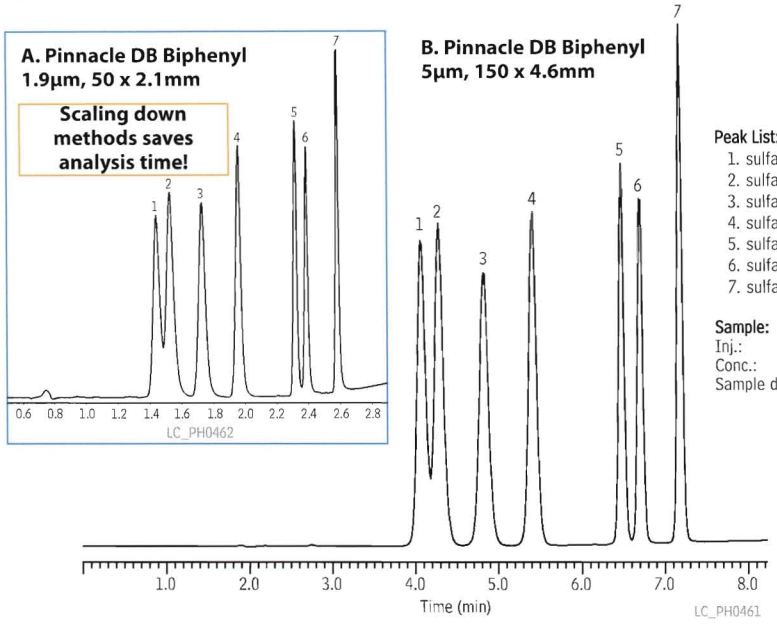
Physical Characteristics:

particle size: 1.9µm or 5µm, spherical
pore size: 140Å
carbon load: 8%
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

1.9µm Column, 2.1mm	cat. #
50mm	9409252
5µm Column, 4.6mm	**cat. #**
150mm	9409565

For other dimensions and guard cartridges for these columns, visit our website at **www.restek.com**.

Figure 3 Restek's Pinnacle™ DB 1.9µm columns can easily be scaled from HPLC to UHPLC and vice versa.



Peak List:
1. sulfadiazine
2. sulfathiazole
3. sulfamerazine
4. sulfamethazine
5. sulfachlorpyridizine
6. sulfamethoxazole
7. sulfamethoxine

Sample:
Inj.: 10µL
Conc.: 100µg/mL
Sample diluent: starting mobile phase (80:20 A:B)

Column: A. 1.9µm Pinnacle™ DB Biphenyl
Cat.#: 9409252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm

Column: B. Pinnacle™ DB Biphenyl
Cat.#: 9409565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 140Å

Conditions:
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

Time(min.)	%B
0.0	20
1.0	20
6.0	80
8.0	80

Flow: 1.0mL/min.
Temp.: 30°C
Det.: UV @ 254nm

NEW! Waste Overflow Indicator for HPLC Systems

- Avoid messy pooling around mobile phase waste containers.
- Audible alarm instantly alerts user, preventing overflow.
- Compact, battery operated unit.

The new Restek Waste Overflow Indicator will help to keep your mobile phase waste where it belongs—in the waste container! Compact, battery operated unit fits securely on 4-liter solvent bottles and accommodates two waste streams. An audible alarm is given as the solvent waste container approaches capacity, giving you time to empty or change the container. Another innovative design from Restek!

Description	qty.	cat.#
Waste Overflow Indicator for HPLC Systems	kit	26543
Replacement AA Battery for the Waste Overflow Indicator	ea.	26544
Replacement AA Batteries for the Waste Overflow Indicator	3-pk.	26545

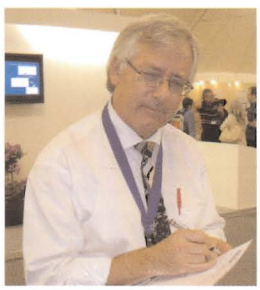
For more information about HPLC Accessories, visit us online at www.restek.com/HPLC

How Many Plates?

Winners of Restek's Column Contest from the *32nd International Symposium on Capillary GC* Announced

The *International Symposium on Capillary GC* is one of the leading symposia on capillary separation techn ogy in the world. Restek contributed to this event with many technical posters and papers, but we also had ti for a little fun!

Prof. Marriot challenging his brain...



At Restek's booth, a game was played where the participant had to guess the plate number of a GC c an LC column. The prize was a free GC or LC column. The GC column chosen for the challenge, w 0.18mm Rxi-5 Sil MS. The LC column was a 5 cm x 2.1 mm. 1.9 um Pinnacle DB. Many visitors guess by looking at the chromatogram or calculating efficiency from column dimensions.

The winner on the GC column was Prof. Philip Marriot, RMIT University, Melbourne, Australia. His of 112.000 theoretical plates was within 2% of the real value!

The winner for the closest plate number guess for the LC column was Pavel Karasek, from the Analytical Chemistry, Brno, Czech Republic.

Congratulations to both scientists!

Visit **http://www.restek.com/ts_riva2008.asp** for electronic copies of Restek's posters and paper at the *32nd International Symposium on Capillary GC.*

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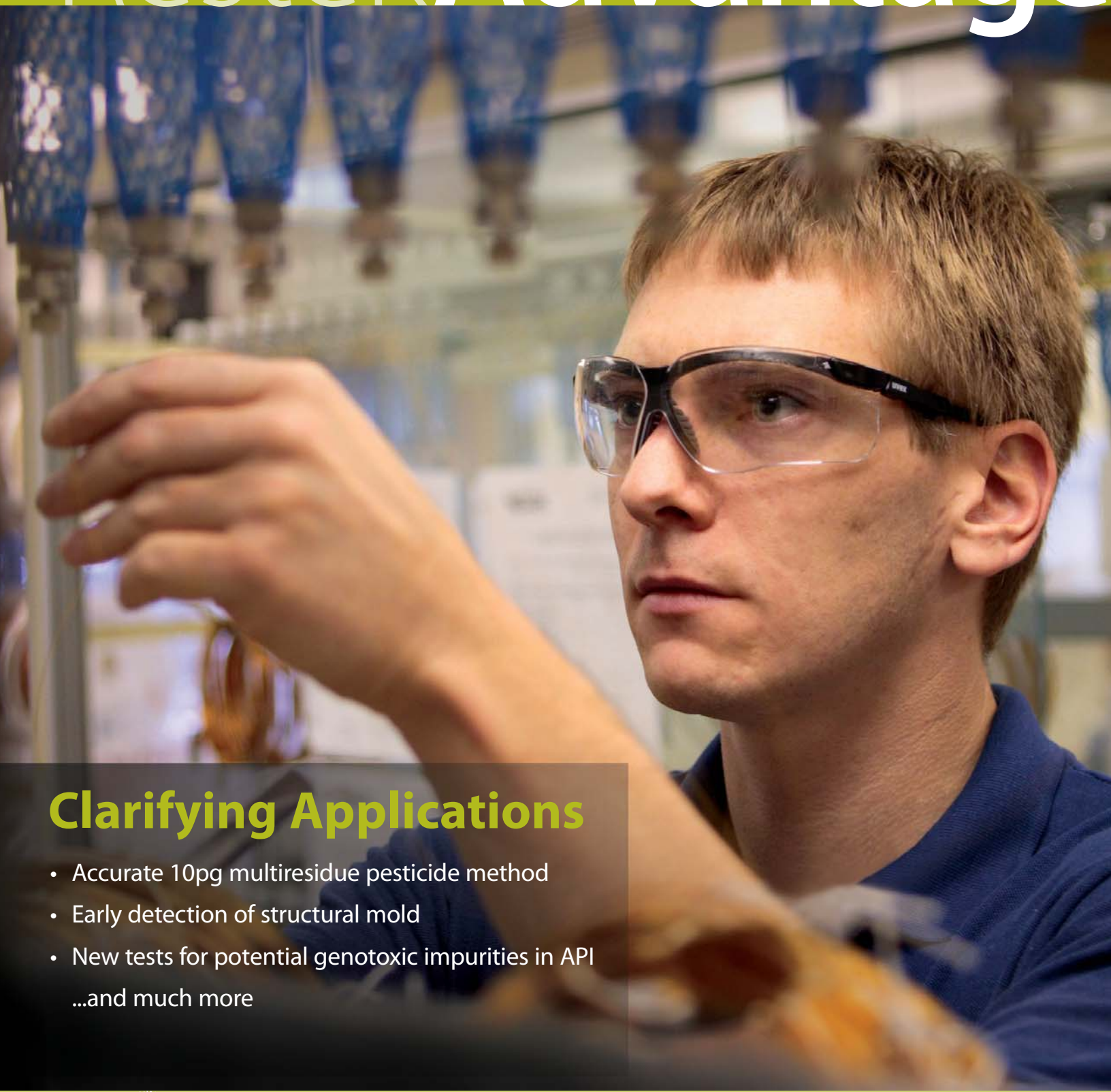


Clarifying Applications

- Accurate 10pg multiresidue pesticide method
- Early detection of structural mold
- New tests for potential genotoxic impurities in API

...and much more

in this issue

2008.03

Erratum:
In Advantage 2008.02, Figure 1 on page 19 was incorrect. The corrected figure can be seen at www.restek.com/aoi_fff_A016.asp

Editorial

Achieving Faster GC

Hans-Gerd Janssen, Ph.D., Unilever Food and Health Research Institute

Numerous articles have been published in the scientific literature regarding faster methods for gas chromatography (GC), yet confusion remains on how best to speed up separations. A significant source of this confusion is the fact that authors often neglect to define the terms "analysis speed" and "analysis time". Does the analysis time include sample preparation time? Or is it just the run time between injection and last time point on the chromatogram? Does it include reconditioning, paperwork, or interpretation? Is it the instrument time or the operator time? Numerous questions often are left unanswered and it is these questions that are to blame for the chaos in fast GC. Here I will try to clarify this confusion.

A chromatographic analysis consists of four steps: sample preparation, chromatographic separation, detection, and data interpretation. Clearly these steps are related and can not be considered in isolation. Changes in the sample preparation might affect the performance of the separation, and more sensitive and selective detectors may allow simpler sample preparation. It is these very strong interactions among the four steps that make it very difficult to describe the consequence of a change somewhere in the procedure on the total analysis time. The next problem to consider is the fact that the term "total analysis time" also is not very well defined. Is it the time-to-result for a sample, or is it the total operator time for the analysis of 100 samples divided by 100? Because of all this confusion, information from the literature on how to speed up GC analyses should be interpreted and used with great care. It is the author's sincere belief that these undefined terms have been, and still are, major obstacles, to the success of faster GC. People have tried solutions towards faster GC that too often did not work. This made people lose their confidence in fast GC. However, we should not forget there are almost 20 methods for speeding up a GC separation![1] If one selects the wrong route, all too often the conclusion is that fast GC does not work, rather than that the analyst was wrong in his or her selection. Fast GC works if—and only if—the correct route is selected. Doing that is much simpler than one might expect. Simple guidelines can be followed to select the best option, if we restrict ourselves to the chromatographic separation itself.

The selection of the best route to speed up a separation starts with an understanding of why a chromatographic separation takes time. The total time a chromatogram takes is the sum of all empty baseline segments plus the sum of the width of all baseline peaks. How can we minimize the total time? Very simple: Get rid of the baseline, only separate those peaks that need to be separated and make the peaks as narrow as possible. This sentence summarizes the three main routes to faster GC. In correct scientific terms, and in the correct order of implementation, one would describe them as 1) minimize resolution to a value just sufficient, 2) maximize the selectivity of the chromatographic system, and 3) implement a method that reduces analysis time while holding resolution constant.

If your chromatogram contains baseline or over-resolved peaks, the first step in making the separation faster is to eliminate this over-resolution. The options to do this include:

- shortening the column.
- working at an above optimum carrier gas velocity.
- increasing the initial temperature or the temperature programming rate.
- converting an isothermal separation to a programmed method.
- using flow programming.
- using a thinner film.

Only after having eliminated all baseline and situations of over-resolution should one continue to step 2. But more importantly, if one does not have baseline or over-resolved peaks, do not even consider using these options! Faster temperature programming has been described as a universal solution for faster GC. But if your chromatogram is full of peaks all just separated without any excess resolution, faster programming will ruin your

Eliminate Column Breakage in High Temperature Biodiesel Analysis

By Barry L. Burger, Petroleum Innovations Chemist, Jaap de Zeeuw, International GC Specialist

*Beat high temperature breakage with Restek **MXT®-Biodiesel TG columns**. More stable than fused silica, for accurate, reliable performance and longer column lifetime. Available with either factory- coupled or fully-integrated retention gaps.*

Restek has raised the bar with a new high-temperature MXT®-Biodiesel TG column line to complement our fused silica column line for biodiesel analysis. These new MXT®-Biodiesel TG columns are stable to 430°C and offer unique retention gap options that minimize dead volume and leaks. Choose either a 0.32mm column factory-coupled to a 0.53mm retention gap, or select a single unit 0.53mm column featuring Integra-Gap™, a built-in retention gap that eliminates the need for a connector. Both designs are extremely stable at high temperatures and produce fast elution times and sharp peaks for high molecular weight glycerides.

Eliminate Column Breakage in High Temperature Biodiesel Analysis

Unsurpassed Stability

The high temperature programs required for analysis of biodiesel oils (B100) by either ASTM D-6584 or EN-14105 methodology present a significant challenge to the analytical column. High-temperature fused silica tubing breaks down under these extreme conditions, but the metal MXT® tubing does not degrade, even at temperatures up to 430°C (Figure 1). This allows analysts to bake out any residue eluting after the triglycerides, preventing carryover without damaging the column.

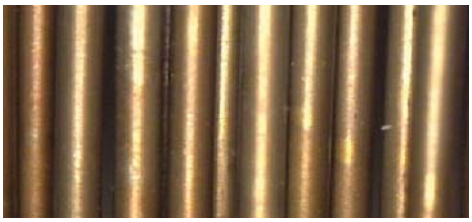
So how well do the MXT®-Biodiesel TG columns perform? We conducted a benchmarking experiment comparing an MXT®-Biodiesel TG column with an Integra-Gap™ retention gap to a high-temperature fused silica column which was coupled to a conventional 0.53mm retention gap. Methodology followed ASTM method D-6584, except the final temperature was modified to 430°C. Both columns were subjected to 100 temperature cycles up to 430°C and then derivatized B100 was injected to check column performance.

MXT®-Biodiesel TG columns are undamaged by the high temperatures required for biodiesel analysis and easily outperform high temperature fused silica columns.

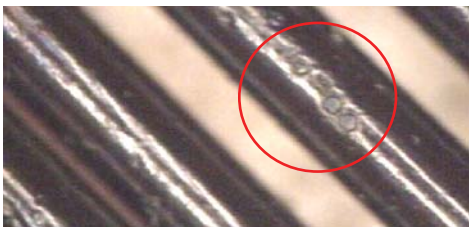
This evaluation was performed using a Shimadzu 2010 gas chromatograph equipped with a flame ionization detector, a model AOC 20i + S autosampler with a 10µL SGE syringe and 42mm 26-gauge needle, and a cold on-column programmable injector with a stainless steel injector insert. A Parker hydrogen generator supplied the carrier gas. Peak symmetry and retention time were evaluated as indicators of thermal stability.

Peak symmetry of butanetriol on a commercial high-temperature fused silica column deteriorates after just 20 injections, compared to the excellent symmetry that is maintained on the MXT®-Biodiesel TG column (Figure 2). In addition to peak shape, retention time stability was used to evaluate column performance. The decrease in retention time seen on the high-temperature fused silica column indicates the liquid phase is being lost (Figure 3). In contrast, the consistent retention times obtained on the MXT®-Biodiesel TG column demonstrate its stability. Practically, this translates into reliable performance and longer column lifetimes.

Figure 1 MXT®-Biodiesel TG columns are undamaged by high thermal cycles compared to high-temperature fused silica columns, which break down under the same conditions.



MXT®-Biodiesel TG columns are undamaged by high thermal cycles.



HT fused silica columns, labeled as stable to 430°C, show pitting and breakdown.

100 temperature cycles to 430°C totaling 500 minutes at maximum temperature.

Figure 2 Stable and consistent peak shape for the internal standard butanetriol gives you more accurate quantitation.

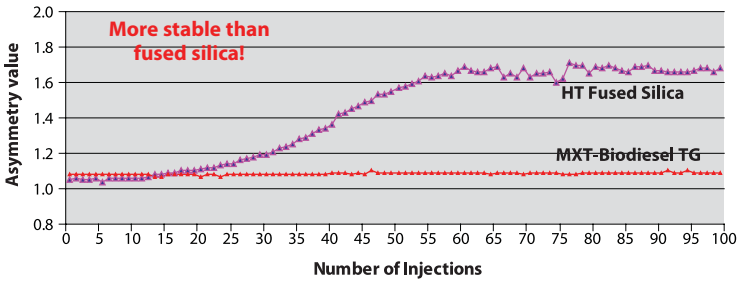
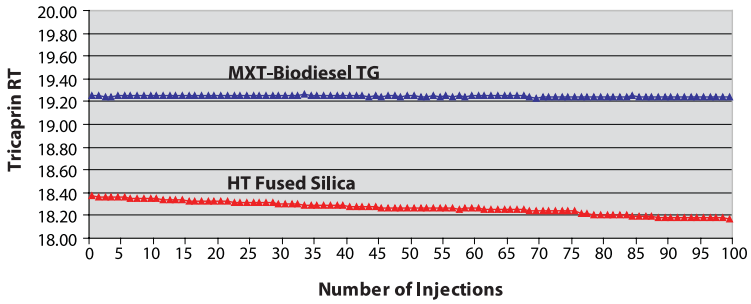


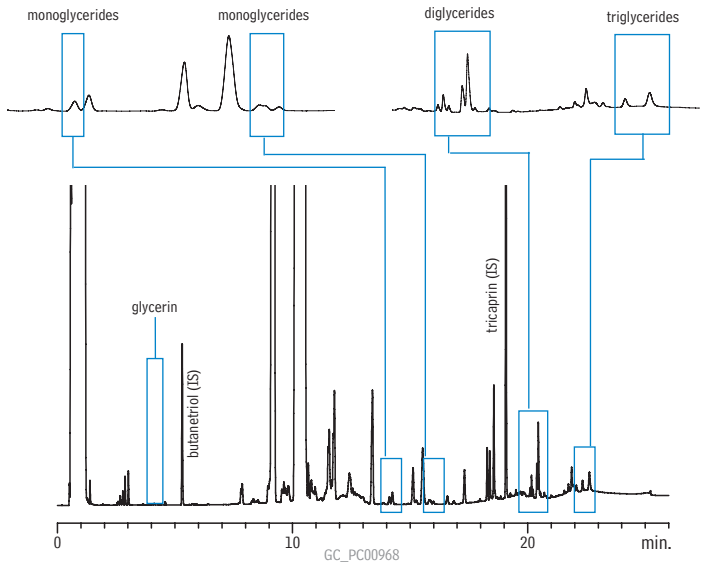
Figure 3 Retention time is stable on a metal MXT®-Biodiesel TG column, even after 100 cycles up to 430°C.



thank **you**

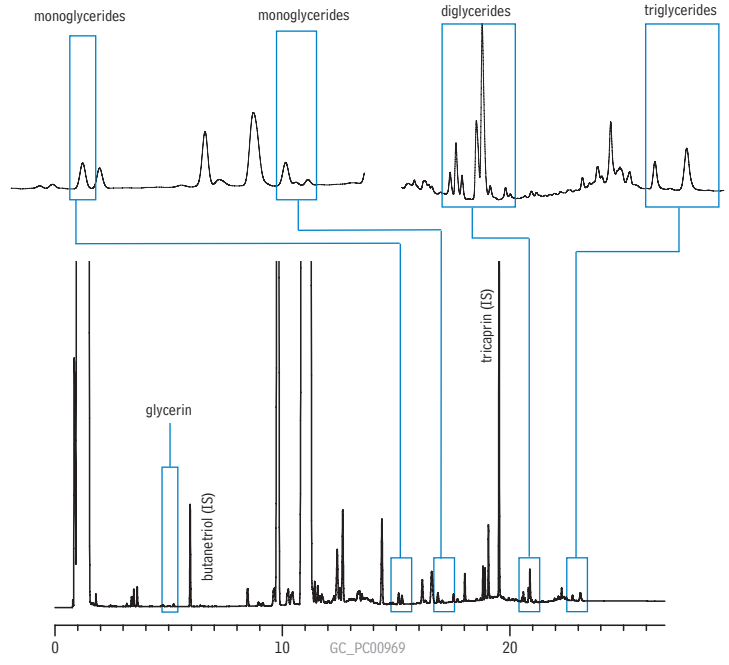
Instrument provided courtesy of Shimadzu **www.shimadzu.com**

Figure 4 Derivatized B100 samples resolve well on the 0.32mm MXT®-Biodiesel TG column, which is factory-coupled to a 0.53mm MXT® retention gap using an MXT® low-dead-volume.



Column: MXT®-Biodiesel TG, 15m, 0.32mm ID, 0.10µm (cat.# 70291) with a 2m x 0.53mm MXT® retention gap connected with an MXT™ low dead-volume connector (17m total length)
Sample: biodiesel (B100), derivatized; Inj.: cool on-column injection 1µL in heptane; Inj. temp.: oven track; Carrier gas: hydrogen, constant flow; Flow rate: 3mL/min.; Oven temp.: 50°C (hold 1 min.) to 180°C @ 15°C/min. to 230°C @ 7°C/min. to 380°C @ 30°C/min. (hold 5 min.); Det.: FID @ 380°C

Figure 5 Excellent chromatographic quality and resolution on the 0.53mm MXT®-Biodiesel TG analytical column with a built in Integra-Gap™ retention gap.



Column: MXT®-Biodiesel TG, 14m, 0.53mm ID, 0.16µm (cat.# 70289) with a 2m x 0.53mm with Integra-Gap™ retention gap (16m total length)
Sample: biodiesel (B100), derivatized; Inj.: cool on-column injection 1µL in heptane; Inj. temp.: oven track; Carrier gas: hydrogen, constant flow; Flow rate: 4mL/min.; Oven temp.: 50°C (hold 1 min.) to 180°C @ 15°C/min. to 230°C @ 7°C/min. to 380°C @ 30°C/min. (hold 5 min.); Det.: FID @ 380°C

Analytical Alternatives

Factory connected 0.32mm MXT®-Biodiesel TG columns & 0.53mm retention gaps

For accurate analysis of heavy triglycerides, on-column injection is required. ASTM D-6584 describes the use of a 0.32mm analytical column coupled with a 0.53mm retention gap. The 0.53mm ID retention gap allows the cool on-column technique to be used, but care must be taken to minimize dead volume and to establish a leak-tight connection. Restek's 0.32mm MXT®-Biodiesel TG columns are factory-coupled to a 0.53mm MXT® retention gap with an MXT® low-dead-volume connector, ensuring a leak-tight connection. Target analytes resolve well and the solvent and triglyceride peaks show excellent symmetry (Figure 4).

0.53mm MXT®-Biodiesel TG columns

The 0.53mm MXT®-Biodiesel TG columns are a simpler alternative to using a 0.32mm column coupled to a 0.53mm retention gap. Restek applied Integra-Gap™ technology to the 0.53mm MXT®-Biodiesel TG columns, eliminating the column coupling. These single unit leak-proof columns feature a built-in retention gap, reducing the risk of peak broadening and tailing. Chromatography from the 0.53mm MXT®-Biodiesel TG with Integra-Gap™ technology (Figure 5) is excellent and comparable to that obtained on the 0.32mm ID column in Figure 4.

Conclusion

As demonstrated, for high temperature GC analysis, the metal MXT®-Biodiesel TG column is a rugged column that withstands the harsh temperatures required for total residual glycerin analysis. The column has the resolution needed for accurate, reliable results and is more stable at high temperatures than competitive fused silica columns, leading to longer column lifetimes. To improve the reliability and robustness of your biodiesel analyses, try a Restek MXT®-Biodiesel TG column.

Product Listing

MXT®-Biodiesel TG Columns (Siltek® treated stainless steel)

Description	cat.#	price
14m, 0.53mm ID, 0.16 w/2m Integra-Gap	70289	
10m, 0.32mm ID, 0.10	70292	
10m, 0.32mm ID, 0.10 w/2m x 0.53mm retention gap	70290	
15m, 0.32mm ID, 0.10	70293	
15m, 0.32mm ID, 0.10 w/2m x 0.53mm retention gap	70291	

temp limits: -60 to 380/430°C *Total column length=16 meters.

Restek Tubing Scorer for MXT® Columns

Designed to make perfectly round cut every time!

Description	qty.	cat.#	price
Restek Tubing Scorer for MXT Columns (0.25-0.53mm ID & 0.5-0.8mm OD)	ea.	20523	
Replacement Scoring Wheel	ea.	20522	

Reliably Detect Pesticides Down to 10pg with Sensitive SIM GC/MS Multiresidue Method

*Market demands are increasing for multiresidue pesticide methods that are both sensitive and effective across a broad range of compound chemistries. The **Rxi®-5Sil MS** column gives accurate low level results for a wide variety of analytes in a single run.*

By Jason Thomas, Environmental Innovations Chemist

As labs operate in an extremely competitive market, the demand for more sensitive multiresidue pesticide methods is increasing. A GC/MS method is a logical choice, as this instrument provides a high degree of specificity, yet is relatively inexpensive and easy to operate, compared to LC/MS/MS, high resolution MS, or GC/MS/MS. However, to take full advantage of GC/MS, careful column selection is critical. The column used must be of the proper selectivity to separate compounds that share common spectra, and also exhibit a high degree of inertness and minimal bleed. Here we demonstrate the effectiveness of an Rxi®-5Sil MS column for low level analysis of a wide variety of pesticides differing in volatility, compound class, and degree of activity.

Excellent Response for Difficult Active Compounds

Column inertness, selectivity, and bleed are key considerations and often determine the success or failure of analytical runs. Inertness can be assessed through the behavior of active compounds, which often exhibit disproportionately poor responses at low concentrations. Although the compound list analyzed here contains many compounds with a high degree of activity, low level linearity (10-1,000ng/mL) was established with an r^2 value of 0.990 or above for many of these challenging compounds (Table I). In addition, the notoriously problematic compounds of EPA Method 8081, endrin and 4,4'-DDT, were among the least troublesome tested here, attaining values of 0.997 and 0.998, respectively. Note that standards were analyzed for this study and some compounds with r^2

Table I The Rxi®-5Sil MS column provides excellent linearity, and thus more accurate results, for a wide range of pesticide chemistries down to 10pg.

Compound	Retention time (min.)	Quant. ion	Qual. ion 1	Qual. ion 2	IS	r^2 (10-1,000 ppb)
methamidophos	5.77	141	95	94	BNB	0.997
dichlorvos	6.02	185	79	109	BNB	0.998
bromonitrobenzene (IS)	7.21	203	201	157	IS	—
mevinphos	8.26	192	127	109	BNB	0.995
acephate	8.30	136	95	94	BNB	0.982
o-phenylphenol	9.44	170	169	141	BNB	0.997
omethoate	10.23	156	110	109	BNB	0.976
dimethoate	11.77	125	143	93	BNB	0.981
pentachloronitrobenzene (IS)	12.13	295	249	237	IS	—
diazinon	12.45	179	304	137	PCNB	0.994
chlorothalonil	12.55	266	264	268	PCNB	0.983
vinclozin	13.48	285	198	212	PCNB	0.998
carbaryl	13.65	144	116	115	PCNB	0.996
metalaxyl	13.69	206	160	132	PCNB	0.997
dichlofluanid	14.17	123	167	224	PCNB	0.954
malathion	14.19	173	125	127	PCNB	0.992
thiabendazole I	15.34	201	202	174	PCNB	0.958
captan	15.34	79	119	149	PCNB	0.987
folpet	15.46	260	130	104	PCNB	0.964
imazalil	16.10	215	175	173	PCNB	0.982
myclobutanil	16.34	206	179	150	PCNB	0.973
endrin	16.82	265	279	317	PCNB	0.997
fenhexamid	17.79	177	179	97	PCNB	0.969
4,4'-DDT	17.79	237	235	165	PCNB	0.998
propargite	18.04	173	150	135	PCNB	0.999
triphenylphosphate (IS)	18.09	325	215	326	IS	—
iprodione	18.47	314	316	187	TPP	0.991
bifenthrin	18.64	181	166	165	TPP	0.998
fenpropathrin	18.82	265	208	181	TPP	0.985
dicofol	18.89	139	251	253	TPP	0.788
permethrin I	20.41	183	165	163	TPP	0.998
permethrin II	20.54	183	163	165	TPP	0.995
deltamethrin	22.87	253	251	181	TPP	0.995

Standard curve: 10, 25, 75, 150, 500, and 1,000 ng/mL mixed standards, single 1µL injections.

Figure 1 Outstanding peak symmetry for active compounds demonstrates column inertness (500pg).

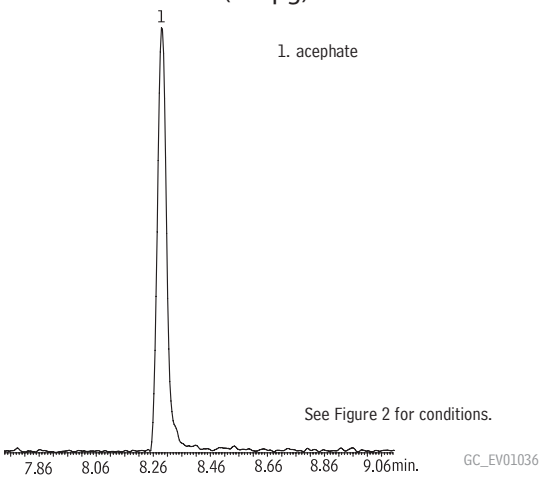
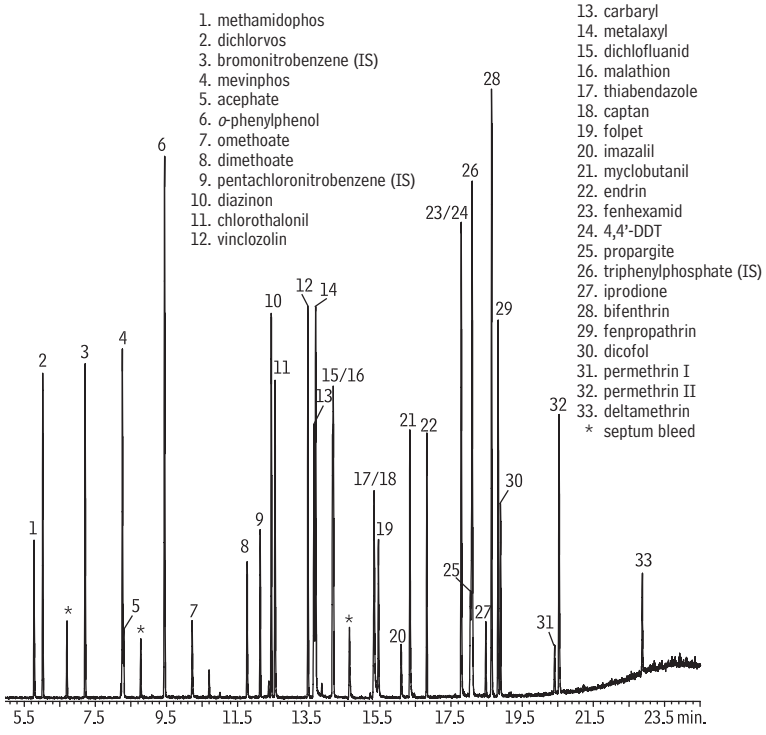


Figure 2 Use an Rxi®-5Sil MS column to easily resolve a broad range of pesticide chemistries in a single run (500pg).



Column:	Rxi®-5Sil MS, 30m, 0.25mm ID, 0.25µm (cat.# 13623), with 5m x 0.25mm ID Rxi® deactivated guard tubing (cat.# 10029)
Instrument:	PerkinElmer Clarus 500 GC/MS
Sample:	1ppm each compound (custom mixes) 1ppm triphenylphosphate (cat.# 32281) 1ppm 1-bromo-2-nitrobenzene (cat.# 32279) 1ppm pentachloronitrobenzene (cat.# 32091)
Inj.:	0.5µL splitless (hold 1 min.), PSS Drilled Uniliner® (hole near bottom) inlet liner (cat.# 22989)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Linear velocity:	32cm/sec. @ 90°C
Oven temp.:	90°C (hold 1 min.) to 310°C @ 10°C/min. (hold 5 min.)
Det.:	MS
Transfer line temp:	300°C
Scan range:	50-350amu
Ionization:	EI
Mode:	scan

The inertness of the Rxi®-5Sil MS column ensures linear performance down to 10pg on-column, allowing more accurate low level quantification.

values less than 0.990, such as acephate, omethoate, and dicofol, show a more linear response when analyzed in matrix. As shown in Figure 1, the Rxi®-5Sil MS column is also highly inert, producing excellent peak shape even for difficult compounds such as acephate. The linearity, sensitivity, and inertness demonstrated here, make the Rxi®-5Sil MS column ideal for more accurate low level quantification of active compounds.

Low Bleed, High Selectivitity

Another crucial characteristic for multiresidue pesticide methods is column bleed. Minimizing bleed is critical in preventing interference with target compounds, even in SIM analysis, as some compounds may share ions and have similar bleed spectra. As shown in the TIC chromatogram in Figure 2, the ultra-low bleed of the Rxi®-5Sil MS column allows full scan analysis with minimal interference from column bleed. The Rxi®-5Sil MS column provides excellent separation for the wide range of chemistries tested and the column is also selective enough to easily separate isomers, such as permethrin I and II.

In summary, many of the difficulties associated with multiresidue methods are simplified by using the Rxi®-5Sil MS column. Its outstanding inertness, low bleed at high temperatures, and unique selectivity provide a robust capillary column with the sensitivity and longevity needed to address the tough challenges inherent to low level multiresidue pesticide analysis.

Product Listing

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623	

Rxi® Guard/Retention Gap Column

Nominal ID	Nominal OD	5-Meter
0.32mm	0.45 ± 0.04mm	10039

PSS Liners for PerkinElmer GCs

ID* x OD & Length (mm)	qty.	cat.#	price
PSS Drilled Uniliner (hole near bottom)			
20mm x 4.0mm x 86.2mm	5-pk.	22989	

PTV On-Column Liner Gives You Two Inlets in One

Why pay for a separate injection port when a simple liner change can convert your programmable temperature vaporization (PTV) inlet to allow for true cold on-column injections? Save time and money by using Restek's ***PTV On-Column Liner****.*

By Scott Grossman, Innovations Chemist, Jack Cochran, Director of New Business and Technology, and Jaap de Zeeuw, International GC Specialist

While PTV is popular internationally, it is an emerging technique in US laboratories and is expected to grow with the awareness of this versatile technique. Now, using a PTV On-Column liner, the capabilities of PTV can be expanded to include true on-column injections, which normally would have required a separate injection port. Why incur the additional expense of a separate injection port when the same results can be achieved with a simple liner change? Restek's PTV On-Column liner, available for Agilent PTVs and the Gerstel CIS4, allows you to perform true cold on-column injections with a PTV port, saving you money and retaining the versatility of the PTV inlet

A Simple Solution

Figure 1 illustrates how this liner works. A 0.53mm ID retention gap column is pressed into the bottom restriction of the liner, forming a leak-free seal between the retention gap's polyimide coating and the inner wall of the liner. The liner's top restriction guides a 26-gauge needle down into the 0.53mm ID retention gap, allowing samples to be injected directly on-column.

Protect Sensitive Compounds

By operating the inlet at low temperatures, an initial flash vaporization is eliminated, protecting thermally labile compounds. Injecting the sample directly into the column also helps avoid injection port activity issues and increases transfer of lower volatility compounds. Both of these features help decrease sample degradation, increase sensitivity, and improve reproducibility. Figure 2 illustrates the outstanding reproducibility that can be achieved with this liner using an example of explosives as probes. Absolute standard deviations were just 2.6% (500pg/µL nitroglycerin) and 1.5% (100pg/µL TNT) for relative peak areas over 5 replicate injections. Variation in realative area was similarly low for both compounds.

Increase Injection Volume

An additional advantage of this liner configuration is the increased analytical sensitivity that can be obtained by injecting a larger sample volume. When the sample needs to be flash vaporized, sample volume expansion in the liner quickly becomes a concern, limiting injection volume to 1-2µL of sample. However, with cold on-column injections, larger sample volumes can be used because the solvent can be gradually vaporized and eluted before the analytes. Using a larger sample volume means more analyte is loaded on-column, giving greater overall sensitivity. The data in Figure 3 demonstrate the excellent linearity achievable using the PTV On-Column liner across a range of injection volumes. Instead of a traditional calibration curve that plots response vs. increasingly concentrated standards, this plot illustrates response vs. increasing volumes of the same standard, in effect producing the same result of more mass on-column. The correlation between peak area and injection volume (5 -100µL) was evaluated and r^2 values of

Using the PTV On-Column liner decreases sample degradation, increases sensitivity, and improves reproducibility.

Figure 1 Use a PTV On-Column liner to perform cold on-column column injections in a PTV injection port.

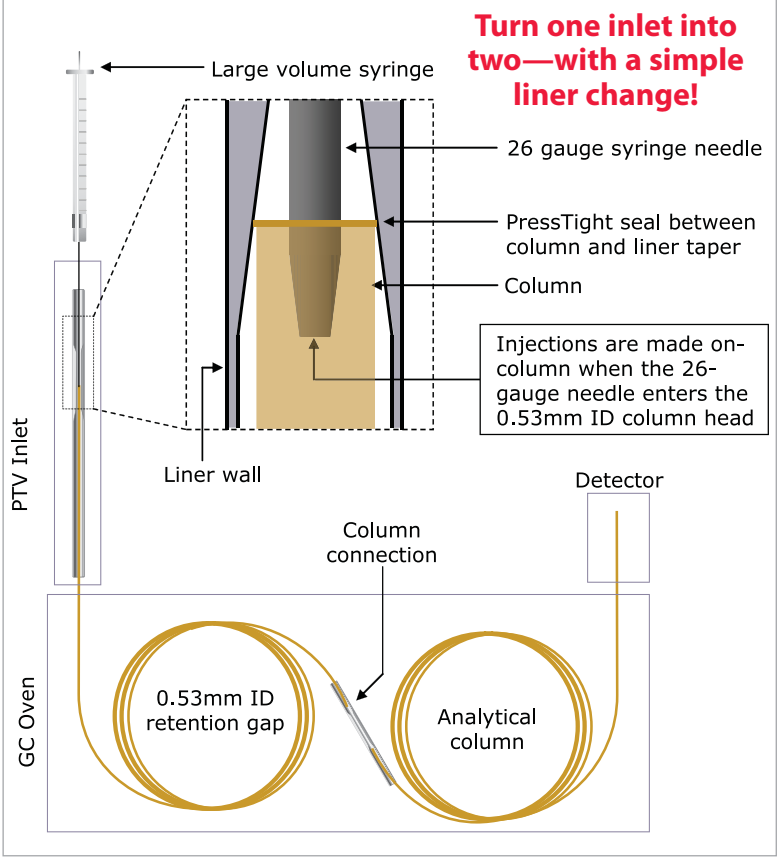
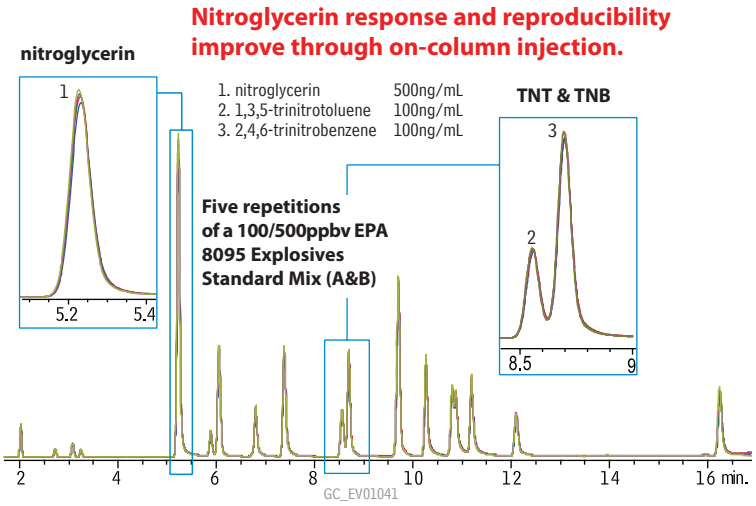


Figure 2 Increase reproducibility and sample integrity with a PTV On-Column liner.



Absolute area reproducibility improves for all compounds, and sensitive compound responses improve dramatically because of the lack of contact with the injection port.

nitroglycerin: Absolute Area % RSD = 2.6% • Relative Area % RSD = 1.6%
TNT: Absolute Area % RSD = 1.5% • Relative Area % RSD = 1.4%

Column: Rxi®-5ms, 6m, 0.53mm ID, 0.5µm (cat.# 563153) with 5m x 0.53mm IP guard tubing (cat.# 10045), connected using PTV On-Column liner (cat.# 24976); Sample: 8095 Calibration Mix A and 8095 Calibration Mix B diluted in acetonitrile; Inj.: PTV injection port splitless (15mL/min. @ 0.35 min.); Inj. temp.: 55°C to 285°C @ 10°C/min. (hold 10 min.); Carrier gas: helium, constant flow; Linear velocity: 60cm/sec. @ 300°C; Oven temp.: 50°C to 280°C @ 10°C/min. (hold 10 min.); Det.: µECD @ 300°C, nitrogen make-up gas @ 60mL/min.

0.9986 (TNB) and 0.9997 (TNT) were obtained. Note that a linear response is maintained—even for high injection volumes.

Why pay for two injection ports when a simple liner change gives you the benefits of having two inlets in one? Using a PTV On-Column liner saves you money and gives you flexibility in the lab. Use this liner and reliably perform true cold on-column injections with your PTV injection port.

Product Listing

PTV Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#	price
PTV On-Column Liner			
1.7mm x 3.0mm x 71mm	ea.	24976	
1.7mm x 3.0mm x 71mm	5-pk.	24977	

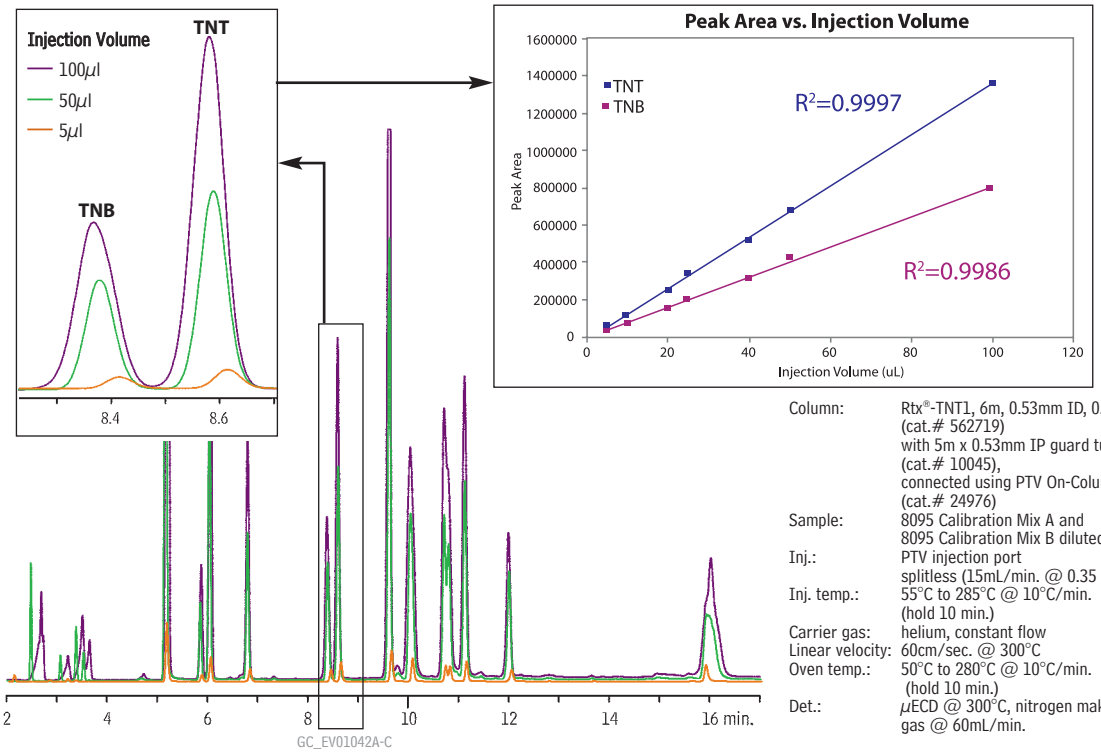
*Nominal ID at syringe needle expulsion point.

Get More!

Visit www.restek.com/environmental and download:

- Explosives and Brominated Flame Retardant Analysis by Gas Chromatography with a New On-Column Injector Liner for a Programmable Temperature Vaporizing Injector
- Using Guard Columns and Retention Gaps in GC

Figure 3 Improve sensitivity with larger injection volumes using a PTV On-Column liner.



Column: Rtx®-TNT1, 6m, 0.53mm ID, 0.5µm (cat.# 562719) with 5m x 0.53mm IP guard tubing (cat.# 10045), connected using PTV On-Column liner (cat.# 24976)
Sample: 8095 Calibration Mix A and 8095 Calibration Mix B diluted in acetonitrile
Inj.: PTV injection port splitless (15mL/min. @ 0.35 min.)
Inj. temp.: 55°C to 285°C @ 10°C/min. (hold 10 min.)
Carrier gas: helium, constant flow
Linear velocity: 60cm/sec. @ 300°C
Oven temp.: 50°C to 280°C @ 10°C/min. (hold 10 min.)
Det.: µECD @ 300°C, nitrogen make-up gas @ 60mL/min.

Early Detection of Structural Mold with SilcoCan™ Air Sampling Canisters

*Early detection of mold growth in buildings is critically important to protecting human health and property values. Restek **SilcoCan™ canisters** allow low levels of mold to be detected in air samples—before it can be seen—providing an opportunity for structural repair and safer living conditions.*

By Silvia Martinez, Innovations Chemist

Mold growth in homes has been linked to serious human health and property value issues; thus, early detection is of increasing importance. Mold releases microbial volatile organic compounds (MVOCs) which can be sampled in air and identified by GC/MS analysis, even prior to visual detection methods. MVOCs attributed to fungal growth include terpenes, ethers, ketones, alcohols, aldehydes, aromatic and chlorinated hydrocarbons, sulfur-based compounds, and amines. These compounds are not unlike other volatile organic compounds commonly analyzed in environmental and industrial hygiene laboratories, and the same equipment can be used to collect, positively identify, and quantify MVOCs.

Due to the polar nature of many MVOCs, and the low concentrations found in early detection, a passivated, large volume collection device is needed for sampling. SilcoCan™ canisters are an excellent choice for sampling and analyzing MVOCs. The canister surface, passivated with a chemically bonded fused silica layer, has been shown to provide the stability and inertness needed for collecting and storing low level volatiles (ppbv) such as those analyzed by EPA methods TO-14A and TO-15, including sulfur-containing compounds and microbial VOCs. Here we show a successful application of highly inert SilcoCan™ canisters and GC/MS for monitoring low level mold growth in building structures.

Table I Boiling points of low volatility MVOCs.

MVOC	bp (°C)
1-octanol	194
isoborneol	212
α-terpineol	214
geosmin	270

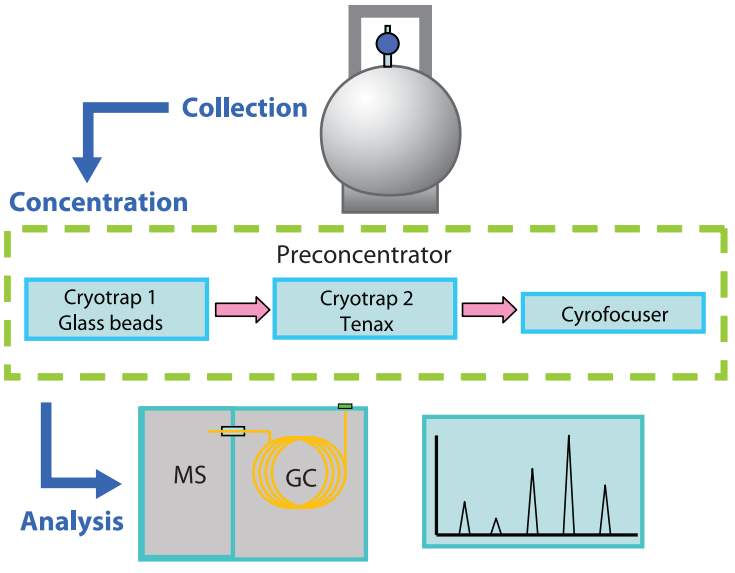
Sample Set-up

For our analysis, we began with standard solutions of 23 MVOCs in methanol at 100µg/mL. The compounds were separated by chemistry into four solutions to prevent degradation reactions: alcohols, ketones, 2-isopropyl-3-methoxypyrazine, and geosmin. After cleaning and evacuating a SilcoCan™ canister, 210µL of water were added to the canister to simulate natural humidity and aid recovery. After equilibration, 2µL of each solution were added to the canister. Finally, the canister was pressurized to 30psig with dry nitrogen to yield a final concentration of 2.2ng/200mL for each MVOC, or 1.4 to 3.8ppbv of each MVOC. (The final ppbv concentration is molecular weight-dependant.) To boost recoveries of the higher-boiling compounds, we used a Restek Air Canister Heating Jacket set to 75°C. The sample was heated to 75°C for 30 minutes prior to, and during testing. Boiling points of some of the lower volatility MVOCs are shown in Table I.

23 MVOCs Identified in Less than 30 Minutes

Sample analysis was conducted using standard air analysis equipment such as is used in environmental laboratories. In our case, we used a Nutech 8900DS autosampler and preconcentrator attached to an Agilent 6890/5973 GC/MS. Volatiles in the sample are concentrated by a cryogenic trap followed by an adsorbent trap, then cryofocused for injection into the GC/MS. Figure 1 shows a schematic of the sampling and preconcentration process. An Rxi®-1ms column was used to provide separation at the ultra-low bleed levels required for spectroscopic analysis. The MVOC sample was analyzed by concentrating 200mL of the 0.011ng/mL gaseous mix using a 1:1 split for only 1ng on column of each MVOC. The resulting chromatogram, shown in Figure 2, shows excellent peak response and resolution for the 23 compounds in less than 30 minutes.

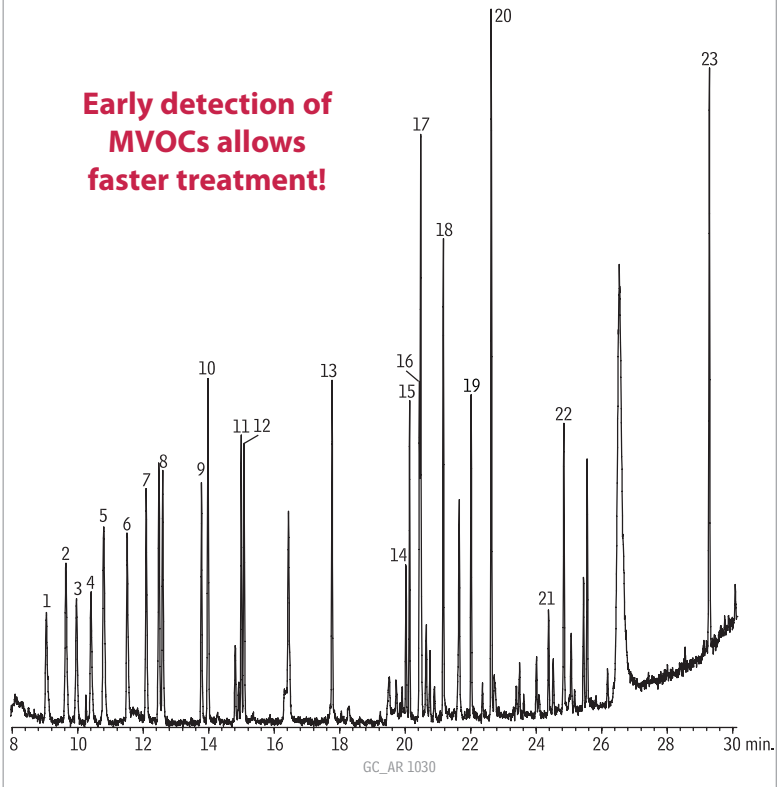
Figure 1 Sample set-up for low level MVOC analysis. Excellent response was seen, even for polar and high boiling point compounds.



Passivated SilcoCan™ canisters are ideal for sampling low concentrations of MVOCs. The inertness of these canisters provides an exceptional storage environment, particularly for polar and high boiling point compounds.

SilcoCan™ canisters easily provide the inertness and stability required for the collection, storage, and analysis of MVOCs, especially for polar and high-boiling compounds. Air sampling of MVOCs using SilcoCan™ canisters allows for early detection of fungal growth, providing an opportunity for structural treatments to eradicate damaging mold.

Figure 2 Detect low levels of structural mold using SilcoCan™ canisters for air sampling (1ng on-column).



Compound	Rt (min.)
1. 2-butanone	9.047
2. 2-methyl-furan	9.640
3. 3-methyl-furan	9.962
4. 2-methyl-1-propanol	10.405
5. 2-methyl-2-butanol	10.791
6. 1-butanol	11.506
7. 3-methyl-2-butanol	12.092
8. 2-pentanol	12.592
9. 2-methyl-1-butanol	13.779
10. dimethyl disulfide	13.979
11. 3-hexanone	14.994
12. 2-hexanone	15.080
13. 2-heptanone	17.767
14. 1-octen-3-ol	20.019
15. 3-octanone	20.133
16. 3-octanol	20.433
17. 2-pentyl-furan	20.476
18. 2-ethyl-1-hexanol	21.163
19. 1-octanol	22.013
20. 2-isopropyl-3-methoxypyrazine	22.628
21. isoborneol	24.379
22. α-terpineol	24.844
23. geosmin	28.347

Column: Rxi®-1ms, 60m, 0.25mm ID, 1.00μm (cat.# 13356)
Sample: microbial volatile organic compounds (MVOCs), 2ppbv, 60% RH
Inj.: 1.0μL split (split ratio 1:1), 1mm split inlet liner (cat.# 20972)
Inj. temp.: 200°C
Carrier gas: helium, constant flow
Flow rate: 1.5mL/min.
Oven temp.: 10°C (hold 1 min.) to 235°C @ 8°C/min.
Det: Agilent 6890/5973 GC/MS 5 min. solvent delay

Transfer line temp.: 260°C
Scan range: 35 to 350amu
Ionization: EI
Mode: scan
Other: Nutech 8900DS Preconcentrator Conditions:
Sample = 200mL from canister
Cryotrap1 = -160°C
Desorb = 20°C
Cryotrap2 = 20°C
Desorb = 200°C
Cryofocuser = 200°C
Desorb = 200°C

Product Listing

Rxi®-1ms Columns (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (μm)	temp. limits	length	cat. #	price
0.25mm	1.00	-60 to 330/350°C	60-Meter	13356	

1mm Split Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#	price
1mm Split			
1.0mm x 6.3mm x 78.5mm	ea.	20972	

SilcoCan™ Air Monitoring Canisters

Ideal for low-level reactive sulfur (1-20ppb), TO-14A, or TO-15 compounds

Canisters are the gold standard for ambient VOC monitoring.

Description	Volume	qty.	cat.#	price
SilcoCan Canister, 1/4" Valve	1L	ea.	24180	561
SilcoCan Canister, 1/4" Valve	3L	ea.	24181	581
SilcoCan Canister, 1/4" Valve	6L	ea.	24182	602
SilcoCan Canister, 1/4" Valve	15L	ea.	24183	923

Air Canister Heating Jacket

The ultimate in controlled heating, for reliably cleaning your air canisters!

Description	qty.	cat.#	price
Air Canister Heating Jacket (110 volt)	ea.	24123	

*Not CE certified.

Prepare Samples in Half the Time Using a Fraction of the Solvent with dSPE

*Simplify and speed up sample preparation with **Resprep dSPE tubes**! Here we show the extraction and clean-up of pesticide residues from olive oil samples—twice as fast as GPC, with only a fraction of the solvent required for conventional SPE.*

By Michelle Misselwitz, Environmental Innovations Chemist, Julie Kowalski, Ph.D., Food Flavors, and Fragrances Innovations Chemist, Mark Crawford*, Applications Chemist, Michael Halvorson Ph.D.*, Senior Product Specialist, and Joan M. Stevens Ph.D.*, Applications Manager
*Gilson, Inc.

Olive oil is considered a healthy fat source and is a staple in many recommended diets. However, concerns about potentially negative health effects associated with pesticide residues have increased consumer interest in testing. While organophosporus pesticides are currently used in olive orchards to control pests, organochlorine pesticides are still tested for as persistent organic pollutants (residues), even though they are no longer in commercial use. There are several existing methods for measuring pesticide residues in olive oil, all of which involve sample extraction and clean-up.[1] The common goal of these methods is to remove lipids that are harmful to the analytical system.[2] Efficient sample clean-up procedures are critical to maximizing sample throughput and minimizing labor and material costs. Here we demonstrate the efficiency of a dSPE clean-up procedure, as well as the capabilities of both method-specific and general purpose analytical columns.

Simple Procedure Uses Half the Time and Minimal Solvent

Sample extraction and clean-up can be accomplished with gel permeation chromatography (GPC), solid phase extraction (SPE), or dispersive solid phase extraction (dSPE) methods. However the dSPE method shown here is much less expensive than GPC (which requires specialized equipment) and uses substantially less solvent than comparable GPC or SPE methods (Table I).[3] The method is simple to use and allows sample extraction and clean-up to be accomplished in half the time of other techniques (Table II).

Extraction and dSPE Clean-up for Pesticide Residues in Olive Oil

Test sample: A 1.5mL sample of commercially obtained virgin olive oil was spiked with a standard organochlorine pesticide mix. The spiked sample was processed as follows.

1. Dilute with 1.5mL hexane.
2. Add 6mL of acetonitrile (ACN).
3. Mix for 30 minutes on a shaker.
4. Allow layers to separate (approximately 20 minutes), then collect the top (ACN) layer.
5. Repeat the liquid-liquid extraction (steps 2-4) and combine both ACN extract layers.
6. Place 1mL of the combined ACN extract in a 1.5mL tube containing 150mg magnesium sulfate and 50mg PSA.
7. Shake the tube for 2 minutes.
8. Centrifuge at 3,000 U/min. for approximately 5 minutes.
9. Remove the top layer and inject directly into the gas chromatograph system.

Extracts were analyzed using both Rtx®-CLPesticides2 and Rxi®-5Sil MS columns (Figure 1). The Rtx®-CLPesticides2 column is a method specific column that resolves all compounds. The Rxi®-5Sil MS column is a general purpose column that has one coelution that can easily be extracted by a mass

Table I Resprep dSPE method uses 42% and 89% less solvent than SPE and GPC methods respectively.

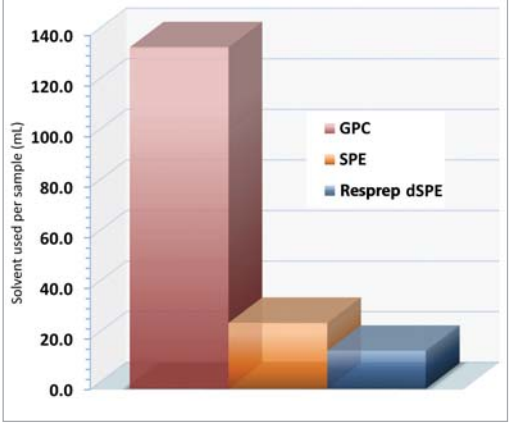


Table II Cut extraction/clean-up time by 50% using a Resprep dSPE method.

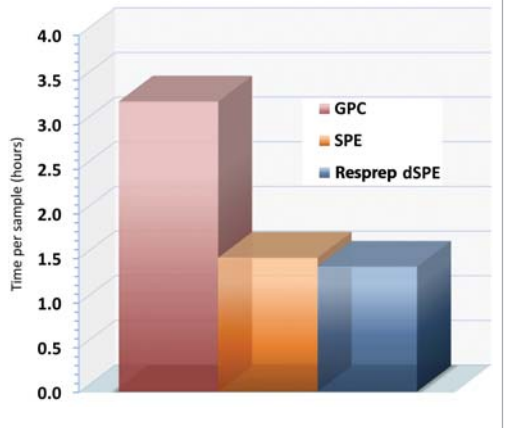
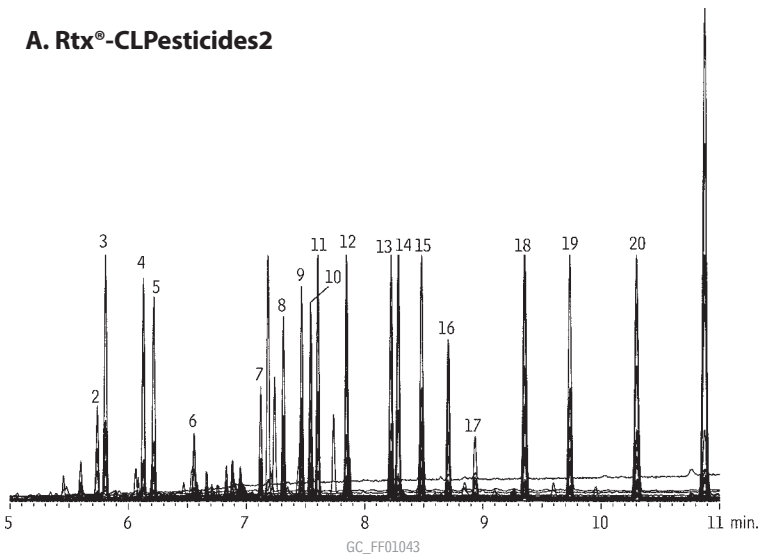


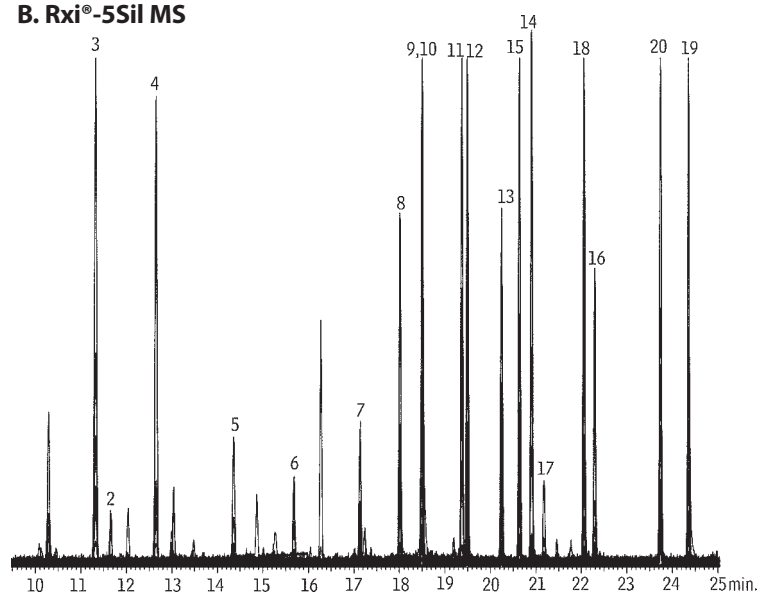
Figure 1 Chlorinated pesticide residues in olive oil are easily separated on either Rtx®-CLPesticides2 or Rxi®-5Sil MS columns.

A. Rtx®-CLPesticides2



GC_FF01043

B. Rxi®-5Sil MS



GC_FF01044

Column:	A. Rtx®-CLPesticides2, 30m, 0.25mm ID, 0.20μm (cat.# 11323) B. Rxi®-5Sil MS, 30m, 0.25mm ID, 0.25μm (cat.# 13623)
Sample:	10μg/mL Organochlorine Pesticide Mix AB # 3 (cat.# 32415) in olive oil
Inj.:	1μL, splitless (hold 0.5 min.), 3.5mm single gooseneck liner (cat.# 20962) packed with wool
Inj. temp.:	225°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	A. 140°C (hold 0.5 min.) to 268°C @ 20°C/min. to 290°C @ 3°C/min. to 330°C (hold 5 min.) @ 20°C/min. B. 130°C (hold 0.5 min.) to 330°C @ 5°C/min
Det:	MS
Transfer line temp.:	320°C
Ionization:	EI
Mode:	SIM

Compound	Quant. ion	Qual. ion 1	Qual. ion 2
*1. α-BHC	219	181	109
2. γ-BHC	219	181	109
3. β-BHC	219	181	109
4. δ-BHC	219	181	109
5. heptachlor	272	237	100
6. aldrin	263	293	220
7. heptachlor epoxide	263	237	81
8. δ-chlordane	272	237	65
9. α-chlordane	272	237	65
10. endosulfan I	195	207	241
11. 4,4'-DDE	246	318	176
12. dieldrin	79	263	277
13. endrin	263	281	81
14. 4,4'-DDD	235	165	199
15. endosulfan II	195	207	-
16. 4,4'-DDT	235	165	199
17. endrin aldehyde	67	250	345
18. endosulfan sulfate	272	229	239
19. methoxychlor	227	274	-
20. endrin ketone	67	317	281

* not present

spectrometer detector (MSD). Only α-BHC was not detected, a subject of further investigation, however either column can be used effectively. Recoveries of 70%-80% were obtained, levels comparable to conventional SPE—without the necessity of vacuum manifolds or high pressure systems. The GPC method attained recoveries of > 95%. However this method requires large amounts of solvent and takes over twice as long as other methods.

The dSPE method shown here is an efficient, cost-effective way to clean up chlorinated pesticide residues in olive oil. With good recoveries and minimal matrix interference, it is an easy way to reduce solvent usage, compared to conventional SPE, and is more cost-effective than GPC.

References
1. C. Lentza-Rizos, E.J. Avramides, Rev. Environ. Contam. Toxicol. 141 (1995) 111.
2. S. Cunha, S. Lehotay, K. Mastovska, J. Sep. Sci. 30 (2007) 620.
3. M. Crawford, M. Halvorson, J. Stevens, The Examination and Automation of GPC, SPE and QuEChERS Utilized in Extracting Pesticides from Olive Oil. HPLC 2008 poster presentation.

Product Listing

dSPE Tube for Clean-Up of Pesticide Residue Samples

Description	Material	Methods	qty.	cat#	price
2mL Microentrifuge Tubes for dSPE					
Resprep	150mg $MgSO_4$,	AOAC			
Q250	50mg PSA	2007.1	100-pk.	26124	

PSA—primary and secondary amine exchange material.

Organochlorine Pesticide Mix AB # 3

(20 components)

aldrin	dieldrin
α-BHC	endosulfan I
β-BHC	endosulfan II
δ-BHC	endosulfan sulfate
γ-BHC (lindane)	endrin
α-chlordane	endrin aldehyde
γ-chlordane	endrin ketone
4,4'-DDD	heptachlor
4,4'-DDE	heptachlor epoxide (isomer B)
4,4'-DDT	methoxychlor

2,000μg/mL each in hexane:toluene (1:1), 1mL/ampul
cat. # 32415 (ea.)

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (μm)	temp. limits	length	cat. #	price
0.25mm	0.20	-60 to 320/340°C	30-Meter	11323	

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (μm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623	

Prevent Fraud in Egg Pasta with Simple Analysis of Cholesterol and Glycerides

*Eliminate the uncertainty of using cholesterol alone to authenticate egg content. Determine both glycerides and cholesterol in a single run using an **Rtx®-65TG** column and get definitive, fraud-identifying results.*

By Julie Kowalski, Ph.D., Food Flavors, and Fragrances Innovations Chemist, Gary Stidsen, Product Marketing Manager, Daniele Naviglio*, Professor, Analytical Chemist, and Fabiana Pizzolongo*, Ph.D., Food Technologist
*Dipartimento di Scienza degli Alimenti – Università degli Studi di Napoli "Federico II" – Via Università, 100 - 80055 Portici (NA) – Italia

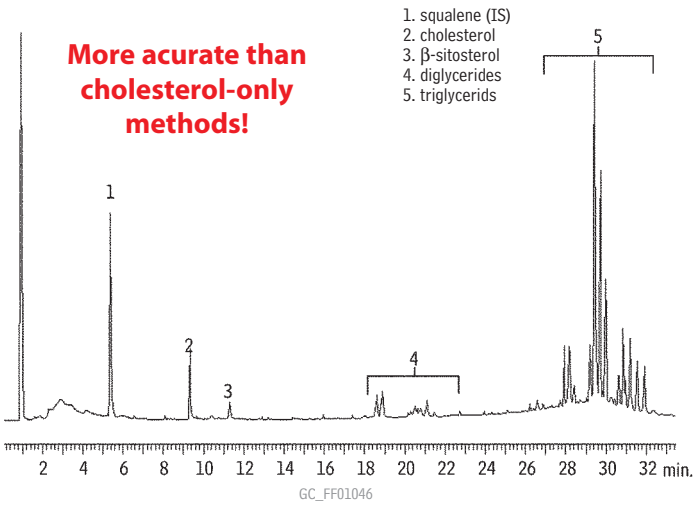
Eggs enhance the nutritional and commercial value of pasta, and thus many countries have established minimum egg content levels (based on either counts or weights) for pasta and other egg-containing products. Although egg content standards have been established, methods are not usually specified and a number of procedures may be applied. Cholesterol methods are often used to authenticate products claimed on the label to be made with eggs; however, since cholesterol can be added using non-egg sources, its presence alone is not a reliable marker of egg content. Also, even if egg is the source of the cholesterol in the product, it is difficult to correlate quantitatively to egg content levels, because the levels of cholesterol found naturally in eggs are highly variable. The method presented here allows the use of glycerides, in addition to cholesterol, to assess egg content in pasta. This method provides chromatographic separation of cholesterol, diglycerides, and triglycerides, allowing fraudulent (non-egg) sources of cholesterol to be easily and accurately determined, so qualitative and quantitative comparisons can be made.

Simple Extraction Method

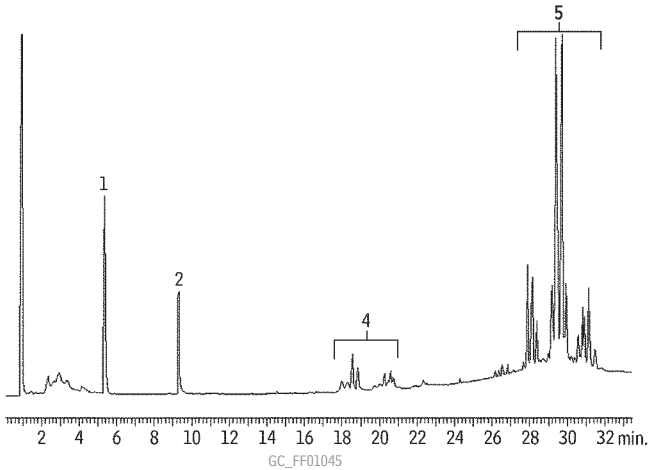
Current methods used for the extraction of fat from flour components generally involve either a 24-hour diethyl ether extraction or an 8-hour Soxhlet extraction. The extraction described here is rapid by comparison. In this simple procedure, fat is extracted from egg pasta dough and freeze-dried egg product by homogenizing the samples and pouring them into glass columns filled with sodium sulfate. The fat phase is eluted with 100mL diethyl ether and then evaporated with nitrogen. Approximately 50mg of the dried fat extract is then dissolved in 1mL internal standard solution (3,000 ppm squalene in diethyl ether). The extracted samples are analyzed by gas chromatography (GC) using an Rtx®-65TG column, which is specifically tested for triglyceride performance.

Figure 1 Easily detect fraud by comparing cholesterol and glyceride profiles in one run on the Rtx®-65TG column.

A. Extracted egg pasta fats



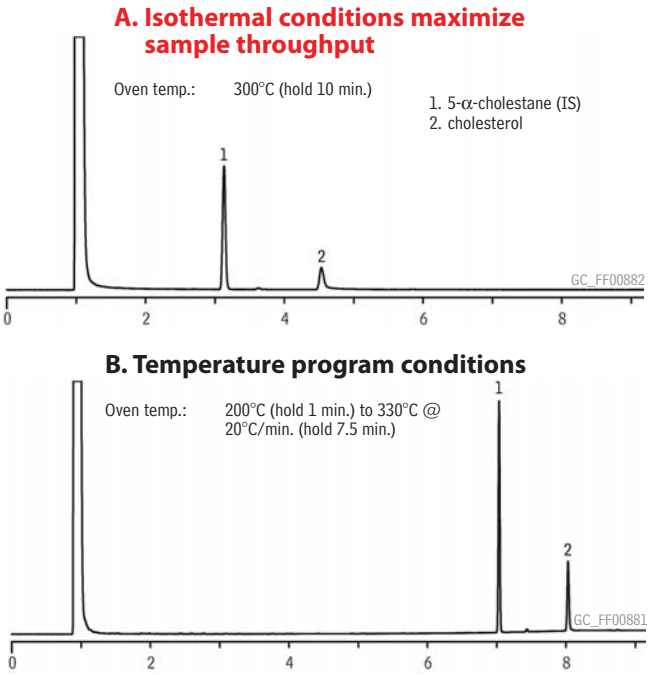
B. Extracted egg fats



Column:	Rtx®-65TG, 30m, 0.25mm ID, 0.10μm (cat.# 17008)
Sample:	A. 50μg/mL fat extract from egg pasta in diethyl ether solution with 3,000ppm squalene (IS) B. 50μg/mL fat extract from egg in diethyl ether solution with 3,000ppm squalene (IS)
Inj.:	0.5μL, split (1:80), 70°C (hold 12 sec.) at 99°C up to 370°C (hold 5 min.)
Carrier gas:	hydrogen
Flow rate:	1.5mL/min.
Oven temp.:	220°C (hold 2.0 min.) to 360°C @ 5°C/min. (hold 5 min.)
Det:	FID @ 370°C

Fraudulent label claims of egg content in egg pasta can be detected more accurately by evaluating glycerides and cholesterol, compared to analyzing cholesterol alone. This simple method determines both in a single run.

Figure 2 5-minute run times benefit cholesterol methods requiring high sample throughput instead of source confirmation.



Column:	Rxi®-5ms, 15m, 0.25mm ID, 0.25µm (cat.# 13420)
Sample:	1,000µg/mL cholesterol in DMF, 1,000µg/mL 5-α-cholestane in hexane; 25ng cholesterol, 150ng 5-α-cholestane on column
Inj.:	1.0µL, split (20:1), single gooseneck inlet liner w/wool (cat.# 22405)
Inj. temp.:	250°C
Carrier gas:	helium, constant pressure (9.7psi @ 200°C)
Linear velocity:	24cm/sec.
Oven temp.:	see above
Det.:	FID @ 340°C

Easy Identification of Fraudulent Product

Excellent chromatographic separation of cholesterol, squalene, diglycerides, and triglycerides was obtained (Figure 1). Once separated, these fractions can be used to confirm the addition of egg fat by comparing the glyceride profiles of the egg pasta extract with those from the egg sample. Egg pasta products adulterated with non-egg sources of cholesterol will not show comparable patterns. Note, while cholestane often is used as an internal standard in cholesterol testing, the use of squalene instead in this method is advantageous as it allows both cholesterol and the glyceride profiles to be analyzed. Squalene is highly stable and similar to cholesterol, but the compounds are well-resolved on the Rtx®-65TG column. Cholestane is not sufficiently separated from cholesterol on this polar phase, however, for methods that recommend cholestane, separations can be accomplished on the less polar Rxi®-5ms column (Figure 2). In fact, for methods with a goal of high throughput cholesterol determination, rather than source authentication, using the Rxi®-5ms column under isocratic conditions can cut analysis time by nearly 50%.

In summary, estimating cholesterol in food products is often part of the authentication testing of label claims regarding egg content. However, the presence of cholesterol in a product may be due to a non-egg source, and the natural variability of cholesterol levels in eggs further complicates quantitative conclusions. The method shown here simplfies fraud detection by incorporating glyceride testing. Easy comparision of the chromatographic profiles of egg and egg product (pasta) samples can be made using an Rtx®-65TG column, which is specifically tested to assure excellent separations and a reliable performance for glycerides.

Product Listing

Rtx®-65TG Columns (fused silica)

(Crossbond® 65% diphenyl/35% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.10	40 to 370°C	30-Meter	17008	

Rxi®-5ms Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	15-Meter	13420	

Fast Screening and Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine

Maximize your analytical options with this versatile GHB extraction method. No derivatization means faster sample preparation. Extracts are amenable to both liquid injection GC/FID and headspace GC/MS methods.

By Amanda Rigdon, Pharmaceutical Innovations Chemist and Kristi Sellers, Clinical/Forensic Innovations Chemist

Gamma-hydroxybutyrate (GHB) and its precursor, gamma-butyrolactone (GBL), are controlled substances associated with drug-facilitated sexual assault. Criminal cases often hinge on lab results, which can include screening urine samples and then quantifying GHB using GC/MS. In its native state, GHB is extremely difficult to chromatograph and must be analyzed as a trimethylsilyl derivative or converted to GBL. The headspace (HS) procedure described here (adapted from an FBI Chemistry Unit method) eliminates time-consuming derivatization.[1] This procedure reduces sample preparation time and minimizes both column contamination from derivatization reagents and contamination from sample matrix caused by liquid injections.

Eliminate Derivatization and Reduce System Contamination

Samples were spiked in urine and extracted according the procedure in Table I, using alpha-methylene-gamma-butyrolactone (AMGB) as an internal standard. GHB is converted to GBL with sulfuric acid, eliminating the need for derivatization (Figure 1). Note the unconverted sample shows comparable levels of GBL and AMGB, whereas GBL levels in the converted sample are significantly higher, due to the conversion of GHB to GBL.

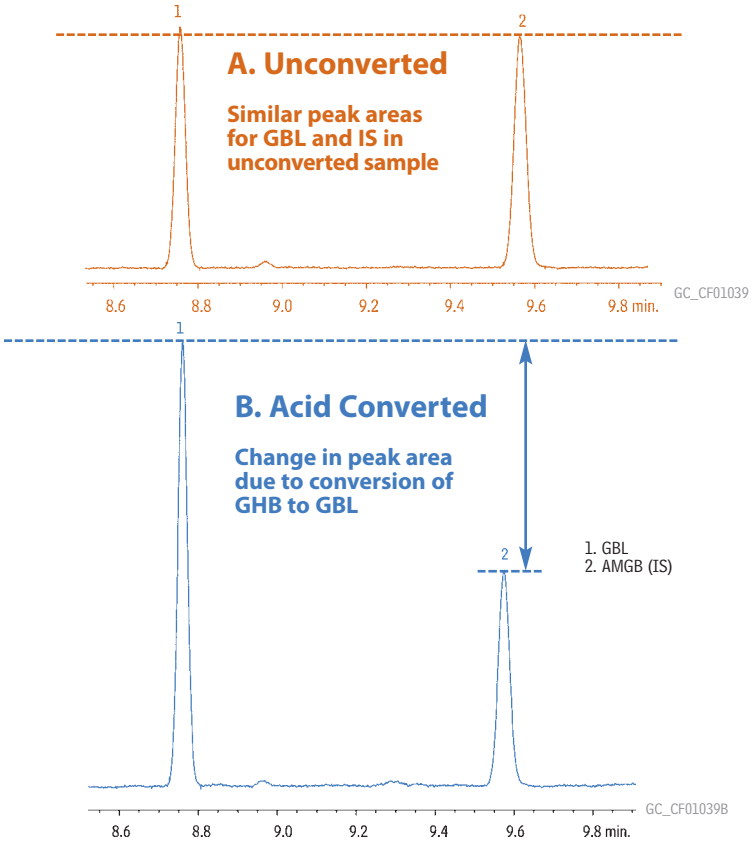
Reliably Screen Samples Using Existing Blood Alcohol Testing Set-Up

Headspace injections (using the total vaporization technique) of the final urine extracts were screened by GC/FID using an Rtx®-BAC1 column in a blood alcohol headspace GC system. This system is com-

Table I Extraction procedure for GHB and GBL.

1. Label two screw top test tubes per specimen. One for total GHB, the other for GBL only.
2. Add 1mL of sample (urine) to each tube.
3. Add 50µL of AMGB to each tube.
4. Add 150µL concentrated sulfuric acid only to tubes used for analysis of total GHB.
5. Vortex all tubes and allow them to sit 5 minutes.
6. Add 5mL methylene chloride to each tube. Shake 10 minutes to extract.
7. Centrifuge samples at 3,000 rpm for 5 minutes.
8. Transfer bottom (methylene chloride) layer to a clean test tube for drying.
9. Concentrate samples to ~100µL at 30°C under nitrogen.
10. For headspace analysis, inject 15µL of sample into a capped headspace vial. Or, for liquid injection, transfer extract to a limited volume insert.

Figure 1 GHB can be converted to GBL for analysis, saving time by eliminating derivatization.



Column:	Rtx®-BAC1, 30m, 0.32mm ID, 1.8µm (cat.# 18003)	Headspace conditions:	Equilibration temp.: 100°C Equilibration time: 10 min. Injection volume: 1mL
Sample:	50µg/mL GHB, GBL, and AMGB (IS) in urine A: unconverted B: converted with sulfuric acid	Oven temp.:	50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.)
Inj.:	1mL headspace injection, split (10:1), 1mm split inlet liner (cat.# 20972)	Det.:	FID @ 240°C
Inj. temp.:	200°C	Hydrogen:	40mL/min.
Carrier gas:	helium, constant flow	Air:	400mL/min.
Flow rate:	1.0mL/min.	Makeup:	40mL/min.

Sample: urine spiked with 50µg/mL each GHB, GBL, and AMGB (IS), extracted according to procedure in Table I, and analyzed using headspace (total vaporization technique).

This versatile extraction and headspace method improves lab efficiency and reduces both contamination and matrix effects by eliminating the need for derivatization and liquid injections.

Figure 2 GHB (analyzed as GBL) confirmation method calibration curve for headspace GC/MS analysis (10-300µg/mL in urine).

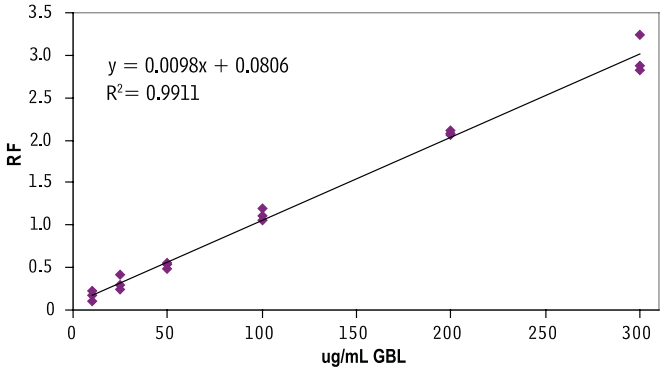
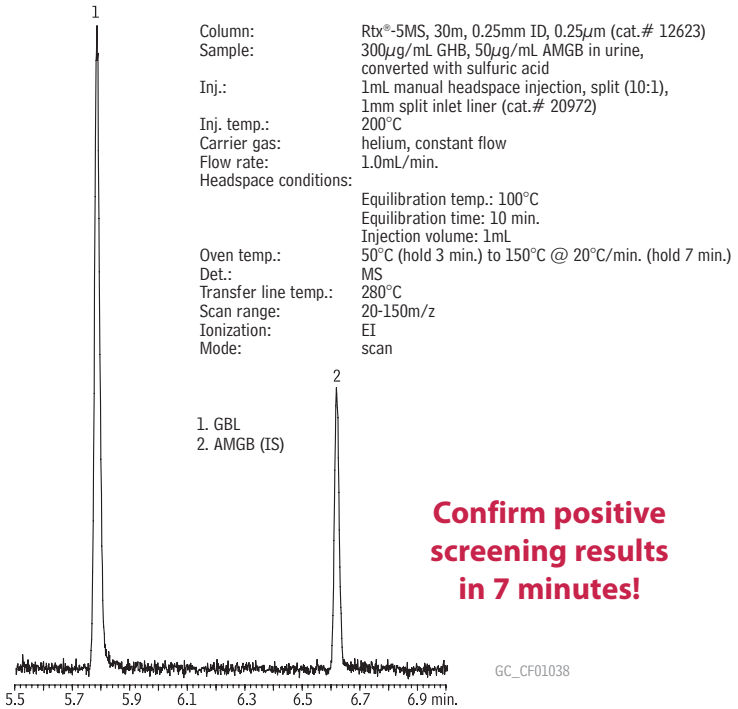


Figure 3 Confirmation headspace GC/MS analysis of 300µg/mL converted GHB (analyzed as GBL) standard in urine.



Confirm positive screening results in 7 minutes!

monly used in clinical/forensic labs, eliminating the need for additional equipment. Excellent linear response was obtained from both unconverted (r^2 = 0.9992, 10-100µg/mL 4-point curve) and converted GHB in matrix (r^2 = 0.9910, 20-200µg/mL 4-point curve) with AMBG at 50µg/mL.

Fast, Definitive Confirmation Analysis by Headspace GC/MS

Positive screening results were quickly confirmed on an Rtx®-5MS column by headspace GC/MS; several quantification and qualifier ions were identified for each compound (GBL: 42, 56, 86; AMBG: 40, 68, 98). Again, excellent linearity was achieved (Figure 2) and analysis time was less than 7 minutes (Figure 3).

In summary, the versatile extraction and headspace method shown here saves lab time and minimizes contamination by eliminating the need for derivatization and by reducing matrix effects. Rapid screening is accomplished on commonly used blood alcohol GC columns, allowing labs to reduce costs by using existing equipment. Confirmation testing using the Rtx®-5MS column, provides the definitive results needed in court with a fast analysis time of less than 7 minutes.

References
1. M.A. LeBeau, M.A. Montgomery, M.L Miller, S. G. Burmeister, J. Anal. Toxicol. 24 (2000) 421.

Product Listing

Rtx®-BAC1 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	1.80	-20 to 240/260°C	30-Meter	18003	

Rtx®-5MS—Low-bleed GC/MS Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623	

Exempted Drug of Abuse Reference Materials

Concentration is µg/mL. Volume is 1mL/ampul.

Compound	CAS#	Solvent Code	Conc.	cat.#	price
GHB					
γ-butyrolactone (GBL)	96-48-0	ACN	1,000	34077	
α-methylene-γ-butyrolactone (AMGBL)	547-65-9	ACN	1,000	34079	

ACN=acetonitrile

1mm Split Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#	price
1mm Split 1.0mm x 6.3mm x 78.5mm	ea.	20972	

Beyond C18—Increase Retention of Hydrophilic Compounds Using Biphenyl Columns

*Searching for a better way to retain hydrophilic aromatic drug compounds? Biphenyl phases, such as the **Pinnacle® DB Biphenyl** column, provide greater retention than alkyl phases. Use a Biphenyl column to separate difficult-to-retain polar aromatics from unretained matrix contaminants.*

By Amanda Rigdon, Pharmaceutical Innovations Chemist and Rick Lake, Pharmaceutical Market Development Manager

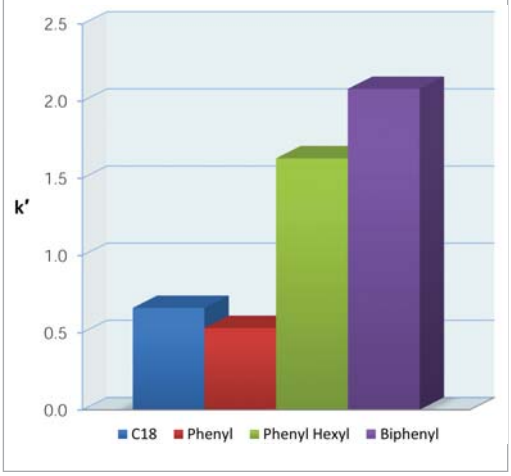
Many drug classes include compounds with aromatic ring structures, some of which also contain a sulfone or sulfoxide group. Both sulfur groups have dipole moments, adding a hydrophilic character to compounds containing these functional groups. The analysis of hydrophilic compounds on a traditional alkyl column (e.g., C18) can be problematic, since alkyl columns depend on hydrophobic (dispersive) interactions for retention. Since the sulfone and sulfoxide groups contain π bonds, the Biphenyl column's affinity toward compounds containing these bonds makes it a logical choice when increased retention of compounds containing these groups is desired.

To explore the selectivity of the biphenyl phase towards sulfur-containing aromatic compounds, phenyl sulfone, a simple probe, was analyzed on alkyl (C18), phenyl, phenyl hexyl, and Biphenyl columns to determine the relative retention of each phase, as measured by capacity factor (k'). In order to ensure separation of analytes from unretained contaminants, a minimum k' value of 2 is recommended for most analyses, however in cases where there is little to no matrix interference, a k' of 1 may be acceptable. The data in Figure 1 show that phenyl sulfone is retained to a much greater degree on the Pinnacle® DB Biphenyl column, than on the other phases tested (k' = 2.08). This is due to the unique retention mechanism of the biphenyl stationary phase, which can interact with both the hydrophobic aromatic ring and the hydrophilic sulfone group through π-π interactions. Although the phenyl stationary phase also allows for the use of π-π interactions, the biphenyl phase has a larger electron cloud and is significantly more retentive.

To further test the retention of the Biphenyl column, a second set of probes, consisting of compounds in the NSAID family, was analyzed. Tenoxicam, which contains a sulfone group, and sulfinpyrazone, which contains a sulfoxide group, were analyzed along with a void marker (uracil). Although these compounds are more complex than the probe used in the first experiment, the same pattern of retention was observed (Figure 2). The Pinnacle® DB Biphenyl column exhibited the greatest retention for tenoxicam. With k' values of 0.33 on the C18 and 0.49 on the phenyl columns, tenoxicam shows almost no retention on these stationary phases. The phenyl hexyl phase performed slightly better with a k' value of 1.52 for tenoxicam. However, when tenoxicam was analyzed on the Biphenyl column under the same conditions, the k' value increased to 2.22, a value much more likely to provide adequate resolution from matrix components. Sulfinpyrazone, a less polar compound, also followed the same pattern of retention (Table I).

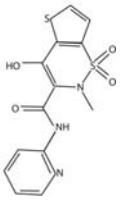
The improved retention for hydrophilic aromatics shown here is due to the unique π-π interaction retention mechanism of the Biphenyl phase. This mechanism is particularly useful for analysis of sulfone- and sulfoxide-containing drug compounds, which are not easily retained on alkyl or phenyl phases. The Biphenyl phase provides greater retention than alkyl and phenyl phases and is ideal for separating difficult-to-retain polar aromatics from unretained matrix contaminants.

Figure 1 The Biphenyl phase is more retentive for phenyl sulfone than other alkyl and phenyl phases.

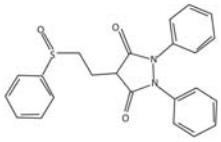


Biphenyl columns are much more effective than alkyl, phenyl, or phenyl hexyl phases when increased retention of hydrophilic aromatics is desired.

Figure 2 Only the Biphenyl phase retains both test probes to k' > 2, the level recommended to ensure separation from unretained matrix contaminants.



2. tenoxicam



3. sulfinpyrazone

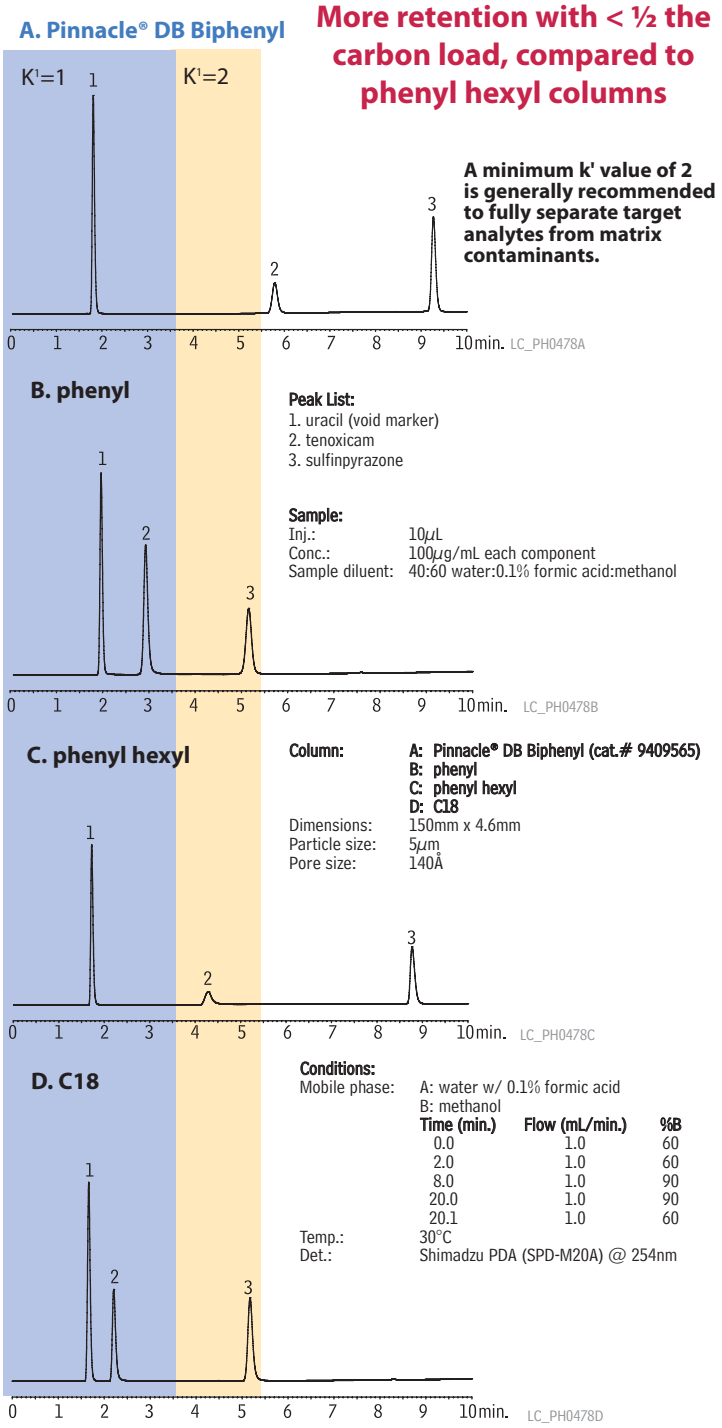


Table I Biphenyl columns show improved retention of sulfone- and sulfoxide-containing aromatic drugs.

K' Value	Biphenyl	Phenyl hexyl	Phenyl	C18
Tenoxicam	2.23	1.39	0.637	0.235
Sulfinpyrazone	4.18	3.90	1.88	1.89

Product Listing

Pinnacle® DB Biphenyl Columns (USP L11)

particle size: 1.9µm, 3µm or 5µm, spherical
pore size: 140Å
carbon load: 8%
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

3µm Column, 1.0mm	cat. #	price
30mm	9409331	
50mm	9409351	
100mm	9409311	
150mm	9409361	
3µm Column, 2.1mm	**cat. #**	**price**
30mm	9409332	
50mm	9409352	
100mm	9409312	
150mm	9409362	
3µm Column, 3.2mm	**cat. #**	**price**
30mm	9409333	
50mm	9409353	
100mm	9409313	
150mm	9409363	
3µm Column, 4.6mm	**cat. #**	**price**
30mm	9409335	
50mm	9409355	
100mm	9409315	
150mm	9409365	
5µm Column, 1.0mm	**cat. #**	**price**
30mm	9409531	
50mm	9409551	
100mm	9409511	
150mm	9409561	
200mm	9409521	
250mm	9409571	
5µm Column, 2.1mm	**cat. #**	**price**
30mm	9409532	
50mm	9409552	
100mm	9409512	
150mm	9409562	
200mm	9409522	
250mm	9409572	
5µm Column, 3.2mm	**cat. #**	**price**
30mm	9409533	
50mm	9409553	
100mm	9409513	
150mm	9409563	
200mm	9409523	
250mm	9409573	
5µm Column, 4.6mm	**cat. #**	**price**
30mm	9409535	
50mm	9409555	
100mm	9409515	
150mm	9409565	
200mm	9409525	
250mm	9409575	

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Two Options for Analyzing Potential Genotoxic Impurities in Active Pharmaceutical Ingredients

*Laboratory needs for analyzing PGIs in API vary. Here we developed both a fast analysis of sulfonate esters on the **Rxi®-5Sil MS column**, and a comprehensive method for both sulfonate esters and alkyl halides on the **Rtx®-200 column**.*

By Amanda Rigdon, Pharmaceutical Innovations Chemist, Rick Lake, Pharmaceutical Market Development Manager, Claire Heechoon*, Research Chemist, Roy Helmy*, Ph.D., Research Fellow, Christopher Strulson*, Research Assistant, and Margaret Figus*, Research Chemist
*Merck & Co., Inc.

Compounds that are used in the synthesis of active pharmaceutical ingredients (API), or reaction byproducts that form during synthesis, have the potential to remain as impurities in API. Some of these compounds are potentially genotoxic impurities (PGIs) and may raise concern about cancer and/or birth defects. Because of the toxicity of these compounds, it is essential that they be controlled to low levels in API after synthesis. In January of 2007, the European Medicines Agency (EMEA) released guidance on acceptable limits of PGIs in APIs (Guideline on the Limits of Genotoxic Impurities (EMEA/CHMP/QWP/251344/2006)). Developing new methods for sensitive detection of impurities is an increasingly active area of research across the pharmaceutical industry.

Scientists from Merck, in collaboration with Restek, have developed a fast method for the analysis of sulfonate esters on the Rxi®-5Sil MS column.

Figure 1 Sulfonate ester PGIs. Differences between sulfonate esters and alkyl halides make the analysis of mixtures challenging.

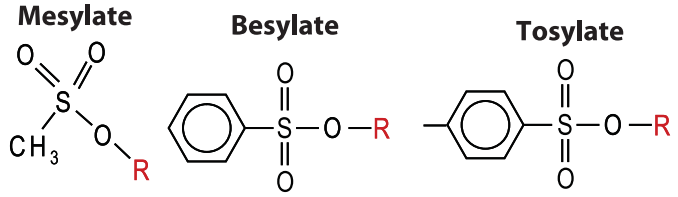
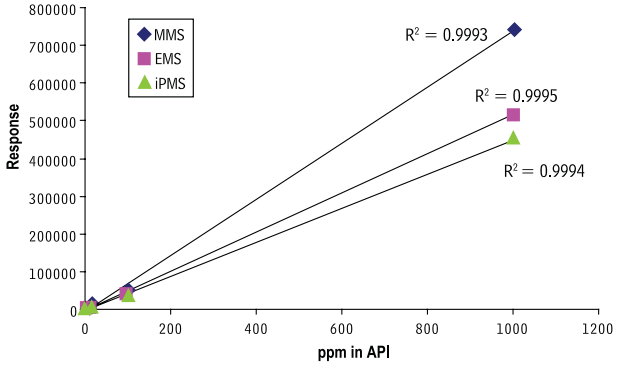


Figure 2 Linearity of fast GC/MS analysis for selected sulfonate esters.



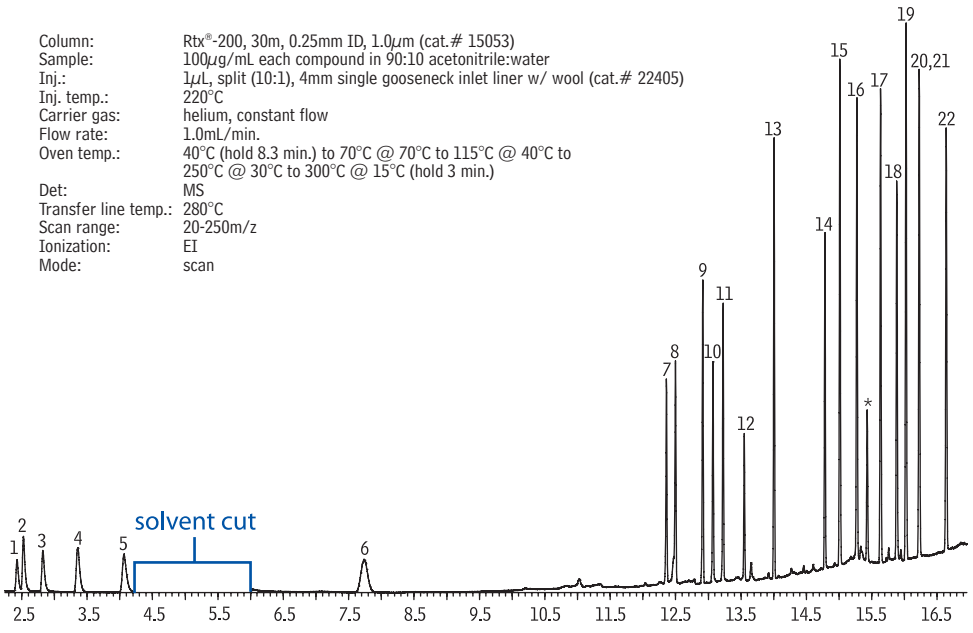
Four structural classes of PGIs are discussed in this article. The first three classes, known collectively as sulfonate esters, include mesylates, besylates, and tosylates (Figure 1). These alkylating sulfonic acid esters may form when sulfonic acid reacts with an alcohol solvent. The first three classes are differentiated by the group that forms an ester with the sulfur: mesylates contain a methyl group, besylates contain a phenyl (benzyl) group, and tosylates contain a toluene group. The fourth class of PGIs tested here, alkyl halides, consists of short alkyl chains with halogen constituents. Since alkyl halides are polar and very volatile, they are not retained well on thin film stationary phases. This can make analysis of a mixture of sulfonate esters and alkyl halides quite problematic.

Two options for the analysis of PGIs in API have been developed to meet different laboratory needs. The first option is a fast method for the analysis of sulfonate esters on the Rxi®-5Sil MS column. The second option is a comprehensive method for the analysis of both sulfonate esters and alkyl halides on the Rtx®-200 column. Both methods require very little sample preparation, which helps increase laboratory productivity.

Option 1: Fast Analysis of Sulfonate Esters

Scientists from Merck, in collaboration with Restek, have developed a fast method for the analysis of sulfonate esters on the Rxi®-5Sil MS column. The use of a thin film Rxi®-5Sil MS column allows for speedy analysis of these active compounds. Since the Rxi®-5Sil MS column is very selective toward sulfonate esters, a fast oven program can be used to speed analysis. This method allows for the analysis of selected sulfonate esters in less than 4.5 minutes. A linearity study performed by Merck shows that this method is linear for sample concentrations from 1ppm to 1,000ppm in API (Figure 2). Depending on the dose of API to the patient, it may be necessary to detect levels of impurities as low as 1 ppm in order to meet EMEA requirements. The 1ppm spike represents the threshold for toxicological concern (TTC) as set by the EMEA for PGIs.

Figure 3 Small, polar alkyl halides are well-retained on the fluorinated Rtx®-200 column, as are less volatile sulfonate esters.



1. 1-chloropropane
2. bromoethane
3. 2-chloropropane
4. 1-bromopropane
5. 2-bromopropane
6. 1-bromobutane
7. dimethyl sulfate (DMS)
8. methyl methanesulfonate (MMS)
9. ethyl methanesulfonate (EMS)
10. isopropyl methanesulfonate (iPMS)
11. diethyl sulfate (DES)
12. di-isopropyl sulfate (DPS)
13. di-*n*-propyl sulfate (DNS)
14. dibutyl sulfate (DBS)
15. methylbenzene sulfonate (MBS)
16. ethylbenzene sulfonate (EBS)
17. methyl toluenesulfonate (MTS)
18. ethyl toluenesulfonate (ETS)
19. *n*-butyl benzenesulfonate (nBBS)
20. *n*-propyl toluenesulfonate (nPTS)
21. isopropyl toluenesulfonate (iPTS)
22. *n*-butyl toluenesulfonate (nBTS)

*ortho isomer of ETS

Option 2: Comprehensive PGI Method

Although the thin film Rxi®-5Sil MS column allows for fast analysis of sulfonate esters, the smaller, more polar alkyl halides are not well retained. To take advantage of the halogen constituents on the alkyl halides, a thick film Rtx®-200 column was used to develop a comprehensive method for both volatile alkyl halides and less volatile sulfonate esters. Since the Rtx®-200 column has a fluorinated stationary phase, the alkyl halides are well-retained (Figure 3). Note that all of the alkyl halides elute at a low temperature and some of the more volatile compounds elute prior to the sample solvent (acetonitrile). Because of this, the solvent cut time must be carefully measured. The Rtx®-200 column is also selective for sulfonate esters, providing baseline resolution for 20 out of 22 of the compounds analyzed (Figure 4). Additionally, the increased polarity of the fluorinated Rtx®-200 phase allows for the use of splitless injection of more polar sample solvents, such as methanol.

Conclusion

Since potential genotoxic impurities are of increasing concern for both regulatory bodies and consumers, the importance of effective methods for detection and quantitation of these compounds is growing. As a result of collaboration between Merck and Restek, two easy, sensitive options are now available for the analysis of PGIs in API using inert, selective columns from Restek.

Product Listing

Rtx®-200 Columns (fused silica)

(Crossbond® trifluoropropylmethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.20	-20 to 310/330°C	20-Meter	45002	
0.25mm	1.00	-20 to 290/310°C	30-Meter	15053	

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.18	-60 to 330/350°C	20-Meter	43602	

Reduce Downtime with Robust Lipidomics Method

*Why lose days to downtime? Restek columns, such as the 10,000 injection **Rxi®-5ms** column shown here, are rugged and built for consistent long-term performance.*

By Julie Kowalski, Ph.D., Innovations Chemist, and John Hanley Jr.*, Ph.D., Platform Development Manager
*Lipomics Technologies

Lipidomic studies of cholesterol synthesis, absorption, and excretion, provide information central to the investigation of cardiovascular disease and other disorders. High-throughput methods are critical to lipidomics and are used to screen thousands of samples in order to identify biomarkers and clinical diagnostics with significant predictive power. Labs can save days of downtime by using an Rxi®-5ms column in assays similar to our test method for cholesterol and low-level sterol metabolites.

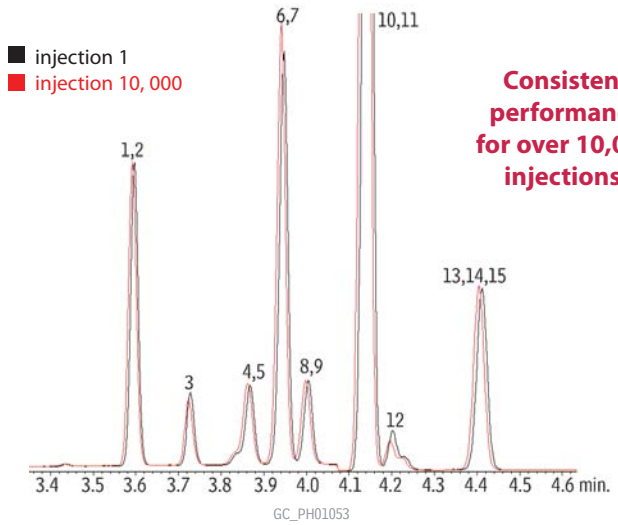
Here, extremely reproducible results were obtained using an Rxi®-5ms column, which gave highly consistent separations—even after 10,000 injections (Figure 1). In our method, biological samples were treated to form trimethylsilyl derivatives. Two injections were made: one to quantify minor sterols using a 10:1 split, and another, using a 100:1 split, to analyze cholesterol. To achieve maximum sensitivity for low-level sterols, multiple SIM retention time windows were set up.

Reduced downtime for column changes and revalidation significantly increased lab productivity.

Stable retention times are critical to our testing program as revalidation is required if significant drift occurs. Revalidation requires days of downtime because inter-day variability must be assessed. The Rxi®-5ms column was chosen for this method, in part, because its long lifetime and stable performance reduced the number of column changes and revalidations, resulting in more days of productive analyses.

We found the performance of the Rxi®-5ms column to be remarkably consistent and reliable for high-throughput testing. The Rxi®-5ms column should be considered by labs running similar lipidomic methods that would benefit from a highly reproducible performance—or by any lab interested in reducing downtime and increasing productivity.

Figure 1 Stable, highly reproducible results on the Rxi®-5ms column mean less downtime and more productive days.



Compound:	m/z
1. coprostanol	370.40
2. d5-cholestanol (IS)	220.20
3. 7α-hydroxycholesterol	456.40
4. cholestanol	306.30
5. d5-epicholestanol (IS)	220.20
6. 7-dehydrocholesterol	325.25
7. desmosterol	343.25
8. lathosterol	458.35
9. d4-lathosterol (IS)	462.40
10. campesterol	382.35
11. 4-cholestenone	382.30
12. stigmasterol	394.15
13. lanosterol	393.35
14. β-sitosterol	396.35
15. d7-β-sitosterol (IS)	403.45

Column:	Rxi®-5ms, 30m, 0.25mm ID, 0.25µm (cat.# 13423)
Sample:	lipid plasma extract as trimethylsilyl derivatives
Inj.:	1µL, split (10:1), 4mm gooseneck inlet liner w/wool
Inj. temp.:	310°C
Carrier gas:	helium, constant flow
Flow rate:	1.0mL/min.
Oven temp.:	250°C (hold 1 min.) to 320°C @ 30°C/min. (hold 1.6 min.)
Det:	MS
Transfer line temp.:	330°C
Ionization:	EI
Mode:	SIM

Product Listing

Rxi®-5ms Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13423	

Achieving Faster GC

Continued from page 2

separation (and another disappointed user is born!). Please also bear in mind that the above options will reduce all baseline segments in your chromatogram to the same extent. So, if you have over-resolution throughout your chromatogram except for one critical peak pair that is just barely resolved, forget about these options. In general however, all of the above options are low-risk options that could be tried before moving on to the more elaborate steps discussed below.

Now that you have eliminated all the empty parts of the baseline you can move to step 2, maximizing the selectivity of the system. Selectivity is the ability to distinguish between compounds. This can be done through the separation or through detection (once the method for sample preparation has been selected). Options for improving selectivity include:

- using a more selective stationary phase or coupled columns.
- using conventional 2-dimensional or comprehensive 2-dimensional GC.
- using selective detection, with mass spectrometry (MS) being particularly attractive.
- backflushing.

Because the above options are all rather expensive and require special instruments and expertise, the only really widely used option is the use of MS detection. Indeed MS is a marvellous way to get selectivity in an easy and quick way.

You have now gone through the two initial steps of speeding up your method. You have selected a system that offers you the required resolution, yet not more resolution than really needed. If the analysis time in this "minimum acceptable resolution" situation still exceeds the desired or permitted time, options that reduce the analysis time at constant resolution should be exploited. Possibilities include:

- reducing the column inner diameter.
- using hydrogen as the carrier gas.
- appling vacuum-outlet conditions.
- using turbulent flow conditions.

Of these options the first two always work; however, vacuum operation only works if you have a separation on a short wide-bore column, and turbulent flow operation in practice is of little use.

Mea culpa, with more than 20 papers published on fast GC, I have also contributed to the chaos in faster GC. I hope the above discussion helps resolve at least part of the confusion. Faster GC is possible, it is always possible, and the need for it is actually still increasing as a result of recent trends in process control and high-throughput experimenting.

1. P. Korytár, H.-G. Janssen, E. Matisová, U.A.Th. Brinkman, Trends in Analytical Chemistry 21 (2002) 558-572.

Hans-Gerd Janssen received his Ph.D. in analytical chemistry from Eindhoven University in 1991. After having worked at Eindhoven as an associate professor for eight years, he joined Unilever Research to work as the group leader for chromatography and mass spectrometry. In 2004, Hans-Gerd was appointed part-time professor at the University of Amsterdam, focusing on biomacromolecular separations.

Restek On-the-Road

Tradeshow Schedule

October, 2008

Show: **2008 NIH Research Festival Exhibit**
Date: Oct. 16-17
Location: National Institutes of Health, Bethesda, MD

Show: **Society of Forensic Toxicologists (SOFT)**
Date: Oct. 27-31
Location: Arizona Grand Hotel, Phoenix, AZ

Show: **COLACRO XII**
Date: Oct. 28-30
Location: Florianopolis Convention Center, Florianopolis, Brazi

November, 2008

Show: **2008 AAPS Annual Meeting & Expo**
Date: Nov. 16-20
Location: Georgia World Congress Center, Atlanta, GA

Show: **Eastern Analytical Symposium (EAS)**
Date: Nov. 17-20
Location: Garden State Convention Center, Somerset, NJ

Show: **Symposium on Air Quality Methods & Technology**
Date: Nov. 3-6
Location: Chapel Hill, NC

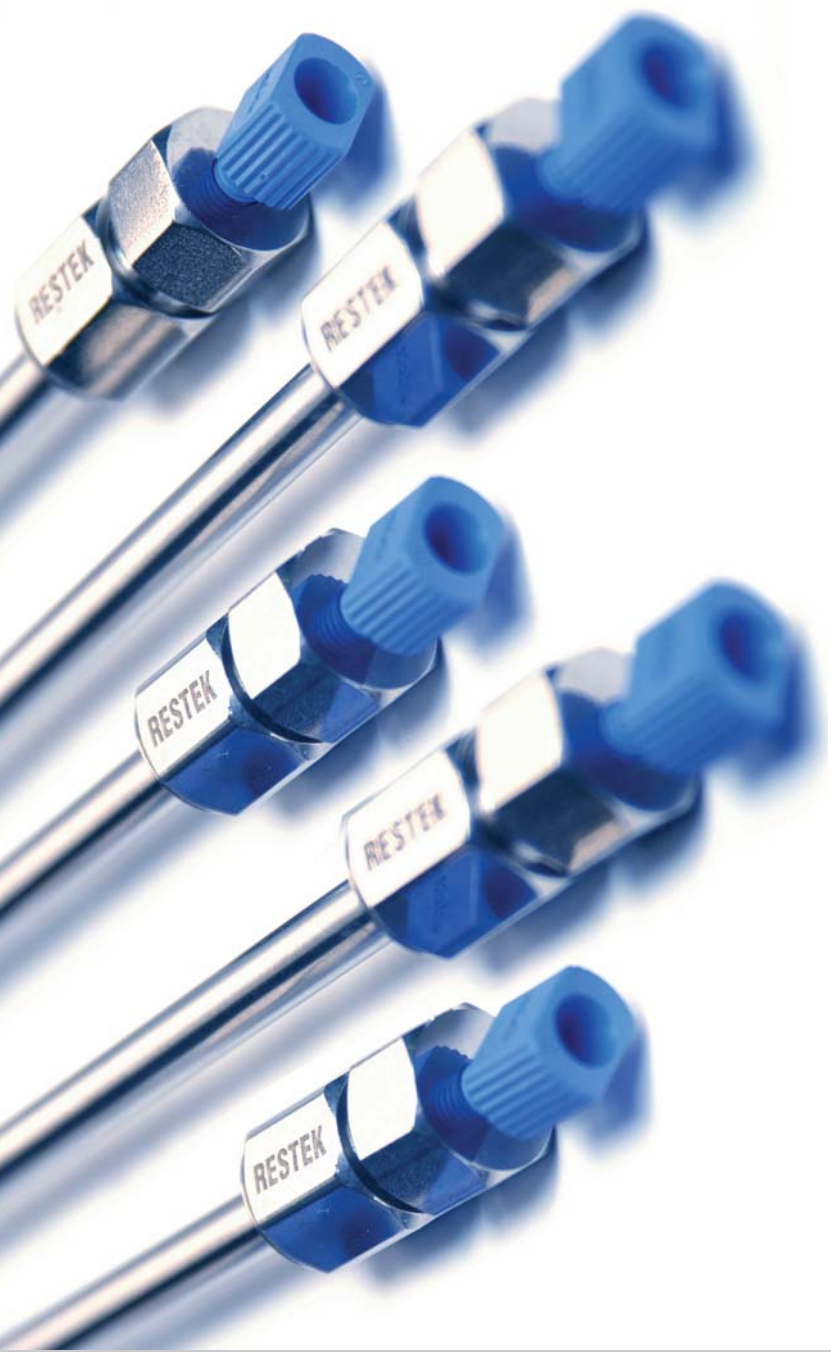
Show: **LC/MS Montreux Symposium**
Date: Nov. 12-14
Location: Montreux Convention Center, Montreux, Switzerland

January, 2009

Show: Gulf Coast Conference
Date: Jan. 20-21
Location: Moody Gardens Convention Center, Galveston, TX

Seminar Schedule

Date	Cat. #	City	State
Petrochemical Seminar			
10/27	65746	Corpus Christi	TX
10/29	65747	Houston	TX
10/31	65748	Oklahoma City	OK
Comprehensive HPLC			
11/3	65749	Seattle	WA
11/5	65750	San Francisco	CA
11/7	65751	San Jose	CA



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- Accurately monitor PEGylation reactions
- Quality control in metabolomics
- Faster PAH sample throughput

...and much more

in this issue

2008.02

Quality Control in Metabolomics

Oliver Fiehn, UC Davis Genome Center

Comprehensive analysis of small molecule metabolites (30-1500 Da) is a challenging task for quality control. Metabolites are found in very different concentrations in complex biological matrices, from which they have to be extracted without compromising the structural integrity and relative abundances. There are metabolites which are transformed extremely rapidly if enzymatic activity is not stopped completely at the time of sample collection, such as the ratio of the energy metabolites ATP to ADP. Similarly, redox carriers such as NADH and NADPH are very sensitive to oxidative degradation during sample preparation. Consequently, quality control in metabolomics means more than just taking care of chromatographic or mass spectrometry parameters. Quality control is an attitude towards gaining reliable data, rather than an automatic procedure implemented in instrument software.

The first issue critical to obtaining valid metabolomic data is understanding the question behind a study. This means that communication with the partners of the metabolomic laboratory is an essential part of any metabolomic study. Most often, at least one other partner will be involved in a study (e.g. another laboratory focused on understanding the effect of a particular genetic alteration in an organism), and these partners may already have hypotheses on specific metabolic pathways that should be pursued. These hypotheses may then lead to suggestions for analytical procedures. For example, many secondary metabolites are easier to analyze by LC/MS methods whereas most primary metabolites can readily be quantified by GC/MS procedures. Therefore, communication with the partners should focus on the chemical classes of compounds that should be targeted. It is also critical for the analytical laboratory to understand that unbiased analysis of mass spectrometric data sets does not constitute metabolomics. A multivariate statistical differentiation of 'test' versus 'control' samples is meaningless if no identified metabolites can be reported that allow biological interpretation! Unidentified signals in metabolite analysis are as useless as unscored peptide peaks in proteomic experiments. Metabolomics is not a number game of detection of m/z features, but must be regarded as an extension of classical target-driven analytical chemistry. Only if the quantification and identification of known compounds empowers biological interpretations, can unknown peaks be further investigated and pulled into statistical tests.

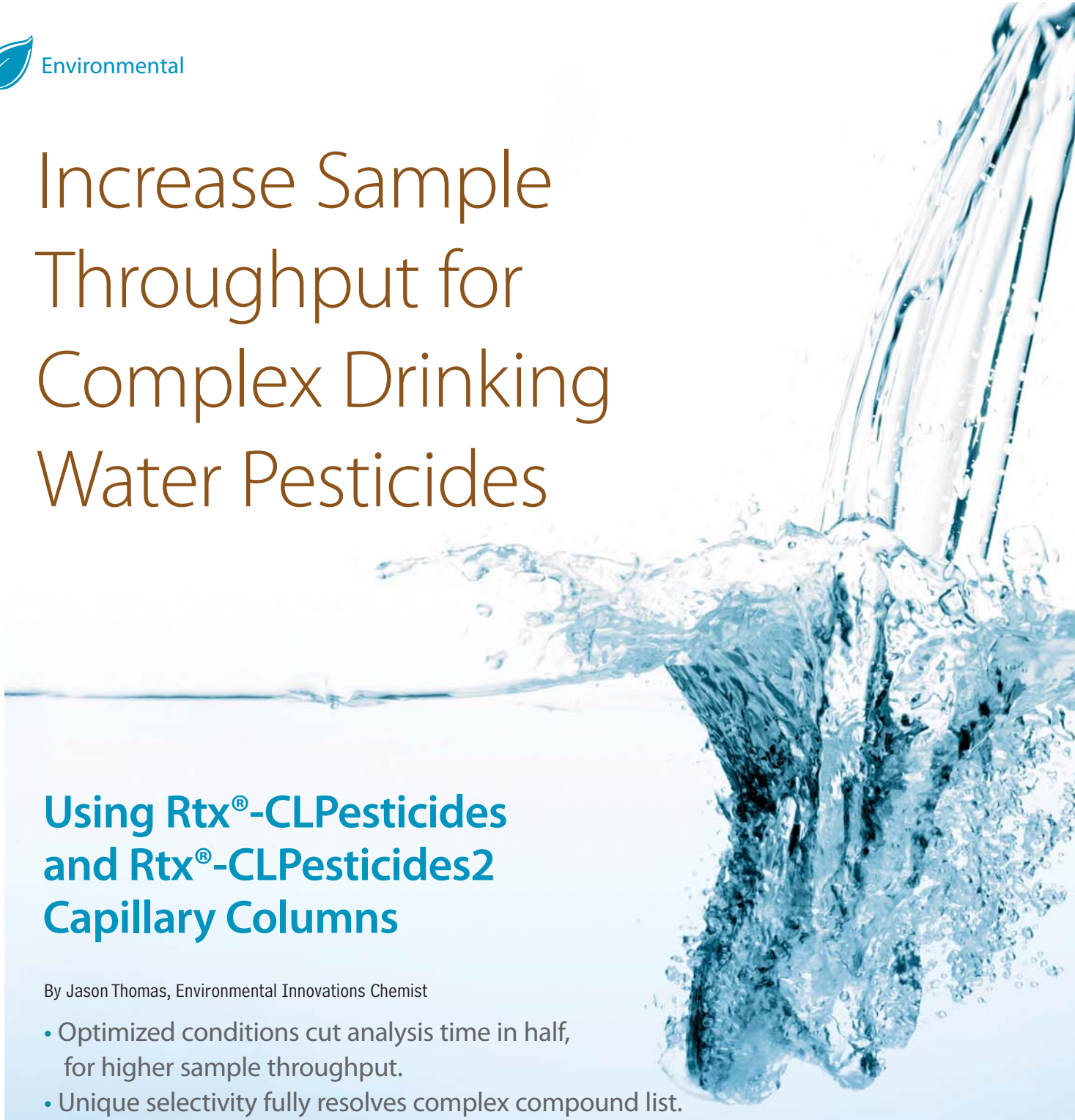
> Metabolomics is not a numbers game of detection; it is an extension of classical target-driven analytical chemistry.

There is a fundamental problem associated with metabolomics analyses, that is, the lack of clean up steps. If metabolomics means a comprehensive analysis of a wide range of small molecules, varying in molecular size, functional moieties, lipophilicity, volatility, or other physicochemical parameters, then the analytical laboratory faces tough choices. One option is to employ a variety of fractionation steps, but this can cause biases in metabolite coverage, require a number of different analytical procedures (raising the subsequent challenge of integrating the data sets), and also may result in analyte loss or degradation. Alternatively, the whole extract is subjected to one or several analytical methods; however, certain matrix components may lead to deterioration of analytical quality. In such cases, literally dirt is injected into the instrument! It is critical, therefore, to acknowledge that each matrix type requires validation and that procedures that worked for microbial organisms may be very inadequate for more complex samples such as blood plasma. For example, nonvolatile material will remain in the liner and other parts of the injector in GC/MS systems, causing problems with cross-contamination, progressing pyrolysis of material, and ultimately the formation of adsorptive materials, or catalytically active sites, in the injector system. Therefore, frequent liner changes are highly recommended.

Correspondingly, for LC/MS procedures, matrix components may be irreversibly adsorbed onto stationary phases, giving rise to similar challenges as described for GC/MS. Additionally, the soft electrospray ionization in LC/MS is a more selective or vulnerable

Continued on page 23

Increase Sample Throughput for Complex Drinking Water Pesticides



Using Rtx®-CLPesticides and Rtx®-CLPesticides2 Capillary Columns

By Jason Thomas, Environmental Innovations Chemist

- Optimized conditions cut analysis time in half, for higher sample throughput.
- Unique selectivity fully resolves complex compound list.
- Meets all method QA requirements, reducing rework.

With the advent of modern agriculture, and its vast selection of chemical pest control measures, the farming community has made significant increases in productivity and efficiency. Crop yield per acre is at an all time high, due in part to the role of pesticides and herbicides in mitigating the devastating effects of many plant and insect pests.[1] However, the use of these chemicals can have drawbacks, including surface and ground water contamination. EPA Methods, such as 508.1, are used to monitor pesticides and herbicides in drinking and ground water. The optimized dual column method shown here satisfies all method requirements in half the analysis time, significantly improving sample throughput.

Continued on page 4.

Increase Sample Throughput for Complex Drinking Water Pesticides

Continued from page 3.

EPA Method 508.1 includes many of the components as Method 505, a similar GC/ECD method, but also contains several others, expanding the list to 38 compounds. This method calls for solid phase extraction and extract concentration, followed by analysis using a GC/ECD system. In order to increase sample throughput, an optimized method was developed using a dual column configuration with the Rtx®-CLPesticides/Rtx®-CLPesticides2 column pair. These columns, used under the conditions shown, offer a unique selectivity that allows the target analytes to be resolved in approximately half the analysis time of the original method (Figure 1). There was one coelution on the primary column, but these compounds were separated on the second column. Both columns easily passed the comprehensive system performance criteria adapted from 508.1 (Table I).[2]

In conclusion, due to the complexity of the compound list in Method 508.1, a very high degree of selectivity is required of the capillary column in order to achieve adequate resolution of all target analytes in a reasonable time. The optimized dual column method shown here offers a significantly faster analysis time, while maintaining excellent resolution of challenging drinking water pesticides and herbicides.

References

1. http://www.usda.gov/nass/pubs/trackrec/track00a.htm#principal
2. US EPA Method 508.1, James W Eichelberger Rev 1.0 1994.

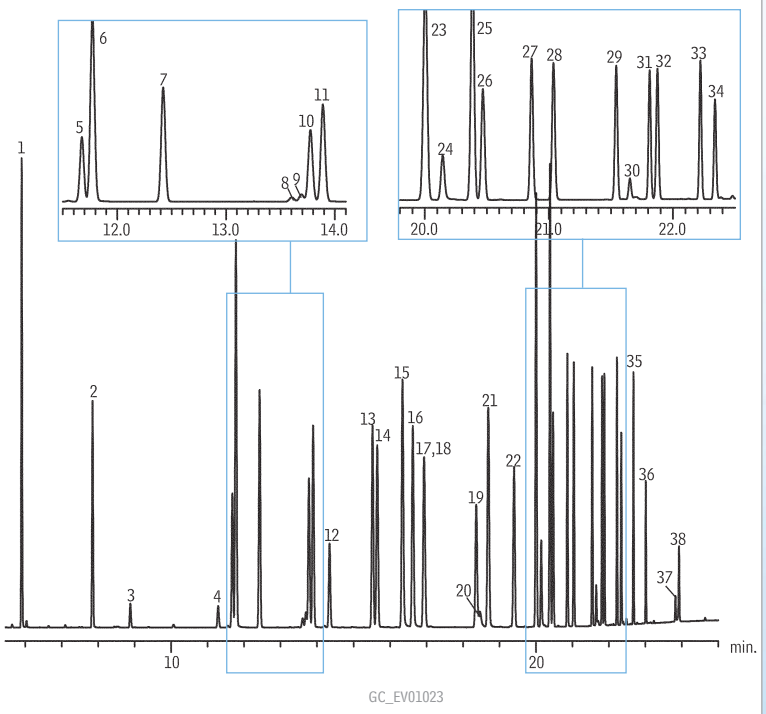
Conditions for Figure 1

Column:	Rtx®-CLPesticides2, 30m, 0.32mm ID, 0.25µm (cat.# 11324) and Rtx®-CLPesticides, 30m, 0.32mm ID, 0.32µm (cat.# 11141) with 5m x 0.32mm ID Rxi® deactivated guard tubing (cat.# 10039), connected using Universal "Y" Press-Tight® Connector (cat.# 20405-261)
Sample:	50ng/mL 508.1 Calibration Mix #1 (cat.# 32094), 100ng/mL 508.1 Calibration Mix #2 (cat.# 32095), 100ng/mL 508.1 Calibration Mix #3 (cat.# 32096), 50ng/mL 508.1 Internal Standard (cat.# 32091), 250ng/mL 508.1 Surrogate (cat.# 32092), 500ng/mL Atrazine (cat.# 32208), 500ng/mL Simazine (cat.# 32236) in ethyl acetate
Inj.:	2µL splitless (hold 0.75 min.), 4mm cyclo double gooseneck liner (cat.# 20896)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Linear velocity:	26cm/sec. @ 80°C
Oven temp.:	80°C (hold 0.5 min.) to 155°C (hold 1 min.) @ 19°C/min. to 210°C @ 4°C/min. to 310°C (hold 0.5 min.) @ 25°C/min.
Detector temp.:	ECD @ 325°C

Figure 1 Resolve all critical pairs using Rtx®-CLPesticides and Rtx®-CLPesticides2 columns.

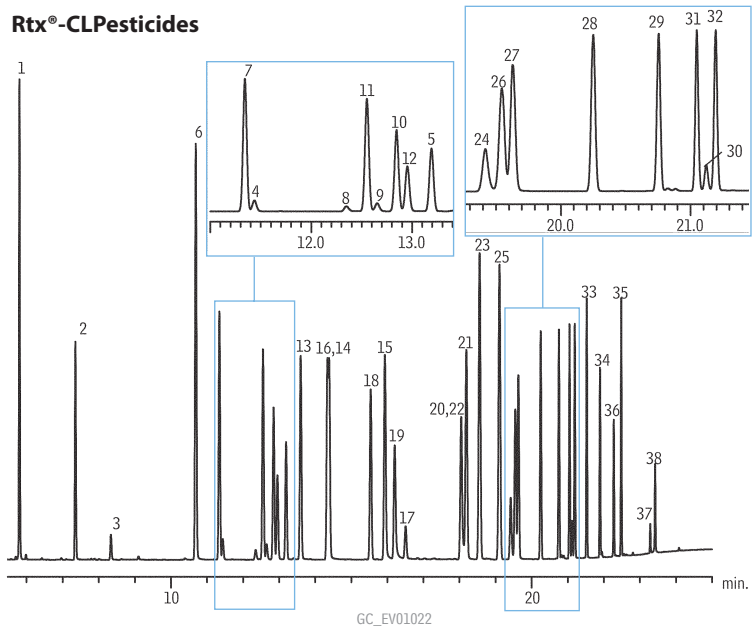
1. hexachlorocyclopentadiene
2. etridiazole
3. chlorneb
4. propachlor
5. trifluralin
6. hexachlorobenzene
7. α-BHC
8. simazine
9. atrazine
10. pentachloronitrobenzene (IS)
11. γ-BHC
12. β-BHC
13. δ-BHC
14. heptachlor
15. chlorothalonil
16. metribuzin
17. alachlor
18. aldrin
19. 4,4'-dibromobiphenyl (SS)
20. metachlor
21. DCPA
22. heptachlor epoxide
23. γ-chlordane
24. cyanazine
25. α-chlordane
26. endosulfan I
27. 4,4'-DDE
28. dieldrin
29. endrin
30. chlorobenzilate
31. 4,4'-DDD
32. endosulfan II
33. 4,4'-DDT
34. endrin aldehyde
35. endosulfan sulfate
36. methoxychlor
37. *cis*-permethrin
38. *trans*-permethrin

Rtx®-CLPesticides2



GC_EV01023

Rtx®-CLPesticides



GC_EV01022

Satisfy all method requirements in half the time!

Table I Rtx®-CLPesticides and Rtx®-CLPesticides2 columns easily pass EPA Method 508.1 performance criteria.

Test/Requirement	Analyte	Concentration (ppb)	Rtx®-CLPesticides2	Rtx®-CLPesticides
Inertness (breakdown <20%)	endrin	50	0.9%	1.4%
Inertness (breakdown <20%)	4,4'-DDE	100	1.0%	1.1%
Sensitivity (S/N>3)	chlorpyrifos	2	12.0	6.2
Chromatographic performance (0.8<PGF<1.15)	DCPA	50	1.03	1.06
Column performance (resolution>0.50)	chlorothalonil	50	9.9	26.8
Column performance (resolution>0.50)	gamma-BHC	40	9.9	26.8

Rxi® Guard/Retention Gap Columns (fused silica)

Nominal ID	Nominal OD	5-Meter	5-Meter/6-pk.	10-Meter	10-Meter/6-pk.
0.25mm	0.37 ± 0.04mm	10029	10029-600	10059	10059-600
0.32mm	0.45 ± 0.04mm	10039	10039-600	10064	10064-600
0.53mm	0.69 ± 0.05mm	10054	10054-600	10073	10073-600

Universal "Y" Press-Tight® Connectors

Description	ea.	3-pk.
Universal "Y" Press-Tight Connector	20405	20406
Deactivated Universal "Y" Press-Tight Connector	20405-261	20406-261
Siltek Treated Universal "Y" Press-Tight Connector	20485	20486

Rtx®-CLPesticides Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.32	-60 to 320/340°C	30-Meter	11141

508.1 Calibration Mix #1 (17 components)

aldrin
α-BHC
β-BHC
δ-BHC
γ-BHC (lindane)
4,4'-DDD
4,4'-DDE
4,4'-DDT
dieldrin
endosulfan I
endosulfan II
endosulfan sulfate
endrin
endrin aldehyde
heptachlor
heptachlor epoxide (isomer B)
methoxychlor

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32094

508.1 Calibration Mix #2 (11 components)

chlorobenzilate
α-chlordane
γ-chlordane
chlorneb
DCPA (Dacthal®)
etridiazole
hexachlorobenzene
cis-permethrin*
trans-permethrin*
propachlor
trifluralin

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32095

*1000µg/mL total permethrin. Exact content of each isomer listed on certificate of analysis.

508.1 Calibration Mix #3 (8 components)

alachlor
atrazine
chlorthalonil
cyanazine
hexachlorocyclopentadiene
metolachlor
metribuzin
simazine

500µg/mL each in ethyl acetate, 1mL/ampul
cat. # 32096

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	-60 to 320/340°C	30-Meter	11324

508.1 Internal Standard

pentachloronitrobenzene
100µg/mL in ethyl acetate, 1mL/ampul
cat. # 32091

508.1 Surrogate

4,4'-dibromobiphenyl
500µg/mL in ethyl acetate, 1mL/ampul
cat. # 32092

Atrazine

1,000µg/mL in acetone, 1mL/ampul
cat. # 32208

Simazine

1,000µg/mL in acetone, 1mL/ampul
cat. # 32236

Splitless Liners for Agilent GC

ID* x OD & Length	qty.	cat.#
Cyclo Double Gooseneck (4mm)		
4.0mm x 6.5mm x 78.5mm	5-pk.	20896

*Nominal ID at syringe needle expulsion point.

Resprep™-C18 SPE Disks

Description	qty.	cat.#
Resprep-C18 47mm SPE Disks	20-pk.	24004

One Stop Shop for EPA Method 535

Reliably Analyze Acetamide Herbicide Degradates by LC/MS/MS

By Jason Thomas, Innovations Chemist, Julie Kowalski, Ph.D., Innovations Chemist, and Christopher Borton, Applied Biosystems

- Full package: reference standards, SPE cartridges, and HPLC columns.
- Chromatographic resolution of alachlor ESA and acetochlor ESA isomers.
- Meet method requirements, with superior sensitivity.

Acetamide herbicides are used in large quantities to suppress weed growth in corn and soybean fields. However, due to the polar nature of ethanesulfonic acid (ESA) and oxanilic acid (OA) degradation products, contamination of drinking water sources is a concern. EPA Method 535 is designed to monitor drinking water for ESA and OA breakdown products of these herbicides. Chromatographic analysis is extremely important for this method because two analytes, alachlor ESA and acetochlor ESA, are isomers that share the same mass spectral multiple reaction monitoring (MRM) transitions, and thus must be separated chromatographically.

Resolution of all Method 535 analytes, including alachlor ESA and acetochlor ESA isomers, can easily be accomplished using Restek's full line of Method 535 products, which includes reference standards, solid phase extraction (SPE) cartridges, and HPLC columns that meet method guidelines. In the procedure shown here, 6mL CarboPrep™ 90 SPE cartridges were used for sample preparation, both to help extend the lifetime of the analytical column as well as to prevent matrix enhancement or suppression. LC/MS/MS analysis was performed on an Ultra C18 column coupled to an Applied Biosystems API 3200™ LC/MS/MS system equipped with a TurboIonSpray® probe for the Turbo V™ source.

Figure 1 Easily resolve Method 535 herbicide degradates on an Ultra C18 HPLC column.

Sample: acetamide herbicides
Inj.: 25µL
Conc.: 50ng/mL
Sample diluent: 5mM ammonium acetate in water

Column: Ultra C18
Cat.#: 9174312
Dimensions: 100mm x 2.1mm
Particle size: 3µm
Pore size: 100Å

Conditions:
Mobile phase: A: 5mM ammonium acetate
B: methanol

Time:	%B
0.0	20%
4.0	30%
10.0	30%
15.0	50%
17.0	85%
18.0	85%
18.1	20%
28.0	20%

Flow: 250µL/min.
Temp.: ambient

Det.: API 3200™ LC/MS/MS system
Ion Source: TurboIonSpray® probe for Turbo V™ source (negative)
Mode: MRM
Curtain gas: 25
Collision gas: medium/5
IonSpray voltage: -4,500
Temperature: 500°C
Ion source gas 1: 50
Ion source gas 2: 50
Interface heater: on
Dwell time: 50
Vertical probe position: 2

Analytes	RT (min)	Quantifer MRM	Qualifier MRM
dimethachlor ESA	7.9	300/80	300/121
alachlor OA	14.5	264/160	264/158
acetochlor OA	14.8	264/146	264/144
1. alachlor ESA	16.1	314/80	314/121
2. metolachlor OA	16.4	278/206	278/174
3. acetochlor ESA	16.4	314/80	314/121
4. metolachlor ESA	16.9	328/80	328/121
butachlor ESA	19.7	356/80	356/121

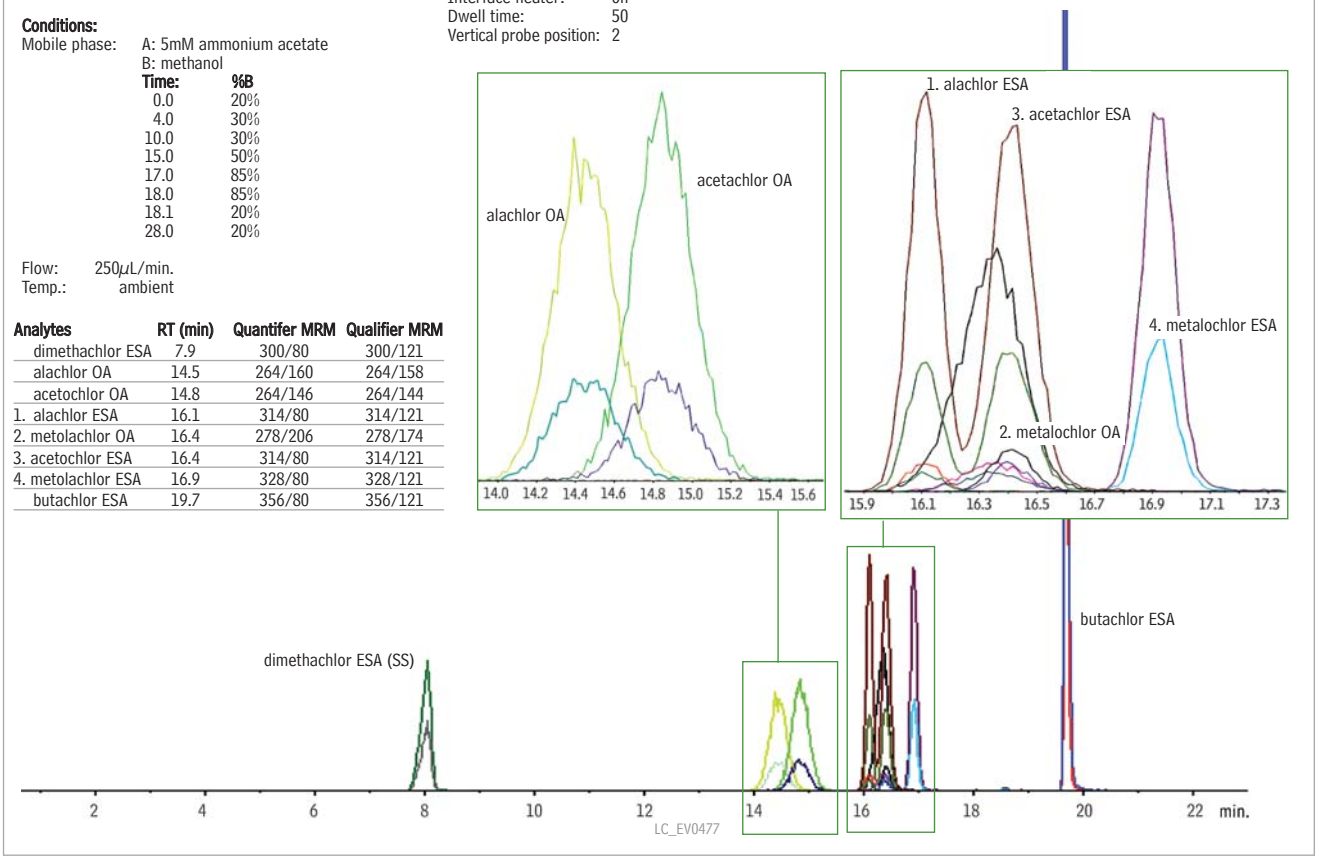


Table I Reliably achieve minimum detection limits of 0.004µg/L or less.

Analyte	LCMRL (µg/L)	Standard Deviation	Calculated Detection Limit in Matrix (µg/L)
alachlor OA	0.013	0.28	0.003
acetochlor OA	0.014	0.27	0.003
alachlor ESA	0.013	0.18	0.002
metolachlor OA	0.013	0.21	0.003
acetochlor ESA	0.012	0.29	0.004
metolachlor ESA	0.012	0.18	0.002

Seven matrix spikes prepared at 0.013µg/L (proposed MRL).

Table II Outstanding accuracy and precision using Ultra C18 HPLC columns.

Analytes	Average Recovery (%)	%RSD
dimethachlor ESA	100.1	9.2
metolachlor OA	95.0	8.5
metolachlor ESA	94.8	8.9
alachlor OA	96.6	8.5
acetochlor OA	97.0	8.9
alachlor ESA	92.5	8.6
acetochlor ESA	94.3	8.0

Four lab fortified blanks spiked at 0.2µg/L.
Method requirements: average recovery ±30% of the true value, %RSD ≤20%.

CarboPrep™ SPE Cartridges
Nonporous graphitized carbon

SPE Cartridge	Tube Volume, Bed Weight	qty.	cat#
CarboPrep 90	6mL, 500mg	30-pk.	26092

Ultra C18 Columns (USP L1)
Excellent for a wide range of analyses

Physical Characteristics:

particle size: 3µm, spherical
pore size: 100Å
carbon load: 20%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

3µm Column, 2.1mm	cat. #
100mm	9174312

Method 535 Individual Compounds

Volume is 1mL/ampul. Concentration is µg/mL.

Compound	Solvent	Conc.	cat.#
acetochlor ESA sodium salt	M	100	33092
acetochlor OA	M	100	33094
alachlor ESA sodium salt	M	100	33096
alachlor OA	M	100	33099
metolachlor ESA sodium salt	M	100	33200
metolachlor OA	M	100	33201

M=methanol

Method 535 Internal Standard

butachlor ESA sodium salt
100µg/mL in methanol, 1mL/ampul
cat. # 33202

Method 535 Surrogate Standard

dimethachlor ESA sodium salt
100µg/mL in methanol, 1mL/ampul
cat. # 33203

Consistent chromatographic resolution of 3.5 or greater for alachlor ESA and acetochlor ESA was easily achieved as shown in Figure 1. Surrogate recoveries, matrix spikes, minimum detection limits, and internal standard recoveries produced consistently acceptable results at low concentrations and showed no interferences from the drinking water matrix. The method reporting limits (MRL) listed in Table I are based on seven replicate fortified blanks prepared at the proposed MRL of 0.013µg/L in drinking water. An LCMRL of 0.012 to 0.014µg/L was established and validated with a calculated detection limit of 0.004µg/L or less. Precision and accuracy were demonstrated using four replicate fortified blanks at 0.2µg/L; recovery and RSD values easily met method requirements (Table II). All analytes were detected in laboratory blanks at ≤1/3 MRL values demonstrating low system background levels.

Resolution of all target analytes, including alachlor ESA and acetochlor ESA isomers, can easily be achieved.

The optimized method developed here shows superior sensitivity for the ESA and OA degradates of chloroacetanilide herbicides alachlor, acetochlor, and metolachlor, as well as reliable resolution between isomers alachlor ESA and acetochlor ESA. This method is simplified by Restek's suite of Method 535 products. All of the reference materials, sample preparation products, and HPLC columns needed are now available from a single source, to facilitate successful Method 535 analysis.

References

1. C. Borton. EPA Method 535; Detection of Degradates of Chloroacetanides and other Acetamide Herbicides in Water by LC/MS/MS. Applied Biosystems, Foster City, CA, 2008.

Breaking Down? Improve BDE-209 Response

Using a New Rtx®-1614 Column for PBDE Analysis

By Jason Thomas, Environmental Innovations Chemist, and Jack Cochran, Director of New Business and Technology

- Higher sensitivity and inertness for BDE-209 than the method-specified column, for more accurate, reproducible results.
- Meets all method requirements for resolution, tailing factors, and retention.
- Optimized short column conditions give improved BDE-209 response 3 times faster.

Polybrominated diphenyl ethers (PBDEs) are ubiquitous in humans and in the environment. Rapid and accurate PBDE methods are increasingly in demand as adverse effects have been associated with PBDE exposure. EPA Draft Method 1614 presents a considerable challenge to the analytical column due to the large number of PBDE compounds and stringent activity guidelines. One target compound, decabromodiphenyl ether (BDE-209), is of particular concern as it is frequently encountered and is the primary component in the only remaining commercial PBDE mixture. Column inertness is critical for BDE-209 analysis, as the breakdown mechanism is predominately column-related.

EPA Draft Method 1614 stipulates a 5% phenyl methyl column in a 30m x 0.25mm x 0.10µm format with a shorter 15m column option. Here we compare the performance of a method-specified column (DB-5HT) to the new Rtx®-1614 column, a 5% phenyl methyl column with a unique deactivation for maximum inertness to BDE-209. Although this method requires analysis on a high-resolution mass spectrometer, the columns were evaluated first on an Agilent 6890 GC with µ-ECD to assess inertness and general chromatographic performance. Columns were then analyzed on an Agilent 7890/5975 GC/MS to verify separation requirements under vacuum outlet conditions.

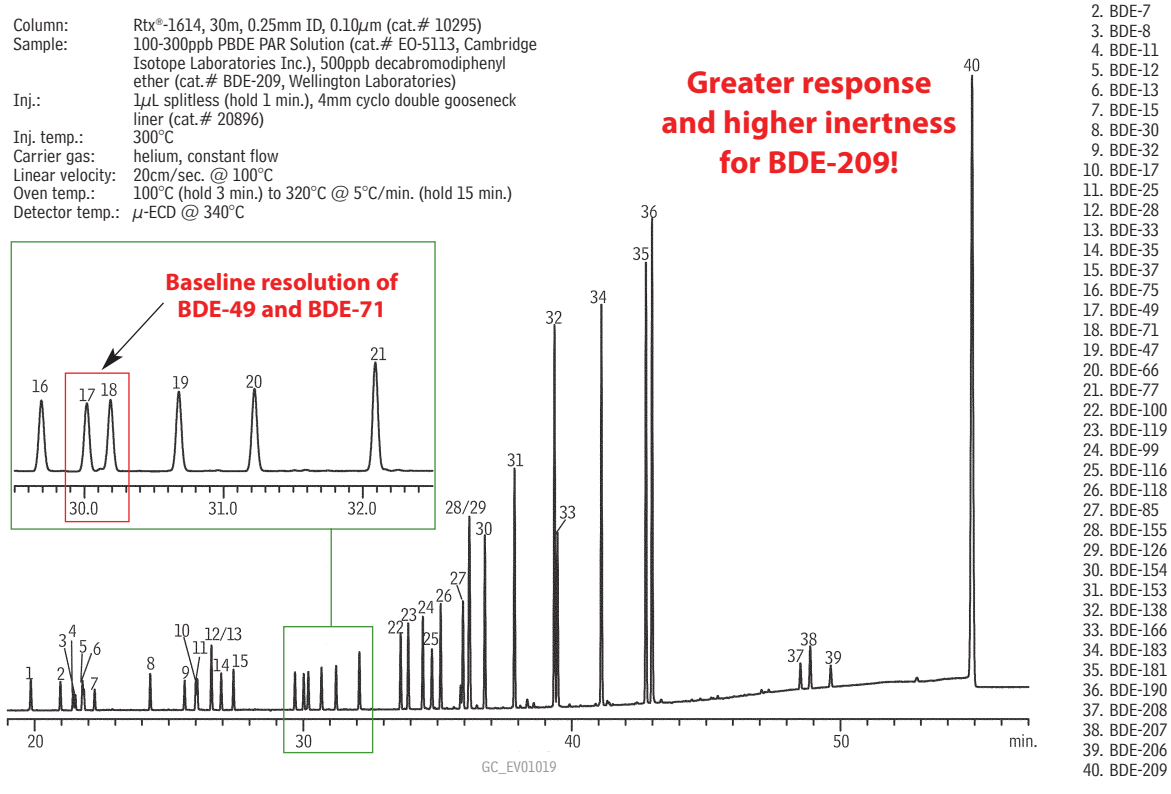
Table I Maximize BDE-209 response with an Rtx®-1614 column, in 15 or 30m lengths!

Column	BDE-209 Average RRF*
Rtx®-1614 (15m)	0.681
Rtx®-1614 (30m)	0.636
DB-5HT (30m)	0.502

*Relative response factors based on internal standard hexabromobiphenyl (n=5). Analyses run under optimized conditions.

The Rtx®-1614 column meets the method requirements for the resolution of critical pairs, tailing factors, and retention. The data in Figure 1 demonstrate the separation of a large list of PBDEs on the Rtx®-1614 column; note the baseline resolution of congeners 49 and 71, which are required to have a 40% valley height of the smallest peak. The Rtx®-1614 column also performed exceptionally well for inertness to BDE-209 (Table 1). Compared to the performance of the DB-5HT, shown in Figure 2, the Rtx®-1614 column

Figure 1 Separate PBDEs accurately and reliably on an Rtx®-1614 column.



shows a greater response for BDE-209 and less peak fronting, indicating less on-column breakdown.

Although the method originally stipulated that BDE-209 must elute at least 48 minutes from injection, eliminating the possibility of much method optimization, a new revision provides a short column option which can greatly improve analysis time and BDE-209 response. Since BDE-209 breaks down primarily in the column, reducing column residence time by using a shorter 15m column, in combination with higher flows and quicker ramp rates, dramatically improves performance. Even applying optimized parameters to a 30m column results in greatly enhanced analyses, relative to the original method-stipulated operating conditions. To further optimize this method, BDE-209 degradation was reduced by using a maximum oven temperature of less than 300°C and setting the injection temperature at 340°C, to ensure complete vaporization, resulting in a consistent and high response (Figure 3).

In conclusion, the Rtx®-1614 is an excellent column choice for analyzing EPA Draft Method 1614, as well as any routine screening analysis of PBDEs, due to its selectivity, sensitivity, and inertness, specifically with respect to BDE-209.

Rtx®-1614 Columns (fused silica)

(5% phenyl methyl)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.10	-60 to 330/360°C	15-Meter	10296
0.25mm	0.10	-60 to 330/360°C	30-Meter	10295

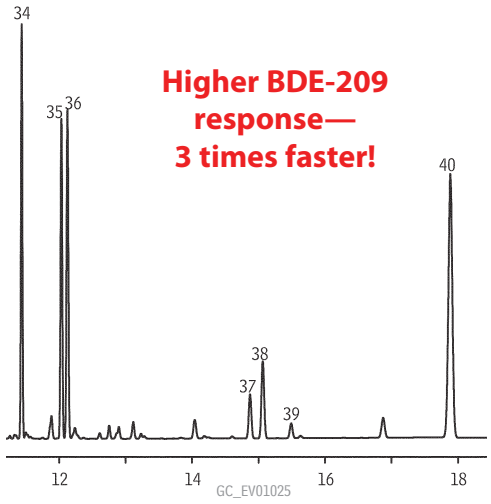
Splitless Liners for Agilent

ID* x OD & Length	qty.	cat.#
Cyclo Double Gooseneck (4mm)		
4.0mm x 6.5mm x 78.5mm	5-pk.	20896

*Nominal ID at syringe needle expulsion point.

Figure 3 Improve BDE-209 response and analysis times with optimized conditions using the short column option.

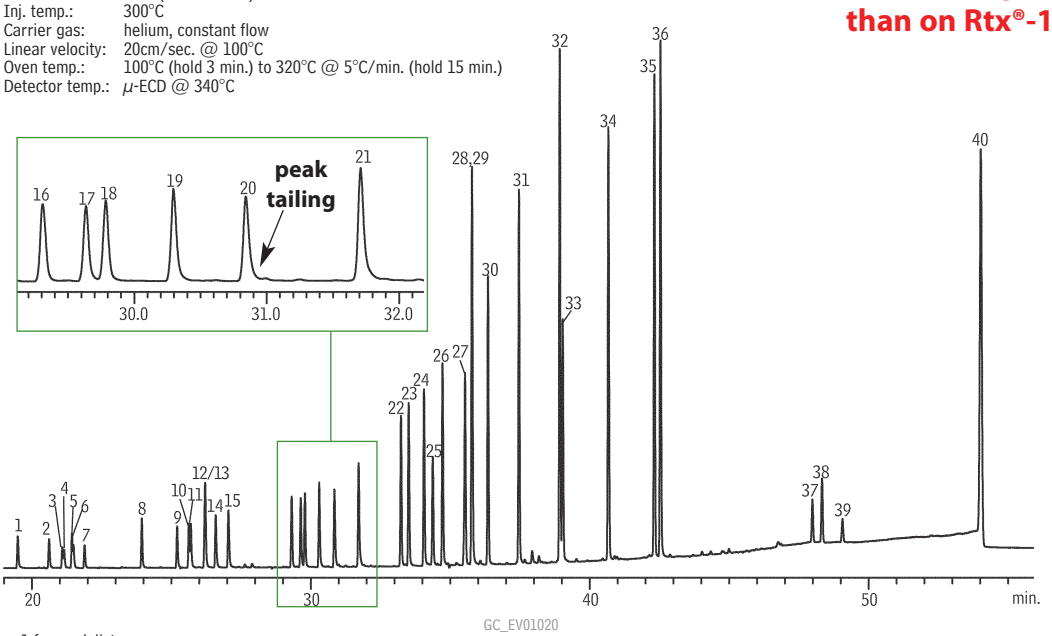
Column: Rtx®-1614, 15m, 0.25mm ID, 0.10µm (cat.# 10295)
Sample: 100-300ppb PBDE PAR Solution (cat.# EO-5113, Cambridge Isotope Laboratories Inc.), 500ppb decabromodiphenyl ether(cat.# BDE-209, Wellington Laboratories)
Inj.: 1µL splitless (hold 1 min.), 4mm cyclo double gooseneck liner (cat.# 20896)
Inj. temp.: 340°C
Carrier gas: helium, constant flow
Linear velocity: 60cm/sec. @ 120°C
Oven temp.: 120°C (hold 1 min.) to 275°C @ 15°C/min. to 300°C @ 5°C/min. (hold 5 min.)
Detector temp.: µ-ECD @ 345°C



See Figure 1 for peak list.

Figure 2 More BDE-209 peak fronting on the method-specific column indicates greater on-column breakdown.

Column: DB-5HT, 30m, 0.25mm ID, 0.10µm
Sample: 100-300ppb PBDE PAR Solution (cat.# EO-5113, Cambridge Isotope Laboratories Inc.), 500ppb decabromodiphenyl ether (cat.# BDE-209, Wellington Laboratories)
Inj.: 1µL splitless (hold 1 min.), 4mm cyclo double gooseneck liner (cat.# 20896)
Inj. temp.: 300°C
Carrier gas: helium, constant flow
Linear velocity: 20cm/sec. @ 100°C
Oven temp.: 100°C (hold 3 min.) to 320°C @ 5°C/min. (hold 15 min.)
Detector temp.: µ-ECD @ 340°C



See Figure 1 for peak list.

Increase Polycyclic Aromatic Hydrocarbon Sample Throughput

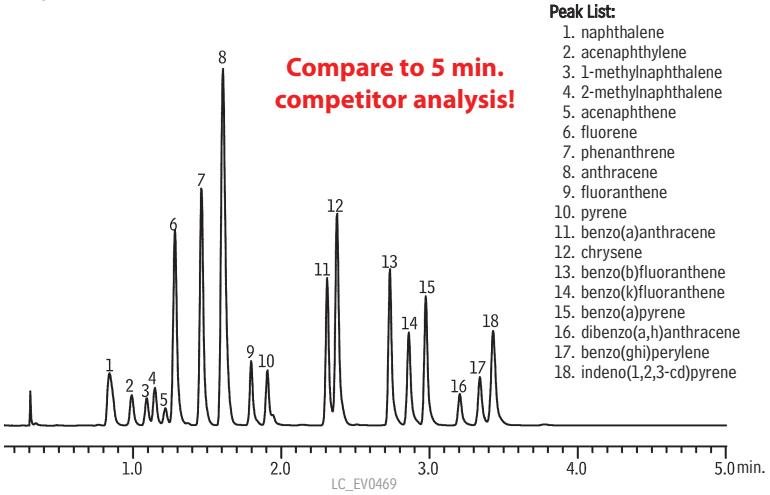
With UHPLC and HPLC Column Options

By Michelle Long, Environmental Innovations Chemist

- Two stationary phases optimized for PAH resolution.
- 3.5 minute EPA 610 and 6 minute EU PAH analyses by UHPLC.
- Portugal PAHs resolved by isocratic HPLC in 4 minutes.

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants resulting primarily from the incomplete combustion of organic materials. PAHs are an increasing human health concern, as this group of chemicals includes several known or suspected carcinogens. Exposure usually occurs by eating charbroiled foods, inhaling fumes from automobile or industrial emissions, or from other sources such as burning coal, wood, and tobacco. PAHs are also present in some medicines, plastics, and pesticides. National and international regulatory agencies provide target analyte lists and, although these lists are not identical, a number of compounds are common across the recommended lists. Here we analyze target compounds from the United States Environmental Protection Agency (EPA), European Union (EU), and Portugal lists by UHPLC and HPLC. Procedures shown use optimized stationary phases and provide analysis times of 3.5 to 6 minutes, allowing labs to achieve significantly faster sample throughput.

Figure 1 Baseline resolve EPA 610 PAHs in less than 3.5 minutes on 1.9µm Pinnacle™ DB PAH columns.



Sample:
Inj.: 2µL
Conc.: 20µg/mL each component
Sample diluent: acetonitrile

Column: Pinnacle™ DB PAH
Cat.#: 9470252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm
Pore size: 140Å

Instrument: JASCO X-LC

Conditions:
Mobile phase: A: water
B: acetonitrile

Time (min.)	%B
0	50
1	60
3	100
5	100

Flow: 0.6mL/min.
Temp.: 30°C
Det.: UV @ 264nm

Two Phases Optimized for PAHs

Although most HPLC methods recommend a C18 column, the Pinnacle™ II PAH and Pinnacle™ DB PAH stationary phases both have been optimized specifically for polycyclic aromatic hydrocarbons and offer greater selectivity for these compounds. Pinnacle™ II PAH columns are available in standard formats, while the Pinnacle™ DB PAH columns are offered on 1.9µm silica. To demonstrate the fast analysis times and optimal selectivity of these phases, US, EU, and Portugal lists were analyzed on 1.9µm Pinnacle™ DB PAH columns using ultra-high pressure liquid chromatography (UHPLC). Portugal PAHs were also analyzed isocratically on a Pinnacle™ II PAH (50mm x 3.2mm, 4µm) column. Conventional HPLC was used for the Portugal list because, since only five analytes are included on the target list, fast analysis times and high sample throughput can be achieved without the high backpressures associated with UHPLC.

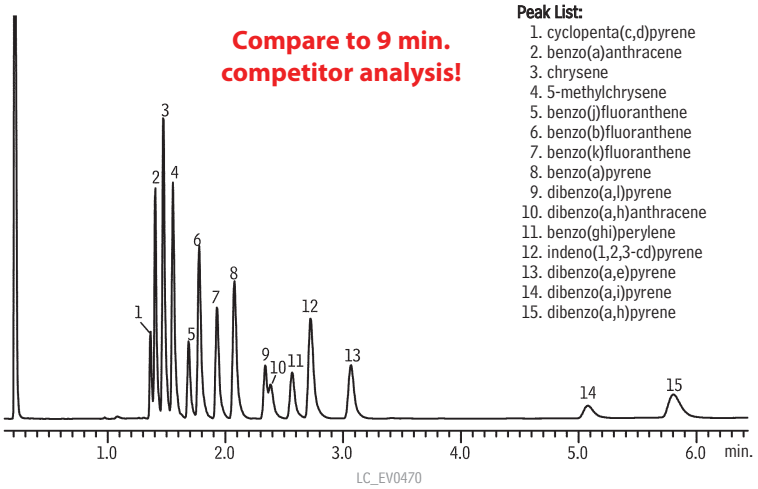
Fully Resolve PAHs in 3.5 to 6 Minutes

The 1.9µm Pinnacle™ DB PAH column resolved all 18 US EPA 610 analytes in less than 3.5 minutes (Figure1). The column was held at a constant temperature of 30°C to improve overall peak shape. The priority PAHs included in EU recommendation 256/2005 were also analyzed on the 1.9µm Pinnacle™ DB PAH column and were separated in less than 6 minutes (Figure 2). Using the 1.9µm Pinnacle™ DB PAH column pairs the stationary phase's high selectivity for PAHs with the increased efficiency and fast analysis times of UHPLC. The Portugal PAH list was analyzed by UHPLC (data not shown), but was also analyzed by conventional HPLC using a 4µm Pinnacle™ II PAH column. All target analytes were resolved in less than 4 minutes (Figure 3).

For the analysis of polycyclic aromatic hydrocarbons, two stationary phases provide optimum results. The Pinnacle™ II PAH phase is available in standard column dimensions while the Pinnacle™ DB PAH phase is available in 1.9µm particle size dimensions. Both alkyl phases have been optimized specifically for PAHs and offer exceptionally fast analysis times, providing a significant opportunity to labs interested in increasing sample throughput.

Acknowledgement
Thanks to JASCO for supplying the JASCO X-LC system used for this work.

Figure 2 Excellent resolution of EU PAHs on 1.9µm Pinnacle™ DB PAH columns.



Sample:
Inj.: 2µL
Conc.: 20µg/mL each component
Sample diluent: acetonitrile

Column: Pinnacle™ DB PAH
Cat.#: 9470252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm
Pore size: 140Å

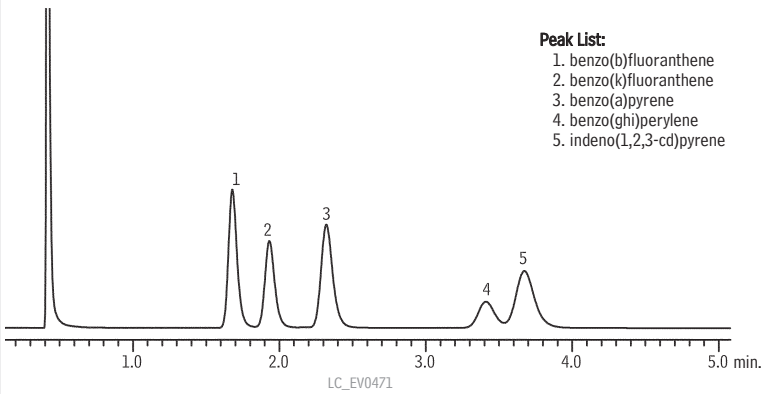
Instrument: JASCO X-LC

Conditions:
Mobile phase: A: water
B: acetonitrile

Time (min.)	%B
0	50
1	90
2	95
5	100
7	100

Flow: 0.6mL/min.
Temp.: 30°C
Det.: UV @ 264nm

Figure 3 Simple, isocratic separation of Portugal PAHs on 4µm Pinnacle™ II PAH columns.



Sample:
Inj.: 2µL
Conc.: 20µg/mL each component
Sample diluent: acetonitrile

Column: Pinnacle™ II PAH
Cat.#: 9219453
Dimensions: 50mm x 3.2mm
Particle size: 4µm
Pore size: 110Å

Instrument: JASCO X-LC

Conditions:
Mobile phase: water:acetonitrile, 5:95 (v/v)
Flow: 0.6mL/min.
Temp.: 30°C
Det.: UV @ 264nm

Pinnacle™ II PAH Columns

Physical Characteristics:

particle size: 4µm, spherical
pore size: 110Å
endcap: fully endcapped
pH range: 2.5 to 10
temperature limit: 80°C

4µm Column, 3.2mm	cat. #
50mm	9219453

Pinnacle™ DB PAH UHPLC Columns

Physical Characteristics:

particle size: 1.9µm
pore size: 140Å
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

1.9µm Column, 2.1mm	cat. #
50mm	9470252

ordering note

For guard cartridges for these columns, visit our website at **www.restek.com**.

Characterizing all 136 Tetra- to Octachlorinated Dioxins and Furans

Using the Rtx®-Dioxin2 Column

By Jack Cochran, Director of New Business and Technology

- Known elution orders for all tetra- through octachlorinated dioxin and furan congeners.
- Resolve 14 of 17 tetra- through octachlorine 2,3,7,8-substituted dioxins and furans.
- TCDD and TCDF specificity, with a column stable up to 340°C.

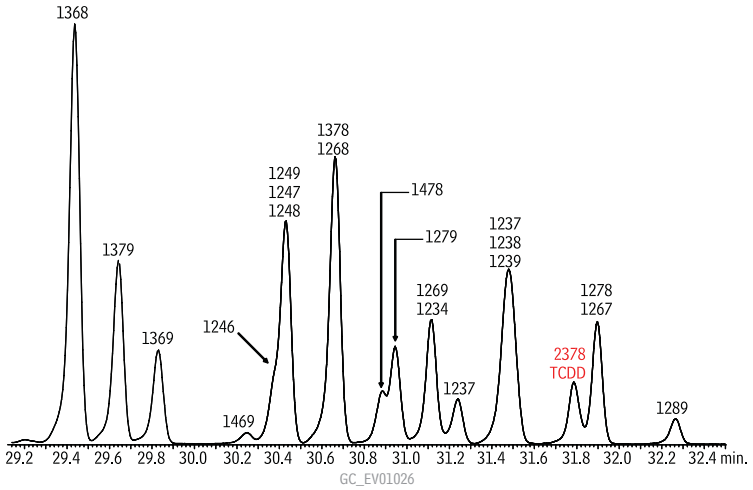
Successful analyses of dioxins and furans are critical because of the extremely toxic nature of these compounds. However, confidently resolving the most toxic congeners, 2,3,7,8-substituted tetrachlorinated dibenzodioxin (TCDD) and tetrachlorinated dibenzofuran (TCDF), is often complicated by the presence of the many other possible congeners. Even with high resolution GC/high resolution MS methods, the proper choice of chromatographic column is essential for separating 2,3,7,8-substituted dioxins and furans from the less toxic congeners and matrix-related compounds.

Complete Column Characterization

It is rare that a column's performance is characterized against all possible 136 tetra- through octachlorinated dioxins and furans. These standards are difficult to obtain, and testing can be time consuming. However, here the Rtx®-Dioxin2 column is characterized against all 136 compounds using standards from Cambridge Isotope Laboratories, Inc. When compared to industry standard stationary phases, a unique selectivity is observed for the Rtx®-Dioxin2 column, and specific resolutions and coelutions are noted. Very few coelutions involving the toxic 2,3,7,8-substituted congeners are observed, making the Rtx®-Dioxin2 column an excellent choice for single column analyses of dioxins and furans (Tables I and II.)

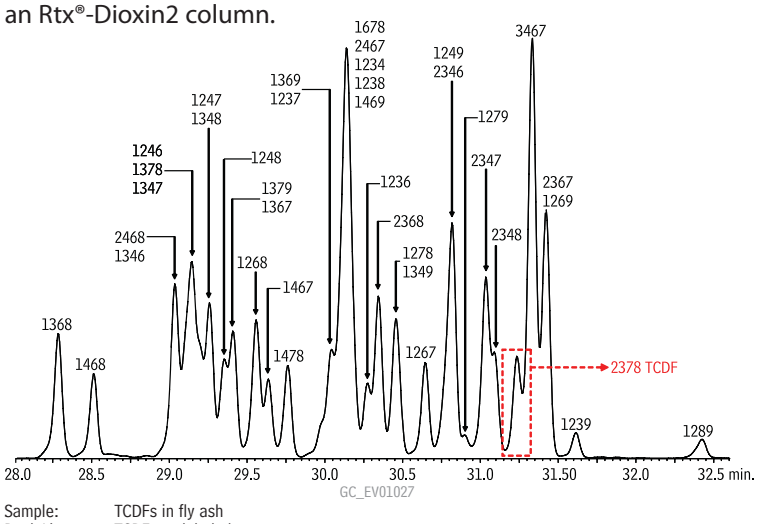
Figure 1 shows fly ash samples, run under the same chromatographic conditions used to characterize the column. 2,3,7,8-tetrachlorodibenzofuran is not resolved under these conditions. However, the characterization study used simple linear temperature programming, and additional work exploring nonlinear oven programs and different flow parameters yielded better resolution between some congeners, especially 2,3,7,8-TCDF (data available upon request). The value in this work is not necessarily to show complete separation of all the congeners on a single column, but to show where all of the 136 compounds of interest elute, making all possible coelutions known.

Figure 1 GC/HR-MS analysis of tetrachlorinated dioxins in fly ash on an Rtx®-Dioxin2 column.



Column:	Rtx®-Dioxin2, 40m, 0.18mm ID, 0.18μm (cat.# 10759)
Sample:	TCDDs in fly ash
Inj.:	1μL splitless
Inj. temp.:	280°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	120°C (hold 1 min.) to 160°C @ 10°C/min. to 320°C @ 4°C/min. (hold 4 min.)
Det.:	Waters AutoSpec Ultima HRMS
Ionization:	EI
Mode:	SIM
Peak List:	TCDDs as labeled

Figure 2 GC/HR-MS analysis of tetrachlorinated furans in fly ash on an Rtx®-Dioxin2 column.



Sample: TCDFs in fly ash
Peak List: TCDFs as labeled
See Figure 1 for conditions

The Rtx®-Dioxin2 column is an excellent column for the analysis of dioxin and furan congeners. It has a unique selectivity for the toxic congeners, including specificity for 2,3,7,8-TCDD and 2,3,7,8-TCDF. Here we characterized all 136 tetra- through octachlorine dioxins and furans and defined all possible coeutions. While commonly used cyanopropyl columns are limited by a low maximum operating temperature of 240°C, the Rtx®-Dioxin2 column is stable up to 340°C, extending column lifetime and improving analyses of dioxins and furans.

Rtx®-Dioxin2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.18	20°C to 340°C	40-Meter	10759
0.25mm	0.25	20°C to 340°C	60-Meter	10758

Stable up to 340° for extended column lifetime!

Table I Retention times (RT) and relative retention times (RRT) for all tetra- through octachlorinated dioxins on an Rtx®-Dioxin2 column.

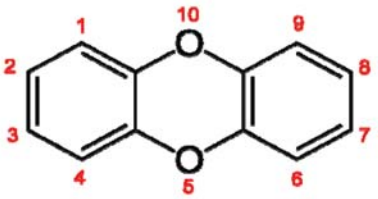
tetrachlorinated			pentachlorinated			hexachlorinated			heptachlorinated			octachlorinated		
Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)
1368	29.43	0.8198	12468	33.75	0.9401	124679	37.89	1.0554	1234679	42.44	1.1822	**12346789**	**46.93**	**1.3069**
1379	29.64	0.8256	12479	33.80	0.9415	124689	37.89	1.0554	**1234678**	**43.34**	**1.2072**			
1369	29.84	0.8312	12469	34.17	0.9515	123468	38.53	1.0730						
1469	30.25	0.8424	12368	34.67	0.9657	123679	38.79	1.0805						
1246	30.38	0.8462	12478	34.83	0.9702	123689	38.82	1.0813						
1249	30.42	0.8474	12379	34.92	0.9727	123469	38.90	1.0833						
1247	30.43	0.8476	12467	35.02	0.9755	**123478**	**39.55**	**1.1017**						
1248	30.44	0.8479	12369	35.08	0.9769	**123678**	**39.66**	**1.1047**						
1378	30.64	0.8535	12489	35.08	0.9772	123467	39.78	1.1081						
1268	30.68	0.8546	12346	35.36	0.9850	**123789**	**39.98**	**1.1136**						
1478	30.88	0.8599	12347	35.40	0.9858									
1279	30.95	0.8621	12367	35.89	0.9997									
1269	31.12	0.8669	**12378**	**35.91**	**1.0003**									
1234	31.15	0.8677	12389	36.21	1.0086									
1236	31.25	0.8705												
1237	31.47	0.8766												
1238	31.50	0.8774												
1239	31.51	0.8777												
2378	**31.79**	**0.8855**												
1278	31.90	0.8883												
1267	31.90	0.8886												
1289	32.27	0.8989												

Blue boxes represent coelutions

Red boxes represent coelutions with 2378-substituted congeners

Bold red text indicates congeners with 2378 substitution

Number Sequence for Polychlorinated Dibenzo Dioxins



RRTs were calculated versus 12378 ^{13}C-labeled dioxin.

Table II Retention times (RT) and relative retention times (RRT) for all tetra- through octachlorinated furans on an Rtx®-Dioxin2 column.

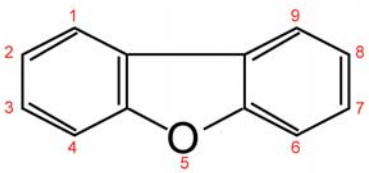
tetrachlorinated			pentachlorinated			hexachlorinated			heptachlorinated			octachlorinated		
Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)	Congener	RT (min)	RRT (min)
1368	28.29	0.8181	13468	32.38	0.9364	123468	37.23	1.0766	**1234678**	**41.99**	**1.2143**	**12346789**	**47.07**	**1.3604**
1468	28.52	0.8243	12468	32.44	0.9378	134678	37.38	1.0807	1234679	42.36	1.2243			
2468	29.03	0.8393	13678	33.53	0.9694	124678	37.40	1.0812	1234689	42.60	1.2319			
1346	29.03	0.8393	13467	33.58	0.9705	134679	37.62	1.0873	**1234789**	**43.92**	**1.2697**			
1246	29.11	0.8413	12467	33.61	0.9717	124679	37.83	1.0876						
1378	29.15	0.8427	14678	33.70	0.9717	124689	38.08	1.1009						
1347	29.19	0.8441	13478	33.69	0.9743	123467	38.45	1.1116						
1247	29.26	0.8459	12368	33.71	0.9746	**123478**	**38.58**	**1.1154**						
1348	29.27	0.8459	12478	33.76	0.9760	**123678**	**38.70**	**1.1191**						
1248	29.35	0.8485	13479	33.85	0.9783	123479	38.86	1.1234						
1379	29.40	0.8497	13469	34.00	0.9829	123469	38.96	1.1263						
1367	29.42	0.8503	12479	34.09	0.9858	123679	39.14	1.1315						
1268	29.56	0.8546	12346	34.14	0.9870	123689	39.40	1.1387						
1467	29.64	0.8569	12469	34.25	0.9902	**234678**	**39.42**	**1.1400**						
1478	29.76	0.8604	23468	34.35	0.9928	123489	40.29	1.1651						
1369	29.97	0.8664	12347	34.36	0.9931	**123789**	**40.31**	**1.1654**						
1237	30.03	0.8684	12348	34.39	0.9945									
1678	30.10	0.8702	**12378**	**34.61**	**1.0006**									
2467	30.14	0.8714	12678	34.85	1.0075									
1234	30.16	0.8719	12367	34.86	1.0075									
1238	30.18	0.8725	12379	34.99	1.0116									
1469	30.19	0.8725	12679	35.27	1.0197									
1236	30.27	0.8754	23467	35.48	1.0257									
2368	30.35	0.8772	12369	35.51	1.0266									
1278	30.45	0.8803	12489	35.56	1.0277									
1349	30.48	0.8812	**23478**	**35.68**	**1.0318**									
1267	30.66	0.8864	12349	35.74	1.0335									
1249	30.78	0.8864	12389	36.47	1.0544									
2346	30.83	0.8910												
1279	30.89	0.8930												
2347	31.03	0.8968												
2348	31.10	0.8991												
2378	**31.22**	**0.9028**												
3467	31.33	0.9058												
2367	31.41	0.9081												
1269	31.44	0.9089												
1239	31.61	0.9141												
1289*	32.43	0.9376												

Blue boxes represent coelutions

Red boxes represent coelutions with 2378-substituted congeners

Bold red text indicates congeners with 2378 substitution

Number Sequence for Polychlorinated Dibenzo Furans



RRTs were calculated versus 12378 ^{13}C-labeled furan.

*Note that the 1289 tetra chlorinated congener elutes after the 13468 penta chlorinated congener.

Assure LC/MS/MS System Performance for Drug Analyses

Using a System Suitability Test Mix

By Kristi Sellers, Clinical/Forensic Innovations Chemist and Houssain El Aribi, Ph.D., LC/MS Product Specialist, MDS Sciex

- Increase sample throughput and data quality with easy, reliable verification of LC/MS/MS performance.
- Extensively documented standard preparation assures accurate, consistent solutions.
- Method included in Cliquid® Drug Screen & Quant Software—automatically generates test reports.

Sample throughput is a critical issue in drug toxicology, and it can be adversely affected by inferior system performance. Poor system performance can produce unreliable data, increase downtime, and necessitate sample reanalysis, which ultimately decreases sample throughput. To ensure that your LC/MS/MS system is running properly, a system suitability mix should be analyzed on a regular basis before case samples are analyzed.

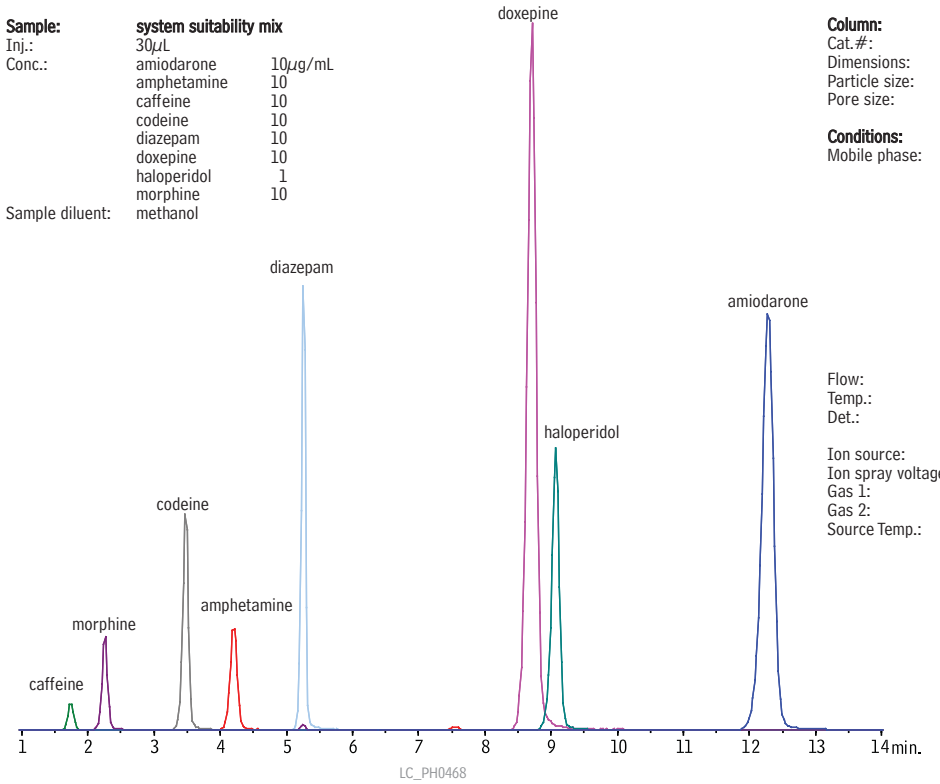
Restek and Applied Biosystems have developed a system suitability mix specifically for drug testing that contains compounds covering a wide range of molecular weights, polarities, and retention times (Table I). This standards mix is designed to verify system performance and identify system problems. Figure 1 shows a representative chromatogram (+MRM transitions) of this suitability mix analyzed on an Applied Biosystems API 3200™ LC/MS/MS system. This simple test evaluates the entire analytical system, including the autosampler, column, HPLC pumps, and mass spectrometer. The data is automatically compared to expected results by Applied Biosystem's Cliquid® Drug Screen & Quant Software to identify system problems.

Table I Mix components vary in chemical properties, providing a rigorous system performance test.

Mass Spectrometer Conditions:

Analyte	MW	RT (min)	Q1	Q3
Amiodarone	645	12.30	646.0	58.0
Amphetamine	135	4.21	136.1	91.1
Caffeine	194	1.72	195.1	122.9
Codeine	299	3.47	300.2	165.2
Diazepam	284	5.25	285.1	193.2
Doxepin	279	8.72	280.2	107.1
Haloperidol	375	9.08	376.1	123.0
Morphine	285	2.24	286.1	165.1

Figure 1 Increase sample throughput by verifying system readiness with a drug standard system suitability mix. (MRM transitions)



Sample: system suitability mix
Inj.: 30µL
Conc.: amiodarone 10µg/mL; amphetamine 10; caffeine 10; codeine 10; diazepam 10; doxepine 10; haloperidol 1; morphine 10
Sample diluent: methanol

Column: Allure® PFP Propyl
Cat.#: 9169552
Dimensions: 50mm x 2.1mm
Particle size: 5µm
Pore size: 60Å

Conditions:
Mobile phase: A: 0.2% formic acid and 2mM ammonium formate in water
B: 0.2% formic acid and 2mM ammonium formate in acetonitrile

Time (min.):	Flow (mL/min.)	%B
0.0	0.5	10
10.00	1.0	90
15.00	1.0	90
15.50	0.5	10
17.50	0.5	10

Flow: see gradient table
Temp.: 40°C
Det.: Applied Biosystems/API 3200™ LC/MS/MS system
Ion source: electrospray, positive
Ion spray voltage: 4000
Gas 1: 40psi
Gas 2: 70psi
Source Temp.: 500°C

The Cliquid® Drug Screen & Quant Software automates this test and generates a verification report which highlights failures. Peak area, peak shape, retention time reproducibility, fragmentation, and library search function all are evaluated through the software by comparing the test mix data to expected results. For example, full scan linear ion trap MS/MS data for diazepam and caffeine are compared to the library to assess fragmentation. A mass spectral match of 80% or more must be achieved to pass this portion of the system suitability test. Otherwise, the failure will be highlighted on the automated report.

Use this system suitability mix for drug analyses to assure system performance and simplify troubleshooting.

Analyzing this system suitability mix for drug analysis on a regular basis assures system performance, improves data quality, increases sample throughput, and simplifies troubleshooting. Moreover, the Cliquid® Drug Screen & Quant Software for Routine Forensic Toxicology enables nonexpert LC/MS/MS users to employ this system suitability test with little effort.

Acknowledgement
Method and data supplied by Applied Biosystems.

References

H. El Arbi, T. Sasaki, A. Schreiber, K. Sellers, K. Herwehe. Development of an LC/MS/MS System Suitability Test for Forensic Toxicology Applications. Applied Biosystems/MDS Sciex, 2007.

Allure® PFP Propyl Columns (USP L43)
Excellent Columns for LC/MS and ELSD

Physical Characteristics:

particle size: 5µm, spherical	endcap: fully endcapped
pore size: 60Å	pH range: 2.5 to 7.5
carbon load: 17%	temperature limit: 80°C

5µm Column, 2.1mm		cat. #
30mm		9169532
50mm		9169552
5µm Column, 3.2mm		**cat. #**
30mm		9169533
50mm		9169553
5µm Column, 2.1mm		**cat. #**
30mm (with Trident Inlet Fitting)		9169532-700
50mm (with Trident Inlet Fitting)		9169552-700
5µm Column, 3.2mm		**cat. #**
30mm (with Trident Inlet Fitting)		9169533-700
50mm (with Trident Inlet Fitting)		9169553-700
Allure® PFP Propyl Guard Cartridges	**qty.**	**cat. #**
10 x 2.1mm	3-pk.	916950212
10 x 4.0mm	3-pk.	916950210
20 x 2.1mm	2-pk.	916950222
20 x 4.0mm	2-pk.	916950220

ordering note

For other dimensions of these columns, visit our website at **www.restek.com**.

ABI/SCIEX Cliquid® Drug Screen Mix

Forensic Drug Screen Test Mixture

amiodarone	10µg/mL	diazepam	10
amphetamine	10	doxepine	10
caffeine	10	haloperidol	1
codeine	10	morphine	10

In P&T methanol, 1mL/ampul
cat. # 36340

Forensic Drug Screen Internal Standard

D5-diazepam D5-doxepine

10µg/mL each in P&T methanol, 10mL/ampul
cat. # 36341

Trident Direct Guard Cartridge System

Easy to Use, Low Dead Volume—The Ultimate Combination of Convenience and Column Protection

Trident Direct 20mm guard cartridge holder with filter
Protection against particulate matter and maximum protection against irreversibly adsorbed compounds.

Trident Direct 10mm guard cartridge holder with filter
Protection against particulate matter and moderate protection against irreversibly adsorbed compounds.

Description	qty.	cat.#
10mm guard cartridge holder with filter	ea.	25084
20mm guard cartridge holder with filter	ea.	25086
Connection tip for Waters-style end fittings	ea.	25088
PEEK tip standard fittings	ea.	25087

Separating NSAIDs through Aromatic Selectivity

Improve Retention by Using An Allure® Biphenyl HPLC Column

By Rick Lake, Pharmaceutical Innovations Chemist, and Benjamin Smith, Applications Technician

- Optimize retention and selectivity of non-steroidal anti-inflammatory drugs, for better separations.
- Orthogonal separations with simple mobile phase changes
- Increased retention requires higher organic content, increasing desolvation efficiency in LC/MS.

Non-steroidal anti-inflammatory drugs (NSAIDs), in either prescribed or over-the-counter formulations, are widely used to treat pain, fever, and inflammation. While steroidal anti-inflammatory drugs all share a similar, four-ring chemical structure, NSAIDs have more diverse chemical structures, complicating their analysis. The work we report here is based on three common classes of NSAIDs: arylalkanoic acids, 2-arylpropionic acids (profens), and oxicams.

NSAIDs have a high carbon to heteroatom ratio and, therefore, historically have been separated through reversed phase HPLC on C18 columns. A conventional C18 stationary phase separates compounds based mainly on their overall hydrophobicity. Considering the carbon to heteroatom ratio, this is an effective separation mechanism for NSAIDs. Newer stationary phases are available, however, and we set out to determine if other phases, using other separation mechanisms, such as π-π interactions, could be more effective for assaying NSAIDs.

When selecting a stationary phase, it is advantageous to exploit inherent differences in the target analytes' chemical structures. Among these three classes of NSAIDS, there are some common functional groups, like halogens, amines, and carboxylic acids, but no one group is shared across the entire list of analytes (Figure 1). However, all of the target analytes do share one basic structural component – the six-carbon aromatic ring. Aromatic rings are common components of drug molecules, and they can be targeted using a phenyl-based stationary phase.

As a retention mechanism, phenyl stationary phases employ π-π interactions between the phenyl groups in the stationary phase and any unsaturated bonds in the analyte. The use of conventional phenyl phases has been somewhat limited due to their moderate retention capacity, relative to that of a C18 phase. Figure 2 illustrates the relative retention capacities of NSAID test probes on an Allure® Biphenyl column, a conventional phenyl column and a C18 column. Note that, in all cases, as commonly seen in practice, the conventional phenyl phase yields only moderate retention compared to that of a C18 column. However, the Allure® Biphenyl phase, which is a stationary

Figure 1 Aromatic rings make NSAIDs candidates for separation through π-π interactions.

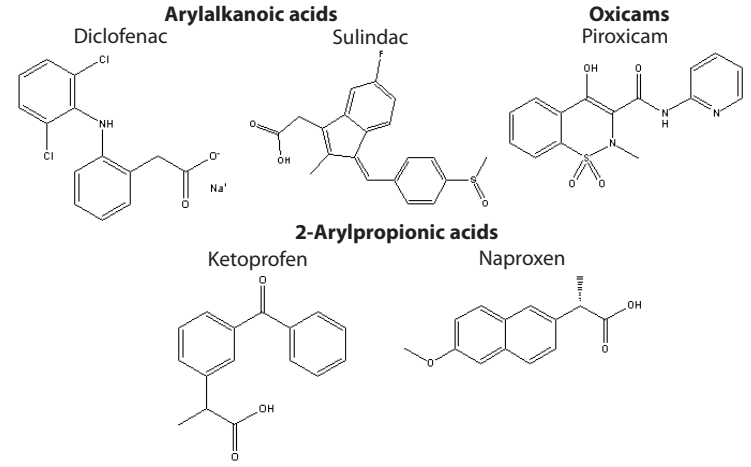
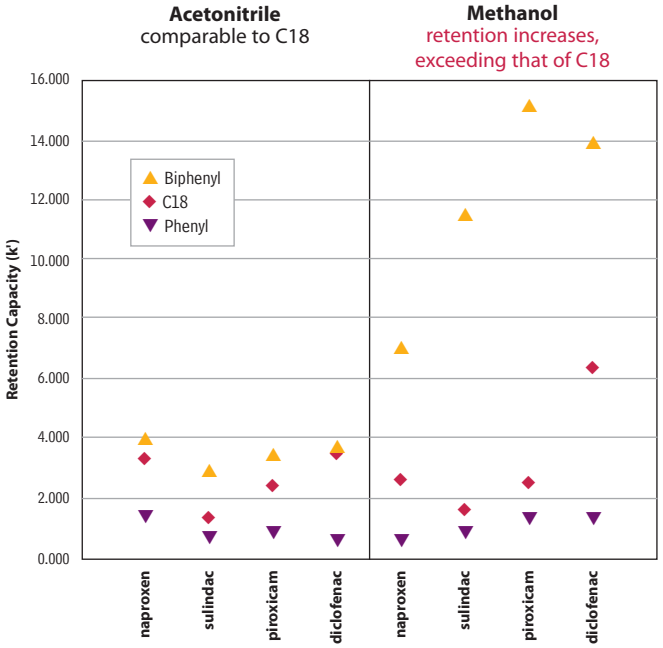


Figure 2 The retention capacity of the Allure® Biphenyl phase far exceeds that of conventional phenyl phases.



For each analyte all columns were assayed under identical isocratic conditions. The equivalent elutropic strength between acetonitrile and methanol was determined by the relative retention capacities of the C18 phase.

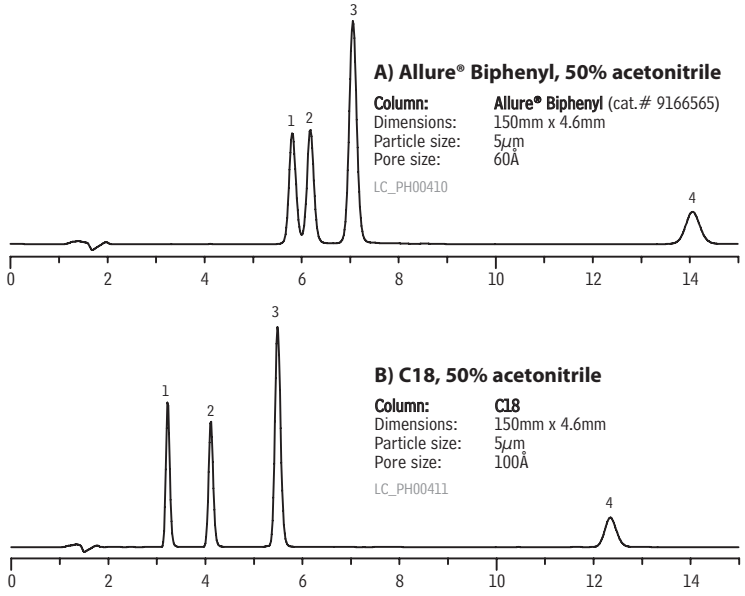
Columns: 5μm, 4.6mm x 150mm
Mobile Phase: 10mM potassium phosphate (pH 2.5): acetonitrile or methanol
Det.: UV @ 254nm
Flow: 1.0 mL/min.

Figure 3 The versatility of the Allure® Biphenyl phase makes it a great alternative to conventional phenyl phase columns, especially in method development.

Sample:
Inj.: 5µL
Conc.: ~300µg/mL each component
Sample diluent: mobile phase

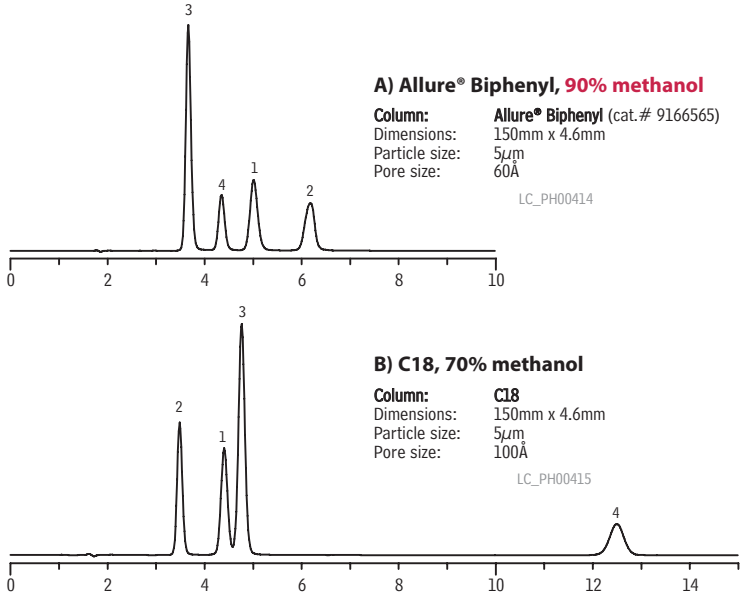
1. sulindac
2. piroxicam
3. ketoprofen
4. diclofenac

In acetonitrile, retention of NSAIDs on an Allure® Biphenyl column is comparable to retention on a C18 column and elution order is the same.



Conditions:
Mobile phase: 0.5% formic acid in water (pH 2.25):0.1% formic acid in acetonitrile, 50:50 (v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

In methanol, retention capacity & selectivity of NSAIDs are much greater on an Allure® Biphenyl column, compared to a C18 column, and elution order changes.



Conditions:
Mobile phase: 0.5% formic acid in water (pH 2.25):0.1% formic acid in methanol, 30:70 or 10:90 (v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

phase composed of two phenyl groups bonded end-to-end, easily achieves retention capacities similar to, and even greater than, those of a C18 column when used with a highly organic mobile phase. For this reason, we evaluated the enhanced retention of the Allure® Biphenyl column for assaying NSAIDs through aromatic selectivity.

First, we compared the retention characteristics of a conventional C18 column and an Allure® Biphenyl column, using acetonitrile as the organic modifier. As expected, the Allure® Biphenyl column exhibited similar retention under equivalent analytical conditions (Figure 3). But, when we assayed the same analytes, using methanol as the organic modifier, we found retention on the Allure® Biphenyl column was *greatly increased*. To maintain the same retention capacities (k') between the columns, we had to increase the organic content by 20% (Figure 3). In addition, selectivity between the two columns became dramatically different. Based on these results, we conclude that methanol in the mobile phase enhances π-π interactions between aromatic compounds and the biphenyl stationary phase, leading to greater retention and superior selectivity.

An Allure® Biphenyl column, in combination with a methanol-containing mobile phase, significantly improves separations of NSAIDs, or other aromatic drug compounds. Increased retention capacity creates a need for a higher percentage of organic solvent in the mobile phase, to elute the analytes in a timely manner. Increasing the organic content, in turn, increases sensitivity in LC/MS methods, because it optimizes the desolvation efficiency in electrospray interfaces. And this, in turn, makes an Allure® Biphenyl column the best choice for separating aromatics.

Allure® Biphenyl Columns (USP L11)

Physical Characteristics:

particle size: 5µm, spherical
pore size: 60Å
carbon load: 23%
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

5µm Column, 4.6mm	cat. #
150mm	9166565

For other dimensions of these columns, visit our website at **www.restek.com**

Allure® Guard Cartridges

Allure Biphenyl	qty.	cat. #
10 x 2.1mm	3-pk.	916650212
10 x 4.0mm	3-pk.	916650210
20 x 2.1mm	2-pk.	916650222

Easily Resolve Oxytocin PEGylation Reaction Products

Using Viva Wide Pore HPLC Columns

Julie Kowalski, Ph.D., Bioanalytical Innovations Chemist

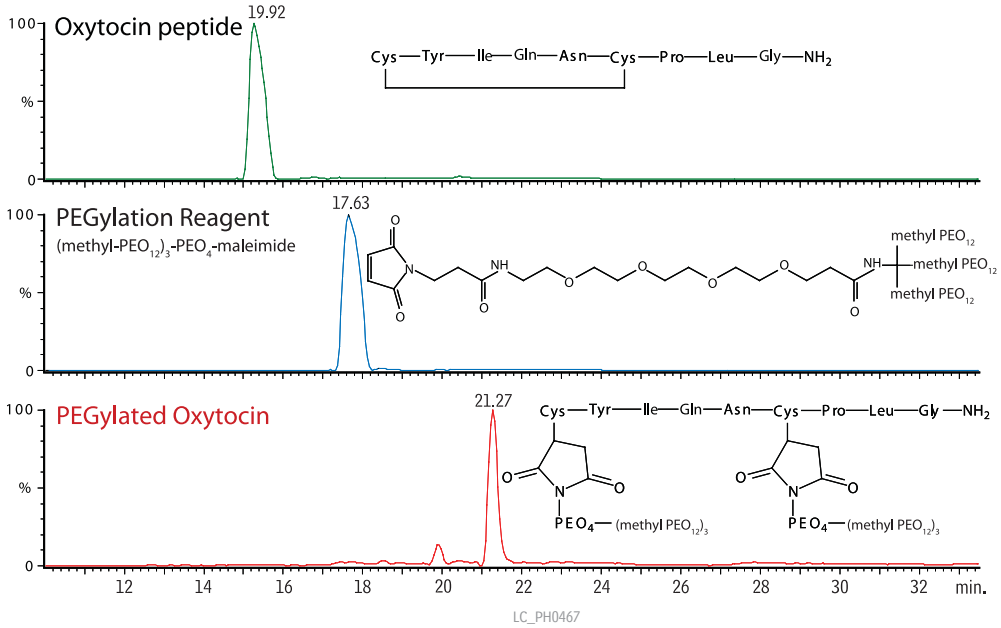
- Ideal for PEGylation reaction monitoring.
- Easy isocratic method saves time, eliminating column equilibration time between injections.
- Largest available surface area in 250-350Å pores; engineered for proteins, peptides, and other large biomolecules.

PEGylation, the covalent attachment of polyethylene glycol (PEG) units to proteins and peptides, is an important tool in drug discovery. PEGylation is used to enhance drug delivery, while maintaining the therapeutic function of the active compound. Viva Wide Pore HPLC columns are ideal for the separation of large molecules, such as oxytocin PEGylation reaction products, as the target analytes can enter the larger pores and access more surface area, increasing retention and overall resolution. For analytes with molecular weights larger than 3,000, pore diameters of 250-350Å offer the best combination of retention and pressure stability, and Viva Wide Pore silica has the greatest available surface area in 250-350Å pores. Here we demonstrate the suitability of Viva Wide Pore HPLC columns for PEGylation reaction monitoring.

Viva columns reliably separate large, closely related compounds.

The PEGylation reaction mixture consisted of oxytocin with an excess of reducing agent tris(2-carboxyethyl)phosphine (TCEP) and (methyl-PEO_{12})$_3$-PEO_4-maleimide. The oxytocin solution was mixed with ammonium bicarbonate buffer to pH 8. Excess TCEP was added and the resulting solution incubated at 60°C for 1 hour. The test solution was cooled to room temperature and a molar excess of (methyl-PEO_{12})$_3$-PEO_4-maleimide was added, followed by incubation in a water bath at 40°C for 1 hour. Approximately 6 nmoles of oxytocin was injected in 20µL of deionized water with 0.1% formic acid. The extracted ion chromatograms in Figure 1 show excellent resolution for the three compounds of interest. The added retention power of Viva columns allows separation of large, closely related compounds, making it an ideal column for monitoring PEGylation reactions.

Figure 1 Excellent resolution of oxytocin PEGylation reaction products on Viva Wide Pore HPLC columns.



Conditions:

Mobile phase:	0.1% formic acid in water:0.1%formic acid in acetonitrile, 60:40
Flow:	0.1mL/min.
Temp.:	ambient
Det.:	Micromass Quattro II
Interface:	ESI
Ion mode:	positive
Temp.:	200°C
Capillary:	2.25kV
Cone:	40V

Sample:	**oxytocin PEGylation reaction products**
Inj.:	20µL
Conc.:	300pmoles/µL
Sample diluent:	0.1% formic acid in water (v:v)

Column:	**Viva Wide Pore C18**
Cat.#:	9514561
Dimensions:	150mm x 1.0mm
Particle size:	5µm
Pore size:	300Å

Viva C18 Columns (USP L1)

5µm Column, 1.0mm	cat. #
150mm	9514561

ordering note

For other dimensions and guard cartridges for these columns, visit our website at **www.restek.com**.

Rapid Screening Method for Carbamates in Orange Oil

Using an Ultra Carbamate HPLC Column

Julie Kowalski, Ph.D., Innovations Chemist

- Fast analysis times, for increased sample throughput.
- Simple methodology saves time—no sample preparation.
- Accurate mass identification, for definitive results.

Concern over the presence of pesticides in food products, particularly citrus, is growing, resulting in an increasing number of countries regulating insecticides such as carbamates. EPA Method 531.1 describes a method for the analysis of carbamates in water, but not in other commodities. Matrices like citrus oil contain numerous interferences and often require time-consuming sample preparation. However, the method described here requires no sample preparation and provides fast analysis times, significantly increasing sample throughput.

Carbamates are most easily determined via HPLC analysis because derivatization is required for GC analysis. The rapid screening method shown here uses the Ultra Carbamate HPLC column, which is designed specifically for analyzing carbamates and is compatible with both traditional detectors and mass spectrometry. This column works well with mass spectrometry amenable buffers and allows an initial mobile phase composition of 20% organic, which promotes complete ionization at the electrospray source.

Orange oil was spiked at 10ppm with a carbamate mix and analyzed (Figures 1-2). The monoisotopic masses and retention times were compared to an injected standard and found to match closely (Table I). The high mass accuracy of the Leco Unique TOF-MS allowed positive analyte identification, even in a complex mixture containing compounds with the same nominal mass (within 1 amu) as the target carbamate. By using the Ultra Carbamate column in conjunction with the Leco Unique TOF-MS, we were able to develop a quick, easy, and accurate screening method for carbamates in a complex matrix such as orange oil.

References:

B. Mayer-Helm, L. Hofbauer, J. Muller. Rapid Communications in Mass Spectrometry, 20 (2006), page 529-536

Ultra Carbamate Column

3µm Column, 2.1mm	cat. #
50mm	9177352

Figure 1 Reference standard carbamates resolve quickly on an Ultra Carbamate HPLC column. (extracted ion chromatograms)

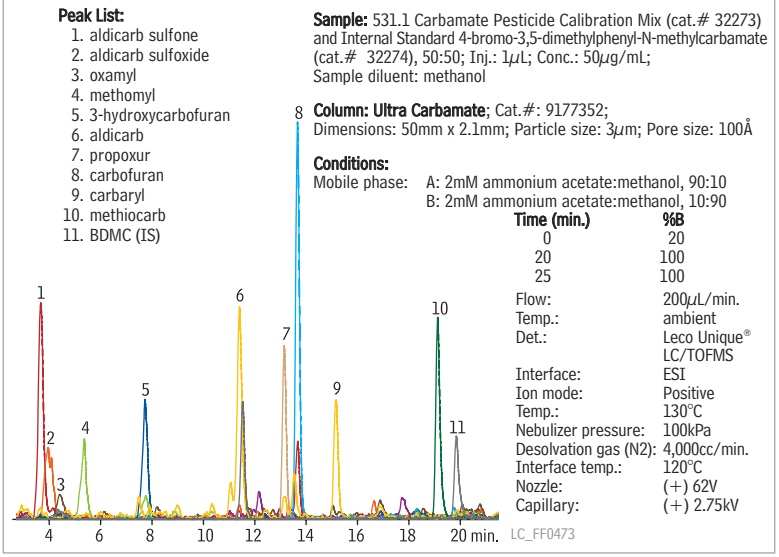


Figure 2 Positive identification of carbamates in orange oil injected with no sample preparation. (extracted ion chromatograms)

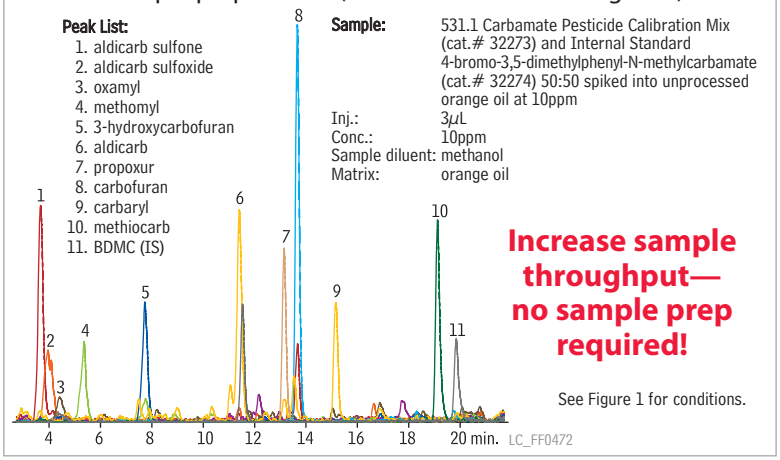


Table I Carbamates were positively identified in matrix using both retention time and mass.

		calculated ion monoisotopic mass	standard ion monoisotopic mass	standard retention time (min.)	orange oil ion monoisotopic mass	orange oil retention time (min.)
aldicarb sulfone	[M+H]+	223.075	223.099	3.81	223.142	3.67
aldicarb sulfoxide	[M+H]+	207.080	207.103	4.31	207.122	4.09
oxamyl	[M+NH4]+	237.102	237.085	4.97	237.110	4.41
methomyl	[M+H]+	163.054	163.074	5.84	163.086	5.36
3-hydroxycarbofuran	[M+H]+	238.108	238.121	8.32	238.128	7.73
aldicarb	[M+H]+	191.085	191.0728 116.0751*	11.92	116.052*	11.53
propoxur	[M+H]+	210.113	210.152	13.53	210.153	13.14
carbofuran	[M+H]+	222.113	222.140	13.98	222.120	13.66
carbaryl	[M+H]+	202.087	202.084	15.48	202.101	15.17
methiocarb	[M+H]+	226.090	226.097	19.22	226.060	19.12
BDMC	[M+H]+	258.013	258.042	19.89	258.005	19.84

* m/z 116.052 is a fragment ion with higher intensity than the [M+H]+ ion and was used for identification in orange oil

Using Thermal Desorption to Enhance Aroma Profiling by GC/MS

Lower Detection Limits with Latest Technology

By Irene DeGraff, Product Marketing Manager, Lara Kelly, Markes International, and Liz Woolfenden, Markes International

- Accommodates a wide range of sampling methods.
- Allows sample re-collection, for repeat analysis and result verification.
- Eliminates extraction solvents, purges volatile interferences, and concentrates sample vapors, for enhanced low-level detection.

Flavor and fragrance profiling by GC/MS presents significant analytical challenges, as profiles typically comprise hundreds of volatile organic compounds (VOCs), often with the lowest concentration analytes having the most profound effects on perceived aroma. Conventional sample preparation methods (solvent extraction, steam distillation, etc.) do not meet sensitivity requirements and often distort the vapor profile so that it is not representative of what the consumer experiences. Recently, thermal desorption (TD) has emerged as a useful complement to GC/MS, enabling more aroma profiling applications to be carried out using quantitative, automatic instrumentation. TD combines automated sample preparation with selective analyte enrichment, allowing VOCs to be injected into the GC/MS as a narrow concentrated band, free of most or all sample matrix effects.

Many Sampling Options, No Extraction Interferences

One of the strengths of thermal desorption for food, flavor, and fragrance profiling is that it offers a versatile range of sampling methodologies including sorbent tubes/traps, on-line sampling, direct desorption, and off-line thermal extraction (dynamic headspace) sampling. Whichever of these approaches is used, the compounds of interest are separated from the sample matrix and focused on a small, electrically-cooled sorbent trap (Figure 1). This focusing trap is subsequently desorbed by heating it rapidly in a reverse flow of carrier gas causing the VOCs to be injected into the GC/MS system as a narrow band of vapor. Since samples are extracted directly

Thermal desorption is an automatic, high-sensitivity alternative to conventional liquid extraction.

Figure 1 Thermal desorption is compatible with a wide range of sampling strategies.

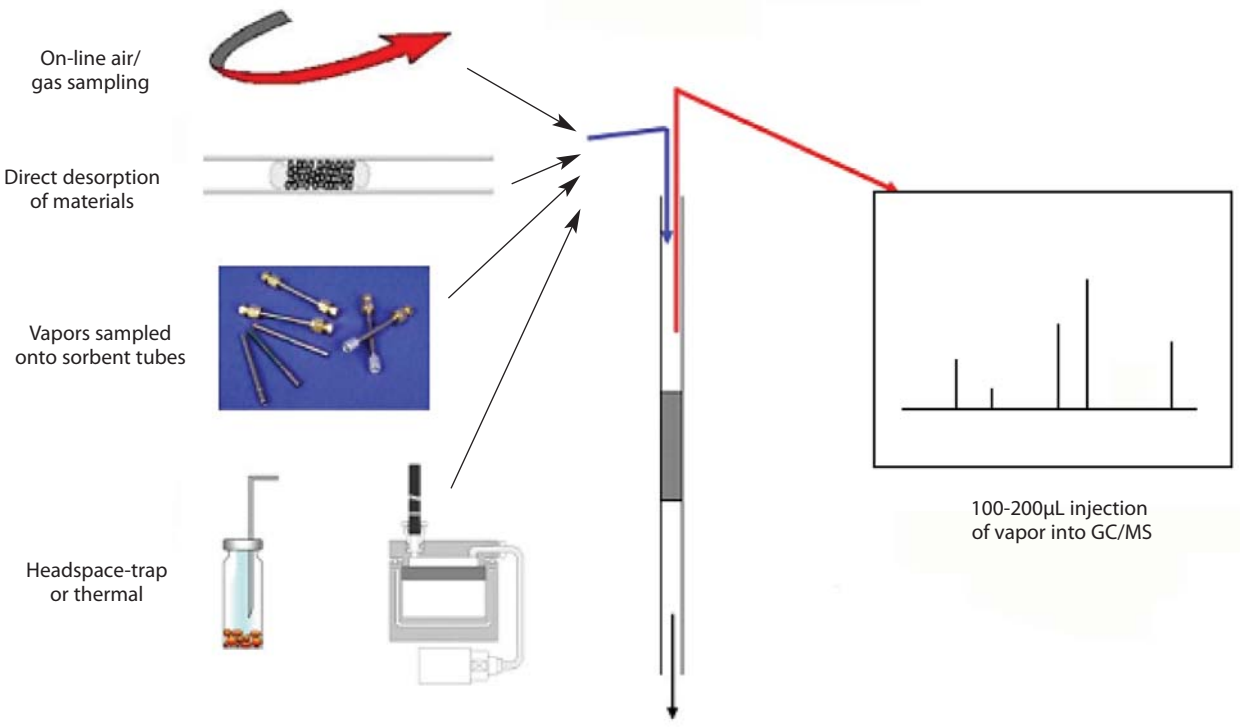
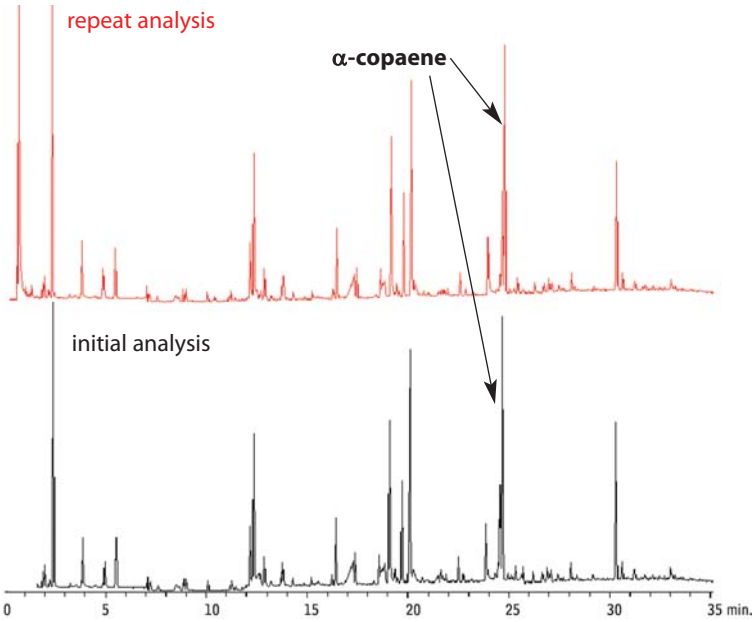
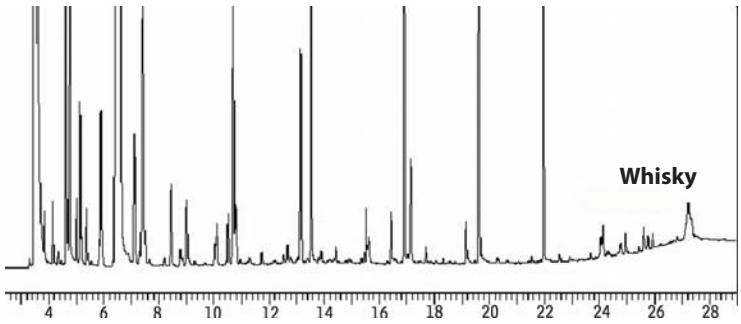


Figure 2 Verify initial results by analyzing re-collected samples.



Analysis of headspace collected above boiling, genetically-modified potatoes. Repeat analysis of the re-collected sample demonstrated excellent recovery of reactive monoterpenes, such as α-copaene.

Figure 3 Thermal desorption allows selective elimination of water and >99% of ethanol vapor, enhancing the determination of key olfactory components.



Analysis of whisky headspace by GC/FID.

Thermal Desorption Unit Tubes, Unconditioned
Fits Markes ULTRA-UNITY, PerkinElmer, and Shimadzu thermal desorbers.

		Unconditioned	
		Stainless Steel	Glass
Description	qty.	cat.#	cat.#
TDU Tubes, Tenax TA	10-pk.	24056	24062
TDU Tubes, Graphitized Carbon	10-pk.	24057	24063
TDU Tubes, Tenax GR/Carbopack B	10-pk.	24058	24064
TDU Tubes, Carbopack B/Carbosieve SIII	10-pk.	24059	24065
TDU Tubes, Tenax TA/Graphitized Carbon/Carboxen 1000	10-pk.	24060	24066
TDU Tubes, Carbopack C/Carbopack B/Carbosieve SIII	10-pk.	24061	24067

into the GC carrier gas stream, no manual sample preparation is required and the problems associated with solvents—masking of peaks of interest, loss of volatiles, and variable extraction efficiency—are eliminated.

Lower Detection Limits and Repeat Analysis

The latest TD systems use thin-walled quartz traps capable of heating at rates over 100°C/sec., maximizing desorption efficiency and lowering detection limits. They also incorporate split re-collection for repeat analysis and simple validation of recovery (Figure 2) through the analytical system. Newer thermal desorption systems are also capable of transferring the vapor profile constituents into the GC capillary column in volumes of carrier gas as low as 100µL. This means that significant concentration enhancement factors can be achieved—typically from 103 to 106—depending on the number of concentration/desorption steps. TD also allows volatile interferences such as water and ethanol to be purged to vent prior to analysis, making it easier to discriminate between samples according to the key olfactory components (Figure 3).

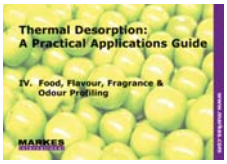
Summary

Thermal desorption offers an automatic, high-sensitivity alternative to conventional liquid extraction methods for aroma profiling by GC/MS. It allows vapor profile constituents to be cleanly separated from the sample matrix and facilitates selective purging of volatile interferences in many cases. This helps to ensure that the vapor profile analyzed is most representative of the aroma perceived by consumers and that key olfactory compounds can be identified and measured at the lowest levels possible.

free literature

Thermal Desorption: A Practical Applications Guide
Download your free copy from
www.restek.com

Technical Guide
lit. cat.# FFTG1037



Thermal Desorption Tube Sorbent	Applications
Tenax TA	Vapor phase organics from C6/7 to C26
Graphitized Carbon	Vapor phase organics from C5/6 to C14
Tenax GR/Carbopack B	Vapor phase organics from *n*-C5/6 to *n*-C20 (EPA Methods TO-14/TO-15/TO-17)
Carbopack B/Carbosieve SIII	Vapor phase organics from *n*-C2/3 to *n*-C12/14 (EPA Methods TO-14/TO-15/TO-17)
Tenax TA/Graphitized Carbon/Carboxen 1000	Vapor phase organics from C2/3 to C20
Carbopack C/Carbopack B/Carbosieve SIII	Vapor phase organics from *n*-C2/3 to *n*-C16/20 (EPA Methods TO-14/TO-15/TO-17)

Tech Tip

Under Pressure?

Reduce System Stress by Backflushing Your HPLC Column

By Tim Herring, Technical Service

Experiencing a higher pump pressure than usual? Or perhaps a complete pressure shut-down of the system has occurred, even after replacing the in-line frit and guard column. High pump pressures can be caused by heavily retained impurities building up within the head of the analytical column. Such contamination can cause poor chromatography, usually in the form of broad, split, or misshapen peaks, and ultimately can compromise results. Backflushing a contaminated analytical column using the following procedure can help restore column performance and reduce pump pressure and system strain.

If back pressure is abnormally high, first take the column out of the equation by disconnecting it from the system altogether. Install a union and run the pumps to verify that the back pressure problem is due to the column, and not to the HPLC system. If the pressure is normal, then the column is most likely the cause of the high back pressure. To address this, reverse the column flow and rinse (backflush) the column to remove the contaminants from the inlet frit and column head. This will move the contaminants down the path of least resistance, instead of forcing them further into the analytical column. Reverse rinse into a waste beaker at low flow (e.g. 0.5mL/min. for a 4.6mm ID column) for 10-15 minutes initially, and then increase the flow to 1.5-2 times the optimal flow (1.5 to 2.0mL/min. for a 4.6mm ID column). Do not reconnect to the detector when backflushing the column. Rather, flush the waste stream into a beaker so that the detector cell is not contaminated by impurities or obstructed by particulate build-up.

Solubility is a key issue when backflushing columns, so remember the old adage, "like dissolves like". For example, if the contaminants are suspected to be oily or hydrophobic in nature, then backflush with a strong, nonpolar solvent such as hexane. If the contamination is polar (a salt for instance), then use a polar solvent, such as water or methanol. Solvent miscibility also needs to be considered, so be sure to use solvents that are miscible with one another. If in doubt, use isopropanol (IPA) as an intermediary solvent between solvent wash steps, as it is miscible with all common solvents. This is particularly true when switching from typical normal phase solvents (such as hexane) to reverse phase solvents (such methanol, acetonitrile, or water) and vice versa. Note that 10 to15 column volumes are generally necessary at each step to remove all traces of immiscible solvents prior to the next step.

If the contaminants are unknown, or vary in chemistry, a series of solvent washes will provide an array of differing chemical interactions and maximize the removal most types of contamination. The solvent order presented in Table I considers miscibility, polarity, and eluotropic strength and is a very effective series for removing most contaminants. Column backflushing, with proper solvent selection, is a simple way to regenerate analytical columns, improving column performance and reducing system stress.

Table I Restore column performance by backflushing with recommended solvent washes.

Reversed phase series:	Normal phase:
A. 1% glacial acetic acid in methanol and water (50:50)	A. isopropanol
B. methanol	
C. chloroform	
D. hexane (or heptane)	
E. methylene chloride (dichloromethane)	
F. methanol	

Contact Restek Technical Service

at support@restek.com or 800-356-1688 with questions on backflushing, or any other technical area. At Restek, we are here to help you!

Quality Control in Metabolomics

Continued from page 2

process than the hard electron impact ionization in GC/MS. It is insufficient to declare that in LC/MS no major matrix effect is apparent with respect to ion suppression just based on quenching of signal intensity of a single infused compound. This single compound may have characteristics that make it less vulnerable to matrix effects, and thus unsuitable to explore matrix effects. Far better suited are classical approaches, most importantly the use of isotope labeled internal standards. Quality control in metabolomics means that the short-term and long-term influence of matrix effects is carefully evaluated by comparing the metabolite coverage and their relative quantification levels to expected values from background knowledge. Only if quantification of a range of well-known target metabolites validates a specific analytical protocol, can unbiased analysis be furthered to the level of metabolomics and comprise novel metabolite signals. Such integration of classical analytical strategies with modern unbiased data analysis should also include randomized sample sequences, blank controls, and bracketing samples with external calibration standards.

Among the most difficult challenges in metabolomics is the annotation of unknown metabolic signals. The Metabolomics Standards Initiative (MSI) has issued a variety of suggestions for reporting minimal experimental parameters to ensure that metabolomic data can be used and reproduced by other laboratories. Importantly, the identification of metabolites must always be based on at least two orthogonal physicochemical characteristics, such as retention index and mass spectrum. Identifications that are based on authentic chemical standards are generally more trustworthy than annotations based on calculated characteristics. Nevertheless, the metabolome itself is an unrestricted entity that clearly comprises more than the suite of known compounds to be found in classical textbooks or that can be purchased from chemical manufacturers. The metabolome cannot be simply computed from reconstructed biochemical pathways due to enzymatic diversity, substrate ambiguity, and variation in regulatory mechanisms. Hence, the finding of many unknown signals in metabolomic surveys comes as no surprise to biochemists. The sheer complexity of natural products, including isomeric compounds, renders the use of accurate masses and database queries insufficient for annotation of metabolites. Instead, novel algorithms are needed to score metabolic signals based on all available information, from calculated physicochemical characteristics to presence in biochemical databases. Such algorithms might ultimately boost the quality of metabolomic data in a similar way as SEQUEST® did for proteomic analysis. Yet, no software is available to perform this much-needed task.

Dr. Oliver Fiehn is a leading researcher in the field of metabolomics. He is a Professor in the Genome Center at the University of California, Davis. Dr. Fiehn's research focuses on developing and applying analytical and bioinformatic methods, primarily GC/MS and LC/MS, in order to unravel the changes in metabolic networks in sets of biological situations.

Restek On-the-Road

Tradeshow Schedule

July, 2008

Show: **Florida Pesticide Residue Workshop (FPRW)**
Date: July 20-23
Location: TradeWinds Island Grand, St. Pete Beach, FL

Show: **18th IAFS Triennial Meeting (International Association of Forensic Sciences)**
Date: July 21-26
Location: New Orleans Marriott Hotel, New Orleans, LA

Show: **NSRA -- 39th Street Rod Nationals**
Date: July 31-Aug. 3
Location: Kentucky Expo Center, Louisville, KY

August, 2008

Show: **28th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2008)**
Date: Aug. 17-22
Location: ICC, Birmingham England UK

September, 2008

Show: **122nd AOAC International Annual Meeting & Exposition**
Date: Sep. 21-24
Location: Hyatt Regency Dallas, Dallas, TX

Show: **Northeastern Association of Forensic Scientists (NEAFS)**
Date: Sep. 30-Oct. 4
Location: Renaissance Westchester Hotel, White Plains, NY

Seminar Schedule

Date	Cat. #	City	State
Comprehensive HPLC			
7/22	65733	Linden	NJ
7/23	65734	Melville	NY
7/24	65735	Parsippany	NJ
GC/MS Training Seminar			
7/28	65736	Blue Ash	OH
7/29	65737	Lexington	KY
7/31	65738	Research Triangle Park	NC
Petrochemical Seminar			
9/8	65739	Seattle	WA
9/9	65740	Richmond	CA
9/11	65741	Long Beach	CA
9/12	65742	Salt Lake City	UT
9/30	65743	Edison	NJ

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2008.01

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- Accurately quantify PAHs down to 5pg on-column using SIM analysis.
- Quantify benzodiazepines by LC/MS/MS at 10ng/mL in matrix in less than 10 minutes.
- Easily monitor air quality at ppt levels with thermal desorption.
- and much more inside.

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IN THIS ISSUE

Using Guard Columns and Retention Gaps in GC (Part 2)

Jaap de Zeeuw, International GC Consumables Specialist, Restek Corporation

Guard columns and retention gaps are used widely in gas chromatography (GC). Many users have difficulty understanding the difference between these two products, even though there is a significant difference in application. In Part 1 of this article we reviewed retention gaps, which mainly are used for focusing the sample components when introducing a large (liquid) sample directly onto the column. In contrast, guard columns are used to protect the analytical column from contamination. Guard columns and retention gaps both must be coupled to the analytical column, and this connection introduces a potential point of risk. A new approach is to integrate the retention gap directly into the analytical column tubing. By applying a "segment" coating technology the stationary phase can be deposited only in a certain part of the column allowing a deactivated section at the beginning. Column coupling is not required and maintenance is greatly simplified. Here we will review guard columns and discuss the new segment coating technology.

Use of guard columns

The purpose of using guard columns is to protect the analytical column from contamination since the sample that is introduced is not always pure. Although the best chromatography is obtained with "clean" samples, the practical situation is that sample clean-up procedures are minimized and relative "dirty" samples are introduced onto the column. Samples can contain particulates, heavy components, derivatization reagents, ionic residues, acids, bases… all these compounds can interfere with the stationary phase and they will influence the separation process. Usually the degradation of column performance is a slow process but it will happen.

Most of the time the impurities accumulate in the first meter(s) of the column and by cutting off this section adequate separation is restored. Many users choose to connect a guard column in front of the analytical column. Such a guard column is deactivated and can be trimmed when contaminated and eventually replaced. Depending on the application, guard columns have a lifetime of 1 week up to 6 months. One has different choices for guard columns; a guard column can consist only of deactivated capillary, or it can be a coated capillary.

Deactivated capillary tubing: Deactivated fused silica tubing can be purchased by the meter and then a defined length can be coupled in front of the analytical column. Upon contamination, a section of the guard column is removed. When the whole guard is "consumed" a new guard column can be coupled. The disadvantage of cutting parts off of the guard column is that the column becomes shorter and this may affect retention times. However, if a similar length is always cut from the guard column, the change in retention time becomes very predictable. A deactivated guard column will also result in band focusing. If the injection is not optimal, there will be a focusing effect similar to that of a retention gap.

Coated capillary tubing: As the guard column needs to prevent contamination of the analytical column, a coated guard column can help as it has both the surface deactivation and also the stationary phase layer. The easiest and most economical way of using coated guard columns (or precolumns) is to buy two analytical columns. One we will use as a separation column and the second one will be used to make coated guard columns. From this second column we will cut 2m sections and couple a section in front of the analytical separation column. We can run our samples until contamination affects peak shape/response and then we can replace the guard with a new 2m section.

The system we have created will produce reproducible retention times as we always will replace the entire 2m coated guard column. Since the stationary phase is the same on the guard as on the analytical column, there will be no surprises. The coated guard column also will allow more aggressive samples/more contamination before it will give up. Lastly, we are able to cut 15 coated guard columns from a full 30m analytical column...that's also economical! However, if using a coated guard column, there will be no focusing effects.

Continued on page 31.

Accurately Quantify PAHs Down to 5pg On-Column

GC/MS SIM Analysis with the **New** Rxi®-5Sil MS Column

By Robert Freeman, Environmental Innovations Chemist

- Excellent linearity across a broad calibration range.
- Ideal for trace level analyses.
- Low bleed at high temperatures, for better overall response and lower detection limits.

Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants, affecting air, water, and soil quality. Although naturally occurring, human impact has created a steady increase in environmental levels of PAHs and their byproducts. PAHs are typically formed through the incomplete combustion of organic materials, such as wood, coal, and oil, but are also used in manufacturing of some medicines, plastics, and pesticides. Many chromatographic methods are available to analyze these pollutants. Laboratories performing low-level PAH analyses often utilize the single ion monitoring (SIM) function of GC/MS because of the sensitivity required to achieve typical regulatory or monitoring levels.

Continued on page 4.

Accurately Quantify PAHs Down to 5pg On-Column *Continued from page 3.*

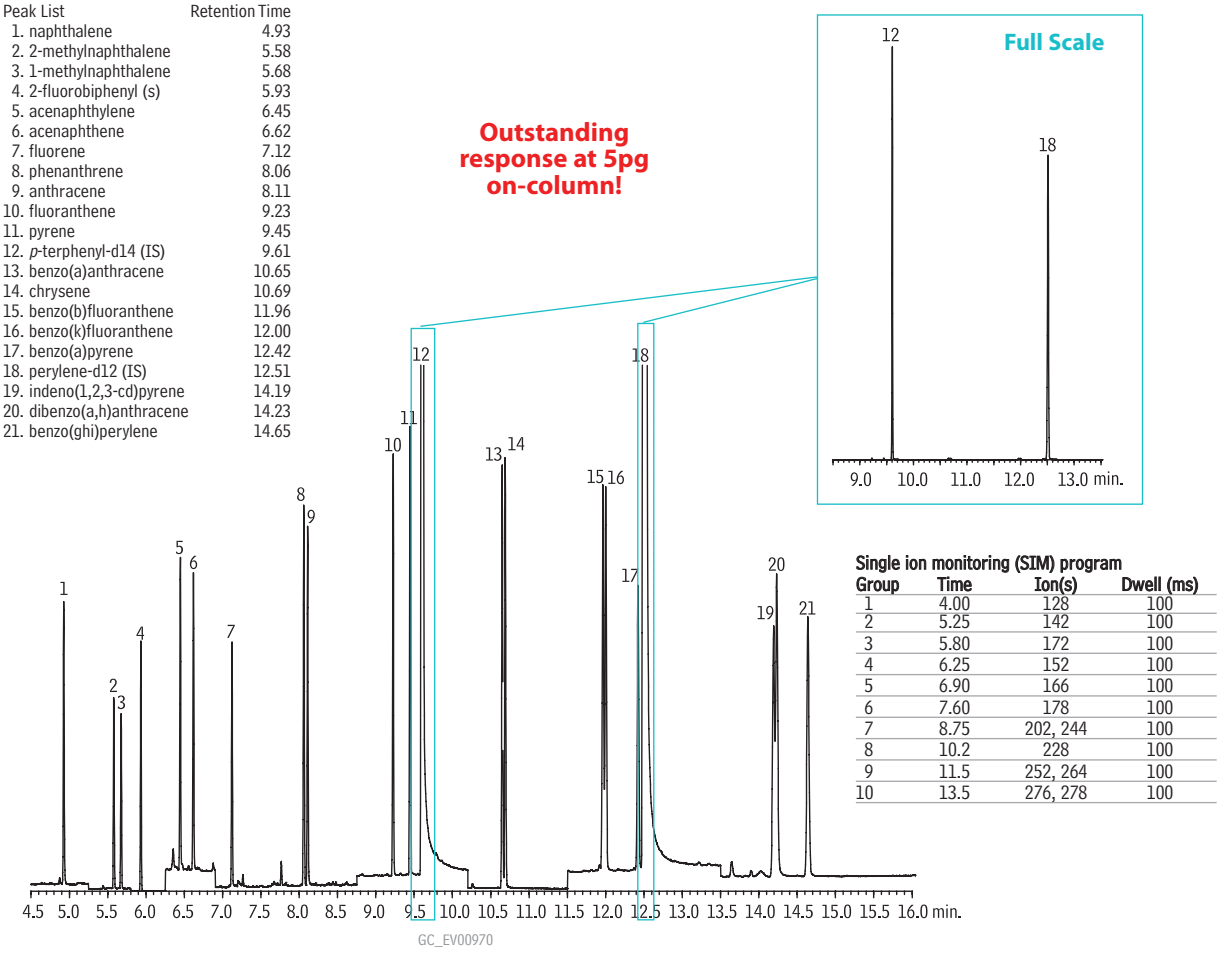
Method Parameters

For our SIM method we chose to use the new Rxi®-5Sil MS column. This stationary phase incorporates phenyl rings in the polymer backbone, which strengthens the siloxane chain, preventing thermal breakdown. This low bleed column is similar in selectivity to 5% diphenyl/95% dimethyl phases, but offers improved signal-to-noise ratios, resulting in increased sensitivity and subsequently lower detection limits. The silarylene polymer not only exhibits improved thermal stability and reduced bleed, but it also shows improved separation for aromatic compounds, such as PAHs.

Analytical conditions were set to optimize resolution of critical pairs and reduce discrimination of high molecular weight analytes. We chose a 4mm Drilled Uniliner® inlet liner with wool, since direct injection using this liner provides near complete transfer of sample analytes to the column. To improve the quantification of high molecular weight compounds we chose a thin film thickness (0.25µm) and set the injection port temperature to 300°C. A pulsed splitless injection technique was used to maximize the transfer of analytes onto the column. The pressure pulse is an effective injection technique for trace level analyses and also helps minimize discrimination against the high molecular weight components. Finally, the ion source and quadrupole temperatures were set at 290°C and 180°C, respectively. This increase in detector temperatures, from the defaults of 230°C and 150°C, yields better peak shapes and responses for the PAHs.

Figure 1 Excellent response and resolution of PAHs at 5pg on column in SIM mode

Peak List	Retention Time
1. naphthalene	4.93
2. 2-methylnaphthalene	5.58
3. 1-methylnaphthalene	5.68
4. 2-fluorobiphenyl (s)	5.93
5. acenaphthylene	6.45
6. acenaphthene	6.62
7. fluorene	7.12
8. phenanthrene	8.06
9. anthracene	8.11
10. fluoranthene	9.23
11. pyrene	9.45
12. *p*-terphenyl-d14 (IS)	9.61
13. benzo(a)anthracene	10.65
14. chrysene	10.69
15. benzo(b)fluoranthene	11.96
16. benzo(k)fluoranthene	12.00
17. benzo(a)pyrene	12.42
18. perylene-d12 (IS)	12.51
19. indeno(1,2,3-cd)pyrene	14.19
20. dibenzo(a,h)anthracene	14.23
21. benzo(ghi)perylene	14.65



Single ion monitoring (SIM) program

Group	Time	Ion(s)	Dwell (ms)
1	4.00	128	100
2	5.25	142	100
3	5.80	172	100
4	6.25	152	100
5	6.90	166	100
6	7.60	178	100
7	8.75	202, 244	100
8	10.2	228	100
9	11.5	252, 264	100
10	13.5	276, 278	100

Column: Rxi®-5Sil MS, 30m, 0.25mm ID, 0.25µm (cat.# 13623)
Sample: PAH mix, 1µL of 0.005µg/mL (IS 2µg/mL)
SV Calibration Mix #5 (cat.# 31011)
1-methylnaphthalene (cat.# 31283)
2-methylnaphthalene (cat.# 31285)
2-fluorobiphenyl (cat.# 31091)
Inj.: 1.0µL (5pg on-column concentration),
4mm Drilled Uniliner® (hole near top) inlet liner (cat.# 21055-200.5),
pulsed splitless: pulse 20psi @ 0.2 min., 60mL/min. @ 0.15 min.

Inj. temp.: 300°C
Carrier gas: helium, constant flow
Flow rate: 1.4mL/min.
Oven temp.: 50°C (hold 0.5 min.) to 290°C @ 25°C/min. to 320°C @ 5°C/min.
Det.: MS
Transfer line temp: 290°C
Ionization: EI
Mode: SIM

Results

These run conditions produced excellent resolution and response for all of the target analytes in a run time of less than 16 minutes. Figure 1 shows the SIM trace at 0.005µg/mL (5pg on column). The system was calibrated at eight levels, from 0.005 to 10µg/mL in single ion monitoring mode. The SIM acquisition program used for this analysis is shown in Figure 1. Each calibration standard contained eighteen target PAHs, two internal standards (*p*-terphenyl-d14 and perylene-d12), and the surrogate (2-fluorobiphenyl). At each level, the relative response factor (RRF) was calculated for all compounds and linearity was determined by calculating the percent relative standard deviation (%RSD) for all response factors, as shown in Table II. The %RSDs for all compounds are in the low single digits with an average for all compounds of 4.7%.

The Rxi®-5Sil MS column allows for a very broad calibration range, in this case 2000-fold from 5pg to 10ng while maintaining exceptional linearity. Using the Rxi®-5Sil MS column and an optimized temperature program is an excellent solution to the challenges posed by SIM PAH analyses.

Table I Relative response factors and %RSD for calibration standards (0.005-10µg/mL).

	Relative Response Factor									
Compound	**0.005**	**0.01**	**0.05**	**0.1**	**0.5**	**1**	**5**	**10**	**Avg**	**%RSD**
***p*-Terphenyl-d14 (IS)**	-	-	-	-	-	-	-	-	-	-
Naphthalene	0.825	0.778	0.822	0.785	0.760	0.774	0.771	0.721	0.779	4.28
2-Methylnaphthalene	0.539	0.518	0.556	0.525	0.512	0.524	0.521	0.495	0.524	3.42
1-Methylnaphthalene	0.503	0.478	0.518	0.483	0.470	0.481	0.476	0.455	0.483	4.05
2-Fluorobiphenyl (SS)	0.689	0.664	0.691	0.680	0.664	0.679	0.669	0.608	0.668	3.93
Acenaphthylene	0.879	0.838	0.917	0.887	0.868	0.899	0.904	0.856	0.881	3.00
Acenaphthene	0.541	0.508	0.544	0.522	0.508	0.522	0.514	0.482	0.518	3.80
Fluorene	0.700	0.662	0.709	0.677	0.659	0.679	0.668	0.627	0.673	3.80
Phenanthrene	1.108	1.049	1.119	1.068	1.028	1.050	1.022	0.953	1.050	4.97
Anthracene	1.052	0.962	1.043	1.003	0.981	1.013	0.993	0.921	0.996	4.27
Fluoranthene	1.239	1.161	1.254	1.206	1.166	1.195	1.171	1.093	1.185	4.25
Pyrene	1.364	1.254	1.355	1.295	1.256	1.284	1.247	1.155	1.276	5.20
Perylene-d12 (IS)	-	-	-	-	-	-	-	-	-	-
Benzo(a)anthracene	1.111	0.980	1.086	1.054	1.048	1.087	1.090	1.017	1.059	4.12
Chrysene	1.153	1.041	1.116	1.073	1.057	1.078	1.043	0.951	1.064	5.59
Benzo(b)fluoranthene	1.282	1.039	1.183	1.146	1.139	1.185	1.204	1.144	1.165	5.92
Benzo(k)fluoranthene	1.327	1.119	1.223	1.189	1.183	1.229	1.225	1.136	1.204	5.35
Benzo(a)pyrene	1.037	0.967	1.146	1.083	1.038	1.089	1.134	1.080	1.072	5.36
Indeno(1,2,3-cd)pyrene	1.457	1.224	1.379	1.366	1.333	1.387	1.471	1.424	1.380	5.69
Dibenzo(a,h)anthracene	1.195	1.027	1.150	1.180	1.094	1.164	1.233	1.173	1.152	5.56
Benzo(ghi)perylene	1.331	1.118	1.238	1.263	1.140	1.192	1.244	1.190	1.215	5.68

SV Calibration Mix #5 / 610 PAH Mix

(16 components)

acenaphthene
acenaphthylene
anthracene
benzo(a)anthracene
benzo(a)pyrene
benzo(b)fluoranthene
benzo(k)fluoranthene
benzo(ghi)perylene
chrysene
dibenzo(a,h)anthracene
fluoranthene
fluorene
indeno(1,2,3-cd)pyrene
naphthalene
phenanthrene
pyrene

2,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 31011

1-Methylnaphthalene

1,000µg/mL in methanol, 1mL/ampul
cat. # 31283

2-Methylnaphthalene

1,000µg/mL in methylene chloride, 1mL/ampul
cat. # 31285

2-Fluorobiphenyl

2,000µg/mL in methylene chloride, 1mL/ampul
cat. # 31091

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623

Direct Injection Liners for Agilent GCs (For 0.25/0.32/0.53mm ID Columns)

ID* x OD & Length (mm)	qty.	cat.#
Drilled Uniliner® (hole near top)		
4.0 ID x 6.3 OD x 78.5	ea.	21054
4.0 ID x 6.3 OD x 78.5	5-pk.	21055
4.0 ID x 6.3 OD x 78.5	25-pk.	20998
Drilled Uniliner® (hole near top) w/ Wool		
4.0 ID x 6.3 OD x 78.5	ea.	21054-200.1
4.0 ID x 6.3 OD x 78.5	5-pk.	21055-200.5
4.0 ID x 6.3 OD x 78.5	25-pk.	20998-214.25

*Nominal ID at syringe needle expulsion point.

NEW— Optimized Film Thicknesses!

13 Minute Chlorophenoxyacid Herbicides Analysis

On New Rtx®-CLPesticides & Rtx®-CLPesticides2 Columns

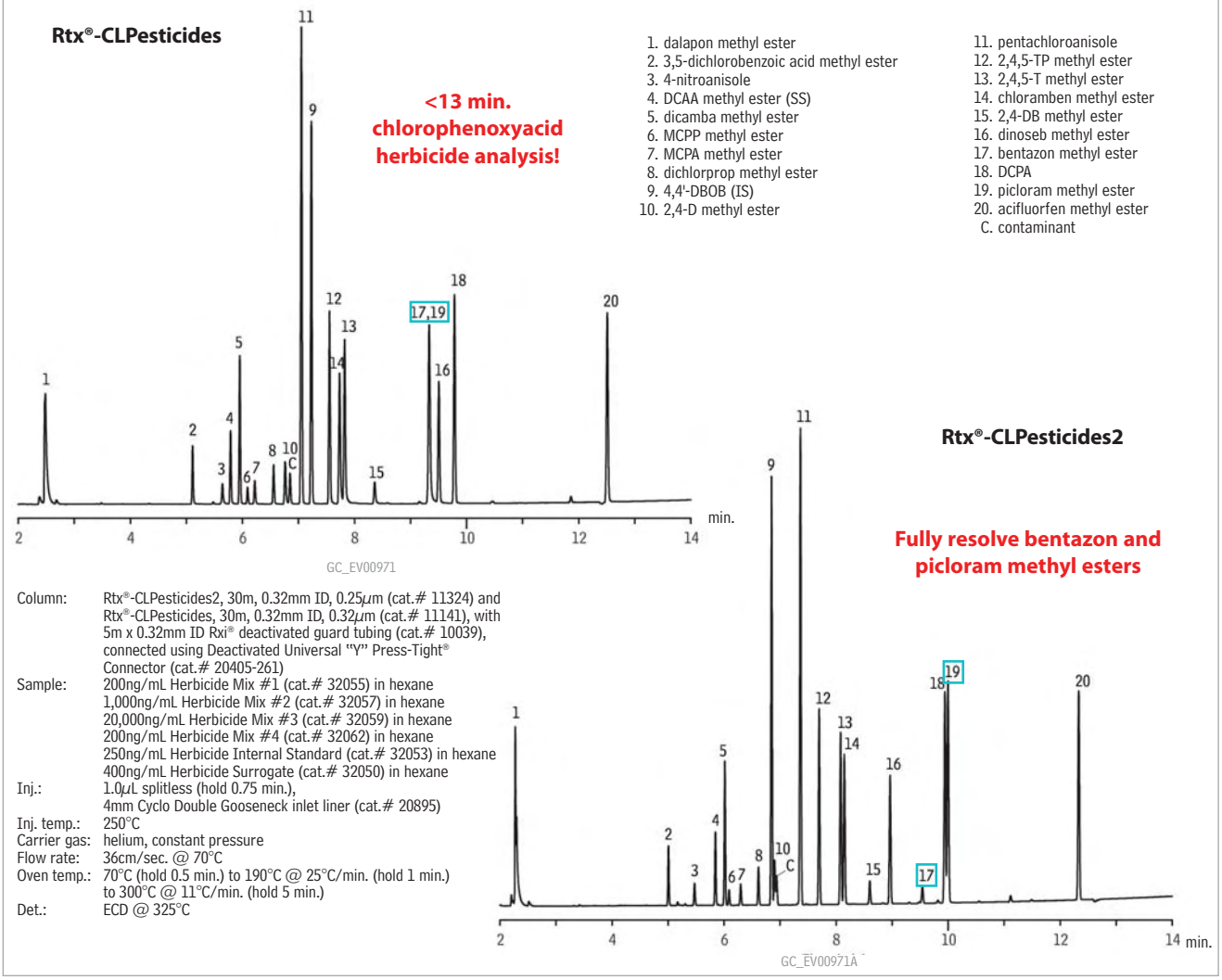
By Jason Thomas, Environmental Innovations Chemist

- Higher throughput compared to typical methods of 20 minutes or more.
- Use one column pair for multiple dual column ECD methods.
- Versatility and durability to harsh samples lead to longer life and less down time.

The analysis of chlorophenoxyacid herbicides is a very common assay performed routinely in most environmental laboratories today. Chlorophenoxyacid herbicides, as a group, are used to prevent the growth of broadleaf plants in agricultural fields. EPA Method 8151A is commonly used for chlorophenoxyacid herbicide analysis and involves extraction and derivatization to methyl ester form. GC analysis using an electron capture detector (ECD) is the analytical procedure of choice, although mass spectrometry is also used. ECD detection requires the use of second column confirmation for quantification of target analytes.

The Rtx®-CLPesticides and Rtx®-CLPesticides2 column pair is an excellent choice for chlorophenoxyacid analysis. Now, with an optimized film thickness for the 0.32mm ID version, this difficult analysis can be made in less than 13 minutes on both the primary and confirmation columns. Near baseline resolution is achieved for all analytes except for bentazon/picloram on the Rtx®-CLPesticides column; however, this pair is fully resolved on the Rtx®-CLPesticides2 column (Figure 1). The Rtx®-CLPesticides and Rtx®-CLPesticides2 column pair is an excellent choice for chlorophenoxyacid herbicide analysis due to the unique selectivity, low bleed, and durability of the columns. The Rtx®-CLPesticides column pair can also be used for other environmental ECD methods, including chorinated pesticide analysis.

Figure 1 Excellent resolution of chlorophenoxyacid herbicides on the Rtx®-CLPesticides column pair.



Column: Rtx®-CLPesticides2, 30m, 0.32mm ID, 0.25µm (cat.# 11324) and Rtx®-CLPesticides, 30m, 0.32mm ID, 0.32µm (cat.# 11141), with 5m x 0.32mm ID Rxi® deactivated guard tubing (cat.# 10039), connected using Deactivated Universal "Y" Press-Tight® Connector (cat.# 20405-261)
Sample: 200ng/mL Herbicide Mix #1 (cat.# 32055) in hexane
1,000ng/mL Herbicide Mix #2 (cat.# 32057) in hexane
20,000ng/mL Herbicide Mix #3 (cat.# 32059) in hexane
200ng/mL Herbicide Mix #4 (cat.# 32062) in hexane
250ng/mL Herbicide Internal Standard (cat.# 32053) in hexane
400ng/mL Herbicide Surrogate (cat.# 32050) in hexane
Inj.: 1.0µL splitless (hold 0.75 min.),
4mm Cyclo Double Gooseneck inlet liner (cat.# 20895)
Inj. temp.: 250°C
Carrier gas: helium, constant pressure
Flow rate: 36cm/sec. @ 70°C
Oven temp.: 70°C (hold 0.5 min.) to 190°C @ 25°C/min. (hold 1 min.) to 300°C @ 11°C/min. (hold 5 min.)
Det.: ECD @ 325°C

Rtx®-CLPesticides Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.32	-60 to 320/340°C	30-Meter	11141

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	-60 to 320/340°C	30-Meter	11324

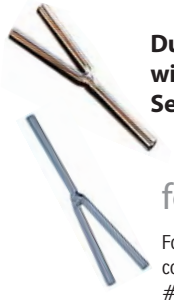
Rxi® Guard/Retention Gap Columns (fused silica)

Nominal ID	Nominal OD	5-Meter	5-Meter/6-pk.	10-Meter	10-Meter/6-pk.
0.32mm	0.45 ± 0.04mm	10039	10039-600	10064	10064-600

Universal "Y" Press-Tight® Connectors

An alternative method of performing dual-column confirmational analyses!

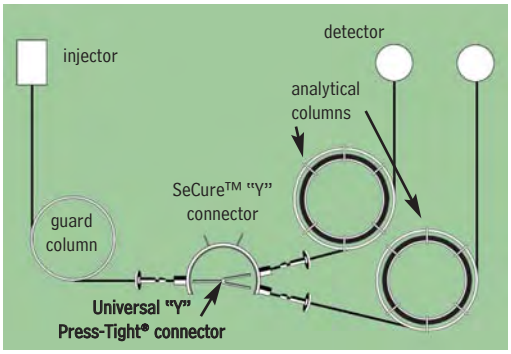
Description	ea.	3-pk.
Universal "Y" Press-Tight® Connector	20405	20406
Deactivated Universal "Y" Press-Tight® Connector	20405-261	20406-261
Siltek® Treated Universal "Y" Press-Tight® Connector	20485	20486



Dual-column confirmational analysis with a single injection—one of the SeCure™ "Y" connector's many uses.

for **more** info

For more information on Restek's Secure™ "Y" connector, download a free copy of lit. cat. #598788A from **www.restek.com**.



Herbicide Mix #1 (7 components)

Derivatized Form:

2,4-D methyl ester
2,4-DB methyl ester
2,4,5-T methyl ester
2,4,5-TP methyl ester
dicamba methyl ester
dichlorprop methyl ester
dinoseb methyl ether

200µg/mL each in hexane, 1mL/ampul
cat. # 32055

Herbicide Mix #2

Derivatized Form:

dalapon methyl ester

2,000µg/mL in hexane, 1mL/ampul
cat. # 32057

1,000µg/mL in methanol, 1mL/ampul
cat. # 32254

Herbicide Mix #3

Derivatized Form:

MCPA methyl ester
MCPP methyl ester

20,000µg/mL each in hexane, 1mL/ampul
cat. # 32059

Herbicide Mix #4 (8 components)

Derivatized Form:

acifluorfen methyl ester
bentazon methyl ester
chloramben methyl ester
DCPA (Dacthal®)
3,5-dichlorobenzoic acid
methyl ester
4-nitroanisole
pentachloroanisole
picloram methyl ester

200µg/mL each in hexane, 1mL/ampul
cat. # 32062

Splitless Liners for Agilent GCs

ID* x OD & Length (mm)	qty.	cat.#
Cyclo Double Gooseneck (4mm)		
4.0 ID x 6.5 OD x 78.5	ea.	20895
4.0 ID x 6.5 OD x 78.5	5-pk.	20896
4.0 ID x 6.5 OD x 78.5	25-pk.	20997

*Nominal ID at syringe needle expulsion point..

Herbicide Internal Standard

4,4'-dibromooctafluorobiphenyl

250µg/mL in hexane, 1mL/ampul
cat. # 32053

2,000µg/mL in methylene chloride, 1mL/ampul
cat. # 31040

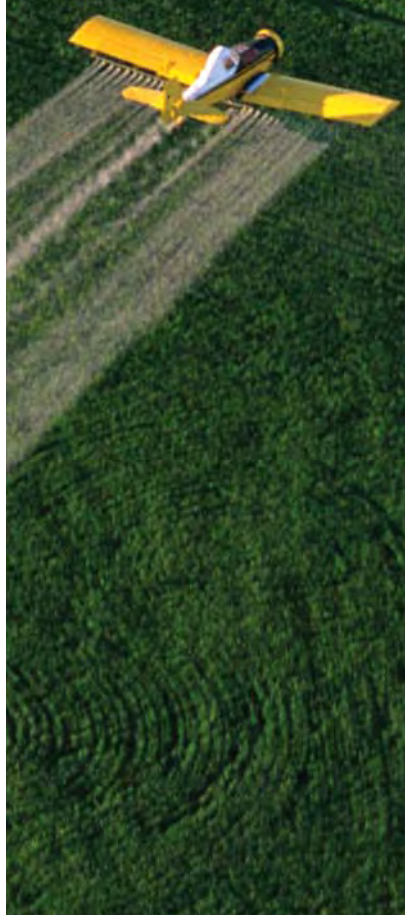
2,000µg/mL in methyl *tert*-butyl ether, 1mL/ampul
cat. # 31856

Herbicide Surrogate

Derivatized Form:

2,4-dichlorophenyl acetic acid methyl ester (DCAA methyl ester)

200µg/mL in hexane, 1mL/ampul
cat. # 32050



Enhancing Air Monitoring Methods with Thermal Desorption

Advantages Over Solvent Extraction Tubes

By Liz Woolfenden, Director, Markes International, UK, and Irene DeGraff, Product Marketing Manager

- Accurately monitor down to ppb/ppt levels.
- Use thermal desorption tubes for either active or passive sampling, without modification.
- Compliant with air sampling methods.

The use of active sampling onto glass tubes packed with charcoal, followed by carbon disulfide (CS_2) extraction and gas chromatography (GC) analysis, was developed as an air monitoring method for vapor-phase organic compounds (VOCs) in the 1970s. The approach is still used today for some personal exposure assessment (occupational hygiene) applications and stack emission testing, but is fundamentally limited with respect to detection limits. Thermal desorption (TD) is a complementary gas extraction technique whereby sorbent tubes (Figure 1) are heated in a flow of carrier gas. Trapped vapors desorb from the sample tubes into the gas stream and are transferred into the GC/MS for analysis. Here, we summarize the key advantages of thermal desorption versus solvent extraction.

Figure 1 A selection of thermal desorption air sampling tubes from Restek's new line.

Sensitivity & Reproducibility

Solvent extraction of charcoal tubes requires at least 1 or 2ml of CS_2 followed by injection of only 1-2µl of extract into the GC/MS, resulting in a 1000-fold dilution of the sample right at the start of the process. Conversely, thermal desorption allows complete transfer of all target analytes to the analytical system, with no dilution or solvent interference. Detection limits offered by thermal desorption methods facilitate ambient monitoring at ppt/ppb levels as well as higher ppm (and %-level) concentrations. In addition to high sensitivity, thermal desorption is highy reproducible, offering efficiency greater than 95%, regardless of ambient conditions and the nature of the target analytes. By comparison, results from solvent desorption tubes may be highly variable.

Passive Sampling Option

While thermal desorption tubes are used extensively for active air sampling, they are also compatible with low-cost passive sampling. Passive samplers eliminate the requirement for personal monitoring pumps making them much less heavy/intrusive. Instead of a pump, each tube is simply fitted with a diffusion cap at the sampling end.

Repeat Analysis & Method Compliance

The historical advantages of solvent desorption tubes over thermal desorption, such as multiple sample injection and method compliance, no longer hold true. Since the advent of the SecureTD-Q™ thermal desoption unit, quantitative re-collection of split flow during both tube and trap desorption is possible. The utility of quantitative sample re-collection for repeat TD-GC/MS analysis has recently been recognized in standard methods as an aid to TD method/data validation.[1] Well-validated thermal desorption methods for many applications are now available from all the major international standards agencies. Key examples include: EN ISO 16017, ISO 16000-6, ASTM D-6196, US EPA Method TO-17, NIOSH 2549, MDHS 72, 80, etc. (UK) and EN 14662.

Conclusion

Thermal desorption technology offers several significant advantages over conventional solvent extraction. TD systems offer better sensitivity, desorption efficiency, and reproducibility compared to charcoal/CS_2 systems. Additionally, tubes may be used for both passive and active sampling without modification. These benefits, in combination with SecureTD-Q™ technology, which allows repeat analysis, make thermal desorption an excellent choice for many air monitoring applications.

References
1. ASTM D6196-03

Thermal Desorption Unit (TDU) Tubes

- Variety of sorbents to collect a wide range of VOCs.
- Use glass tubes for maximum inertness in active sampling.
- Choose stainless steel tubes for either active or passive sampling. No sampling pump necessary for passive sampling with diffusion caps!
- Individually etched with unique serial number for convenient sample identification.
- Available unconditioned or preconditioned and ready to sample. Tubes are Reusable after thermal desorption.

High-quality thermal desorption tubes by Markes International are now available from Restek. These sorbent tubes are suitable for ppt to ppm concentrations of volatile organic compounds (VOCs) in ambient, indoor, and industrial hygiene environments. Available in both stainless steel and glass (for thermally labile VOCs), they fit Markes ULTRA-UNITY, PerkinElmer, and Shimadzu thermal desorbers. Packed tubes come with a report detailing the total mass of sorbent in the tube; conditioned tubes also include a blank chromatogram.

Thermal Desorption Tube Sorbent	Applications
Tenax TA	Vapour phase organics from C6/7 to C26
Graphitized Carbon	Vapour phase organics from C5/6 to C14
Tenax GR/Carbopack™ B	Vapour phase organics from *n*-C5/6 to *n*-C20 (EPA Methods TO-14/TO-15/TO-17)
Carbopack™ B/Carbosieve™ SIII	Vapour phase organics from *n*-C2/3 to *n*-C12/14 (EPA Methods TO-14/TO-15/TO-17)
Tenax TA/Graphitized Carbon/Carboxen™ 1000	Vapour phase organics from C2/3 to C20
Carbopack™ C/Carbopack™ B/Carbosieve™ SIII	Vapour phase organics from *n*-C2/3 to *n*-C16/20 (EPA Methods TO-14/TO-15/TO-17)

method applications

Method	Application
US EPA	TO-17
ASTM	D-6196
NIOSH	2549
DIN EN ISO	16017

Specifications

Dimensions: 1/4" OD x 3-1/2" long
Low sampling rates:
0.01-0.20 L/min.
(<10L total volume)
Long-term storage caps are supplied with conditioned tubes

Thermal Desorption Unit Tubes, Unconditioned and Conditioned & Capped

		Unconditioned		Conditioned & Capped	
		Stainless Steel	Glass	Stainless Steel	Glass
Description	qty.	cat.#	cat.#	cat.#	cat.#
TDU Tubes, Tenax TA	10-pk.	24056	24062	24080	24086
TDU Tubes, Graphitized Carbon	10-pk.	24057	24063	24081	24087
TDU Tubes, Tenax GR/Carbopack™ B	10-pk.	24058	24064	24082	24088
TDU Tubes, Carbopack™ B/Carbosieve™ SIII	10-pk.	24059	24065	24083	24089
TDU Tubes, Tenax TA/Graphitized Carbon/Carboxen™ 1000	10-pk.	24060	24066	24084	24090
TDU Tubes, Carbopack™ C/Carbopack™ B/Carbosieve™ SIII	10-pk.	24061	24067	24085	24091

Stainless Steel, Conditioned and Capped

Thermal Desorption Unit Tubes, Empty

		Stainless Steel	Glass
Description	qty.	cat.#	cat.#
TDU Tubes, Empty	10-pk.	24054	24055

Glass, Unconditioned

Thermal Desorption Unit Tubes, Calibration

		Stainless Steel	Glass
Description	qty.	cat.#	cat.#
TDU Tubes, Calibration, Tenax TA 1cm Bed	10-pk.	24075	24076

Description	qty.	cat.
Calibration Solution Loading Rig	ea.	24077
Calibration Solution Loading Rig 9.5mm Replacement Septa	10-pk.	24078
Certified Reference Standard, 100ng BTX on Tenax TA	10-pk.	24079

Stainless Steel, Unconditioned

Thermal Desorption Unit Tubes, Accessories

Description	Benefits/Uses	qty.	cat.
1/4" Brass Cap and PTFE Ferrules	Use for long-term storage of blank/sampled tubes.	20-pk.	24068
1/4" PTFE Ferrules	Long-term storage caps.	20-pk.	24069
CapLok Tool	Use for tightening long-term storage caps.	ea.	24070
Pen Clip		10-pk.	24071
TubeMate Tool	Assists with tube packing.	ea.	24072
1/4" Stainless Steel Union and PTFE Ferrules	Use for connecting tubes in series.	10-pk.	24073
Diffusion Caps	Required for diffusive sampling with stainless steel tubes.	10-pk.	24074

CapLok Tool

Diffusion Caps

Selecting a GC Column for Glycerin in Biodiesel

By Barry Burger, Petroleum Innovations Chemist, and Gary Stidsen, Product Marketing Manager

- Choose metal MXT®-Biodiesel TG columns for high temp. conditions; low bleed and leak-proof for more accurate results.
- Use Rtx®-Biodiesel TG columns up to 380°C when fused silica is desired; reliable, low bleed performance.
- Innovative Alumaseal™ and Integra-Gap™ technology; choose Restek for leak-proof retention gap options.

Comparing Fused Silica and Metal Columns

Fused silica columns traditionally have been used for GC biodiesel analysis, but metal columns offer significant performance advantages. How can analysts determine which column is best for their lab? Here we compare fused silica and metal column performance for total glycerin analysis of biodiesel and offer guidelines for column selection.

Excellent chromatography can be obtained using Rtx®-Biodiesel TG fused silica columns. However, for high temperature work (>380°C) metal columns are much more rugged because the polyimide resin used to make fused silica hardens at high temperatures, making columns brittle and producing active sites in the column. To maximize column lifetime, tubing choice should be based on the maximum temperature setting in the GC temperature program. If the temperature program will be 400°C or lower, high temperature fused silica tubing is an acceptable choice; for GC temperatures that will exceed 400°C, metal tubing should be used.

Rtx®-Biodiesel TG Fused Silica Columns

Two fused silica GC column dimensions are available for the analysis of total glycerin: 10m x 0.32mm ID or 15m x 0.32mm ID, both of which are connected to a 2m x 0.53mm ID retention gap for cool on-column injection. The retention gap is factory coupled using Restek's unique Alumaseal™ connector (Figure 1). This innovative connector is leak-tight and low dead volume, making it advantageous for high temperature work.

Metal Column Solutions: Two Options for Increased Stability and Performance

- 0.32mm MXT®-Biodiesel TG columns with factory-connected retention gaps.
- 0.53mm MXT®-Biodiesel TG columns with built-in retention gaps.

The primary advantage of using metal MXT® columns is that they are more stable at high temperatures than fused silica columns. This means they will exhibit lower bleed, improving analytical performance, and have longer lifetimes, making them a cost-effective option. High temperature tolerance also means these columns can be brought to high temperatures (430°C) allowing nonvolatile material to be baked off of the column. MXT®-Biodiesel TG columns are available in the same dimensions as their fused silica counterparts:

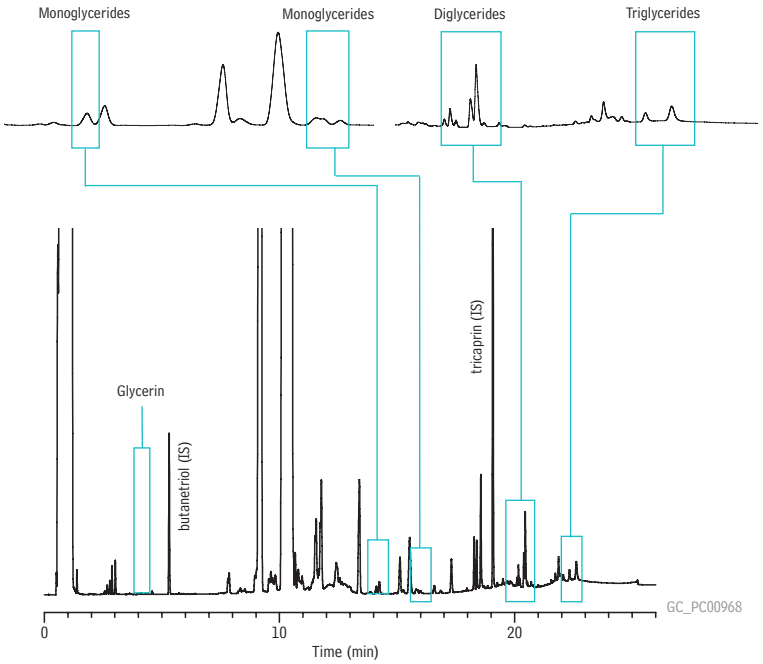
Figure 1 The Alumaseal™ connector

The Alumaseal™ connector is the best column connector for coupling fused silica and metal columns, even columns of different internal diameters. Made of aluminum, it is designed for high temperature performance. These connectors have been factory-coupled and tested using temperature programmed mass spectrometry and have shown no signs of leaks, even at 430°C.

The Alumaseal™ connector offers:

- A leak-tight connection.
- Low dead volume.
- Low thermal mass.
- High inertness.

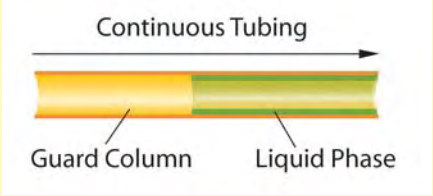
Figure 2 Derivatized B100 samples resolve well on the 15m x 0.32mm MXT®-Biodiesel TG column, which is factory coupled to a 0.53mm retention gap using an Alumaseal™ connector.



Column:	MXT®-Biodiesel TG, 15m, 0.32mm ID, 0.10µm (cat.# 70291) with a 2m x 0.53mm MXT® retention gap connected with an Alumaseal™ connector (17m total length)
Sample:	biodiesel (B100), derivatized
Inj.:	cool on-column injection 1µL in heptane
Inj. temp.:	oven track
Carrier gas:	hydrogen, constant flow
Flow rate:	3mL/min.
Oven temp.:	50°C (hold 1 min.) to 180°C @ 15°C/min. to 230°C @ 7°C/min. to 380°C @ 30°C/min. (hold 5 min.)
Det.:	FID @ 380°C

Figure 3 The Ultimate Biodiesel Solution: MXT®-Biodiesel TG column with Integra-Gap™ integrated retention gap.

The 0.53mm MXT®-Biodiesel TG columns are an innovative alternative to using a 0.32mm column coupled to a 0.53mm retention gap. Restek applied the Integra-Gap™ integrated retention gap technology to the 0.53mm MXT®-Biodiesel TG columns, eliminating the column coupling. These 100% leak-proof columns feature a built-in retention gap, reducing the risk of peak broadening and tailing, and guaranteeing the user many analyses without downtime.



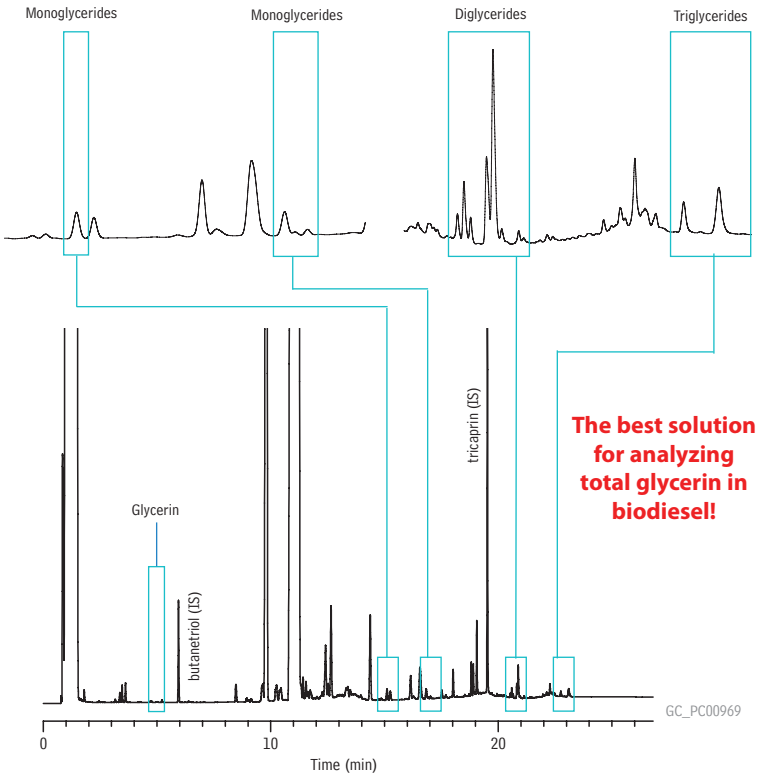
10 x 0.32mm ID and 15m x 0.32mm ID, both of which are factory coupled to a 2m x 0.53mm retention gap using an Alumaseal™ connector. Excellent resolution of all glycerides is achieved, as shown in Figure 2.

Restek has also developed an innovative column where the analytical column includes a built-in retention gap in a continuous section of tubing, requiring no connectors. This column, the MXT®-Biodiesel TG column, is 14m x 0.53mm ID, and features a 2m x 0.53mm ID Integra-Gap™ integrated retention gap (Figure 3). This product eliminates any need for connections because the column and retention gap are one piece of continuous tubing. Target analytes resolve exceptionally well and the solvent and triglyceride peaks show excellent symmetry (Figure 4). Peak shape for butanetriol is very good, demonstrating inertness, and the resolution and response of the glycerides is also excellent.

Conclusion

There are several column options available for analyzing total glycerin in biodiesel fuels. The best chromatographic solution for this analysis is the 14m x 0.53mm ID MXT®-Biodiesel TG column with the 2m x 0.53mm ID Integra-Gap™ integrated retention gap. This column eliminates the column connection and can be used to 430°C allowing for faster analysis times and higher sample throughput.

Figure 4 Excellent chromatographic quality and resolution on the 0.53mm MXT®-Biodiesel TG column, with the Integra-Gap™ integrated retention gap.



Column:	MXT®-Biodiesel TG, 14m, 0.53mm ID, 0.16µm (cat.# 70289) with a 2m x 0.53mm Integra-Gap™ retention gap (16m total length)
Sample:	biodiesel (B100), derivatized
Inj.:	cool on-column injection 1µL in heptane
Inj. temp.:	oven track
Carrier gas:	hydrogen, constant flow
Flow rate:	4mL/min.
Oven temp.:	50°C (hold 1 min.) to 180°C @ 15°C/min. to 230°C @ 7°C/min. to 380°C @ 30°C/min. (hold 5 min.)
Det.:	FID @ 380°C

MXT®-Biodiesel TG Columns (Siltek® treated stainless steel)

Description	temp. limits	cat.#
14m, 0.53mm ID, 0.16 w/2m Integra-Gap™	-60 to 380/430°C	70289
10m, 0.32mm ID, 0.10	-60 to 380/430°C	70292
10m, 0.32mm ID, 0.10 w/2m x 0.53mm retention gap**	-60 to 380/430°C	70290
15m, 0.32mm ID, 0.10	-60 to 380/430°C	70293
15m, 0.32mm ID, 0.10 w/2m x 0.53mm retention gap**	-60 to 380/430°C	70291

**Connected with low-dead-volume Alumaseal™ connector.

Rtx®-Biodiesel TG Columns (fused silica)

Description	temp. limits	cat.#
10m, 0.32mm ID, 0.10	to 330/380°C	10292
10m, 0.32mm ID, 0.10 w/2m x 0.53mm retention gap**	to 330/380°C	10291
15m, 0.32mm ID, 0.10	to 330/380°C	10294
15m, 0.32mm ID, 0.10 w/2m x 0.53mm retention gap**	to 330/380°C	10293

**Connected with low-dead-volume Alumaseal™ connector.

Stable Sulfur & Mercury Sampling in Refineries

Using Siltek® and Sulfinert® Surface Treated Components

By Gary Barone, Restek Performance Coatings, and Irene DeGraff, Product Marketing Manager

- Reliably sample sulfur and mercury compounds at ppb levels.
- Reduce lab costs—obtain accurate results the first time.
- Detect costly process upsets, improving product yield.

Refinery and natural gas samples often contain trace amounts of sulfur- and mercury-containing compounds, which can interfere with reactions, poison catalysts in petrochemical processes, and damage equipment. Because these compounds quickly react with stainless steel surfaces, accurate determination of these compounds is impossible when samples are collected and stored in untreated sample cylinders. Restek's Siltek® and Sulfinert® passivation techniques bond an inert layer into the surface of stainless steel, preventing active compounds from reacting with or adsorbing to the steel.

Accurate sulfur sampling

To characterize Sulfinert® surfaces, we tested the stability of 17ppbv standards of sulfur compounds in three Sulfinert® sample cylinders over a 54-hour period. Dimethyl sulfide, which is not adsorbed by stainless steel, was used as an internal standard. The Sulfinert®-treated cylinders were inert to the reactive sulfur compounds over the 54-hour test period (Figure 1). Hydrogen sulfide exhibited greater than 85% recovery; methyl mercaptan, ethyl mercaptan, carbonyl sulfide, and dimethyl disulfide exhibited greater than 90% recovery.

Sulfinert®-treated gas sampling equipment is ideal for collecting and storing samples containing ppb levels of sulfur compounds, such as natural gas or beverage-grade carbon dioxide. Sulfinert® treatment ensures that sulfur compounds or other highly active compounds remain stable during transport from the field to the laboratory.

Stable Mercury Results

Siltek® surface treatment has been used in a wide variety of applications in which an inert surface is of paramount importance. To measure the impact of Siltek® treatment on adsorption of mercury during storage, we compared the performances of 304 grade stainless steel gas sampling cylinders (Swagelok®, Solon OH) with and without Siltek® treatment.

We filled each cylinder with 8µg/m³ of elemental mercury (approximately 1 part per billion) (Spectra Gases, Alpha NJ) and assessed the mercury concentration in each cylinder over time to determine changes in mercury concentration. Detection was achieved by direct interface gas sampling to an atomic absorption detector. The sample pathway regulator and tubing were Siltek® treated to ensure accurate transfer.

The data in Figure 2 demonstrate that Siltek® treatment provides a stable surface for elemental mercury, and untreated stainless steel does not. Based on these results, we conclude that Siltek® surface treatment for steel or stainless steel components and tubing in CMMS and sorbent tube mercury sampling systems will improve analytical reliability.

Siltek® and Sulfinert® surface treated cylinders and sampling components provide an inert sample path, which prevents adsorption of active compounds and ensures accurate sampling. For more information about these treatments, visit us at www.restekcoatings.com.

Acknowledgement

The authors wish to acknowledge Ted Neeme and Steve Mandel from Spectra Gases for their contributions to this work.

Figure 1 Stability of sulfur compounds is remarkable in Sulfinert®-treated cylinders.

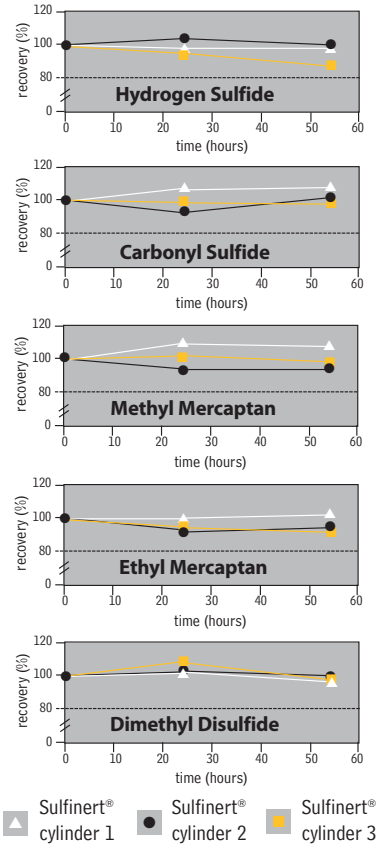
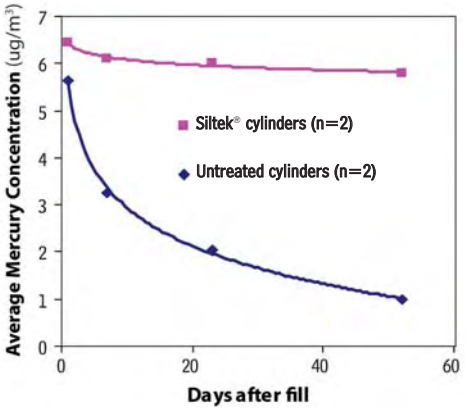


Figure 2 Siltek® treated gas sampling cylinders show very good inertness toward mercury.



Sulfinert® Treated Swagelok® Sample Cylinders

- Stable storage of samples containing ppb levels of sulfur compounds.
- Manufactured by Swagelok®; U.S. D.O.T. rated to 1,800psi (12,411kPa) at room temperature.
- 304 grade stainless steel with 1/4" female NPT threads on both ends.

Description	Size	qty.	cat.#
Sulfinert® Sample Cylinder	75cc	ea.	24130
Sulfinert® Sample Cylinder	150cc	ea.	24131
Sulfinert® Sample Cylinder	300cc	ea.	24132
Sulfinert® Sample Cylinder	500cc	ea.	24133
Sulfinert® Sample Cylinder	1000cc	ea.	24134
Sulfinert® Sample Cylinder	2250cc	ea.	21394

Sulfinert® Treated Alta-Robbins Sample Cylinder Valves

- All wetted parts are Sulfinert® treated for inertness.
- Compatible with Sulfinert® treated Swagelok® sample cylinders.
- Large, durable, Kel-F® seat ensures leak-free operation; temperature range: -40°C to 120°C.

Description	qty.	cat.#
1/4" NPT Exit	ea.	21400
1/4" Compression Exit	ea.	21401
1/4" NPT with Dip Tube*	ea.	21402
1/4" NPT with 2850psi Rupture Disc	ea.	21403
1/4" NPT Male Inlet x 1/4" Female Outlet with 2850psi Rupture Disc	ea.	21404

*To order catalog #21402 (Sulfinert Alta-Robbins Sample Cylinder Valve, 1/4" NPT with Dip Tube), please call Customer Service at 800-356-1688, ext. 3, or contact your Restek representative. Specify dip tube length or % outage when ordering (maximum length = 5.25"/ 13.3cm). Note: End of part will not be treated after cutting tube to length.

Siltek®/Sulfinert® Treated Coiled Electropolished 316L Grade Stainless Steel Tubing

Recommended for:

- high temperatures
- demanding/corrosive environments
- ultimate inertness

OD	ID	cat.#	5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
1/8" (3.18mm)*	0.085" (2.16mm)	22538				
1/4" (6.35mm)**	0.180" (4.57mm)	22539				

Siltek®/Sulfinert® Treated Coiled 316L Grade Stainless Steel Tubing

Recommended for:

- inert applications
- high temperatures
- high pressures
- corrosive environments

OD	ID	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
1/8" (3.18mm)**	0.055" (1.40mm)	22508				
1/4" (6.35mm)**	0.180" (4.57mm)	22509				
3/8" (9.52mm)***	0.277" (7.04mm)	22914				

Siltek®/Sulfinert® Treated Straight Seamless 316L Grade Stainless Steel Tubing

- Individual 6-foot pieces.

6 foot Length			
OD	ID	qty.	cat.#
1/8" (3.18mm)**	0.055" (1.40mm)	ea.	22901
1/4" (6.35mm)**	0.180" (4.57mm)	ea.	22902
3/8" (9.52mm)***	0.277" (7.04mm)	ea.	22903

1/8" OD: 5 ft. to 100 ft. in one continuous coil; 1/4" OD: 5 ft. to 300 ft. in one continuous coil. Longer lengths will be more than one coil. Note: required length in meters x 3.2808 = length in feet.

*0.020" wall thickness
**0.035" wall thickness
***0.049" wall thickness

ordering note

An extra charge is applied for cutting Siltek®/Sulfinert® or Silcosteel®-CR tubing. The charge is calculated from the total number of pieces produced for each line item

High Sensitivity Melamine GC/MS Analysis of Cat Food

Modified Conditions Save Costs and Reduce Maintenance

By Michelle Long, Innovations Chemist and Julie Kowalski, Ph.D., Food Flavor and Fragrance Innovations Chemist

- Excellent results in pet food matrix; lower pyridine background for better sensitivity.
- Easy sample preparation; reduced derivatization reagent volume lowers costs and keeps inlet and column clean.
- Modified conditions reduce maintenance and extend filament lifetime.

A large pet food recall occurred in 2007 when animals became ill or died after eating food contaminated with melamine and related compounds. Melamine is an industrial chemical used in the production of plastics, adhesives, flame retardants, fabrics and other materials. It is not a food ingredient, but since melamine and related compounds are high in nitrogen content—and protein testing methods are based on nitrogen levels—these compounds were used as additives to generate artificially high label values for protein content.

Procedure

The procedure for this experiment was adapted from the U.S Food and Drug Administration (FDA), GC/MS Method for Screening and Confirmation of Melamine and Related Analogs, Version 2, May 7, 2007. Standards were diluted to 10µg/mL and 1µg/mL with 10:40:50 diethylamine:water:acetonitrile. Three 0.5g matrix samples (dry cat food) were prepared: one control, one spiked at 50µg/g and one at 10µg/g.

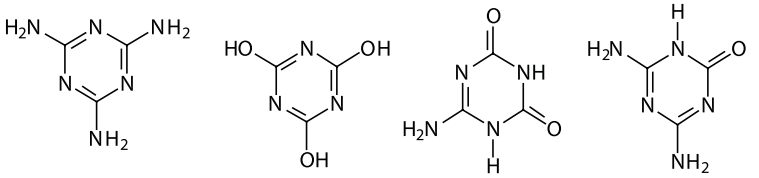
Two modifications were made to the derivatization procedure in the FDA method. The amount of derivitizing reagent was reduced from 200µL to 50µL of BSTFA with 1% TMCS (which is still a molar excess of 50:1). Incubation time was subsequently increased from 45 min. to 120 min.

Analyses were performed on a Shimadzu QP-2010 Plus gas chromatograph mass spectrometer (GC/MS) using a 30m x 0.25mm ID x 0.25µm Rtx®-5MS column. The mass spectrometer data was acquired in SIM acquisition mode with selected ions for each analyte of interest (Table I).

Results

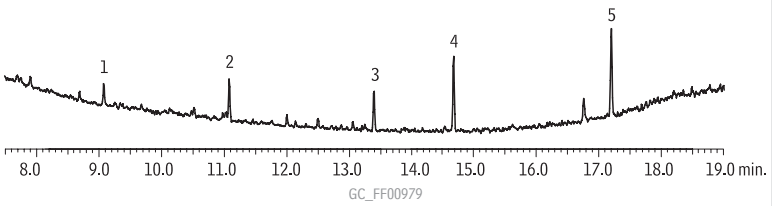
The original method conditions resulted in a significant initial baseline elevation due to the presence of pyridine, which is necessary for the derivatization reaction (Figure 2). Pyridine can increase ion signal background over a long period of time. To combat this, pyridine can be evaporated and the remaining analytes can be dissolved in a more GC amenable solvent, but this is time consuming and can result in analyte loss. A simpler solution is to eliminate the pyridine ion signal by changing the mass range to be scanned. All of the analytes have characteristic ions of interest well above m/z 79 which is associated with pyridine. Therefore,

Figure 1 Melamine and related compounds are nitrogen-rich and can artificially raise labeled protein content when used as an additive.



Melamine | Cyanuric Acid | Ammelide | Ammeline

Figure 2 Original method produces an elevated baseline, compromising integration and reducing sensitivity (10µg/mL standard).



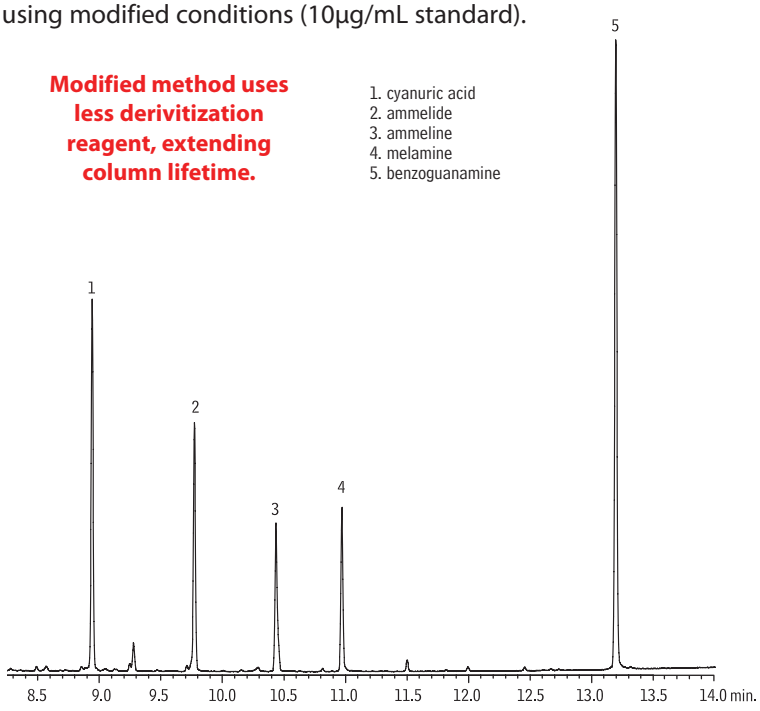
1. cyanuric acid
2. ammelide
3. ammeline
4. melamine
5. benzoguanamine

Column:	Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample:	melamine, cyanuric acid, ammelide, ammeline, benzoguanamine (10µg/mL prederivatized)
Inj.:	1µL, splitless (hold 1 min.), 3.5mm splitless inlet liner (cat.# 22286)
Inj. temp.:	280°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	75°C (hold 1 min.) to 320°C (hold 4 min.) @ 15°C/min.
Det:	MS
Transfer line temp.:	290°C
Scan range:	50-450 m/z
Ionization:	EI
Mode:	scan

Table I MS conditions (SIM mode).

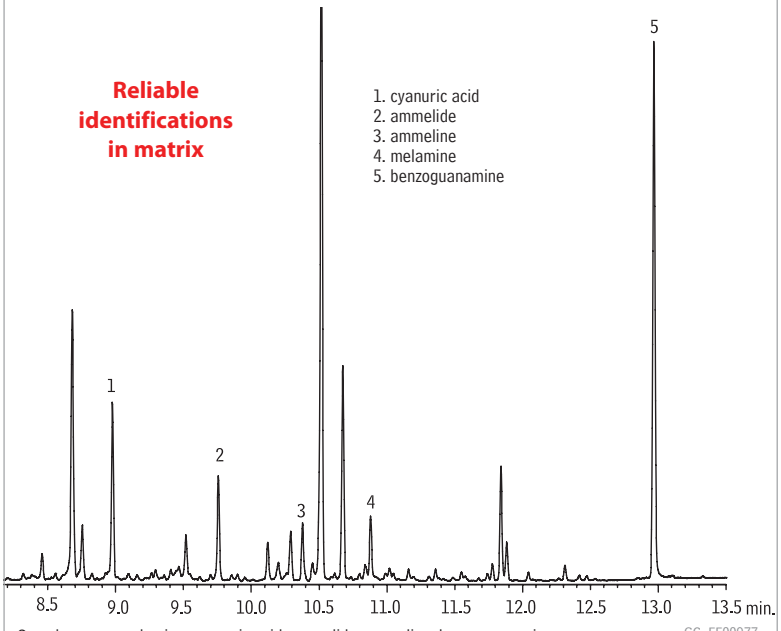
Compound	Retention Time (min.)	Target Ions	Reference Ions	Reference Ions	Reference Ions
Cyanuric Acid	8.97	345 (100)*	330 (36)	346 (30)	347 (15)
Ammelide	9.79	344 (100)	329 (30)	345 (58)	330 (16)
Ammeline	10.44	328 (100)	343 (79)	329 (29)	344 (24)
Melamine	10.97	327 (100)	342 (53)	328 (30)	343 (17)
Benzoguanamine	13.18	316 (100)	331 (68)	332 (20)	330 (9)

Figure 3 Excellent separation of melamine and related compound using modified conditions (10µg/mL standard).



Column:	Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample:	melamine, cyanuric acid, ammelide, ammeline, benzoguanamine (10µg/mL prederivatized)
Inj.:	1µL, splitless (hold 1 min.), 3.5mm splitless inlet liner (cat.# 22286)
Inj. temp.:	280°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	75°C to 320°C @ 15°C/min. (hold 4 min.)
Det:	MS
Transfer line temp.:	290°C
Ionization:	EI
Mode:	SIM

Figure 4 Melamine production analytes are easily identified in cat food using SIM analysis (50µg/g spike).



Sample: melamine, cyanuric acid, ammelide, ammeline, benzoguanamine in dry cat food (10µg/mL prederivatized)

See Figure 3 for conditions.

the scan method was modified to begin scanning at m/z 85. The solvent delay was also increased to approximately 8 min. due to the high background levels. This extra time helps increase the filament lifetime and ensures all the analytes will be detected.

This method provides excellent separation of melamine and cyanuric acid, the suspected toxic compounds, as well as ammelide and ammeline (Figure 3). Reproducible and reliable retention times were obtained for matrix spikes; this, along with SIM mass spectrometric detection, allows easy identification of analytes at both the high and low spike levels (Figure 4).

Conclusions

This work demonstrates that the FDA method is a valuable guideline for analysts screening melamine and related analogs. Using an Rtx®-5MS column and modifying the original method provides additional benefits: 1) decreasing the derivitization reagent volume results in longer column lifetime and less inlet maintenance, and 2) increasing the solvent delay decreases pyridine ion background, resulting in higher sensitivity, approximately 5 times higher, for the analytes of interest.

References

GC-MS Method for Screening and Confirmation of Melamine and Related Analogs, Version 2, May 7, 2007, U.S Food and Drug Administration, http://www.fda.gov/cvm/GCMSscreen.htm.

Rtx®-5MS—Low-bleed GC/MS Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623

Splitless Liners for Shimadzu 17A, 2010, and 2014 GCs

ID* x OD & Length (mm)	qty.	cat.#
3.5mm Splitless		
3.5 ID x 5.0 OD x 95	ea.	22286
3.5 ID x 5.0 OD x 95	5-pk.	22287

*Nominal ID at syringe needle expulsion point.

Silylation Derivatization Reagents

Compound	CAS#	cat.#
BSTFA w/1% TMCS (N,O-bis[trimethylsilyltrifluoroacetamide] w/1% trimethylchlorosilane)		
10-pk. (10x1g)	25561-30-2	35606
25g Flex Tube	25561-30-2	35607

Fast, Simple Sample Cleanup

Using QuEChERS SPE Tubes

By Julie Kowalski, Innovations Chemist, Lydia Nolan, Innovations Chemist,
Jack Cochran, Director of New Business and Technology, and Irene DeGraff, Product Marketing Manager

- Achieve a four-fold increase in sample throughput.
- Significantly reduce material costs.
- Convenient, ready to use centrifuge tubes with ultra pure, pre-weighed adsorbent mixtures.

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe, the QuEChERS ("catchers") method for extracting pesticides from food is based on research by the US Department of Agriculture.[1] In addition to using less solvent and materials versus conventional SPE methods, QuEChERS employs a novel and much quicker dispersive solid phase extraction cleanup (dSPE). QuEChERS methods, including an AOAC Official Method[2] and modifications to the methods, have been posted on the Internet.[3] These methods have several basic steps in common:

Step 1: Sample preparation and extraction–Commodities are uniformly comminuted. Acetonitrile solvent is added for a shake extraction. Salts, acids and buffers may be added to enhance extraction efficiency and protect sensitive analytes. Surrogate standards can be added to monitor extraction efficiencies.

Step 2: Extract cleanup – A subsample of solvent extract is cleaned up using dSPE, a key improvement incorporated in the QuEChERS technique. Small polypropylene centrifuge tubes are prefilled with precise weights of $MgSO_4$ and SPE adsorbents to remove excess water and unwanted contaminants from the extracted samples. After agitation and centrifugation, the cleaned extracts are ready for analysis.

Step 3: Sample analysis – Samples may be pH adjusted to protect sensitive pesticides and/or solvent-exchanged to improve analysis by either GC/MS or LC/MS. Internal standards can be added.

QuEChERS methods are convenient, rugged methods that simplify extract cleanup, reduce material costs, and improve sample throughput. Here we demonstrate the effectiveness of QuEChERS sample cleanup using a multiresidue analysis of pesticides on strawberries.

Experimental

Strawberry extracts were prepared, spiked, and dSPE treated according to Table I. Analytical conditions are presented in Table II.

One microliter splitless injections of the extracts were performed by a Shimadzu AOC-20i autosampler using "mid" injection speed into a Shimadzu QP-2010 Plus GC-MS system operated under the conditions in Table II.

Table I Modified mini-multiresidue QuEChERS for pesticides from strawberries.

Sample preparation and extraction	
Sample:	10g of strawberries were homogenized and placed in a 50mL PTFE centrifuge tube
Solvent:	10mL of acetonitrile were added to homogenate Shake for 1 minute, until uniform
Salts:	4.0g MgSO4 (powder or granular) 1.1.0g NaCl 1.0g trisodium citrate dihydrate 0.5g disodium hydrogencitrate sesquihydrate Salts were added and vigorously shaken for 1 minute. Sample was centrifuged and the supernatant removed for cleanup. Pesticides standards (200ng/mL) were spiked in at this point.
Sample extract cleanup	
QuEChERS tubes:	1mL of supernatant from the previous step was placed into several 2mL polypropylene centrifuge tubes, each containing one of the following adsorbent mixes: A. 50mg PSA + 150mg $MgSO_4$ (cat.# 26124) B. 50mg PSA + 150mg $MgSO_4$ + 50mg C18 (cat.# 26125) C. 50mg PSA + 150mg $MgSO_4$ + 50mg GCB (cat.# 26123)
Cleanup:	Samples were shaken with the adsorbents for 30 seconds (carbon for 2 minutes), then centrifuged to produce a clear supernatant for GC/MS analysis.
Internal standard:	Pentachloronitrobenzene in a formic acid solution, pH 5.

PSA—primary and secondary amine exchange material.
GCB—graphitized carbon black

Table II Instrument conditions.

Column:	Rtx®-CLPesticides2 20m, 0.18mm ID, 0.14μm (cat.# 42302)
Sample:	custom pesticide mix 200μg/mL each pesticide, internal standards: 8140-8141 ISTD, 1000μg/mL (cat.# 32279), 508.1 ISTD 100μg/mL (cat.# 32091), triphenylphosphate 1000μg/mL (cat.# 32281)
Inj.:	1.0μL splitless (hold 1 min.)
Inj. temp.:	250°C
Carrier gas:	helium
Flow rate:	constant linear velocity @ 40cm/sec
Oven temp.:	40°C (hold 1 min.) to 320°C @ 12°C/min.
Det:	Shimadzu GCMS-QP2010 Plus
Transfer line temp.:	300°C
Ionization:	Electron ionization
Mode:	Selected ion monitoring

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (μm)	temp. limits	length	cat. #
0.18mm	0.14	-60 to 310/330°C	20-Meter	42302

Results and Discussion

Primary and secondary amine exchange material (PSA) is the base sorbent used for dSPE cleanup of QuEChERS fruit and vegetable extracts because it removes many organic acids and sugars that might act as instrumental interferences.

A pesticide-spiked strawberry extract (200ng/mL) subjected to dSPE with PSA was used to generate one-point calibration curves. Spiked strawberry extracts subjected to additional dSPE sorbents were analyzed and the results versus PSA dSPE are shown as percent recoveries in Table III. C18 is suggested for use when samples might contain fats; not an issue for a strawberry extract, but it was important to verify that gross losses of more hydrophobic pesticides (e.g. Endrin and DDT) would not occur. GCB is used to remove pigments, and when treated, the pink/red strawberry extract became clear. However, GCB can also have a negative effect on certain pesticides, especially those that can assume a planar shape like chlorothalonil and thiabendazole.

Restek dSPE products in a variety of standard sizes and formats make QuEChERS even simpler. The centrifuge tube format, available in 2mL and 15mL sizes, contains magnesium sulfate (to partition water from organic solvent) and a choice of SPE sorbents, including PSA (to remove sugars and fatty acids), C18 (to remove nonpolar interferences such as fats), and GCB (to remove pigments and sterols). Custom products also are available by request. If you are frustrated by the time and cost involved with your current approach to pesticide sample cleanup, we suggest you try this simple and economical new method.

References

1. Michelangelo Anastassiades, Steven J. Lehotay, Darinka Štajnbaher, Frank J. Schenck. "Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and Dispersive Solid-Phase Extraction for the Determination of Pesticide Residues in Produce." *J. AOAC International*, 2003, vol. 86(22), pp.412-431.
2. AOAC Official Method 2007.01, "Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate."
3. http://www.quechers.com/

References not available from Restek

Table III Pesticide percent recoveries in strawberry extracts treated with C18 or GCB dSPE, relative to PSA only.

Rt (min.)	pesticide	CAS Number	action/Use	classification	C18*	GCB**
9.50	Dichlorvos	62-73-7	Insecticide	Organophosphorus	111	116
9.67	Methamidophos	10265-92-6	Insecticide	Organophosphorus	105	107
11.75	Mevinphos	7786-34-7	Insecticide	Organophosphorus	112	130
12.02	*o*-Phenylphenol	90-43-7	Fungicide	Unclassified	106	97
12.14	Acephate	30560-19-1	Insecticide	Organophosphorus	128	147
13.89	Omethoate	1113-02-6	Insecticide	Organophosphorus	120	119
14.74	Diazinon	333-41-5	Insecticide	Organophosphorus	108	127
14.98	Dimethoate	60-51-5	Insecticide	Organophosphorus	124	151
15.69	Chlorothalonil	1897-45-6	Fungicide	Organochlorine	125	13
15.86	Vinclozolin	50471-44-8	Fungicide	Organochlorine	102	98
16.21	Metalaxyl	57837-19-1	Fungicide	Organonitrogen	105	117
16.28	Carbaryl	63-25-2	Insecticide	Carbamate	114	111
16.60	Malathion	121-75-5	Insecticide	Organophosphorus	124	160
16.67	Dichlofluanid	1085-98-9	Fungicide	Organohalogen	122	103
17.51	Thiabendazole	148-79-8	Fungicide	Organonitrogen	88	14
17.70	Captan	133-06-2	Fungicide	Organochlorine	88	91
17.76	Folpet	133-07-3	Fungicide	Organochlorine	108	63
18.23	Imazalil	35554-44-0	Fungicide	Organonitrogen	115	95
18.39	Endrin	72-20-8	Insecticide	Organochlorine	104	101
18.62	Myclobutanil	88671-89-0	Fungicide	Organonitrogen	119	114
19.07	4,4-DDT	50-29-3	Insecticide	Organochlorine	102	95
19.22	Fenhexamid	126833-17-8	Fungicide	Organochlorine	118	77
19.40	Propargite 1	2312-35-8	Acaricide	Organosulfur	110	95
19.43	Propargite 2	2312-35-8	Acaricide	Organosulfur	121	114
19.75	Bifenthrin	82657-04-3	Insecticide	Pyrethroid	106	81
20.04	Dicofol	115-32-2	Acaricide	Organochlorine	98	54
20.05	Iprodione	36734-19-7	Fungicide	Organonitrogen	118	90
20.21	Fenpropathrin	39515-41-8	Insecticide	Pyrethroid	113	96
21.32	*cis*-Permethrin	52645-53-1	Insecticide	Pyrethroid	106	65
21.47	*trans*-Permethrin	51877-74-8	Insecticide	Pyrethroid	109	71
23.74	Deltamethrin	52918-63-5	Insecticide	Pyrethroid	97	52

*50mg PSA, 50mg C18, **50mg PSA, 50mg GCB

$$\% \text{ recovery} = \frac{\text{RRF C18 or GCB}}{\text{RRF PSA}} \times 100$$

QuEChERS SPE Tubes

AOAC Method 2007.1	Benefits/Uses	qty.	cat#
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate and 50mg PSA	Cleanup of agricultural produce extracts, 1mL sample volume.	100-pk.	26124
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate, 50mg PSA, and 50mg Graphitized Carbon	Cleanup of 1mL sample extract with residual pigments and sterols.	100-pk.	26123
2mL QuEChERS SPE Micro-Centrifuge Tube Contains 150mg Magnesium Sulfate, 50mg PSA, and 50mg C18	Cleanup of 1mL sample extract with residual fat.	100-pk.	26125
15mL QuEChERS SPE Centrifuge Tube Contains 900mg Magnesium Sulfate, 300mg PSA, and 150mg Graphitized Carbon	Cleanup of 6mL sample extract with residual pigments and sterols.	50-pk.	26126

PSA—primary and secondary amine exchange material.

FREE Sample Packs Available!

To receive your free sample pack, add -248 to the item number. (One sample per customer)

Multi-task with an Ultra IBD Column

A Versatile Column with Many Applications

By Rick Lake, Pharmaceutical Innovations Chemist

- Effective in normal or reversed mode; compatible with 100% aqueous mobile phases.
- Excellent base deactivation—superior peak shape for basic compounds.
- Enhanced retention of hydrophilic compounds.

Reversed phase HPLC analyses are predominantly performed on C18 columns, which, in many cases, are suitable. There are, however, situations in which a conventional C18 column produces less than optimal chromatography. For example, C18 columns have little retention for hydrophilic compounds, basic compounds often exhibit peak tailing, and highly aqueous conditions can cause inconsistent retention or even phase collapse.

One way in which column manufacturers attempt to address these issues, and yet maintain the favorable hydrophobic interaction of a C18 column, is to impart polar functionality into an alkyl phase. The Ultra IBD column is an example of such a polar embedded column. Compared to a C18 column, this column offers enhanced retention and selectivity towards a wider range of compounds, orthogonal separations, improved base-deactivation, and compatibility with entirely aqueous mobile phases.

Degree of Polarity

The Ultra IBD column exhibits a high degree of polarity relative to conventional and aqueous C18 phases. Because the Ultra IBD column possesses both nonpolar and highly polar characteristics, it can be used in both normal phase mode and reversed phase mode. The bonding chemistry used in the Ultra IBD column makes it a very adaptable column capable of unique separations.

Base-Deactivation

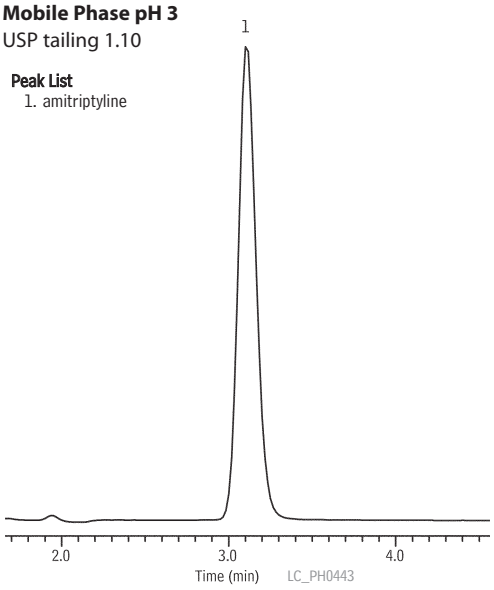
The Ultra IBD column bonding chemistry alleviates one of the common problems associated with alkyl phases—peak tailing of basic analytes. Comparing the analysis of amitriptyline on a conventional C18 column and an Ultra IBD column demonstrates the effectiveness of this bonding chemistry. Amitriptyline is a highly basic, tricyclic antidepressant that commonly tails on silica-based alkyl phases. Even at a neutral pH and, importantly, with no modifiers, the Ultra IBD column exhibits excellent peak shape for amitriptyline (Figure 1). This is advantageous because it provides needed flexibility for method development, especially for analytes that are labile under acidic conditions. In applications where Gaussian peak shape is needed for accurate integrations, such as potency assays, or when tighter system suitability criteria are required, an intrinsically base-deactivated stationary phase offers a benefit that a conventional C18 column cannot—exceptional peak shape with a simplified mobile phase.

Retention and Selectivity

In contrast to conventional C18 columns, the Ultra IBD has a polar functional group embedded within the alkyl chain. Retention, therefore, is attributed not only to hydrophobic interactions (the major retention mechanism of an alkyl (or C18) phase, but also to polar attraction between the analyte and stationary phase. This mixed-mode mechanism results in high retention for hydrophilic compounds or compounds with polar moieties, such as purines (Figure 2).

Orthogonal separations also can be achieved through the Ultra IBD phase chemistry. For example, a small group of hydroxybenzoic acids was also assayed on a C18 and IBD column under identical conditions. The elution order of the analytes differed and dihydroxybenzoic acid was more retained on the Ultra IBD column (Figure 3). Additionally, the unique phase chemistry of the Ultra IBD column makes it suitable for a simultaneous analysis of a wide range of compounds—acidic through basic, as well as zwitterions (Figure 4).

Figure 1 Ultra IBD offers more flexibility in method development giving excellent peak shape for highly basic compounds—even without mobile phase modifiers—over a wide mobile phase pH range.



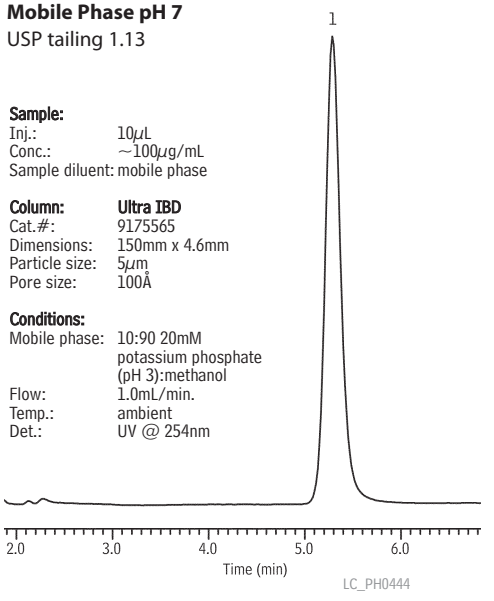


Figure 2 The Ultra IBD column exhibits high retention for hydrophilic compounds or compounds with polar moieties, and is compatible with up to 100% aqueous mobile phases ruggedness in aqueous mobile phases.

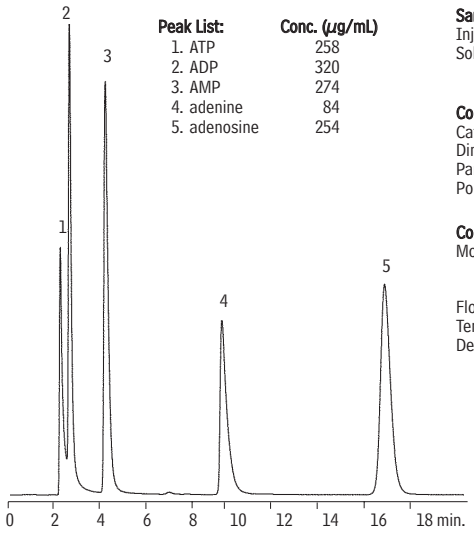


Figure 3 The Ultra IBD column gives needed flexibility for polar compounds; it increases retention, enhances resolution, and creates alternate selectivity.

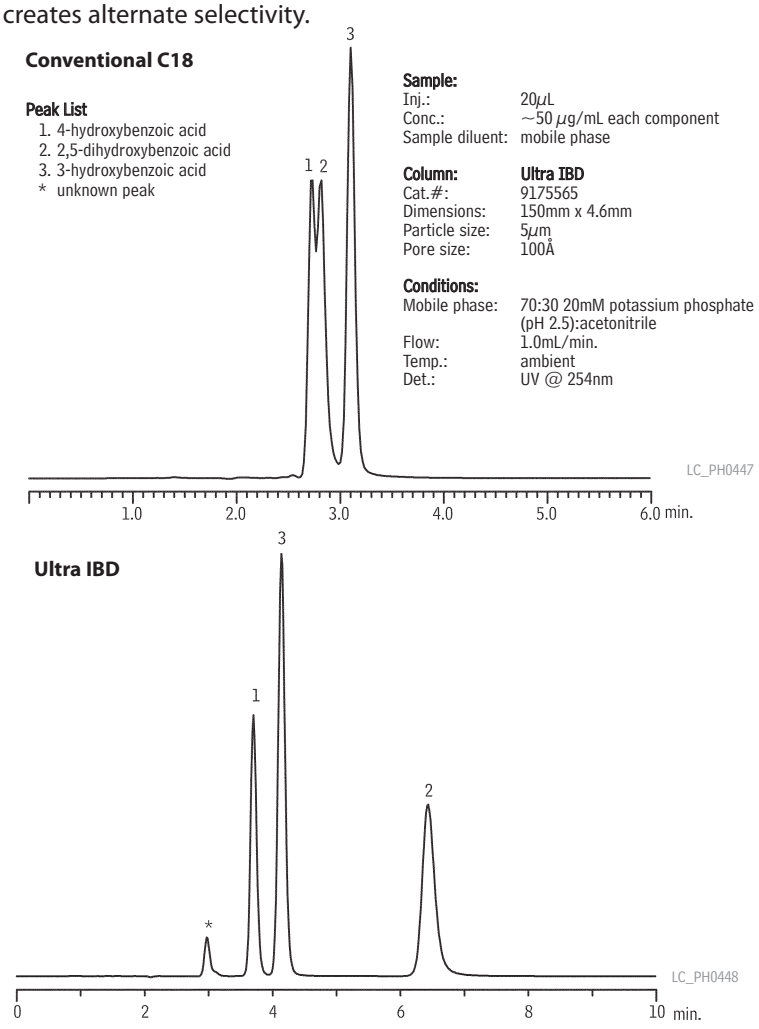
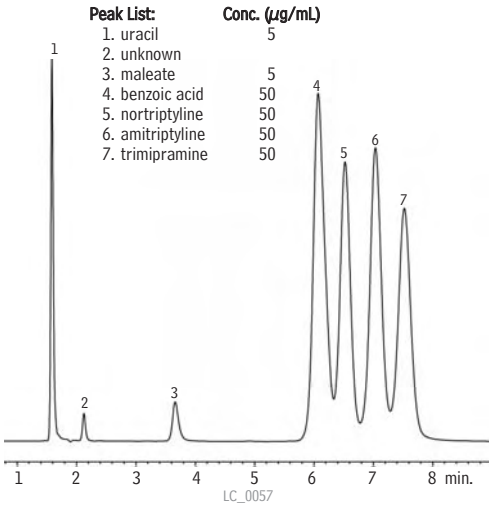


Figure 4 The versatility of the Ultra IBD makes it well-suited for analyzing a wide range of compounds.



Conclusion

The Ultra IBD, through unique bonding chemistry, is an extremely versatile HPLC column. It offers alternate selectivity, and a high degree of both polar and nonpolar retention, making it a powerful tool for analyzing a wide range of compounds. The Ultra IBD also addresses the inherent problems attributed to linear alkyl phases, providing excellent peak shape for basic compounds and heightened retention of hydrophilic compounds. The versatility of the Ultra IBD makes it an excellent tool for the practicing method developer.

Ultra IBD Columns
Specialized Columns for Mixed Polar and Nonpolar Compounds

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 100Å
carbon load: 12%
endcap: no
pH range: 2.5 to 7.5
temperature limit: 80°C

Ultra IBD, 5µm Columns

5µm Column, 4.6mm		cat. #
150mm		9175565
150mm (with Trident Inlet Fitting)		9175565-700
Ultra IBD Guard Cartridges	**qty.**	**cat. #**
10 x 2.1mm	3-pk.	917550212
10 x 4.0mm	3-pk.	917550210
20 x 2.1mm	2-pk.	917550222
20 x 4.0mm	2-pk.	917550220

Fast, Sensitive Analysis of Benzodiazepines by LC/MS/MS

Quantify an Order of Magnitude below Typical Methods

By Kristi Sellers, Clinical/Forensic Innovations Chemist

- Achieve full chromatographic separation of compounds with shared precursor ions
- Quantify compounds at 10ng/mL or less in urine.
- Increase accuracy with improved desolution efficiency from highly organic mobile phase.

Benzodiazepines are widely prescribed drugs used for treating anxiety and sleep disorders. Since addiction and abuse can occur, efficient screening methods are critical to clinical, forensic, and toxicology laboratories. The liquid chromatography tandem mass spectrometry (LC/MS/MS) method presented here offers several advantages over other techniques: minimal sample preparation, fast analysis times, multiple reaction monitoring transitions for quantification and confirmation, and sensitivity down to 0.10-10ng/mL. This method uses the Allure® PFP Propyl stationary phase, which retains compounds long enough to minimize matrix interferences and chromatographically separate compounds that share the same precursor ion.

Procedure

Samples were prepared by adding 100µL of internal standard solution (1µg/mL D5-Diazepam and D3-Dioxepine) to 100µL urine, diluting with 800µL LC grade water, and centrifuging. The samples were then analyzed by LC/MS/MS. Compound separation was achieved using an Allure® PFP Propyl column and a mobile phase gradient program.

A 3200 QTrap® LC/MS/MS system equipped with a Turbo V™ source with electrospray ionization was used to develop and detect the two MRM transitions (Table 1). For each compound, MRM 1 was used to quantify, and the ratio to MRM 2 was used to confirm.

Cliquid™ Drug Screen & Quant Software was used to process data and generate automatic reporting relevant to forensic guidelines. Limits of quantification were determined and the automated reporting allowed for positive confirmation based on the detected MRM ratios.

Results

By diluting the urine samples ten-fold, matrix effects are reduced (reducing ion suppression) and LOQs between 0.10ng/mL and 10ng/mL can be achieved (Table 1). Ion suppression is further reduced by using a retentive column which 1) elutes matrix interferences before the compounds of interest, and 2) allows for better desolvation efficiency due to the ability to use 90% organic in the mobile phase composition. The Allure® PFP Propyl is such a column; it has high retention and selectivity for basic drug compounds, such as benzodiazepines (Figure 1).

Figure 1 MRM transitions of 27 benzodiazepines, 3 nonbenzodiazepine hypnotics, and two internal standards on the Allure® PFP Propyl column.

Sample: **benzodiazepines**
Inj.: 20µL
Conc.: NA
Solvent: NA

Column: **Allure® PFP Propyl**
Cat.#: 9169552
Dimensions: 50mm x 2.1mm
Particle size: 5µm
Pore size: 60Å

Conditions:
Mobile phase: A: 0.1% formic acid and 1mM ammonium formate in water
B: 0.1% formic acid and 1mM ammonium formate in acetonitrile

Time (min.)	Flow (µL/min.)	%B
0.0	500	10
10.00	1000	90
15.00	1000	90
15.50	500	10
17.50	500	10

Flow: see gradient table
Temp.: 40°C
Det.: Applied Biosystems/MDS Sciex
API 3200™ MS/MS system
Ion Source: Electrospray, positive
IonSpray Voltage: NA
Gas 1: NA
Gas 2: NA
Source Temperature: 500°C

Data courtesy of: Applied Biosystems MDS Sciex

Analyze 27 benzodiazepines in less than 10 minutes!

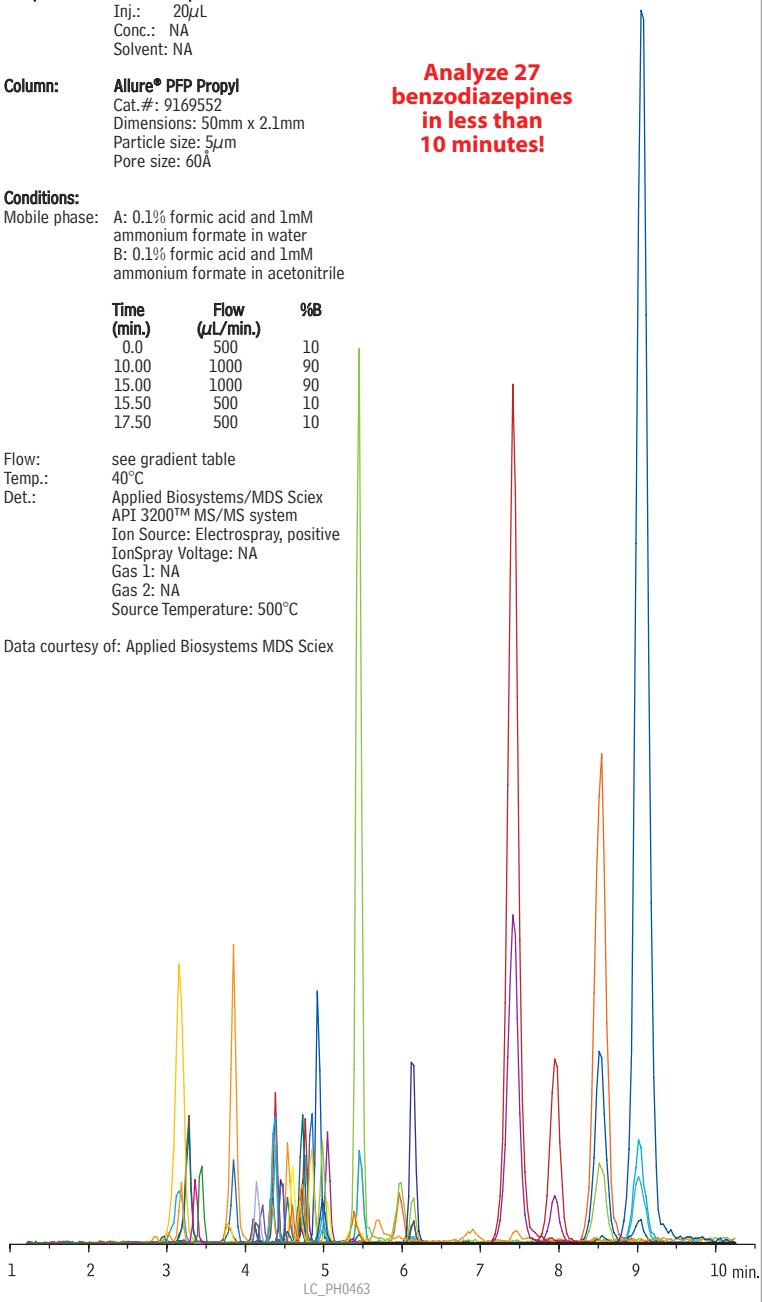


Table I MRM Transitions, retention times, and LOQ values.

Compound Name	Retention Time (min.)	Precursor Ion (amu)	MRM 1 (amu)	MRM 2 (amu)	DP	CE (MRM 1)	CE (MRM 2)	LOQ (ng/mL)
7-aminonitrazepam	3.2	252.1	121.1	94.0	51	35	53	1.0
7-aminoclonazepam	3.3	286.1	121.0	222.2	46	41	35	0.5
7-aminoflunitrazepam	3.8	284.1	135.1	226.0	51	39	49	0.5
Bromazepam	3.8	316.0/318.0	182.1	182.1	51	45	45	5.0
α-hydroxyalprazolam	4.1	325.1	297.2	204.9	51	31	59	2.0
α-hydroxytriazolam	4.1	359.0	239.2	176.0	61	63	37	5.0
Oxazepam	4.2	287.0	241.1	268.9	41	27	19	10.0
Lorazepam	4.3	321.0/323.1	275.0	277.0	41	31	27	5.0
Estazolam	4.4	295.0	205.0	267.1	51	53	31	2.0
Zaleplon	4.4	306.2	236.3	264.2	56	35	27	0.5
2-hydroxyethylflurazepam	4.5	333.1	211.2	109.0	56	51	41	1.0
Desmethylflunitrazepam	4.5	300.1	254.2	198.2	56	35	51	2.0
Nitrazepam	4.6	282.0	236.1	180.2	71	35	51	2.0
Clonazepam	4.7	316.0	270.2	214.0	56	41	51	2.0
Desalkylflurazepam	4.7	289.1	140.1	226.1	71	41	39	2.0
Temazepam	4.7	301.1/303.1	255.1	257.2	35	30	30	5.0
Triazolam	4.7	343.0	238.9	314.9	61	53	37	1.0
Alprazolam	4.8	309.1	205.1	281.1	56	53	35	1.0
Lormetazepam	4.8	335.0/337.1	289.0	291.1	41	29	29	2.0
Clobazam	4.9	301.1	259.1	224.3	46	29	47	1.0
Flunitrazepam	5.0	314.0	268.1	239.1	56	35	49	1.0
Nordiazepam	5.0	271.1	140.2	164.9	46	37	35	2.0
Zolpiclone	5.4	389.1	244.8	217.0	16	25	41	1.0
D5-Diazepam	5.4	290.1	198.2	-	55	41	-	
Diazepam	5.5	285.0	193.2	154.1	55	41	37	1.0
Chlordiazepoxide	6.0	300.1	227.1	283.2	36	31	21	5.0
Prazepam	6.1	325.1	271.1	140.0	81	31	53	2.0
Zolpidem	7.4	308.1	235.1	236.1	56	39	35	0.2
Midazolam	7.9	326.1	291.3	222.0	56	33	63	0.5
Flurazepam	8.5	388.2	315.1	317.1	36	27	27	0.1
Medazepam	9.0	271.0	91.1	207.3	46	41	39	2.0
D3-Doxepine	9.1	283.0	107.1	-	41	35	-	

Bar color indicates shared precursor ions. Note compounds with shared precursor ions are baseline resolved on the Allure® PFP Propyl column, as shown by retention time comparison. Data courtesy of Applied Biosystems MDS Sciex.

The Allure® PFP Propyl stationary phase provides baseline resolution for compounds sharing the same precursor ion, such as nordiazepam and medazepam. The ability to chromatographically separate compounds with similar spectra allows this method to be adapted for single stage MS, however, the LOQ values would be affected. Tandem MS is advantageous since two MRM transitions are collected, allowing quantification and confirmation to be accomplished in a single run, without loss of sensitivity.

Conclusion

The method presented here provides significant advantages over other techniques for benzodiazepine analysis: simple sample preparation, fast analysis time (less than 10 minutes), LOQs of 0.10-10ng/mL in matrix, and quantification and confirmation in a single run. Further, using the retentive Allure® PFP Propyl column eliminates coelution of matrix peaks with target compounds and assures full chromatographic resolution of analytes with shared precursor ions.

Acknowlegement

We sincerely thank Andre Schreiber of Applied Biosystems and Houssain El Aribi and John Gibbons of MDS Sciex for supplying the method and data.

References

Schreiber, Andre PhD, El Arbi, Houssain and Gibbons, John. 2007. A Fast and Sensitive LC/MS/MS Method for the Quantitation and Confirmation of 30 Benzodiazepines and Nonbenzodiazepine Hypnotics in Forensic Urine Samples. Applied Biosystems MDS Sciex.

Trident Direct Guard Cartridge System

Description	qty.	cat.#
High-pressure filter	ea.	25082
10mm guard cartridge holder without filter	ea.	25083
10mm guard cartridge holder with filter	ea.	25084
20mm guard cartridge holder without filter	ea.	25085
20mm guard cartridge holder with filter	ea.	25086

*The standard PEEK™ tip in Trident Direct systems is compatible with Parker®, Upchurch Scientific®, Valco™, and other CPI-style fittings. To use Trident Direct systems with Waters-style end fittings, replace the tip with cat.# 25088.

Allure® PFP Propyl Columns (USP L43) Excellent Columns for LC/MS and ELSD

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 60Å
carbon load: 17%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

5µm Column, 2.1mm	cat. #
50mm	9169552
50mm (with Trident Inlet Fitting)	9169552-700

Allure® PFP Propyl Guard Cartridges	qty.	cat. #
10 x 2.1mm	3-pk.	916950212
10 x 4.0mm	3-pk.	916950210
20 x 2.1mm	2-pk.	916950222
20 x 4.0mm	2-pk.	916950220

Exempted Drug of Abuse Reference Materials: Benzodiazepines

Concentration is µg/mL. Volume is 1mL/ampul.

Compound	CAS#	Solvent Code	Conc.	cat.#
alprazolam	28981-97-7	PTM	1,000	34042
bromazepam	1812-30-2	PTM	1,000	34043
chlordiazepoxide	438-41-5	PTM	1,000	34044
clobazam	22316-47-8	PTM	1,000	34045
clonazepam	1622-61-3	PTM	1,000	34046
diazepam	439-14-5	PTM	1,000	34047
flunitrazepam	1622-62-4	PTM	1,000	34049
flurazepam	1172-18-5	PTM	1,000	34050
lorazepam	846-49-1	PTM	1,000	34051
nitrazepam	146-22-5	PTM	1,000	34053
oxazepam	604-75-1	PTM	1,000	34054
prazepam	2955-38-6	PTM	1,000	34055
temazepam	896-50-4	PTM	1,000	34056
triazolam	28911-01-5	PTM	1,000	34057

PTM=purge & trap grade methanol

Accurate, Reproducible Amphetamines Analysis

Clean Up Procedure Improves Chromatography and Reduces Maintenance

By Kristi Sellers, Clinical/Forensic Innovations Chemist, and Amanda Rigdon, Innovations Chemist

- Derivatization improves peak symmetry, for more accurate results.
- Clean up procedure reduces system contamination, and extends column lifetime.
- Rtx®-5MS column produces a stable baseline for derivatized compounds, ideal for GC/MS analysis.

Introduction

Analyzing amphetamines by GC/MS is challenging whether the compounds are derivatized or underivatized. Underivatized amphetamines appear as irregular and asymmetric peaks, which are difficult to integrate, and may lead to irreproducible results. Derivatized amphetamines result in symmetric peaks, but derivatizing reagents can contaminate the inlet/column. This contamination can shorten column lifetime and cause noisy, elevated baselines that interfere with the analysis of target compounds.

In this study, we evaluated the effects of several sample pretreatment methods. These methods included: 1) no pretreatment, 2) converting the salt forms into free bases, 3) derivatizing the free bases with heptafluorobutyric acid anhydride (HFAA), and 4) derivatizing the free bases with HFAA followed by a clean up. Our objectives were to obtain symmetric shapes, reduce baseline noise, and maintain low column bleed from injection to injection for GC/MS analysis.

Procedure

The first method had no pretreatment. The untreated standard was prepared in methanol and diluted to a final concentration of 100µg/mL. It was then injected without any further preparation. The second pretreatment involved converting the drug standard to the free base form. The free base forms were prepared by mixing the standard (100µg/mL) with water, then adding saturated sodium borate water, and extracting the amphetamines with butylchloride. The resulting sample was then analyzed by GC.

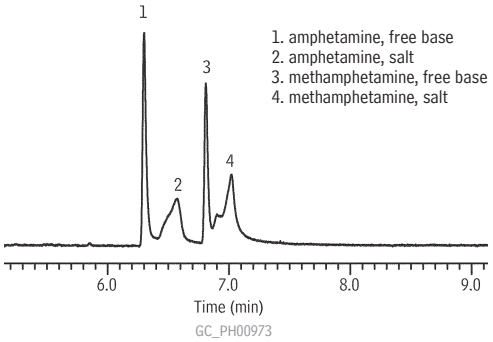
The third pretreatment procedure included both conversion and derivatization. The HFAA derivatized amphetamines were prepared by converting the compounds to free bases (as described above), reacting with derivatizing reagent HFAA, and diluting the sample before injection. The fourth pretreatment procedure consisted of free base conversion, HFAA derivatization, and a clean up step to remove the acidic byproducts of derivatization. The clean up procedure included mixing the sample with a phosphate buffer (pH=7.0) before dilution, removing the butylchloride layer, and then diluting the sample just before injection. An Rtx®-5MS column (30m x 0.25mm ID x 0.25um) was used for analysis; instrument conditions are presented in Figure 1. Repetitive GC/MS runs (over 190 injections) were evaluated to confirm symmetry, baseline, and bleed results.

Results

Analyzing untreated amphetamine and methamphetamine results in peak doublets caused by the presence of both the salt (hydrochloride) and free base forms (Figure 1). Peak doublets were eliminated by conversion to free base form, however, some tailing was still observed (Figure 2). This pretreatment improves reproducibility, but is still not optimal as tailing can cause irreproducible integration and significant variation in peak area counts.

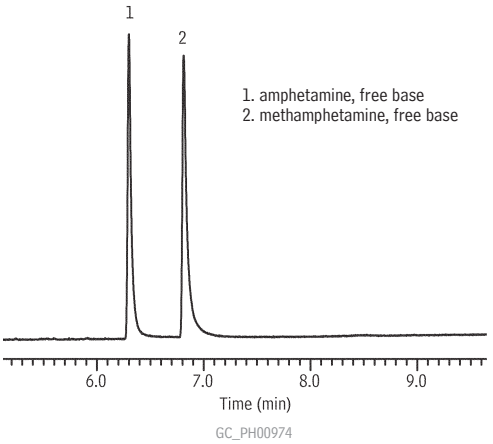
The most symmetric peak shapes were obtained by derivatizing the amphetamines with HFAA (Figure 3). Although peak shape was improved, the acidic derivatization byproducts generated a noisy baseline and shortened column life. This system contamination increases injector and column maintenance.

Figure 1 Untreated standard contains both salt and free base forms causing inaccurate, irreproducible results.



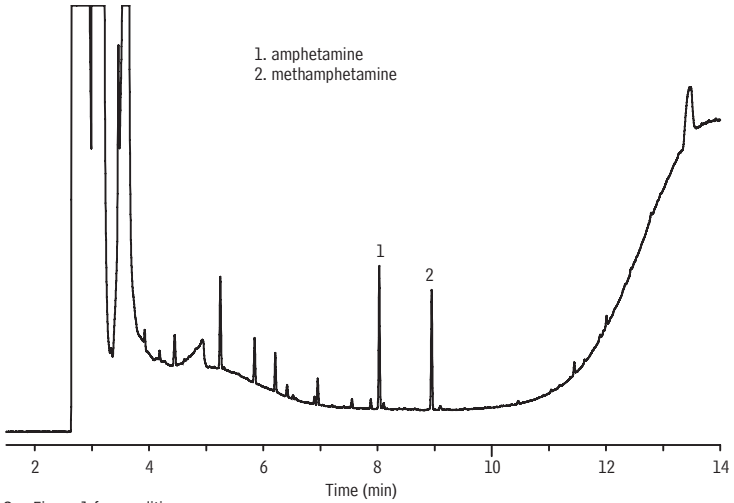
Column:	Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample:	100µg/mL amphetamine and methamphetamine in methanol
Inj.:	1µL, split (split ratio 10:1), 4mm single gooseneck w/ wool inlet liner (cat.# 20798-211.1)
Inj. temp.:	250°C
Carrier gas:	hydrogen, constant flow
Flow rate:	1mL/min.
Oven temp.:	70°C to 250°C @ 15°C/min. (hold 5 min.)
Det:	FID @ 300°C

Figure 2 Conversion to free base form improves chromatography, but produces tailing factors over 2.0.



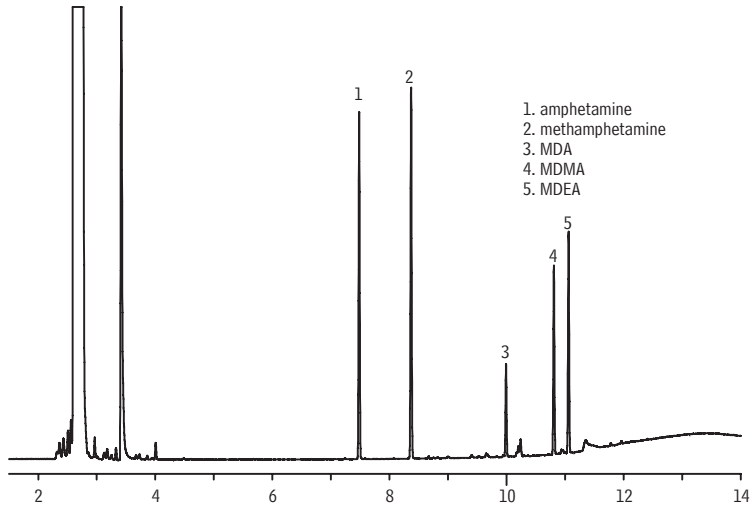
See Figure 1 for conditions.

Figure 3 Derivatizing with HFAA yields symmetric peaks but results in system contamination and a noisy baseline.



See Figure 1 for conditions.

Figure 4 A post-derivatization clean up procedure results in symmetric peaks and a clean baseline.



See Figure 1 for conditions.

Table I Tailing factor comparison of pretreatments.

Pretreatment	TF Amp	TF Meth	TF MDA	TF MDMA	TF MDEA
Sodium Borate Wash (GC/FID)	2.115	2.837	NA	NA	NA
HFAA Only (GC/FID)	1.010	0.989	NA	NA	NA
HFAA w/Post clean Up (GC/FID)	0.981	0.996	1.007	0.997	0.992

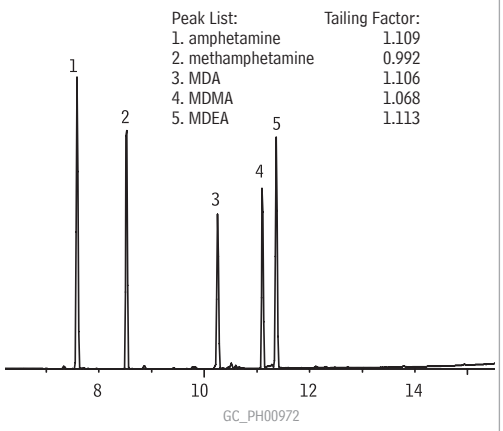
NOTE: A perfectly symmetric peak exhibits a tailing factor of 1.0. Tailing factors shown were generated using the USP tailing factor calculation.

Rtx®-5MS—Low-bleed GC/MS Column (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623

Figure 5 Post-derivatization clean up also produces symmetric peaks and a stable baseline when analyzed by GC/MS.



Column: Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample: 100µg/mL each amphetamine, methamphetamine, MDA, MDMA, and MDEA extracted from methanol and HFAA derivatized
Inj.: 1µL, splitless (hold 0.5 min.), 3.5mm custom splitless inlet liner w/IP deacitvated wool; Inj. temp.: 220°C; Carrier gas: helium, constant flow; Flow rate: 1.25mL/min.; Oven temp.: 70°C (hold 1 min.) to 290°C @ 15°C/min. (hold 4 min.); Det: MS; Transfer line temp.: 280°C; Scan range: 43-450amu; Ionization: EI; Mode: scan.

Incorporating a post conversion/derivatization clean-up procedure removed derivatization contaminants while maintaining chromatographic quality (Table I), thus reducing the need for frequent system maintenance and extending column lifetime. These benefits were also seen when samples were analyzed by GC/MS (Figure 5).

Conclusion

The conversion/derivatization/clean-up procedure presented here produces symmetric peaks while reducing the amount of contamination that can enter the GC system. This method ensures accurate area count reproducibility, a clean GC system, and a stable baseline, even for GC/MS work.

Acylation Derivatization Reagents

Compound	CAS#	cat.#
HFAA (heptafluorobutyric acid anydride)		
10-pk. (10x1g)	336-59-4	35622
25g Flex Tube	336-59-4	35623

Exempted Drug of Abuse Reference Materials: Amphetamines & Metabolites

Concentration is µg/mL. Volume is 1mL/ampul.

Compound	CAS#	Solvent Code	Conc.	cat.#
d-amphetamine	51-63-8	PTM	1,000	34020
3,4-MDA HCl	4764-17-4	M	1,000	34070
3,4-MDEA HCl	82801-81-8	M	1,000	34072
3,4-MDMA HCl	42542-10-9	M	1,000	34071

M=methanol
PTM=purge & trap grade methanol

The Forgotten Septum

How to Correctly Diagnose the Source of Bleed Contamination

By Amanda Rigdon, Innovations Chemist

- Avoid lengthy inlet troubleshooting.
- Reduce interference with correct solvent-septum compatibility.

Septum bleed is not common, but when it occurs it is observed as sharp, repetitive peaks in high temperature portions of an analysis. Bleed peaks can come from either the injection port septum or the vial cap septum. Interfering peaks and inaccurate data can result, so it is important to correctly identify the source and understand how to eliminate or minimize the bleed.

Diagnose the Bleed Source

The bleed from either septum shows a similar pattern (Figure 1), but it is easy to determine the source with a simple test. Isolate the injection port by setting the instrument to perform a run without an injection. Perform an analysis; if the bleed disappears, then the vial cap septum was the source. Determining if the vial cap septum is the source of the bleed can save time by preventing unnecessary troubleshooting and maintenance of the injection port. If the vial cap septum is causing bleed, the problem can be eliminated or minimized with the following considerations.

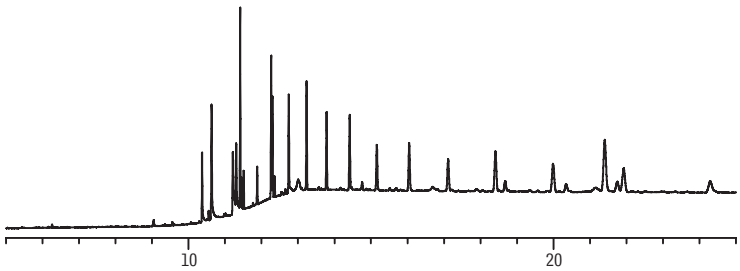
Check Solvent-Septum Compatibility

Most of the time, septum bleed is negligible. However, when a solvent and vial cap septum are incompatible, extreme contamination can occur. Figure 2 compares the first and fifth injections from a vial containing a derivatized amphetamine sample. In this case, the septum bleed peaks are almost as large as the analyte peaks. This level of bleed can interfere with analyses, especially those geared for trace levels. Reduce the risk of septum bleed by using a compatibility chart, such as the one in the on-line version of this article *(www.restek.com/general)* to determine which septum material is compatible with the sample solvent used.

Use Lined Septa

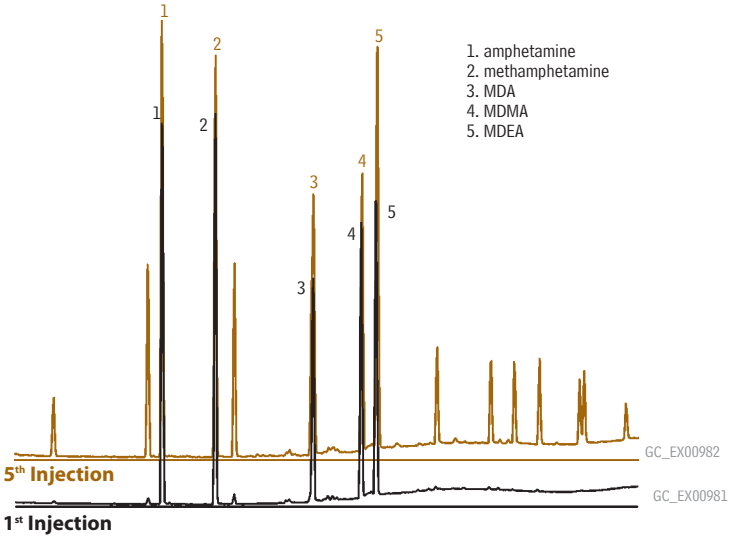
Most vial cap septa are lined with a protective layer of polytetrafluoroethylene (PTFE) to prevent solvent attack. As shown in Figure 3, PTFE effectively prevents septum breakdown due to solvent exposure. In comparison, unlined septa exhibit bleed after just 24 hours at room temperature. Bleed levels for unlined septa varied by material, but even a low level of bleed can interfere with integration and is of particular concern for trace analyses (Figure 4).

Figure 1 Sharp, repetitive peaks are typical of septum bleed from the vial cap or injection port.



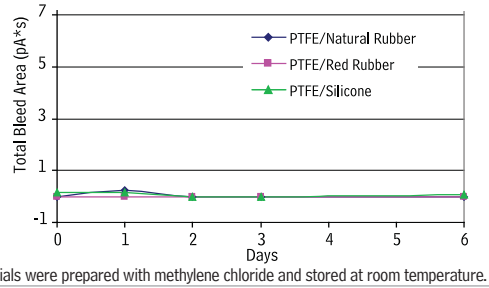
Columns:	Rtx®-5MS, 30m, 0.25mm, 0.25im (cat.# 12623)
Sample:	methylene chloride blank
Inj.:	1.0µL split (split ratio 10:1), 4mm split inlet liner w/ wool (cat.# 20782)
Inj. temp.:	240°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	70°C (hold 1 min.) to 290°C @ 20°C/min. (hold 13 min.)
Det.:	FID @ 250°C

Figure 2 Contamination from septum bleed can cause significant interference with target analytes.



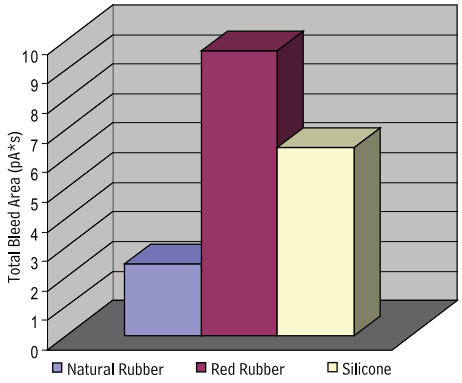
Column:	Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample:	100µg/mL each amphetamine, methamphetamine, MDA, MDMA, and MDEA extracted from methanol and HFAA derivatized
Inj.:	1µL, splitless (hold 0.5 min.), 3.5mm custom splitless inlet liner w/ IP deacitvated wool
Inj. temp.:	220°C
Carrier gas:	helium, constant flow
Flow rate:	1.25mL/min.
Oven temp.:	70°C (hold 1 min.) to 290°C @ 15°C/min. (hold 4 min.)
Det:	MS
Transfer Line temp.:	280°C
Scan range:	43-450amu
Ionization:	EI
Mode:	scan

Figure 3 PTFE lining prevents bleed due to solvent/septum interaction.



Vials were prepared with methylene chloride and stored at room temperature.

Figure 4 Unlined septa show bleed contamination within 24 hours.



Vials were prepared with methylene chloride, sealed with caps containing septa that were inserted upside down in order to expose the non-PTFE lined septum surface to the solvent, and stored at room temperature.

Figure 5 Bleed contamination increases over multiple injections.

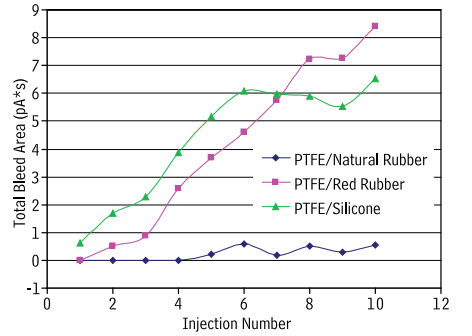
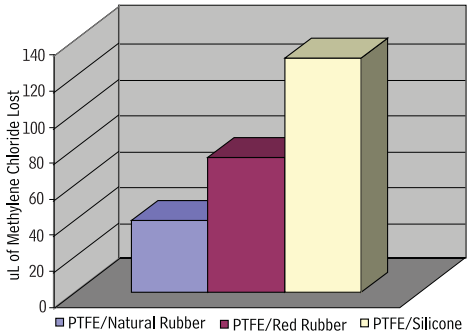


Figure 6 This bar graph shows the volume of solvent lost for three septum types.



Vials containing 300µL of methylene chloride were punctured and left at room temperature for 24 hours.

Consider Resealability

Multiple injections can core the vial cap septum and lead to significant bleed. Resistance to coring varies by septum material (Figure 5). Coring can be minimized by preparing separate vials for replicate injections, when feasible, and by carefully considering the type of septum material when multiple injections are necessary. Septum resealability also affects evaporative loss, which can be a significant source of error for low volume samples. For example, a relatively nonvolatile analyte in a volatile solvent can concentrate significantly due to evaporative loss (Figure 6). Vials should be recapped when necessary for extended runs or long term storage.

Conclusion

Septum bleed is not a very common occurrence, but when bleed does occur, it is easy to assume the injection port septum is the source because the vial cap septum often is not considered. However, correctly diagnosing the source of bleed contamination can save time and effort by preventing unnecessary injection port maintenance. Effectively and efficiently reducing interfering peaks by controlling septum bleed can significantly improve analytical performance, particularly for trace analyses.

Crimp-Top Vials, Snap Seal™ Style—12 x 32mm, 11mm Crimp

Description	100-pk.	1000-pk.
2.0mL Clear Glass Vial w/White Graduated Marking Spot	24383	24384
2.0mL Amber Glass Vial w/White Graduated Marking Spot	24385	24386
2.0mL Clear Glass Vial without Graduated Marking Spot	21152	21153

2.0mL, 11mm Aluminum Crimp Seals with Septa

Description	100-pk.	1000-pk.
Silver Seal, PTFE/Natural Rubber Septa	21174	21175
Red Seal, PTFE/Red Rubber Septa	24355	24356
Silver Seal, PTFE/Silicone Septa	24359	24360

Limited Volume Inserts for 2.0mL Crimp-Top & Short-Cap, Screw-Thread Vials

Description	100-pk.	1000-pk.
350µL Glass, Flat Bottom Insert w/ ID Ring	24692	24693

Rtx®-5MS—Low-bleed GC/MS Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623

Split Liners for Agilent GCs

ID* x OD & Length (mm)	qty.	cat.#
4mm Split w/ Wool		
4.0 ID x 6.3 OD x 78.5	ea.	20781
4.0 ID x 6.3 OD x 78.5	5-pk.	20782
4.0 ID x 6.3 OD x 78.5	25-pk.	20783

*Nominal ID at syringe needle expulsion point.

Selecting the Right HPLC Guard Column

By Terry Reid, Technical Service

HPLC guard columns (cartridges) are installed in front of an analytical column in order to protect it from strongly retained impurities. Understanding the significant factors that affect guard column performance can help you protect your analytical column and save money by extending column lifetime.

Packing & Dimensions

It is best to use a guard column that contains the same packing material as the analytical column. In other words, the best guard column for a Pinnacle™ II C18 analytical column is a Pinnacle™ II C18 guard column. Trident guard cartridges come in two lengths, 10mm or 20mm. The 10mm length is adequate for most applications, but a 20mm guard should be considered for samples that contain a lot of impurities, such as crude extracts. Regarding internal diameter (ID), the general rule is that the guard column ID should be the same as, or one size smaller than, the ID of the analytical column to prevent a loss of efficiency.

Cartridge Holder Options

Trident guard cartridges can be used with three different styles of Trident guard holder: integral, in-line, and direct. Note that guard cartridges from one manufacturer should never be used in another manufacturer's holder.

The Trident Integral guard system is a cost-effective, low dead volume option that is recommended when purchasing a guard and analytical column at the same time. This system includes an analytical column, a guard cartridge, and extra frit, all of which are integrated into a single unit. The advantages of the Trident Integral Guard column system are that it has the lowest dead volume of any of the holders and is also the most cost effective option.

Restek also offers in-line and direct holders. Trident In-Line holders are traditional stand-alone style holders that require an additional piece of HPLC capillary tubing to connect the guard holder to the analytical column. The Trident Direct Holder differs in that it contains a threaded PEEK™ tip. The threaded tip allows the holder to screw directly into the analytical column's inlet end fitting, eliminating the need for any additional tubing. The Trident In-Line and Trident Direct holders both can be connected to any HPLC column, even those from other manufacturers. Both these holders are available in either a "with filter" or "without filter" version. The "with filter" versions have an XF (extra filter) fitting that contains a cap frit that can be changed independently of the guard cartridge.

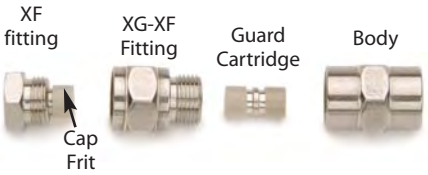
Filters

The ID of the cap frit should match the ID of the guard cartridge; however, frit porosity is largely a matter of preference. The smaller porosity will provide the greatest protection against particles, but also may mean that the frit needs to be changed more frequently. Choosing a filter porosity that matches the porosity of the analytical column protects against particles lodging in the column inlet frit.

Restek offers a wide selection of HPLC guard options. An understanding of the significance of different guard column parameters, including dimensions, holder styles, and extra filters, can simplify the selection process. Choosing the proper guard column will maximize the lifetime of your analytical column by effectively protecting it from sample contaminants.

Trident In-Line 10mm guard cartridge holder with filter

Components



Assembled

Installed onto column

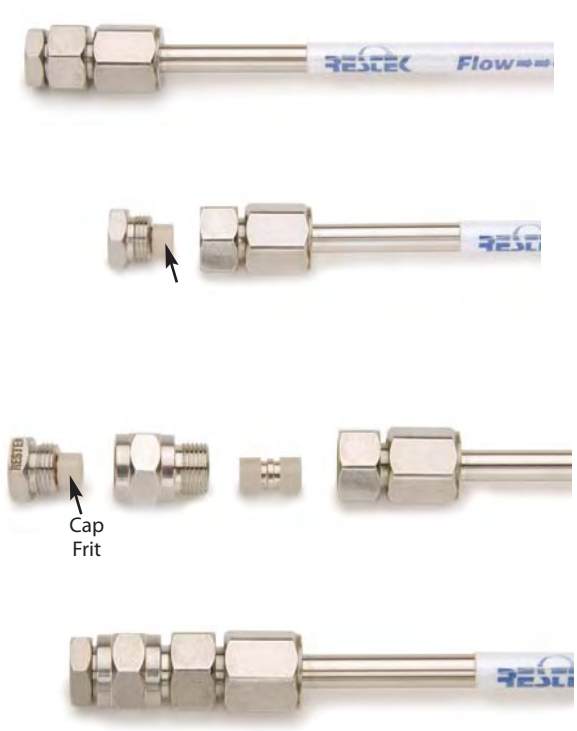
Trident Direct 10mm guard cartridge holder with filter

Components

Assembled

Installed onto column

Trident Integral Guard System



Looking for HPLC guard column options?

Visit us at **www.restek.com**, or call Technical Support at 800-356-1688, to discuss your applications.

NEW! Waste Overflow Indicator for HPLC Systems

By Becky Wittrig, Ph.D, HPLC Product Marketing Manager

- Avoid messy pooling around mobile phase waste containers.
- Audible alarm instantly alerts user, preventing overflow.
- Compact, battery operated unit.

The new Restek Waste Overflow Indicator will help to keep your mobile phase waste where it belongs—in the waste container! Compact, battery operated unit accomodates two lines and fits securely on 4-liter solvent bottles. An audible alarm is given as the solvent waste container approaches capacity, giving you time to empty or change the container. Another innovative design from Restek!

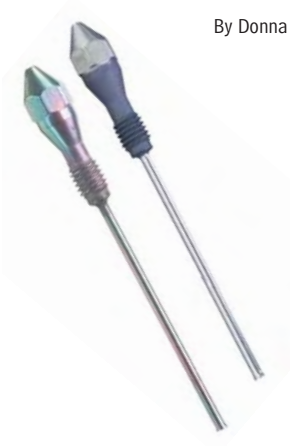
Waste Overflow Indicator for HPLC Systems

Description	qty.	cat.#
Waste Overflow Indicator for HPLC Systems	kit	26543
Replacement AA Battery for the Waste Overflow Indicator	ea.	26544
Replacement AA Batteries for the Waste Overflow Indicator	3-pk.	26545

Peak Performers

Replacement Parts for Agilent FID Detectors

By Donna Lidgett, GC Accessories Product Marketing Manager and Sue Benes, GC Accessories Product Marketing Manager



FID Replacement Jets

Standard Version

- Engineered with a fluted tip to guide the capillary column into the jet.
- Threads specially coated for easy installation and removal.
- Special processing ensures the highest degree of cleanliness.

High-Performance Version

- Similar to the standard version, but Siltek® treated.
- Extremely inert, for use with active compounds.

Capillary Adaptable FID Replacement Jet for Agilent 5890/6890/6850 GCs

0.011-Inch ID Tip	Similar to Agilent part #	qty.	cat.#	qty.	cat.#
Standard, 0.011-Inch ID Tip	19244-80560	ea.	20670	3-pk.	20671
High-Performance Siltek® Treated, 0.011-Inch ID Tip	19244-80560	ea.	20672	3-pk.	20673

Capillary Dedicated FID Replacement Jet for Agilent 6890/6850/7890 GCs

0.011-Inch ID Tip	Similar to Agilent part #	qty.	cat.#	qty.	cat.#
Standard, 0.011-Inch ID Tip	G1531-80560	ea.	21621	3-pk.	21682
High-Performance Siltek® Treated, 0.011-Inch ID Tip	G1531-80560	ea.	21620	3-pk.	21683

Packed Column FID Replacement Jets for Agilent 5890/6890/6850 GCs

0.018-Inch ID Tip*	Similar to Agilent part #	qty.	cat.#	qty.	cat.#
Standard, 0.018-Inch ID Tip	18710-20119	ea.	21694	3-pk.	21695
High-Performance Siltek® Treated, 0.018-Inch ID Tip	18710-20119	ea.	21696	3-pk.	21697
0.030-Inch ID Tip*	**Similar to Agilent part #**	**qty.**	**cat.#**	**qty.**	**cat.#**
Standard, 0.030-Inch ID Tip	18789-80070	ea.	21688	3-pk.	21689
High-Performance Siltek® Treated, 0.030-Inch ID Tip	18789-80070	ea.	21686	3-pk.	21687

* 0.018-inch ID jets: Used for most general-purpose packed column applications.

** 0.030-inch ID jets: For packings that exhibit high bleed and that frequently clog the tip of smaller 0.018-inch jets.

tech tip

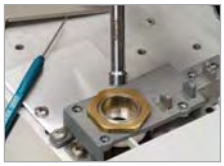
Which FID Jet Should I Use?

There are two FID jet configurations for Agilent GCs. The longer "adaptable" jet fits both 5890 and 6890 GCs, and can be used with capillary or packed columns. The shorter "dedicated" jet is for the FID in the 6890 GC that is designed only for use with capillary columns.

restek **innovation**!

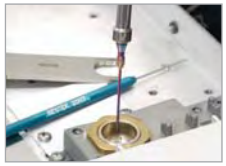
FID Jet Removal Tool for Agilent 5890/6890/6850/7890 FIDs

- Securely grips jet in socket for easy removal or installation.
- Unique, ergonomic handle—easy to hold.



Slip tool over FID jet...

...loosen jet...



...and remove.

Description	qty.	cat.#
FID Jet Removal Tool for Agilent 5890/6890/6850/7890 FIDs	ea.	22328

Rugged design!

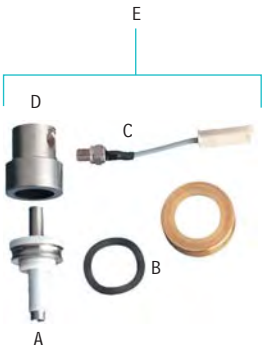
FID Gauge Pack for Agilent 5890 GCs

Pressure regulators and gauges for air & hydrogen. The 1/8-inch bulkhead allows easy hookup to instrument. Rated for inlet pressures to 250psi (1724kPa), outlet pressures of 0 to 60psi (0-414kPa).

Description	qty.	cat.#
FID Gauge Pack for Agilent 5890 GCs	ea.	22071

Direct Replacement FID Collector Assembly Kit for Agilent 5890 GCs

Description	Similar to Agilent part #	qty.	cat.#
E) FID Collector Assembly Kit (includes insulators)	19231-60690	kit	23010
E) FID Collector Assembly Kit w/Siltek® Ignitor Castle	—	kit	21131



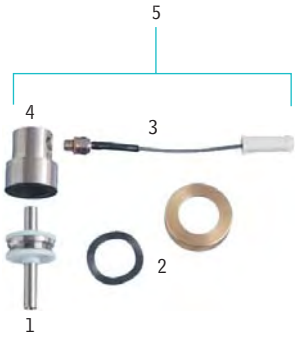
Replacement FID Parts for Agilent 5890 GCs

Description	Similar to Agilent part #	qty.	cat.#
A) FID Collector (includes insulators)	19231-20970 19231-20960 19231-20950	ea.	21138
B) FID Collector Nut and Washer	19231-20940 5181-3311	set	21136
C) FID Ignitor*	19231-60680	ea.	21001
D) FID Ignitor Castle	19231-20910	ea.	21137
Siltek® FID Ignitor Castle	—	ea.	21135

*Also fits OI Analytical 4410 detector (similar to OI part # 191833).

Direct Replacement FID Collector Assembly Kit for Agilent 6890/6850/7890 GCs

Description	Similar to Agilent part #	qty.	cat.#
5) FID Collector Assembly Kit (includes insulator)	G1531-60690	kit	21699
5) FID Collector Assembly Kit w/Siltek® Ignitor Castle	—	kit	21132



Replacement FID Parts for Agilent 6890/6850/7890 GCs

Description	Similar to Agilent part #	qty.	cat.#
1) FID Collector (includes insulators)	G1531-20690 G1531-20700	ea.	21139
2) FID Collector Nut and Washer	19231-20940 5181-3311	set	21136
3) FID Ignitor*	19231-60680	ea.	21001
4) FID Ignitor Castle	19231-20910	ea.	21137
Siltek® FID Ignitor Castle	—	ea.	21135

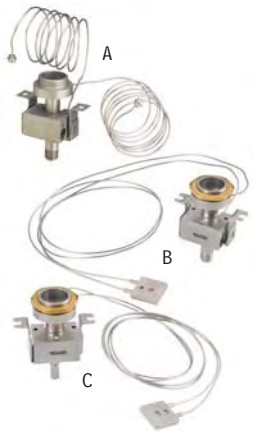
*Also fits OI Analytical 4410 detector (similar to OI part # 191833).

FID Base Weldment for Agilent GCs

- Meets or exceeds manufacturer's performance.
- Includes brass nut.

Description	Similar to Agilent part #	qty.	cat.#
A) FID Base Weldment for Agilent 5890 GCs	19231-80580	ea.	23041
B) FID Base Weldment, Pack Column FID, for Agilent 6850/6890 GCs	G1531-80580	ea.	23052
C) FID Base Weldment, Capillary Column FID, for Agilent 6850/6890 GCs	G1531-80630	ea.	23053

Note: 6890 GC connections to EPC modules are not compatible with the 7890 EPC modules.



Spanner Wrench for Agilent 5890/6890/6850/7890 FID Collector Assemblies

- Easily remove the nut from the FID collector without damaging the nut.
- Unique, ergonomic handle—easy to grip.


Remove FID ignitor castle.


Easily loosen the nut by aligning the two pins on the bottom of the wrench with the two open slots on the nut...


...then turn counter-clockwise...


...and remove.

Description	Similar to Agilent part #	qty.	cat.#
Spanner Wrench for Agilent 5890/6890/6850/7890 FID Collector Assembly	19231-00130	ea.	22329

NEW! Electron Multipliers for Mass Spectrometry

By Sue Benes, GC Accessories Product Marketing Manager

- The multi-dynode approach of all ETP electron multipliers results in longer lifetimes and better sensitivity compared with channel electron multipliers or continuous dynode multipliers.
- Optimized ion and electronic optics and unique dynode shapes for maximum performance.
- Increased surface area for enhanced sensitivity and extended operational life.

Cat# 23074

Features of ETP Electron Multipliers

- Proprietary specialized surface material resulting in very high secondary electron emission.
- Air stable.
- 2-year shelf life guarantee.
- Discrete dynode design results in extended operating life.

The electron multipliers manufactured by ETP use a proprietary dynode material. This material has a number of properties that make it very suitable for use in an electron multiplier. It has very high secondary electron emission, which allows exceptional gain to be achieved from each dynode. This material is also very stable in air. In fact, an ETP multiplier can be stored for years before being used. As a direct result of the high stability of the active materials used in ETP multipliers, they come with a 2-year shelf life warranty (store in original sealed package). Many testing laboratories take advantage of this long shelf life by keeping a replacement ETP multiplier on hand, ready for immediate installation. This keeps the instrument down time to a minimum.

For a typical ETP electron multiplier for GC/MS, the total active dynode surface area is ~1000mm^2. This can be compared to a standard continuous dynode multiplier that has a total channel surface area of only around 160mm^2 (for a channel with 1mm diameter and 50mm length). This increased surface area spreads out the work-load of the electron multiplication process over a larger area, effectively slowing the aging process and improving operating life and gain stability.

ETP Electron Multipliers for Mass Spectrometry

Description	qty.	cat.#
Electron Multipliers for Agilent GC-MS and LC-MS		
For Agilent 5970 GC-MS	ea.	23072
For Agilent 5971, 5972, GCD GC-MS	ea.	23073
For Agilent 5973 & 5975 GC-MS (includes mount for initial installation)*†	ea.	23074
For Agilent 5973 & 5975 GC-MS and LC-MSD (Replacement Multiplier)*†	ea.	23075
For Agilent LC-MSD (includes mount for initial installation)*†	ea.	23076
Electron Multipliers for Applied Biosystems (Sciex)		
For API 300, 3000 & 4000 Applied Biosystems	ea.	23077

*Note: The electron multipliers have been specifically developed to retrofit the original manufacturer's equipment. The detector incorporates a modular design to facilitate ease of replacement and additional innovations intended to enhance performance. First time installation requires a mount which includes the mechanical housing. After initial installation, only the replacement electron multiplier is required.
†This unit is designed for use in the 5975, 5973 GC and the LC/MSD.

for more info

For more information on ETP Electron Multipliers, request **lit. cat.# GNFL1000.**

please note

Other electron multipliers are available upon request. Call 800-356-1688 ext. 4, or contact your local Restek representative, for information on other models.

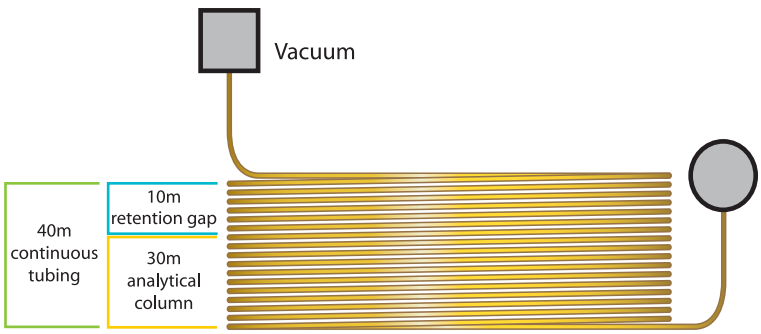
Using Guard Columns and Retention Gaps in GC (Part 2) *Continued from page 2.*

Segment coating technology eliminates problematic connections

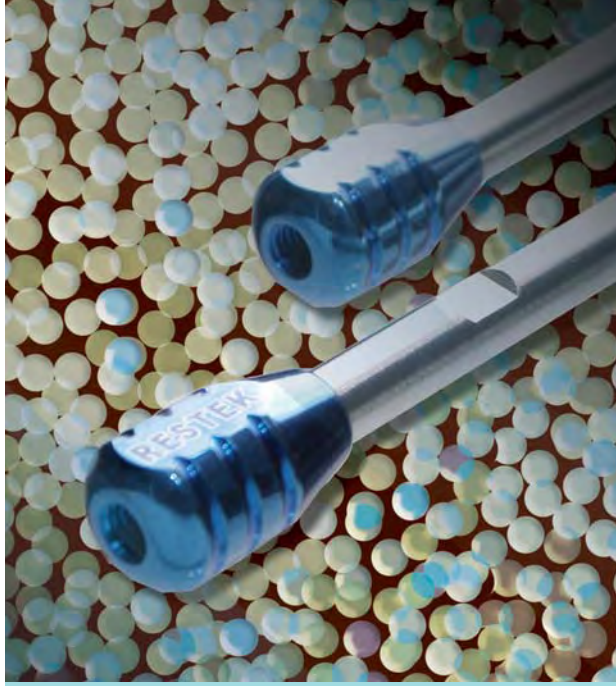
Both retention gaps and guard columns must be coupled to the analytical column. While there are several types of effective coupling devices, all can create dead volume and can be a potential source of leaks and reactivity. Segment coating technology allows the retention gap or guard column to be built directly in the same piece of tubing as the analytical column, eliminating the connector and associated risks. This technology, available from Restek, is termed Integra-Guard™ or Integra-Gap™ and is based on the static coating method. In this process the capillary column is filled with a coating solution of stationary phase in a volatile solvent. The column is sealed on one end and on the other side a vacuum is applied. The solvent is evaporated and the dissolved polymer is deposited on the inside deactivated wall of the fused silica column. The static coating method allows columns to be coated by segment. When filling, for example, a 40m capillary with the coating solution, only 30m are filled. The first 10m remain uncoated, having only the deactivation treatment (Figure 1). This method deposits the stationary phase only in a designated portion of the capillary, creating the Integra-Guard™ or the Integra-Gap™. The advantages of this technology are clear: eliminating the connector removes a potential source of leaks and reduces dead volume. Additionally, maintenance is faster and simpler since there is no manual connection to make.

Guard columns and retention gaps are useful tools to the practicing chemist, and it is important to understand the difference between them. While they help protect analytical columns and focus samples, respectively, they are also a source of potential problems, such as leaks. Segment coating technology offers a better solution—integrated columns containing both the guard or gap section and the analytical column together in a single piece of tubing. These Integra-Guard™ and Integra-Gap™ columns are a simple, effective solution; they eliminate the risks of a separate connection and provide stable, accurate data.

Figure 1 Static coating allows Integra-Gap™ integrated retention gaps to be built directly into the analytical column tubing.



For more information on Integra-Gap™ technology, see "Selecting a GC Column for Glycerin in Biodiesel" on page 10.



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- Speed up semivolatiles analysis with new Rxi®-5Sil MS GC columns.
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2007.04

IN THIS ISSUE

Erratum
The heading of Figure 1 on page 8 of the 2007.03 issue of the Restek Advantage incorrectly describes the column internal diameter as 0.18mm. The correct internal diameter is 0.32mm.

Using Guard Columns and Retention Gaps in GC (Part 1)

Jaap de Zeeuw, International GC Consumables Specialist, Restek Corporation

Guard columns and retention gaps are used widely in gas chromatography (GC). Many users have difficulty understanding the difference between these two products, even though there is a significant difference in application. Retention gaps mainly are used for focusing the sample components when introducing a large (liquid) sample directly onto the column. Guard columns are used to protect the analytical column from contamination. When using a retention gap system, the retention gap will also act as a guard column, but its primary function is to create a focusing effect.

Guard columns and retention gaps both must be coupled to the analytical column, and this connection introduces a potential point of risk. A new approach is to integrate the retention gap directly into the analytical column. By applying a "segment" coating technology, the stationary phase can be deposited in a certain part of the column allowing a deactivated section at the beginning. Column coupling is not required, and maintenance is greatly simplified. In Part 1 of this article, we will explore retention gaps and build a foundation for a comparison to guard columns. In Part 2, we will review guard columns and discuss the new segment coating technology.

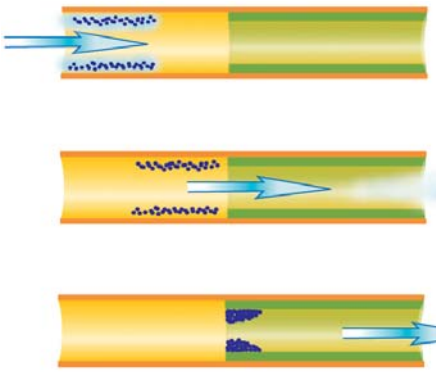
Use of retention gaps

In today's laboratory, GC methods must be simple, fast, and low detection limits are required. Besides that, sufficient precision must also be obtained. It all starts by introducing the sample in the smallest possible injection band and making the band migrate through the capillary with minimal loss of the target components. With on-column injection, a liquid sample is directly introduced into the capillary column as a liquid while the capillary column is kept at a temperature 10-15°C below the boiling point of the solvent. During this process, the sample components are spread in an unreproducible way over the first 20-100cm of capillary while the solvent is evaporating. Parameters like injection speed, carrier gas flow, temperature of solvent and column, type of solvent and pressure all will affect the injection band width. Additionally, when nonbonded stationary phases are used, the direct contact with liquids will result in a distortion of the stationary phase film and very short column lifetime. The majority of today's stationary phases, like the Rtx® and Rxi® phases, are immobilized by cross- and surface bonding techniques.

For proper application of the on-column injection technique, the use of retention gaps is essential.[1,2] The retention gap consists of a 1-3m length of deactivated capillary that is positioned in front of the analytical column. All the processes described will still take place, but now the components are distributed over the retention gap. When the oven temperature is

Continued on page 23.

Figure 1 Retention gaps are used to focus components in a tight band at the beginning of the analytical column



a) Sample introduction: liquid film of solvent and sample are deposited in the first length of capillary.

b) Oven temperature is increased (temp. program run): solvent and target compounds are vaporized and travel unretained through retention gap.

c) When target compunds come in contact with the stationary phase, they are refocused on the analytical column, resulting in a narrow initial band width.

Fast, Accurate Semivolatiles Analysis!

Using **New** Rxi®-5Sil MS GC Columns

By Robert Freeman, Environmental Innovations Chemist

- Ultra-low bleed column saves you time and money with faster baseline stabilization.
- Highly inert for more accurate low-level analysis of active compounds.
- Guaranteed column-to-column reproducibility.

Semivolatiles methods, such as EPA Method 8270, place stringent demands on the analytical system, especially the GC column. 5% diphenyl/95% dimethyl polysiloxane ("5" phase) columns often are used for this GC/MS test method; however, silarylene columns generally perform better with the sensitivity of mass spectrometers. Silarylene phases are lower bleed and produce improved peak efficiencies for difficult compounds while maintaining selectivity that is similar to a conventional "5" phase column. Restek recently improved its silarylene column (Rtx®-5Sil MS) using Rxi® technology. The result is the new Rxi®-5Sil MS column, a more inert, low-bleed column with improved peak shape and resolution for the active compounds found in semivolatiles analysis. *Continued on page 4.*

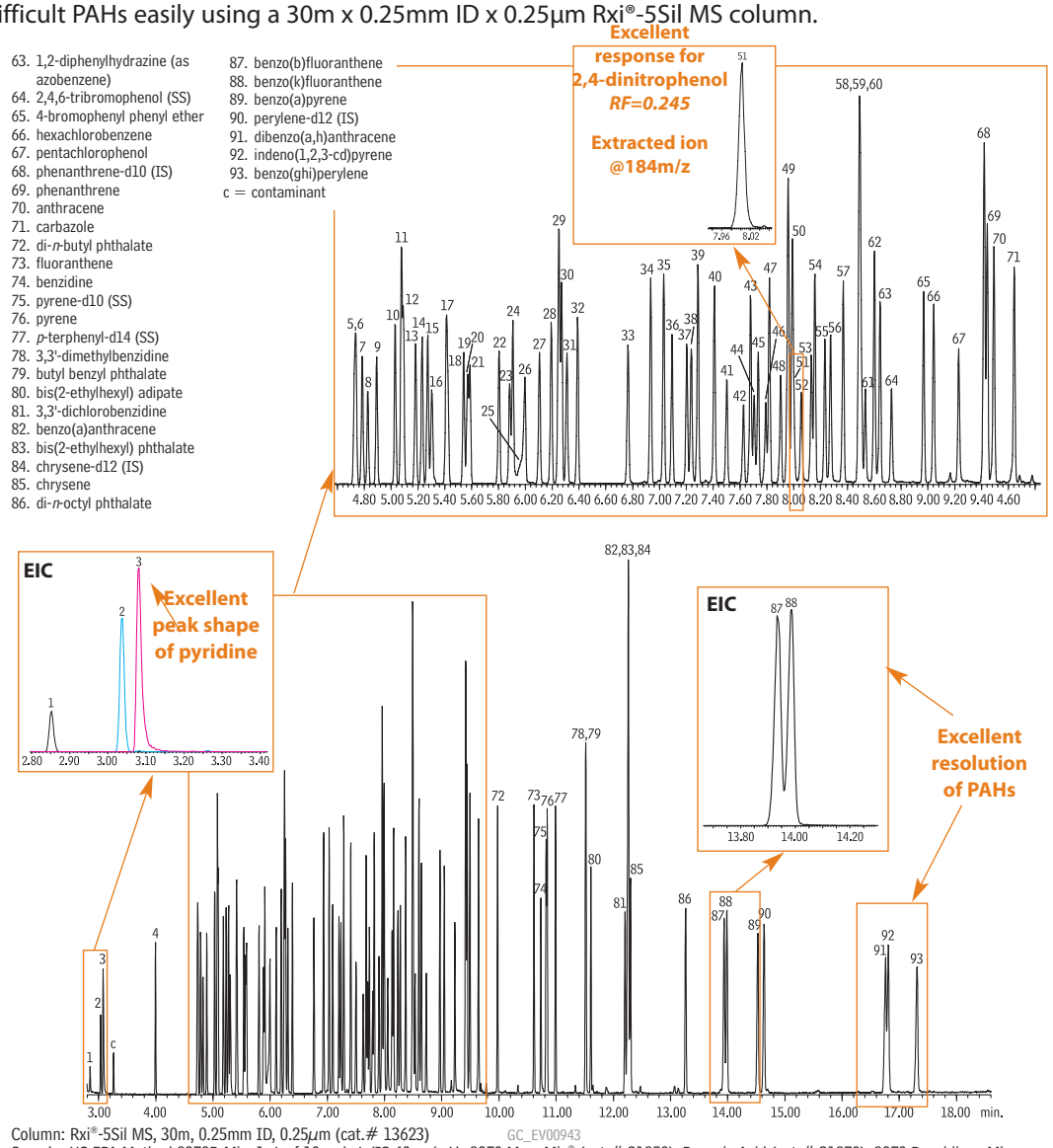
Fast, Accurate Semivolatiles Analysis *Continued from page 3.*

Rxi®-5Sil MS columns are ideal for the analysis of semivolatile analytes such as those found in EPA Method 8270. Low bleed profiles assure accurate quantification of late eluting compounds, such as polycyclic (polynuclear) aromatic hydrocarbons (PAHs), including the challenging separation of benzo(b)fluoranthene and benzo(k)fluoranthene (Figures 1 and 2). The inertness of the Rxi®-5Sil MS column is demonstrated through the peak shapes and responses of active analytes, such as pyridine (basic) and 2,4-dinitrophenol (acidic), at low levels. Peak symmetry is good and analyte responses exceed method requirements even at single ng on-column levels (Figure 3). Chromatography, and thus quantification, of many active semivolatile compounds is improved by the inertness of Rxi®-5Sil MS columns.

The Rxi®-5Sil MS columns most commonly used for semivolatiles analysis are the 30m x 0.25mm ID columns with either 0.25µm or 0.5µm film thicknesses. These dimensions generally offer the best balance of sample capacity, analysis time, and column lifetime. However, if sample throughput is paramount, shorter narrow bore columns, such as the 20m x 0.18mm ID with either 0.18µm or 0.36µm film thicknesses, are preferred. Due to increased peak efficiencies, temperature programs can be accelerated without compromising key separations. Regardless of which dimension you choose, the new Rxi®-5Sil MS columns are ideal for analyzing semivolatile compounds.

Figure 1 Separate difficult PAHs easily using a 30m x 0.25mm ID x 0.25µm Rxi®-5Sil MS column.

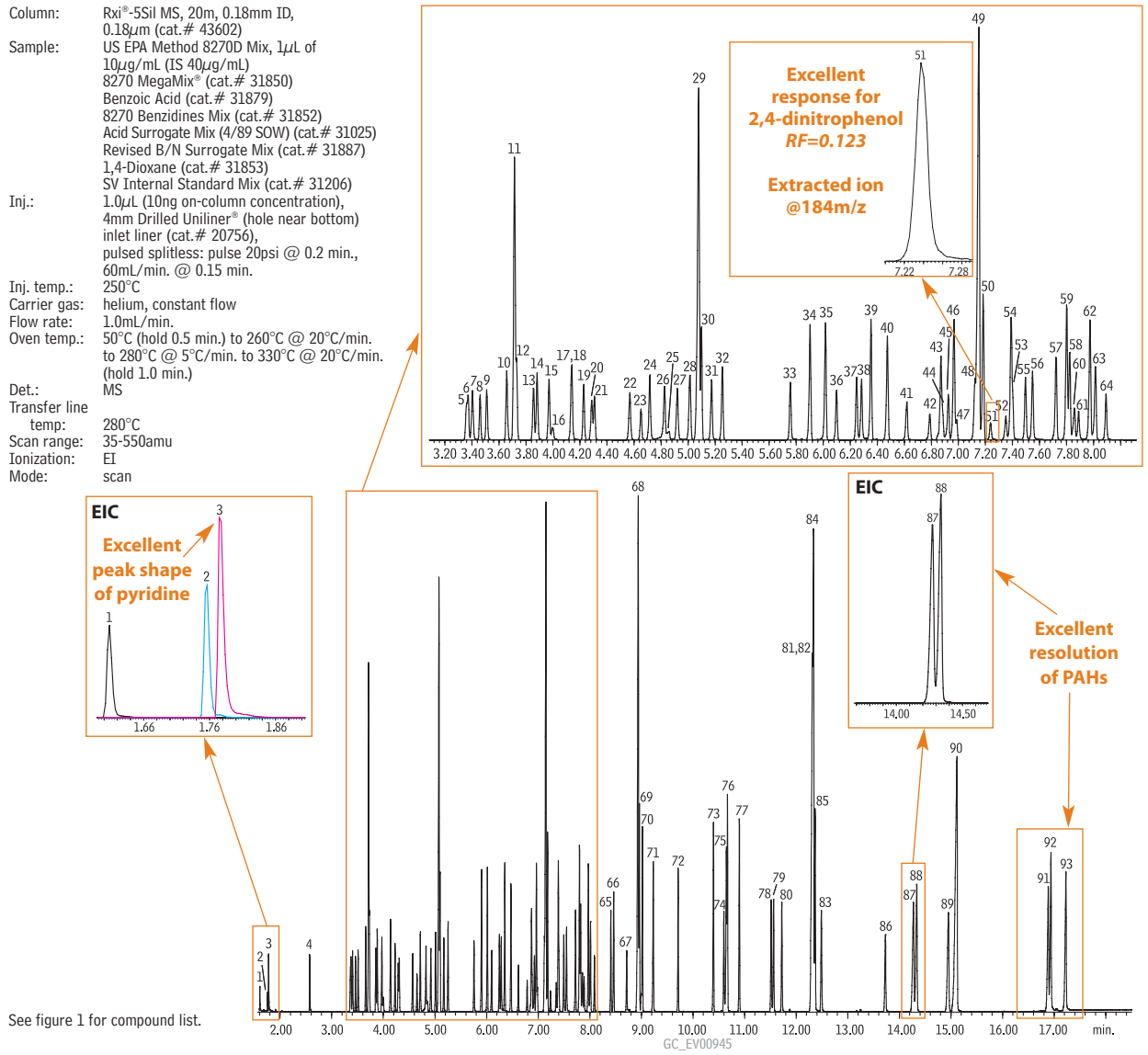
1. 1,4-dioxane
2. *n*-nitrosodimethylamine
3. pyridine
c. toluene
4. 2-fluorophenol (SS)
5. phenol-d6 (SS)
6. phenol
7. aniline
8. bis(2-chloroethyl) ether
9. 2-chlorophenol
10. 1,3-dichlorobenzene
11. 1,4-dichlorobenzene-d4 (IS)
12. 1,4-dichlorobenzene
13. benzyl alcohol
14. 1,2-dichlorobenzene
15. 2-methylphenol
16. bis(2-chloroisopropyl) ether
17. 4-methylphenol/3-methylphenol
18. *n*-nitroso-di-*n*-propylamine
19. hexachloroethane
20. nitrobenzene-d5 (SS)
21. nitrobenzene
22. isophorone
23. 2-nitrophenol
24. 2,4-dimethylphenol
25. benzoic acid
26. bis(2-chloroethoxy)methane
27. 2,4-dichlorophenol
28. 1,2,4-trichlorobenzene
29. naphthalene-d8 (IS)
30. naphthalene
31. 4-chloroaniline
32. hexachlorobutadiene
33. 4-chloro-3-methylphenol
34. 2-methylnaphthalene
35. 1-methylnaphthalene
36. hexachlorocyclopentadiene
37. 2,4,6-trichlorophenol
38. 2,4,5-trichlorophenol
39. 2-fluorobiphenyl (SS)
40. 2-chloronaphthalene
41. 2-nitroaniline
42. 1,4-dinitrobenzene
43. dimethyl phthalate
44. 1,3-dinitrobenzene
45. 2,6-dinitrotoluene
46. 1,2-dinitrobenzene
47. acenaphthylene
48. 3-nitroaniline
49. acenaphthene-d10 (IS)
50. acenaphthene
51. 2,4-dinitrophenol
52. 4-nitrophenol
53. 2,4-dinitrotoluene
54. dibenzofuran
55. 2,3,5,6-tetrachlorophenol
56. 2,3,4,6-tetrachlorophenol
57. diethyl phthalate
58. 4-chlorophenyl phenyl ether
59. fluorene
60. 4-nitroanaline
61. 4,6-dinitro-2-methylphenol
62. *n*-nitrosodiphenylamine (diphenylamine)
63. 1,2-diphenylhydrazine (as azobenzene)
64. 2,4,6-tribromophenol (SS)
65. 4-bromophenyl phenyl ether
66. hexachlorobenzene
67. pentachlorophenol
68. phenanthrene-d10 (IS)
69. phenanthrene
70. anthracene
71. carbazole
72. di-*n*-butyl phthalate
73. fluoranthene
74. benzidine
75. pyrene-d10 (SS)
76. pyrene
77. *p*-terphenyl-d14 (SS)
78. 3,3'-dimethylbenzidine
79. butyl benzyl phthalate
80. bis(2-ethylhexyl) adipate
81. 3,3'-dichlorobenzidine
82. benzo(a)anthracene
83. bis(2-ethylhexyl) phthalate
84. chrysene-d12 (IS)
85. chrysene
86. di-*n*-octyl phthalate
87. benzo(b)fluoranthene
88. benzo(k)fluoranthene
89. benzo(a)pyrene
90. perylene-d12 (IS)
91. dibenzo(a,h)anthracene
92. indeno(1,2,3-cd)pyrene
93. benzo(ghi)perylene
c = contaminant



Column: Rxi®-5Sil MS, 30m, 0.25mm ID, 0.25µm (cat.# 13623) GC_EV00943
Sample: US EPA Method 8270D Mix, 1µL of 10µg/mL (IS 40µg/mL), 8270 MegaMix® (cat.# 31850), Benzoic Acid (cat.# 31879), 8270 Benzidines Mix (cat.# 31852), Acid Surrogate Mix (4/89 SOW) (cat.# 31025), Revised B/N Surrogate Mix (cat.# 31887), 1,4-Dioxane (cat.# 31853), SV Internal Standard Mix (cat.# 31206); Inj.: 1.0µL (10ng on-column concentration), 4mm Drilled Uniliner® (hole near bottom) inlet liner (cat.# 20756), pulsed splitless: pulse 25psi @ 0.2 min., 60mL/min. @ 0.15 min.; Inj. temp.: 250°C; Carrier gas: helium, constant flow; Flow rate: 1.2mL/min.; Oven temp.: 40°C (hold 1.0 min.) to 280°C @ 25°C/min. to 320°C @ 5°C/min. (hold 1 min.); Det.: MS; Transfer line temp: 280°C; Scan range: 35-550amu; Ionization: EI; Mode: scan

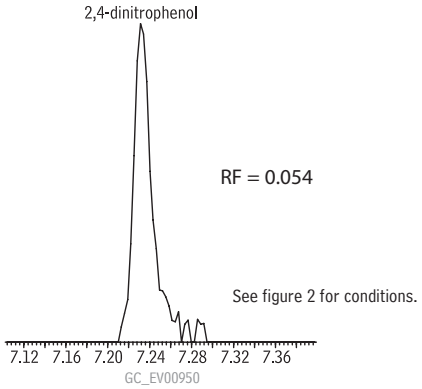
Figure 2 Semivolatile compounds resolved on a 20m x 0.18mm ID x 0.18µm Rxi®-5Sil MS column.

Column:	Rxi®-5Sil MS, 20m, 0.18mm ID, 0.18µm (cat.# 43602)
Sample:	US EPA Method 8270D Mix, 1µL of 10µg/mL (IS 40µg/mL) 8270 MegaMix® (cat.# 31850) Benzoic Acid (cat.# 31879) 8270 Benzidines Mix (cat.# 31852) Acid Surrogate Mix (4/89 SOW) (cat.# 31025) Revised B/N Surrogate Mix (cat.# 31887) 1,4-Dioxane (cat.# 31853) SV Internal Standard Mix (cat.# 31206)
Inj.:	1.0µL (10ng on-column concentration), 4mm Drilled Uniliner® (hole near bottom) inlet liner (cat.# 20756), pulsed splitless: pulse 20psi @ 0.2 min., 60mL/min. @ 0.15 min.
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.0mL/min.
Oven temp.:	50°C (hold 0.5 min.) to 260°C @ 20°C/min. to 280°C @ 5°C/min. to 330°C @ 20°C/min. (hold 1.0 min.)
Det.:	MS
Transfer line temp:	280°C
Scan range:	35-550amu
Ionization:	EI
Mode:	scan



See figure 1 for compound list.

Figure 3 Excellent peak symmetry and response at 1ng on-column.



See figure 2 for conditions.

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.18	-60 to 330/350°C	20-Meter	43602
0.18mm	0.36	-60 to 330/350°C	20-Meter	43604
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623
0.25mm	0.50	-60 to 330/350°C	30-Meter	13638

8270 MegaMix® (76 components)

1,000µg/mL each in methylene chloride, 1mL/ampul*
cat. # 31850

*3-methylphenol and 4-methylphenol concentration is 500µg/mL.
For a complete list of components, visit us at **www.restek.com/standards**

Direct Injection Liners for Agilent GCs

ID* x OD & Length (mm)	qty.	cat.#
Drilled Uniliner® (hole near bottom)		
4.0 ID x 6.3 OD x 78.5	ea.	20756
	5-pk.	20771

Complete Resolution of Benzene from Ethanol in Spark Ignition Fuels

Using a Modified ASTM D3606-06e1 Method and the New D3606 Column Set

By Barry L. Burger, Petroleum Innovations Chemist

- Easy, accurate quantification of aromatics.
- Fully conditioned column set—ready to use out of the box.
- Each column set is tested for method applicability and includes chromatogram.

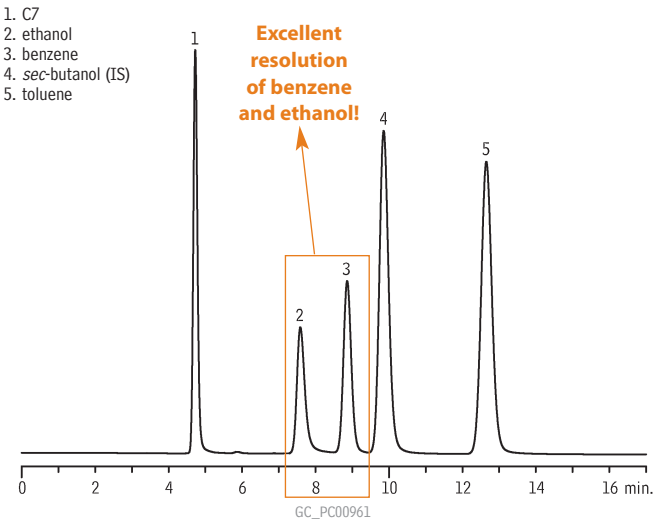
Laboratories analyzing reformulated spark ignition fuels that contain ethanol for the determination of benzene and toluene must use a modified ASTM D3606-06e1 method to prevent the coelution of ethanol and benzene. This method modification is also a requirement of the US EPA. The benzene range of determination is 0.1 to 5% by volume, and the toluene range is 2 to 20% by volume. The primary challenge in this analysis is twofold: the tailing of the ethanol peak, and the retention time shift of the aromatics towards ethanol, specifically benzene merging quickly into the ethanol peak and preventing accurate quantification.

Restek has resolved these issues by developing a new D3606 column set for this modified ASTM D3606-06e1 application. Column 1 is a 6' x 1/8" OD (1.8m x 2mm ID) nonpolar Rtx®-1 phase which separates components by boiling point. After the elution of *n*-octane (C8), Column 1 is backflushed to prevent heavier compounds from entering Column 2, the main analytical column. The light compounds pass into Column 2, a 16' x 1/8" OD (4.9m x 2mm ID) column packed with a new proprietary polymer that fully resolves the aromatic compounds.

To demonstrate the performance of this new column set, we installed it in an Agilent 6890 GC equipped with a flame ionization detector (FID). Helium was used as the carrier gas at 20mL/min. in the constant flow mode. The data in Figure 1 show that the aromatic compounds are fully resolved, and can easily be quantified using the internal standard, sec-butanol.

This column set is fully conditioned and ready to use right out of the box. Only a brief (10 min.) carrier gas purge at ambient temperature, followed by a 30 min. hold at 165°C, is required. If your laboratory has been struggling with ASTM method D3606-06e1 for reformulated fuels containing ethanol, Restek's new column set is the solution.

Figure 1 Complete resolution of benzene from ethanol using a D3606 column set and modified ASTM D3606-06e1 method.



Column:	D3606 Column Set column 1: 6' x 1/8" OD (1.8m x 2mm ID), nonpolar Rtx®-1 polymer column 2: 16' x 1/8" OD (4.9m x 2mm ID), proprietary packing
Sample:	0.05µg/µL; C7 (26%), ethanol (10%) benzene(10%), *sec*-butanol (26%), toluene (26%)
Inj.:	0.05µL, direct injection
Inj. temp.:	200°C
Carrier gas:	helium, constant flow
Flow rate:	20mL/min.
Oven temp.:	135°C, isothermal
Det.:	FID @ 250°C

D3606 Application Column (2 column set) new!

Description	cat.#*
D3606 Application Column (2 column set)**	
Column 1: 6' (1.8m), 1/8" OD, 2.0mm ID, nonpolar Rtx®-1	
Column 2: 16' (4.9m), 1/8" OD, 2.0mm ID, proprietary packing material	83606-

*Please add column instrument configuration suffix number to cat.# when ordering.
**This column set is for a valving system; therefore, packing material is filled to ends of columns.

Column Instrument Configurations

General Configuration **Suffix -800**

Varian 3700, Vista Series, FID: **Suffix -820**

Agilent 5880, 5890, 5987, 6890: **Suffix -810**

PE 900-3920 Sigma 1,2,3: **Suffix -830**

PE Auto System 8300, 8400, 8700 (Not On-Column): **Suffix -840**

Note: Initial 2" of column will be empty, to accommodate a needle. For a completely filled column (not on-column), add suffix -901.

Rapid Characterization of Garlic Volatiles No Sample Prep Required!

Using Headspace GC/MS and an Rxi®-5ms Capillary Column

By Julie Kowalski, Innovations Chemist; Michelle Long, Innovations Chemist;
Jason Thomas, Innovations Chemist; and William Goodman*, GC/MS Applications Specialist

- No sample preparation! Eliminate complicated steps required by other methods.
- Rapid screening of garlic-specific flavor and odor compounds.
- Speedy determination of volatiles profile.

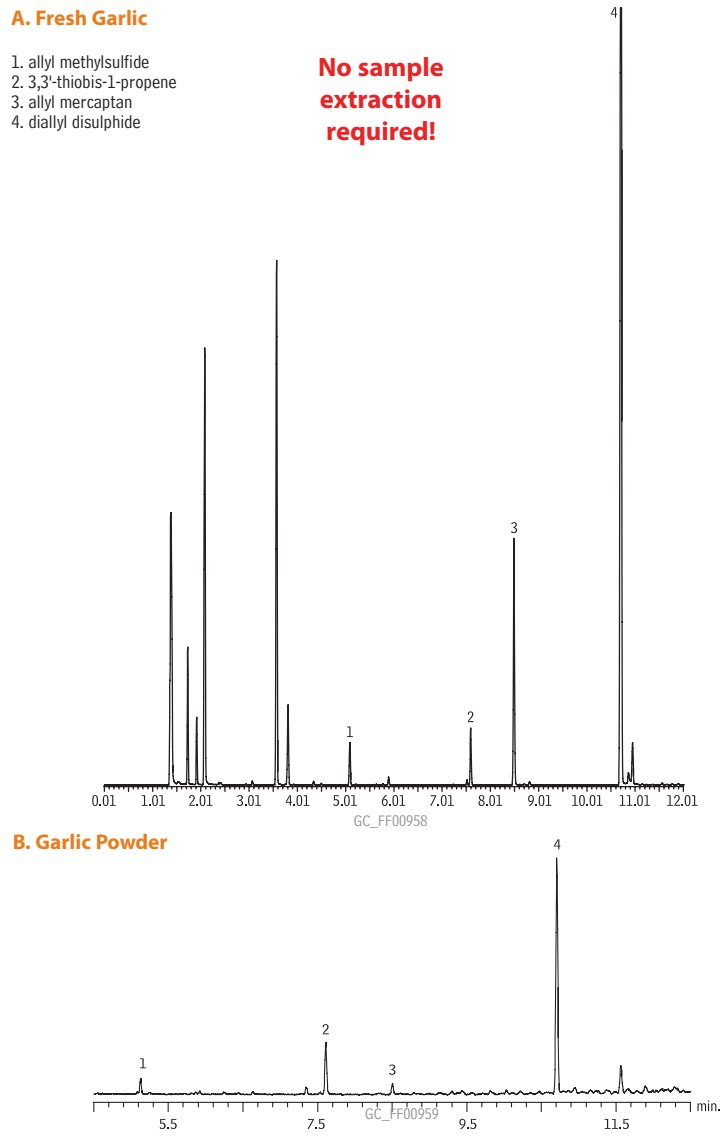
Garlic, *Allium sativum* (L.), has a rich history in cooking and medicinal use. Recently, garlic supplements have gained popularity for boosting immune and cardiovascular health. Chromatographic methods for garlic are used by the dietary supplements industry to detect volatiles, such as sulfide degradents, that may affect the acceptability of supplements to the consumer. The headspace gas chromatography mass spectrometry (HS GC/MS) method for garlic and garlic powder shown here requires no sample preparation—making the bench work simple and fast. Other methods involve steam distillation, solid phase trapping solvent exchange, headspace solid phase microextraction, and simultaneous distillation and solvent extraction, which can be difficult and time-consuming.

This HS GC/MS analysis was done using a 30m x 0.25mm ID x 1.0µm Rxi®-5ms column and a PerkinElmer TurboMatrix 40 Trap Headspace Sampler. Conditions used are shown in the figure and were set to optimize the comparison. Several sulfur components were identified including allyl methylsulfide, 3,3'-thiobis-1-propene, allyl mercaptan and diallyl disulfide. Diallyl disulfide appeared to be the dominant component for both garlic preparations. The fingerprint, or relative ratios, of the other components were distinct for fresh garlic and powdered garlic (Figure 1).

Headspace GC/MS is an effective technique for rapid characterization of garlic and garlic powder samples. The experimental set-up shown here is ideal for both screening and low-level trace analysis. This method provides a fast assessment of garlic quality and is applicable to the determination of low-level sulfur containing compounds from odorless supplements.

* PerkinElmer

Figure 1 Rapid screening of garlic volatiles—analyze samples in less than 11 minutes! (Total ion chromatogram)



Column: Rxi®-5ms, 30m, 0.25mm ID, 1.0µm (cat.# 13453) with a 5m, 0.32mm ID IP deactivated guard column (cat.# 10044); Sample: A. fresh garlic B. garlic powder
Inj.: split (10:1); Inj. temp.: 220°C; Flow rate: 1.5mL/min.; Oven temp.: 35°C (hold 1 min.) to 220°C @ 15°C/min. to 300°C @ 45°C/min.; Det: MS; Scan range: 35-350amu; Ionization: EI; Mode: scan
Headspace Conditions
Instrument: PerkinElmer TurboMatrix 40 Trap Headspace Sampler; Column pressure:15psi (103kPa); Inj. pressure: 30psi (207kPa); Thermostat time: 15 min.; Vial pressurize time: 1 min.; Withdraw time: 0.2 min.; Injection time: 0.02 min.; Oven temp.: 80°C; Needle temp.: 90°C; Transfer temp.: 110°C

Rxi®-5ms Column (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	1.00	-60 to 330/350°C	30-Meter	13453

Simplify and Speed Up Opiates Analysis

Using LC/MS/MS & an Allure® PFP Propyl HPLC Column

By Kristi Sellers, Innovations Chemist

- 7-minute analysis time, for increased sample throughput.
- Faster sample prep—no derivatization required.
- Separate compounds with similar mass spectra.

Opiates are one of the primary drug classes tested in clinical and forensic laboratories, and most confirmation methods use GC/MS. These methods require derivatization of the target compounds, which significantly lengthens sample preparation time. Here we present an alternative confirmation method, using LC/MS/MS, which can increase sample throughput by eliminating derivatization and shortening analysis time. This procedure also provides accurate confirmation and quantification of compounds that have similar mass spectra, by using an Allure® PFP Propyl column to chromatographically separate compounds that share product ions, allowing positive identification based on retention time.

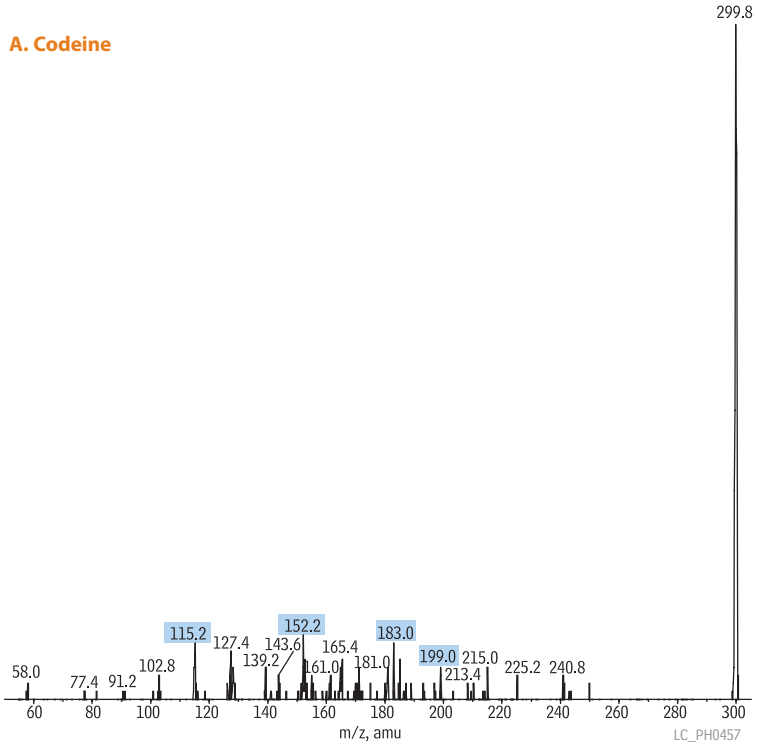
In developing this LC/MS/MS method for the analysis of opiates, our goals were to obtain baseline resolution of compounds having similar mass spectra while providing an analysis time of less than 10 minutes. To accomplish this, mass spectrometer conditions, column selection, mobile phase, and gradient profiling were evaluated and optimized. Several different stationary phases initially were evaluated including an aqueous C18, a biphenyl, a propyl cyano, and a pentaflurophenyl propyl stationary phase. Consistent column dimensions and base silica (50mm, 2.1mm ID, 5µm particle size, and 60Å pore size) were used for all phases; mobile phase conditions were optimized for each stationary phase. Mobile phases tested included: 0.1% formic acid in water, 0.1% formic acid in acetonitrile, and 0.1% formic acid in methanol in various combinations. A variety of gradient profiles also were evaluated.

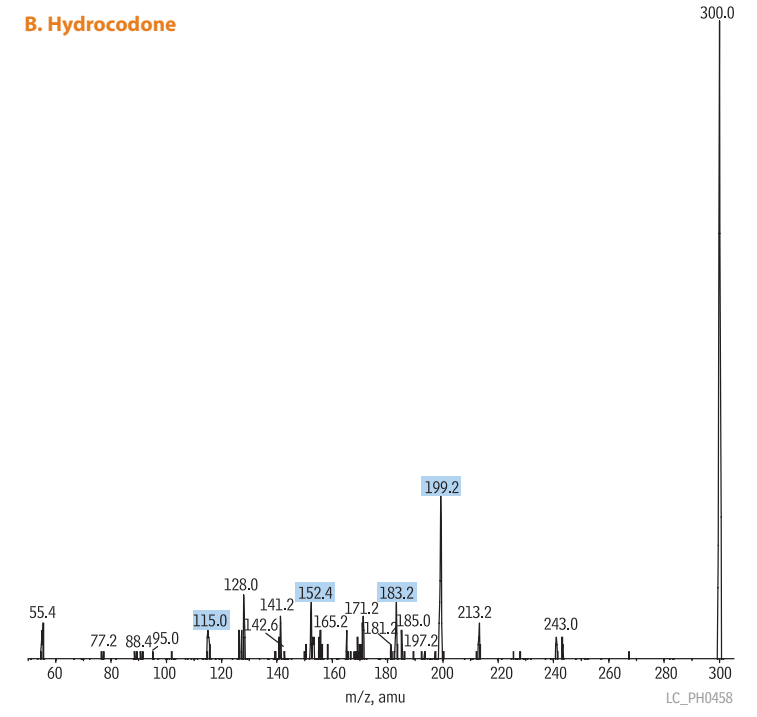
Table I +MRM Transitions for Opiates.

Mass Spectrometer Experiments:

Compound	Q1	Q3	Declustering Potential (V)	Collision Energy (V)
morphine	286	152	46	79
morphine	286	165	46	51
hydromorphone	286	185	46	41
hydromorphone	286	157	46	55
oxymorphone	302	227	36	37
oxymorphone	302	198	36	55
codeine	300	152	46	85
codeine	300	115	46	89
hydrocodone	300	199	46	39
hydrocodone	300	128	46	69
oxycodone	316	240	31	39
oxycodone	316	256	31	33
6-monoacetylmorphine	328	211	51	55
6-monoacetylmorphine	328	193	51	35

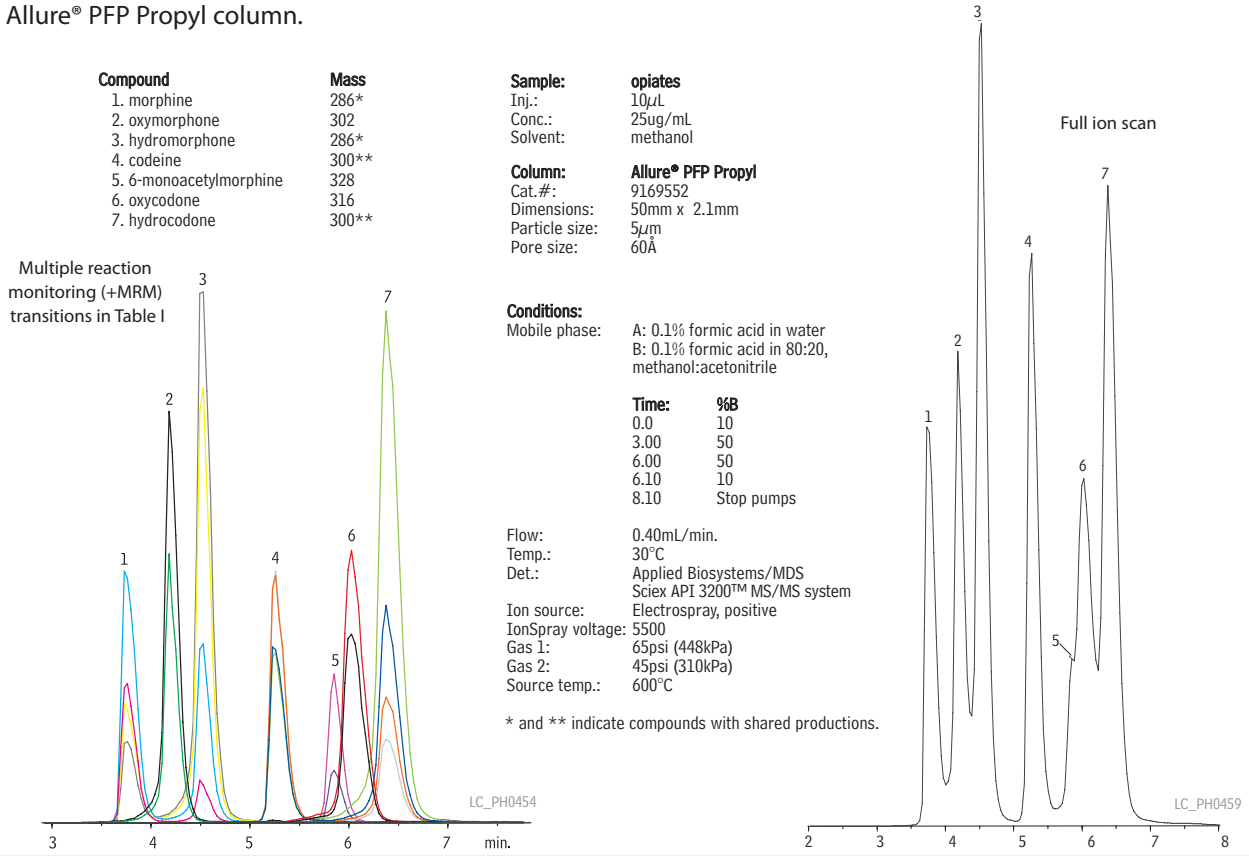
Figure 1 Codeine and hydrocodone share product ions and must be separated chromatographically.





For conditions see Figure 2.

Figure 2 Fully resolve opiates with shared product ions on (morphine/hydromorphone and codeine/hydrocodone an Allure® PFP Propyl column.



After mass spectrometry conditions were optimized for each compound, the resulting mass spectra were used to generate +MRM (multiple reaction monitoring) methods. Since MS/MS was used, we were able to target two +MRM transitions per compound to verify the identity of each compound. Table I shows the +MRM transitions and the mass spectrometer conditions. Standards contained morphine, hydromorphone, oxymorphone, codeine, hydrocodone, oxycodone, and 6-monoacetylmorphine (6-MAM) in methanol. The on-column concentration used for column evaluations was 250ng for all compounds.

Although two +MRM transitions were targeted for each compound, some compounds, such as codeine and hydrocodone, shared all monitored product ions (Figure 1). Since these compounds have similar mass spectra, two peaks appear in the extracted ion chromatograms. This made it necessary to separate codeine and hydrocodone chromatographically and identify compound peaks by retention time. Morphine and hydromorphone present the same challenge. Of the stationary phases tested, pentafluorophenyl propyl phase (Allure® PFP Propyl column) produced the best chromatographic separation and peak shape. Baseline resolution of opiates that shared the same product ions was achieved on an Allure® PFP Propyl column in a total analysis time of 7 minutes (Figure 2). Mobile phase gradient and composition had a significant effect on peak shape and resolution (data not shown) and optimized analytical conditions were used.

The Allure® PFP Propyl column, coupled with an LC/MS/MS, produced positive identification of opiates while reducing sample preparation time and keeping analysis time short. Use of the Allure® PFP Propyl column and the LC/MS/MS method shown here can increase sample throughput and is recommended for routine opiates analysis.

Acknowledgement
The authors wish to thank Applied Biosystems for supplying the Applied Biosystems/MDS Sciex API 3200™ MS/MS system used for this work.

Allure® PFP Propyl Columns (USP L43) Excellent Columns for LC/MS and ELSD

Physical Characteristics:

particle size: 5µm, spherical
pore size: 60Å
carbon load: 17%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

5µm Column, 2.1mm	cat. #
50mm	9169552
50mm (with Trident Inlet Fitting)	9169552-700

Guard Cartridges	qty.	cat. #
10 x 2.1mm	3-pk.	916950212
10 x 4.0mm	3-pk.	916950210
20 x 2.1mm	2-pk.	916950222
20 x 4.0mm	2-pk.	916950220

Exempted Drug of Abuse Reference Materials: Opiates & Metabolites

Concentration is µg/mL. Volume is 1mL/ampul.

Compound	CAS#	Solvent Code	Conc.	cat.#
codeine	76-57-3	PTM	1,000	34000
hydrocodone	34195-34-1	PTM	1,000	34002
hydromorphone	71-68-1	PTM	1,000	34063
morphine	6211-15-0	PTM	1,000	34006
oxycodone	124-90-3	PTM	1,000	34007
oxymorphone	76-41-5	PTM	1,000	34065

PTM=purge & trap grade methanol.

For a full product listing for these columns and reference materials, visit our website at **www.restek.com**.

Easy Transfer of HPLC Methods to UHPLC

Using Fully Scalable Pinnacle™ DB Columns

By Rick Lake, Pharmaceutical Innovations Chemist

- Methods on Pinnacle™ DB columns are easily transferred from 3 and 5µm to 1.9µm, allowing faster analysis without losing separation quality.
- Pinnacle™ DB columns are 100% Restek manufactured–from base silica to final packed column.
- Restek offers the widest selection of stationary phases for UHPLC—more choices mean better selectivity for your analytes.

Ultra High Pressure Liquid Chromatography (UHPLC) is a rapidly growing technique that produces significantly faster analysis times compared to conventional HPLC. While transferring HPLC methods to UHPLC can increase sample throughput, comparable method parameters must be used to maintain equivalent separations. Here we review which column properties and operating conditions should remain consistent and which need to be optimized in order to maintain selectivity.

In this example, we will perform a scale-down method transfer for sulfonamides (Figure 1). For optimal selectivity and faster analysis times, we used a Pinnacle™ DB Biphenyl stationary phase for this application (Figure 2). When performing a scale-down procedure, column pore size, carbon load, and support material must remain the same. Changes to other parameters can be made using a few simple calculations. Let's go through them sequentially.

Adjusting Column Size

The first calculation determines the appropriate column length. Keeping the same column length while decreasing the particle size increases the number of theoretical plates. Therefore, column length can be shortened without losing resolution. By adjusting the column length properly, using Equation 1, we can maintain the same separation.

Adjusting Injection Volume

Once we have determined the proper column length, we can calculate injection volume. Decreasing the column internal diameter and length decreases the overall column volume and sample capacity. Therefore, we must alter the injection volume as described in Equation 2. Note that since overall column volume has decreased, it is important to match the sample solvent to the starting mobile phase composition. Mismatched sample solvents can cause irreproducible retention times, efficiencies, and even changes in selectivity.

Adjusting Flow rate

Next, flow rate must be adjusted to maintain comparable linear velocity through a column with smaller internal diameter. To maintain the same linear velocity (which is important in maintaining

Figure 1 Chemical structures for example sulfonamides.

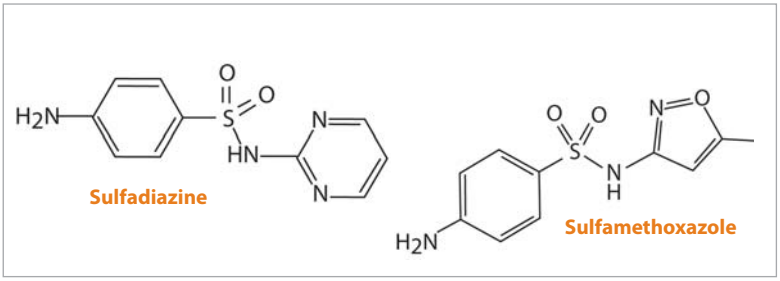
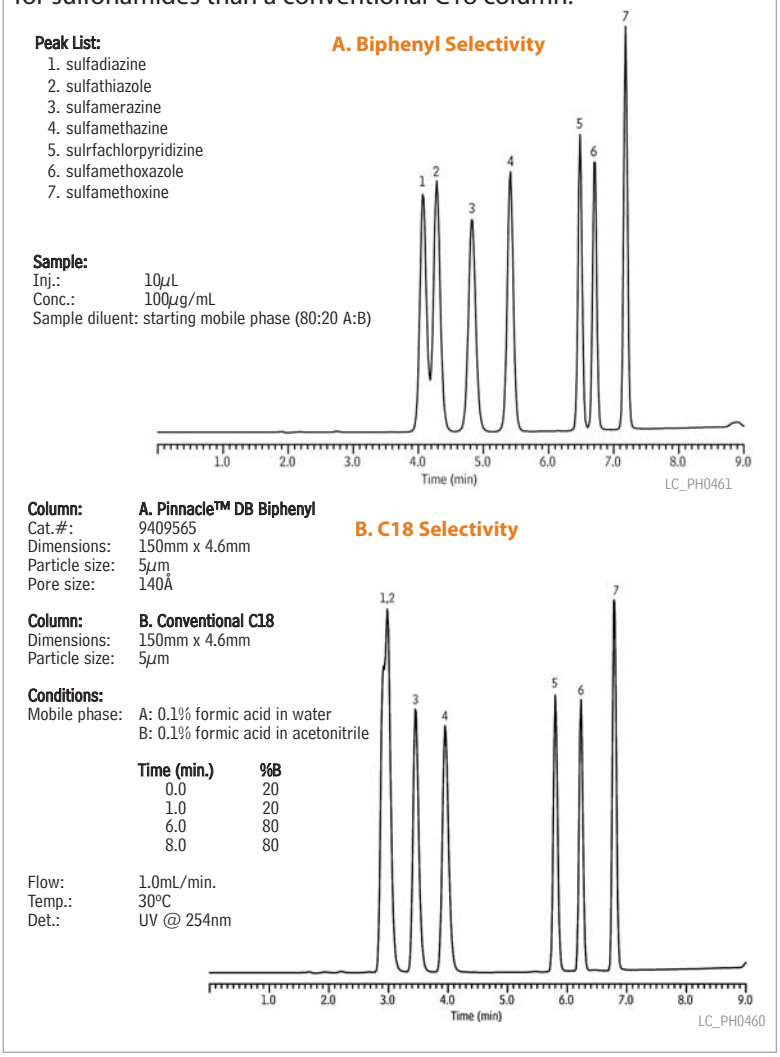


Figure 2 A 1.9µm Pinnacle™ DB Biphenyl column is more selective for sulfonamides than a conventional C18 column.



Equation 1 Adjusted column length can easily be calculated when scaling from HPLC to UHPLC.

$$L_{C2} = \frac{L_{C1} \cdot dp_2}{dp_1}$$

Example:

$$L_{C2} = \frac{150\text{mm} \cdot 1.9\mu\text{m}}{5\mu\text{m}}$$

$$L_{C2} = 57\text{mm}$$

L_C = Column Length
dp = Particle Size

Equation 2 Changing column dimensions requires an adjusted injection volume.

$$V_{I2} = V_{I1} \cdot \left(\frac{d_{C2}^2 \cdot L_{C2}}{d_{C1}^2 \cdot L_{C1}} \right)$$

Example:

$$V_{I2} = 10\mu\text{l} \cdot \left(\frac{2.1\text{mm}^2 \cdot 50\text{mm}}{4.6\text{mm}^2 \cdot 150\text{mm}} \right)$$

$$V_{I2} = 0.69\mu\text{l}$$

V_I = Injection Volume
L_C = Column Length
d_C = Column Diameter

Equation 3 Changing column internal diameter requires using an adjusted flow rate.

$$F_{C2} = \left(\frac{d_{C2}}{d_{C1}} \right)^2 \cdot F_{C1}$$

Example:

$$F_{C2} = \left(\frac{2.1\text{mm}}{4.6\text{mm}} \right)^2 \cdot 1.0\ \text{ml/min}$$

$$F_{C2} = 0.208\ \text{ml/min}$$

F_C = Column Flow
d_C = Column Diameter

Equation 4 When scaling down a gradient method, the time program needs to be adjusted.

$$t_{g2} = t_{g1} \cdot \left(\frac{F_{C1}}{F_{C2}} \right) \cdot \left(\frac{d_{C2}^2}{d_{C1}^2} \right) \cdot \left(\frac{L_{C2}}{L_{C1}} \right)$$

Example:

$$t_{g2} = 5\text{min.} \cdot \left(\frac{1.0\text{ml/min.}}{0.2\text{ml/min}} \right) \cdot \left(\frac{2.1\text{mm}^2}{4.6\text{mm}^2} \right) \cdot \left(\frac{50\text{mm}}{150\text{mm}} \right)$$

$$t_{g2} = 1.7\ \text{min}$$

t_g = Gradient Time
F = Column Flow
L_C = Column Length
d_C = Column Diameter

Pinnacle™ DB Biphenyl Columns (USP L11)

Physical Characteristics:

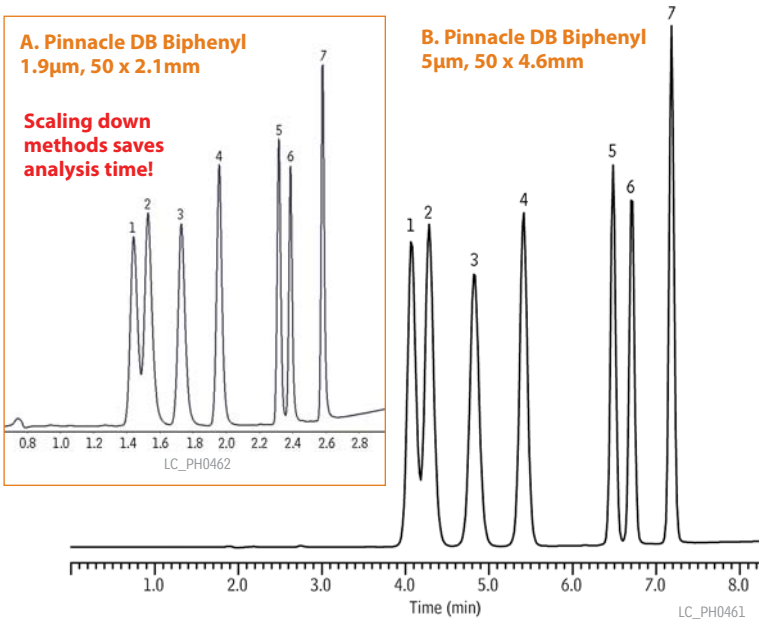
particle size:
1.9µm, & 5µm, spherical
pore size: 140Å
carbon load: 8%

endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

1.9µm Column, 2.1mm	cat. #
50mm	9409252
5µm Column, 4.6mm	**cat. #**
150mm	9409565

For a full product listing, including guard cartridges for these columns, visit our website at **www.restek.com**.

Figure 3 Restek's Pinnacle™ DB 1.9µm columns can easily be scaled from HPLC to UHPLC and vice versa.



Peak List:
1. sulfadiazine
2. sulfathiazole
3. sulfamerazine
4. sulfamethazine
5. sulrfachlorpyridizine
6. sulfamethoxazole
7. sulfamethoxine

Sample:
Inj.: 10µL
Conc.: 100µg/mL
Sample diluent: starting mobile phase (80:20 A:B)

Column: A. 1.9µm Pinnacle™ DB Biphenyl
Cat.#: 9409252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm

Column: B. Pinnacle™ DB Biphenyl
Cat.#: 9409565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 140Å

Conditions:
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

Time(min.)	%B
0.0	20
1.0	20
6.0	80
8.0	80

Flow: 1.0mL/min.
Temp.: 30°C
Det.: UV @ 254nm

efficiencies), flow rates must be decreased. Also, since smaller particle sizes give rise to higher optimal linear velocities, isocratic flow rates should be calculated with particle size taken into account. In this example, a gradient elution was used and, therefore, particle size was not included in the equation. Equation 3 can be used to estimate the adjusted flow rate needed for equivalent chromatography. Also, note that since <2µm particle sizes are less affected by flow rate, faster flow rates can be used in isocratic systems without detrimental effects on peak efficiency.

Adjusting Time Program

After determining the proper column length, injection volume, and flow rate, we can calculate the time needed for gradient or step elutions. As an analytical method is scaled down, the time program also needs to be scaled down to keep the phase interactions the same. Time can be adjusted using Equation 4.

Conclusion

After determining the equivalent conditions for scaling-down the analysis of sulfonamides, we can see that the separations are equivalent, while the analysis time was greatly reduced (Figure 3). By following the procedure described here to ensure that the columns are equivalent, scaling analytical procedures from HPLC to UHPLC can easily be accomplished using Pinnacle™ DB columns.

Complete Resolution of 13 Carbonyls as DNPH Derivatives

Using the New Allure® AK HPLC Column

By Randy Romesberg, HPLC Innovations Chemist, and Becky Wittrig, Ph.D, HPLC Product Marketing Manager

- Superior separation of difficult carbonyls—including butyraldehyde and methyl ethyl ketone—compared to C18 columns.
- Allows the use of a simple water:acetonitrile mobile phase for easier preparation and waste disposal.
- Significantly faster run time than conventional C18 columns—less than 12 minutes.

Carbonyls are collected and measured from a variety of samples, including air, exhaust, and cigarette smoke. For example, the California Air Resources Board (CARB) was established in 1967 to address many aspects of air pollution, including air quality problems caused by motor vehicles. CARB Method 1004 is used by the automotive industry to monitor a range of carbonyl compounds (e.g., aldehydes and ketones) in engine exhaust.

Sample collection cartridges impregnated with 2,4-dinitrophenylhydrazine (DNPH), or impingers containing acidified DNPH, are used to sample air or exhaust. After conversion to DNPH derivatives, the carbonyl compounds are collected and analyzed by HPLC. Since the DNPH derivatives absorb strongly at 360nm, detection limits below 1ppm are easily achievable. The original CARB method uses two C18 columns in series for analysis, although other columns can be used as long as they provide an equivalent or better separation.

The new Allure® AK HPLC column was developed specifically for the analysis of aldehydes and ketones. With a single 200mm column, excellent resolution of these compounds can be achieved in less than 12 minutes (Figure 1). While C18 phases often cannot separate butyraldehyde and methyl ethyl ketone (MEK), the Allure® AK column shows excellent resolution of this difficult pair. In addition, a simple mobile phase gradient of water:acetonitrile can be used with the Allure® AK column, while C18 phases require the addition of THF to achieve acceptable resolution.

When analyzing aldehydes and ketones by HPLC, such as the carbonyls specified in CARB method 1004, the new Allure® AK column will give you the resolution and fast analysis times that you require.

Figure 1 Excellent resolution of carbonyl compounds in less than 12 minutes, using the Allure® AK HPLC column and a simple water:acetonitrile mobile phase gradient.

Peak DNPH derivatives of:	Ret. Time (min.)
1. formaldehyde	4.74
2. acetaldehyde	5.78
3. acrolein	6.86
4. acetone	7.09
5. propionaldehyde	7.31
6. crotonaldehyde	8.19
7. methacrolein	8.55
8. butyraldehyde	8.79
9. methyl ethyl ketone	9.06
10. benzaldehyde	10.03
11. valeraldehyde	10.39
12. *m*-tolualdehyde	11.08
13. hexaldehyde	11.36

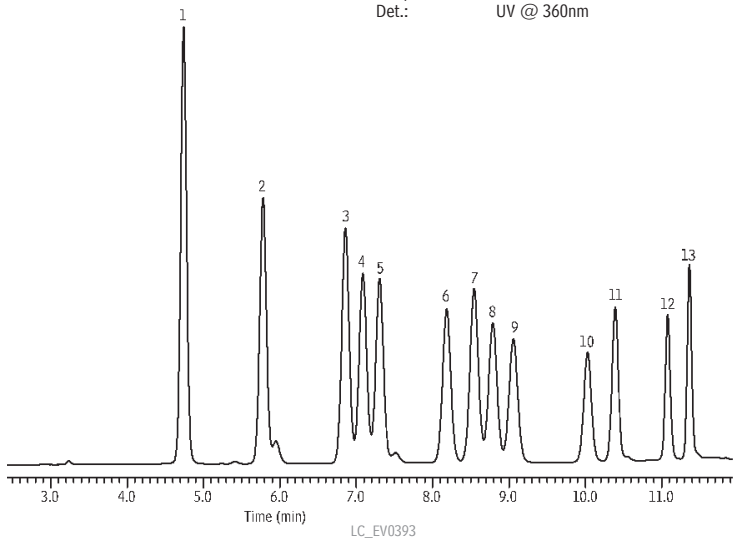
Sample:
Inj.: 10µL
Conc.: 3µg/mL each analyte, as aldehyde/ketone
Sample diluent: acetonitrile

Column: Allure® AK
Cat.#: 9159525-700
Dimensions: 200mm x 4.6mm
Particle size: 5µm
Pore size: 60Å

Conditions:
Mobile phase: A) water : B) acetonitrile

Time (min.)	%B
0	60
8	70
10	100

Flow: 1.5mL/min.
Temp.: 30°C
Det.: UV @ 360nm



CARB 1004 Aldehyde/Ketone-DNPH Calibration Standard (13 components)

acetaldehyde-2,4-DNPH
acetone-2,4-DNPH
acrolein-2,4-DNPH
benzaldehyde-2,4-DNPH
n-butyraldehyde-2,4-DNPH
crotonaldehyde-2,4-DNPH
formaldehyde-2,4-DNPH
hexaldehyde-2,4-DNPH
methacrolein-2,4-DNPH
methyl ethyl ketone-2,4-DNPH
propionaldehyde-2,4-DNPH
m-tolualdehyde-2,4-DNPH
valeraldehyde-2,4-DNPH

3µg/mL each in acetonitrile, 1mL/ampul
cat. # 33093

Allure® AK Columns

Physical Characteristics:

particle size: 5µm
pore size: 60Å
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

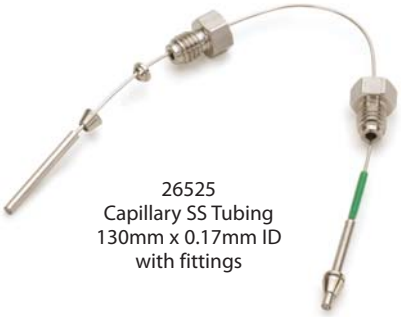
	cat. #
5µm Column, 3.2mm, 200mm (with Trident Inlet Fitting)	9159523-700
5µm Column, 4.6mm, 200mm (with Trident Inlet Fitting)	9159525-700

Guard Cartridge	qty.	cat. #
10 x 4.0mm	3-pk.	915950210

Now Available!
Capillary Stainless Steel Tubing Assemblies

For Agilent HPLC systems

By Becky Wittrig, Ph.D, HPLC Product Marketing Manager



26525
Capillary SS Tubing
130mm x 0.17mm ID
with fittings

- Precut, micropolished tubing and preseated fittings for quick, easy maintenance of your systems.
- Designed and tested for Agilent HPLC systems.
- Restek offers a full range of high-quality replacement parts for your HPLC systems.

Capillary Stainless Steel Tubing Assemblies for Agilent HPLC Systems

Description	Similar to Agilent part #	qty.	cat.#
Capillary SS Tubing, 130mm x 0.17mm ID, with fittings	01090-87305	ea.	26525
Capillary SS Tubing, 800mm x 0.17mm ID, with fittings	01078-87305	ea.	26526
Capillary SS Tubing, 180mm x 0.17mm ID, with fittings	G1313-87305	ea.	26527
Capillary SS Tubing, 700mm x 0.25mm ID, with fittings	01018-67305	ea.	26528
Capillary SS Tubing, 700mm x 0.25mm ID, with fittings	01078-87306	ea.	26529
Seat Capillary, SS Tubing, 0.17mm ID	01078-87303	ea.	26530
Capillary SS Tubing, 105mm x 0.17mmID	5021-1816	ea.	26531
Mixing Capillary Assembly	G1312-67302	ea.	26532
Capillary SS Tubing, Valve to Metering Head	G1313-87301	ea.	26533
Capillary SS Tubing, 150mm x 0.17mm ID	5021-1817	ea.	26534
Capillary SS Tubing, 280mm x 0.17mm ID	5021-1818	ea.	26535
Capillary SS Tubing, 400mm x 0.17mm ID	5021-1819	ea.	26536
1/16" SS Fitting, Front and Back Ferrules	5062-2418	10-pk.	26537

26530
Seat Capillary, SS Tubing
0.17mm ID

26527
Capillary SS Tubing
180mm x 0.17mm ID
with fittings

26533
Capillary SS Tubing
Valve to Metering Head

26529
Capillary SS Tubing
700mm x 0.25mm ID
with fittings

26532
Mixing Capillary
Assembly

26537
1/16" SS Fitting
Front and Back Ferrules

Sulfinert® Treated Systems Preserve ppb Levels of Active Sulfur Compounds

By Gary Barone, Martin Higgins, David Smith (Restek Performance Coatings Division);
Shawn Rowan and Warren J. Gross (O'Brien Corporation); and Phil Harris (Harritec LLC.)

- Sulfinert® treatment prevents adsorption of sulfur compounds, ensuring representative sampling.
- Improved accuracy allows precise control of downstream processes, for better efficiency and profitability.
- Shorter cycles translate directly into increased sample throughput.

Many volatile sulfur compounds adsorb strongly to metal surfaces in sampling, storage, and transfer apparatuses. In addition to causing inaccurate values, adsorption can prolong analysis cycle times. To compare quantitative losses of active sulfur species, we sampled, stored, and transferred low ppmv to low ppbv concentrations of active sulfur gases, using control (untreated) and Sulfinert® treated system components.

Preventing Sulfur Compound Losses During Storage

Figure 1a depicts results from a comparison in which a gas containing 17ppbv of hydrogen sulfide was stored for 7 days in untreated or in Sulfinert® treated stainless steel sample cylinders. The response ratio for hydrogen sulfide, relative to a stable reference material, dimethyl sulfide, is steady at approximately 1:1 for at least 7 days in Sulfinert® treated cylinders. The data show a Sulfinert® treated system will reliably store ppb levels of the active sulfur-containing compound during transport from the sampling site to the analytical laboratory. In contrast, hydrogen sulfide degraded rapidly in the untreated cylinder, and was lost totally within 24 hours. In a similar study in which gas containing 18.8ppbv methyl mercaptan was stored for 60 hours in Sulfinert® treated sample cylinders, recovery of the active sulfur compound was equally high relative to the stable reference material, dimethyl sulfide, as shown in Figure 1b.

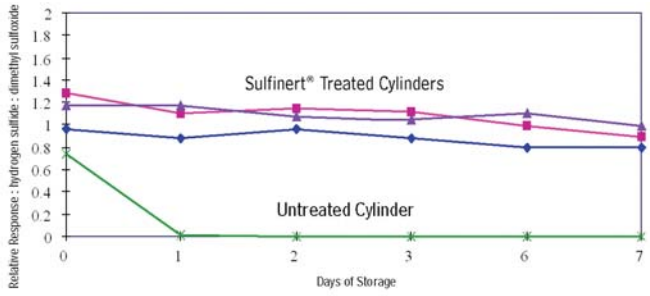
Sample Transfer: Adsorption of Sulfur Compounds to Tubing

Comparison of Sulfinert® treated electropolished stainless steel tubing (TrueTube® EPS tubing, O'Brien Corporation, St. Louis, MO), untreated electropolished stainless steel tubing (TrueTube® EP tubing, O'Brien Corporation), and raw commercial grade 316L stainless steel tubing showed Sulfinert® treated electropolished tubing has the inertness necessary for quantitatively transferring low ppmv to low ppbv concentrations of sulfur compounds.

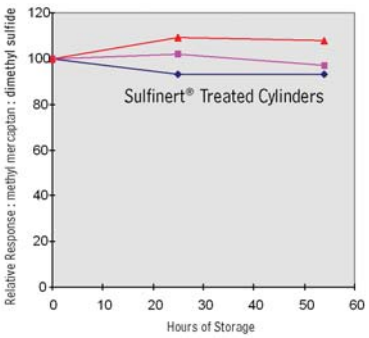
To determine whether an active sulfur-containing compound in a gas stream would adsorb to active sites on the transfer tubing surface, we monitored the length of time that elapsed before recovery values for a sulfur compound exiting a 100-foot (30.5-meter) length of tubing were stable and accurate, using helium containing 0.500ppmv methyl mercaptan as the test material, at a flow rate of 40cc/minute. Figure 2 shows Sulfinert® treated electropolished tubing did not adsorb methyl mercaptan to any measurable extent, delivering a representative sample with no delay. When adsorption of sulfur-containing compounds is prolonged, desorption from the surface also is slow. This "memory" of adsorbed compounds can cause long delays in re-equilibrating a sample pathway. In Figure 3, Sulfinert® treated tubing shows the lowest retention of sulfur compounds, by several orders of magnitude. Samples can be evaluated,with accurate results and with no delay between them.

Figure 1 Sulfur compounds are stable in Sulfinert® treated cylinders, assuring a representative sample is delivered.

a) 17ppbv hydrogen sulfide in 500mL cylinders



b) 18.8ppbv methyl mercaptan in 300mL cylinders



The authors thank Shell Research and Technology Centre, Amsterdam, for the data used in evaluating sulfur gas uptake and the memory effects of tubing substrates.

Figure 2 Sulfinert® treated tubing (red) does not adsorb methyl mercaptan (500ppbv), giving accurate results with no delay.

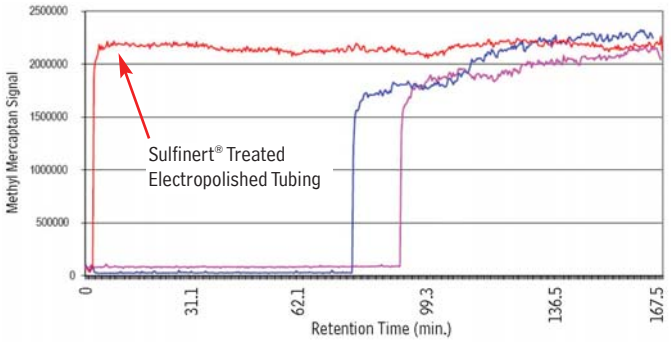
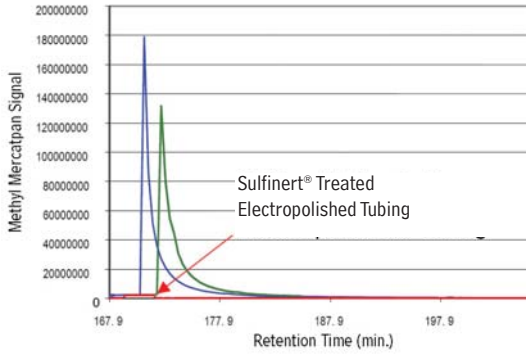


Figure 3 Sulfur memory is prolonged in raw commercial grade stainless steel tubing, increasing cycle time and reducing accuracy.



Conclusion

We obtained more accurate data, with no delay between samples, by using Sulfinert® treated electropolished tubing in the sampling-storage-transport system. In contrast, we obtained significantly less accurate data, with delays of more than two hours between samples, by using untreated tubing. Improved accuracy and reliability of data for sulfur mean downstream processes can be more precisely controlled, with associated cost savings. Shorter cycles translate directly into more samples collected and analyzed in a given period of time. Analysts charged with monitoring sulfur levels can significantly improve efficiency and profitability by using Sulfinert® treated tubing and components.

Acknowledgement

The authors thank O'Brien Corporation for arranging the research studies and supplying the electropolished tubing. To learn more about O'Brien Corporation, visit **www.obrien-analytical.com**.

This article is an analytical summary. For the complete study, visit us at www.restek.com or call 800-356-1688, ext. 5 and request lit. cat.# 59082.

Sulfinert® Treated Alta-Robbins Sample Cylinder Valves

- All wetted parts are Sulfinert® treated for inertness.
- Compatible with Sulfinert® treated Swagelok® sample cylinders.
- Large, durable, Kel-F® seat ensures leak-free operation; temperature range: -40°C to 120°C.

Description	qty.	cat.#
1/4" NPT Exit	ea.	21400
1/4" Compression Exit	ea.	21401
1/4" NPT with Dip Tube*	ea.	21402
1/4" NPT with 2850psi Rupture Disc	ea.	21403
1/4" NPT Male Inlet x 1/4" Female Outlet with 2850psi Rupture Disc	ea.	21404

*To order catalog #21402 (Sulfinert Alta-Robbins Sample Cylinder Valve, 1/4" NPT with Dip Tube), please call Customer Service at 800-356-1688, ext. 3, or contact your Restek representative. Specify dip tube length or % outage when ordering (maximum length = 5.25"/ 13.3cm). Note: End of part will not be treated after cutting tube to length.

Sulfinert® Treated Swagelok® Sample Cylinders

Ideal for collecting and storing samples, such as natural gas or beverage-grade carbon dioxide, because active compounds remain stable during transport.

Size	qty.	cat.#
75cc	ea.	24130
150cc	ea.	24131
300cc	ea.	24132
500cc	ea.	24133
1000cc	ea.	24134
2250cc	ea.	21394

Performance Testing VOC Audit Sample for Air Toxics

By Irene DeGraff, Product Marketing Manager

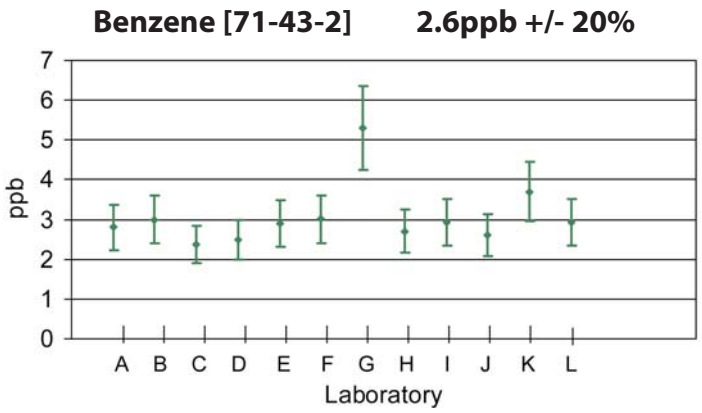
- Demonstrate your lab's competence and compare anonymously to competitors.
- Improve your quality program.
- Applicable to US EPA, ASTM, and DIN EN ISO methods.

Restek is pleased to be a source of Performance Testing/VOC Audit Samples for the Spectra Gases testing program. This is an on-going testing program in which laboratories, or other users of VOC gas standards, are able to evaluate their own capabilities and compare their performance with that of other air toxic labs. US EPA methods TO-14A, TO-15, and TO-17 are used to determine volatile organic compounds in ambient air. Without an air analysis accreditation program available, a performance testing program by Spectra Gases provides an invaluable tool allowing labs to demonstrate their competence with these methods.

As a participant in the program, you will receive a disposable cylinder directly from Spectra Gases containing multiple unknown VOC components at varying concentrations that are to be identified, quantified, and reported via the Spectra Gases PT Audit Program form. The results will then be published and distributed for peer review. The report provided to participants includes a program methodology overview, compound list with actual concentration, and individual and summary results for each compound (Figure 1). To ensure confidentiality, all participating laboratories will be anonymous and only the individual laboratory will know their own results.

The audit sample will be shipped to all labs during the same period, once a year during the fourth quarter. Analytical results need to be returned to Spectra Gases by January 30 to be included in this statistical report.

Figure 1 Example graph from program report lets you anonymously compare your lab to competitors.



Average = 3.06 Std. Dev. = 0.78

cylinder **design**

TO-14A/TO-15/TO-17 Peformance Test Standard:

Size: 5A disposable (3.2" x 12")
Volume/Pressure: 170L @ 2,015psi
CGA 180 outlet fitting
Weight: 2.2lbs.

TO-14A/TO-15/TO-17 Performance Test Standard

170 liters @ 2,015psi
cat. # 34560

Datapack not available.

Don't miss out on this opportunity to confirm your competence in air toxic analysis—order now to participate!

Affected by the Helium Shortage?

Switch Your GC Carrier Gas to Hydrogen

By Al Carusone, Technical Service

Faced with helium shortages and prices that continue to soar upwards like a runaway party balloon? Consider switching your carrier gas to hydrogen. Hydrogen is a safe alternative to helium, and high quality gas is readily available from either cylinders or hydrogen generators.

Switching to hydrogen is cost-effective and can improve GC performance. Hydrogen provides shorter (by half if running isothermally) analysis times than helium and many times yields overall better separations. Also, with splitless injection, hydrogen's higher velocities carry the solutes from the inlet to the column faster and more efficiently, decreasing the potential for band broadening. However, while hydrogen is a great choice for most GC work, it is difficult to remove from the MS source and energizing the source without the pumps running could cause an explosion. Therefore, hydrogen is not typically recommended for mass spectrometry applications.

The most common concern when considering a switch to hydrogen is the risk of explosion. Safety depends largely on whether a GC is back pressure regulated or head pressure regulated. Generally older instruments use a pressure regulator located upstream of the injection port (head pressure regulated). In the event of a leak the upstream pressure regulator will maintain pressure, but overall flow can increase dramatically. This situation can lead to an explosion if hydrogen carrier gas fills the hot GC oven. Check your instrument manual to make sure your instrument is either back pressure regulated or equipped with safety features to prevent major leaks. Many instrument companies also are now recognizing the benefits of using hydrogen as a carrier gas and are manufacturing their latest models with additional safety features designed to prevent hydrogen build-up and reduce the risk of explosion.

Hydrogen is available in cylinders, but it can also be produced on-site using a hydrogen generator. Hydrogen generators are much safer and more cost-effective than high pressure cylinders. All hydrogen generators offered by Restek are equipped with built-in sensing circuits that will automatically shut down the generator in the rare case that a leak is detected. Another advantage is that hydrogen generators produce hydrogen on-demand, meaning only small volumes (50-100mL) are stored at any one time. Producing hydrogen as it is consumed is much safer than using cylinders which each store up to 9,000 liters.

Hydrogen is a safe, dependable alternative to helium, and hydrogen generators are an ideal way to produce the hydrogen your lab requires. They include great safety features and are cost-effective; based on cylinder savings alone, a generator pays for itself in only one or two years. If your lab has been affected by the current helium shortage and you are considering a switch to hydrogen, see the titles in the sidebar for more information. You'll find switching to hydrogen and using a hydrogen generator to supply your lab offers significant financial and performance benefits.

See page 18 & 19 for our listing of Hydrogen Generators.

Get More!

Information on switching from helium to hydrogen.

Visit us on-line at **www.restek.com/outofgas** to see the following technical articles:

"Helium Supply Deflates, Gas Prices Rise Quickly"

"Parker Hydrogen Generators, Is Your Lab Wasting Money on Bottled Gas?"

"Using Hydrogen for Gas Chromatography"

"Loctite Saves Almost $20,000 per Year by Generating Its Own Hydrogen for GC/FIDs"

"Parker Balston® Hydrogen Generators Fast Facts"

Parker Balston® PEM Hydrogen Generators

By Sue Benes, GC Accessories Product Marketing Manager

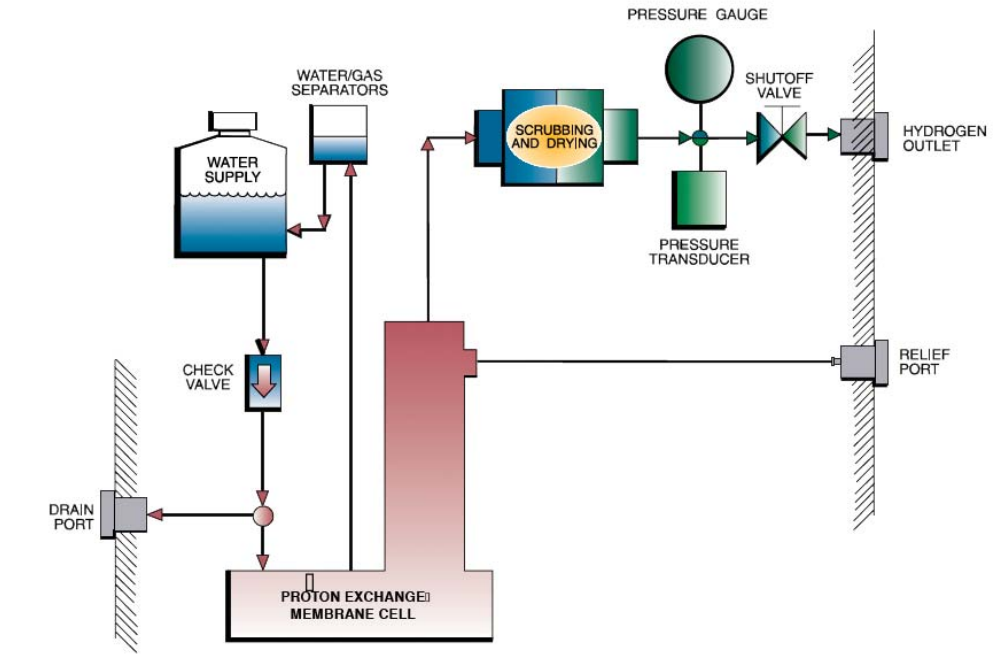
- Cost effective, convenient, and safe alternative to high-pressure gas cylinders.
- Reliably generate 99.9995% pure hydrogen for better chromatography.
- Quick and easy to maintain; require only minutes a year!

Fuel-grade high purity hydrogen generators are a safe, cost-effective alternative to high-pressure gas cylinders. Parker Balston® hydrogen generators are engineered for safety and feature a built-in sensing circuit which shuts down the generator automatically if a hydrogen leak is detected. These generators are also designed for performance and convenience. They include an exclusive water management system and control circuitry to maximize uptime, and also feature indicator lighting, which allows at-a-glance status checks and water level monitoring. Hydrogen generators offer enhanced safety and convenience, and are cost-effective. Based on cylinder gas savings alone, a hydrogen generator pays for itself in only one or two years.

Parker Balston® hydrogen generators are reliable and easy to use and maintain. Deionized water is all that is required to generate hydrogen for weeks of continuous operation. Each generator has an output capacity of up to 510cc/minute—enough to supply 99.9995% pure hydrogen for several GC-FID systems. The new Proton Exchange Membrane (PEM) cell eliminates the need for liquid electrolytes. Maintenance requires only a few moments a year—no inconvenient, extended downtime. Simply change the filters every six months, the hydration pump biannually, and the desiccant cartridge whenever it turns from beige to clear.

These units are compact, requiring only one square foot of bench space, and come with a set of universal power adaptors for U.S., European and Asian plug types. Produced and supported by an ISO 9001 registered organization, Parker Balston® hydrogen generators are the first built to meet the toughest laboratory standards in the world: CSA, UL, cUL, and CE Mark.

Hydrogen generator technology



All Parker Balston® hydrogen generators meet NFPA requirements and OSHA 1910.103 regulations governing the storage of hydrogen.

new & **improved**!

Hydrogen generators now come with a set of universal power adapters for US, European, and Asian plug types.

for **more** info

Looking for more information on Parker Hydrogen Generators? Download free technical literature from **www.restek.com**.

Fast Facts Lit. Cat.# 580053A

Principal Specifications

Model Number	H2PEM-100 (cat. #23065)	H2PEM-165 (cat. #23066)	H2PEM-260 (cat. #23067)	H2PEM-510 (cat. #23068)
Purity:	99.9995%	99.9995%	99.9995%	99.9995%
Flow Rates:	100cc/min	165cc/min	260cc/min	510cc/min
Outlet Port:	1/8" compression	1/8" compression	1/8" compression	1/8" compression
Electrical:	100-230Vac/50-60Hz	100-230Vac/50-60Hz	100-230Vac/50-60Hz	100-230Vac/50-60Hz
Delivery Pressure:	10-100 psig ± 1 psig	10-100 psig ± 1 psig	10-100 psig ± 1 psig	10-100 psig ± 1 psig
Shipping Weight:	40lb (18kg) dry	40lb (18kg) dry	40lb (18kg) dry	40lb (18kg) dry
Dimensions:	17.12"H x 13.46"W x 17.95"D (43.48cm x 34.19cm x 45.6cm)	17.12"H x 13.46"W x 17.95"D (43.48cm x 34.19cm x 45.6cm)	17.12"H x 13.46"W x 17.95"D (43.48cm x 34.19cm x 45.6cm)	17.12"H x 13.46"W x 17.95"D (43.48cm x 34.19cm x 45.6cm)

Description	Capacity	qty.	cat.#
Hydrogen Generator H2PEM-100	100cc/min.	ea.	23065
Hydrogen Generator H2PEM-165	165cc/min.	ea.	23066
Hydrogen Generator H2PEM-260	260cc/min.	ea.	23067
Hydrogen Generator H2PEM-510	510cc/min.	ea.	23068
Replacement and Maintenance Components for Hydrogen Generators (for all models listed above)			
Replacement Desiccant Cartridge for H2PEM Generators		ea.	23069
6-Month Maintenance Kit for H2PEM Generators (Includes: 1 deionizer cartridge, 1 water filter, 3 environmental filters)		kit	23070
24-Month Maintenance Kit for H2PEM Generators (Includes: 1 deionizer cartridge, 1 water filter, 3 environmental filters, 1 water level sensor, 1 water pump, and 1 desiccant cartridge)		kit	23071

Super-Clean™ Gas Filter and Base Plate Kits

Description	qty.	cat.#
Carrier Gas Cleaning Kit (includes mounting base plate, 1/8" inlet/outlet fittings, and oxygen/moisture/hydrocarbon Triple Gas Filter)	kit	22019
Fuel Gas Purification Kit (includes mounting base plate, 1/8" inlet/outlet fittings, and hydrocarbon/moisture Fuel Gas Filter)	kit	22021

All traps measure:
10 5/8" x 1 3/4" (27 x 4.4 cm)
Each base plate unit measures:
4" x 4" x 1 7/8"
(10.2 x 10.2 x 4.8 cm)

Replacement Gas Filters

Description	qty.	cat.#
Replacement Triple Gas Filter (removes oxygen, moisture and hydrocarbons)	ea.	22020
Replacement Fuel Gas Filter (removes moisture and hydrocarbons)	ea.	22022

Gas Filter Bundle Kit

- Kit includes two Fuel Gas Filters for FID fuel gases and one Triple Gas Filter for carrier gas.
- Ideal for use in combination with 3-position base plate—purchase separately.

Description	qty.	cat.#
Gas Filter Bundle Kit	kit	22031

Super-Clean™ Ultra-High Capacity Gas Filters

Description	qty.	cat.#
Ultra-High Capacity Hydrocarbon Filter	ea.	22030
Ultra-High Capacity Moisture Filter	ea.	22028
Ultra-High Capacity Oxygen Filter	ea.	22029

Filter Base Plates

- Standard base plate fittings are 1/8". To adapt to 1/4", order 1/8" to 1/4" tube-end unions.
- Base plates fit all Super-Clean™ gas filters listed above.

Description	qty.	Brass cat.#
Single-Position Filter Base Plate	ea.	22025
2-Position Filter Base Plate	ea.	22026
3-Position Filter Base Plate	ea.	22027

Warm Up Before You Run

Why conditioning your inlet parts after maintenance is good practice

By Scott Grossman, GC Accessories Chemist

- Eliminate background peaks and avoid costly reanalysis.
- Improve reproducibility and system performance.
- Demonstrate system cleanliness.

Every good coach tells athletes to warmup before they run to make sure the body is primed for optimum performance. The same principle applies to maintaining your gas chromatograph—time spent warming up the analytical system after maintenance pays big dividends by improving accuracy and reducing the need for reanalysis. No matter whose products you purchase, inlet parts, just like columns, require a brief conditioning before they are ready for analytical work. Although it is tempting to save time by jumping directly into sample analysis after maintenance, warming up your system helps you ensure accurate results the first time. In this article, we will highlight inlet liners as a perfect example of the need to condition your inlet after maintenance to avoid costly coelutions, irreproducible results, and avoidable reanalysis.

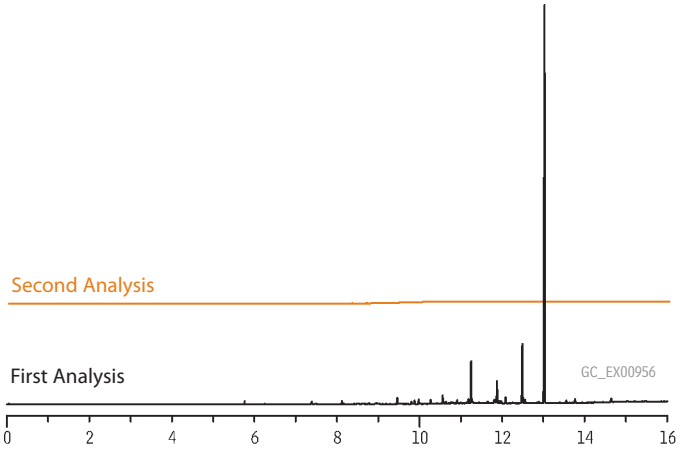
Sources of Contamination

Even the best liner can exhibit a small bleed pattern if it is used immediately after installation. Common sources of contaminants that can cause bleed include plastic packaging (e.g. phthalates used to make plastics more flexible) and fatty acids from finger oils. To evaluate bleed from contaminated liners, we first established a clean baseline with a control liner, then installed a test liner, and ran the instrument without making an injection. Figure 1 illustrates the effect of handling an inlet liner with bare hands. Even some gloves will impart hydrocarbon contamination that can be very prominent and persistent (Figure 2). So, care needs to be taken when handling your new liners. Handling liners with clean forceps or lint-free technical wipes is a good way to prevent liner contamination.

Reduce Noise by Conditioning Your System

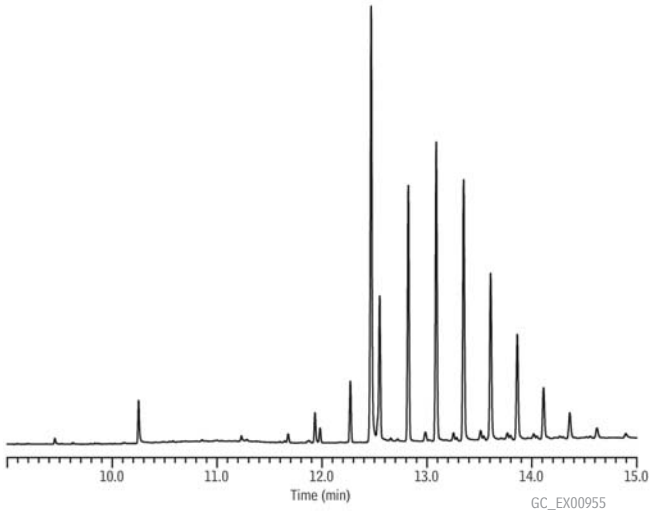
This contamination, also called background "noise," can be eliminated simply by conditioning the GC system prior to use. You can condition the entire inlet a variety of ways. One suggestion is to make a few preliminary runs using the analytical method parameters (inlet temperature, oven program, etc.) to be used in the subsequent analyses. We evaluated several commercially available liners and determined that liner bleed generally will be gone by the second or third run (Figure 3). An

Figure 1 Eliminate contamination peaks from finger oils by warming up the system prior to sample analysis.



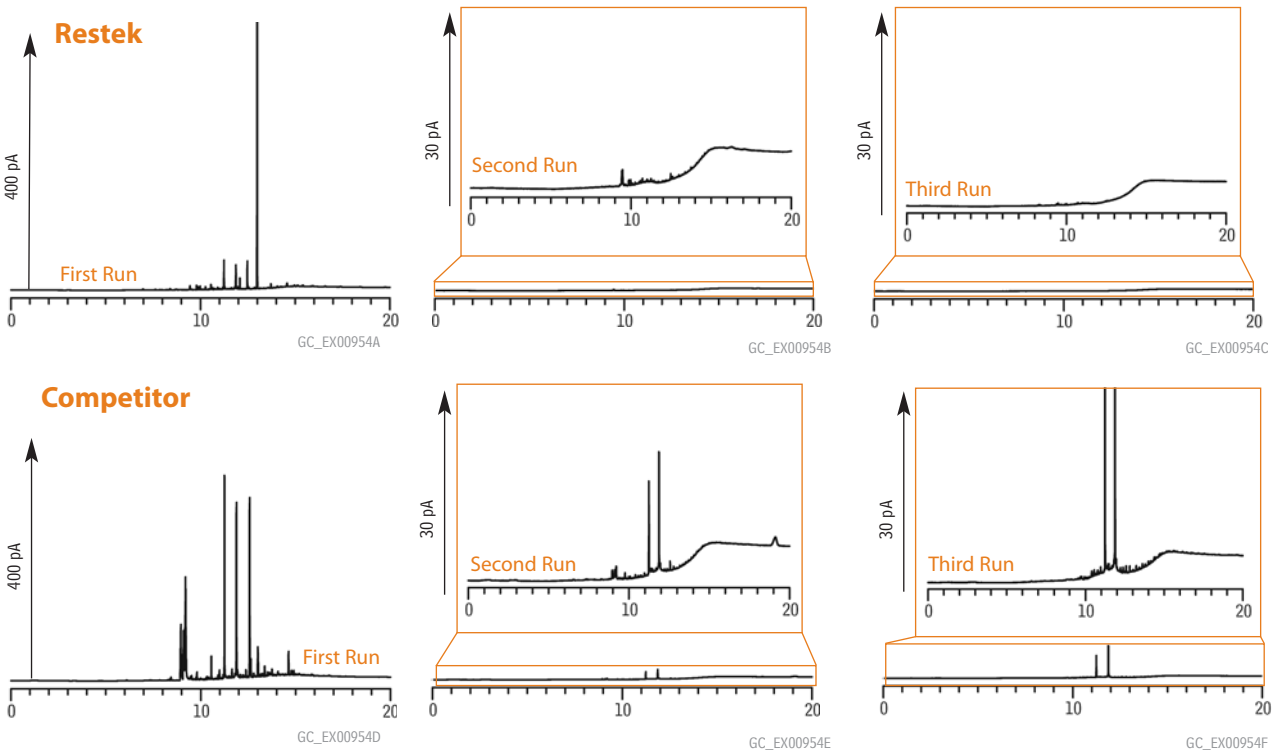
Column:	Rxi®-5ms, 30m, 0.32mm, 0.25µm (cat.# 13424)
Sample:	no sample injected; instrument ran the method conditions with no injection.
Inj.:	no injection; splitless mode (hold 0.5 min., split flow 40mL/min.) 4mm split with wool inlet liner, IP deactivated (cat.# 20782).
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	5.0mL/min.
Oven temp.:	50°C (hold 5 min.) to 320°C @ 30°C/min. (hold 10 min.)
Det.:	FID @ 330°C

Figure 2 Hydrocarbon peaks from nitrile gloves are another example of contamination from maintenance activities that can be eliminated by warming up the system.



Column:	Rxi®-5ms, 30m, 0.32mm, 0.25µm (cat.# 13424)
Sample:	no sample injected; instrument ran the method conditions with no injection.
Inj.:	no injection; splitless mode (hold 0.5 min., split flow 40mL/min.) 4mm single gooseneck liner with wool, IP deactivated (cat.# 22406).
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	5.0mL/min.
Oven temp.:	50°C (hold 5 min.) to 320°C @ 30°C/min. (hold 10 min.)
Det.:	FID @ 330°C

Figure 3 Warmup your system and invest in quality liners to reduce noise and improve accuracy.

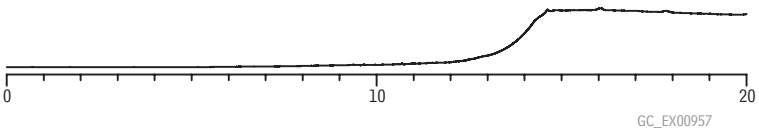


Column: Rxi®-5ms, 30m, 0.32mm, 0.25µm (cat.# 13424)
Sample: no sample injected; instrument ran the method conditions with no injection.
Inj.: no injection; splitless mode (hold 0.5 min., split flow 40mL/min.)
4mm split with wool inlet liner, IP deactivated (cat.# 20782).
Inj. temp.: 250°C
Carrier gas: helium, constant flow
Flow rate: 5.0mL/min.
Oven temp.: 50°C (hold 5 min.) to 320°C @ 30°C/min. (hold 10 min.)
Det.: FID @ 330°C

advantage to this technique is that it doesn't exert any additional thermal stress on the system, which may mean longer lifetimes for some parts, such as inlet O-rings.

Another method is to elevate the thermal zones in your instrument for a set period of time. The data in Figure 4 show that a flat baseline is achieved after just ten minutes of thermal conditioning. If you use thermal conditioning, be sure to use progressively hotter temperatures along the sample flow path. For example, your column should be hotter than your inlet, and your detector should be hotter than your column. This prevents condensation of contaminants in the system which can appear as "ghost peaks" or poorly shaped peaks that elute at irreproducible retention times.

Figure 4 Conditioning your system above method temperatures is an excellent way to remove contaminants.



Column: Rxi®-5ms, 30m, 0.32mm, 0.25µm (cat.# 13424)
Sample: no sample injected; instrument ran the method conditions with no injection.
Inj.: no injection; splitless mode (hold 0.5 min., split flow 40mL/min.)
4mm split with wool inlet liner, IP deactivated (cat.# 20782).
Inj. temp.: 250°C
Carrier gas: helium, constant flow
Flow rate: 5.0mL/min.
Oven temp.: 50°C (hold 5 min.) to 320°C @ 30°C/min. (hold 10 min.)
Det.: FID @ 330°C

Conclusion

We observed that no matter whose product you buy, you can expect some background noise if you install an inlet liner and immediately begin analysis. However, these background peaks easily can be eliminated by either a few warm-up runs or a brief period of thermal conditioning. Before analyzing valuable samples, take the time to warm up your system, ensuring that you are ready to run!

For a full listing of Restek liners, visit us at
www.restek.com

Dual Vespel® Ring Inlet Seals

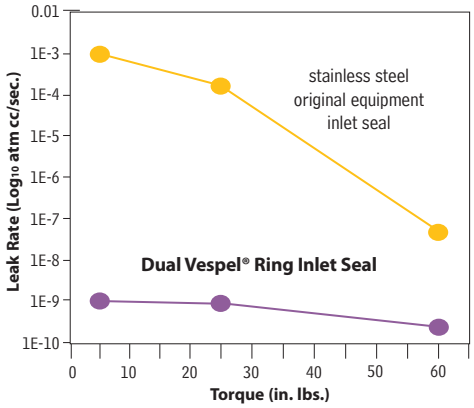
Washerless, Leak-Tight Seal for Agilent GCs

- Prevents oxygen from permeating the carrier gas, increasing column lifetime.
- Vespel® ring in top surface reduces operator variability by requiring minimal torque to seal.
- Vespel® ring in bottom surface simplifies installation—eliminates the washer.

In Agilent split/splitless injection ports, it can be difficult to make and maintain a good seal with a conventional metal inlet disk. The metal-to-metal seal dictates that you apply considerable torque to the reducing nut, and, based on our testing, this does not ensure a leak-tight seal. Over the course of oven temperature cycling, metal seals are prone to leaks, which ultimately can degrade the capillary column and cause other analytical difficulties.

Our patented Dual Vespel® Ring Inlet Seal greatly improves injection port performance—it stays sealed, even after repeated temperature cycles, without retightening the reducing nut! This seal features two soft Vespel® rings, one embedded in its top surface and the other embedded in its bottom surface. These rings eliminate the need for a washer, and ensure very little torque is needed to make a leak-tight seal. The rings will not harm the critical seal in the injector body, or any other surface, and are outside the sample flow path. Tests using a high sensitivity helium leak detector show Dual Vespel® Ring Inlet Seals will seal equally effectively at torques from 5 in. lb. to 60 in. lb. (Figure 1).

Figure 1 The Dual Vespel® Ring Inlet Seal achieves leak-tight seals even at low torque, reducing the chance of leak-related problems.



Why trust a metal-to-metal seal when you can make leak-tight seals quickly and easily—and more reliably—without a washer, with a Restek Dual Vespel® Ring Inlet Seal. Use a stainless steel seal for analyses of unreactive compounds. To reduce breakdown and adsorption of active compounds, use a gold-plated or Siltek®-treated seal. The gold surface offers better inertness than untreated stainless steel; Siltek® treatment provides inertness similar to that of a fused silica capillary column.

Patented.

Washerless, leak-tight seals for Agilent GCs

0.8mm ID Dual Vespel® Ring Inlet Seal	2-pk.	10-pk.
Gold-Plated	21240	21241
Siltek® Treated	21242	21243
Stainless Steel	21238	21239
1.2mm ID Dual Vespel® Ring Inlet Seal	**2-pk.**	**10-pk.**
Gold-Plated	21246	21247
Siltek® Treated	21248	21249
Stainless Steel	21244	21245

Dual Vespel® Ring Inlet Seals are available in gold plating, stainless steel, and Siltek® treated.

Dual Vespel® Ring Cross-Disk Inlet Seals for Agilent GCs new!

- Ideal for high-flow split applications.
- Washerless, leak-tight seals.

0.8mm ID Dual Vespel® Ring Cross-Disk Inlet Seal	2-pk.	10-pk.
Gold-Plated	22083	22084
Siltek® Treated	22085	22086
Stainless Steel	22087	22088

Using Guard Columns and Retention Gaps in GC (Part 1)

Continued from page 2

increased the sample components will start to move (there is very little retention …that's why it's called a retention "gap"). When reaching the analytical column, the components will focus in the stationary phase resulting in a narrowing of injection band width (Figure 1). As these retention gaps are mainly used for on-column injection, the inside diameter is usually 0.32mm up to 0.53mm since the needle of an on-column syringe must be able to enter the retention gap. For coupling the retention gaps to the analytical column, we need generally coupling devices that can deal with different diameter capillary tubing.

Retention gaps and splitless injection

While on-column injection minimizes discrimination and provides the best quantitative data, especially for thermolabile components, it can be challenging to perform. Many laboratories will choose a splitless method for ease of use. For splitless injection we generally do not require a retention gap. The sample is injected in a hot injection port, evaporated, and transported with a carrier gas flow of approximately 1mL/min. into the capillary. The amount of solvent vapor that enters the column per unit time is much smaller than with on-column injection. Although with splitless injection the oven temperature is also 10-15°C below the boiling point of the solvent, there is little chance of the solvent condensing. The high concentration of solvent entering the capillary column will cause a strong focusing effect for the components, generating a narrow injection band. If, in splitless injection, a method is used where the initial (injection) oven temperature is much lower than the boiling point of the solvent, the risk of solvent condensation (forming a liquid plug) will increase. This can cause unwanted broadening of the injection band. Coupling a retention gap will also fix this problem.

Wettability of the retention gap

An important factor for good performance is the wettability of the retention gap surface. It is critical that the solvent spread evenly over the surface. This means that nonpolar solvents (hexane, methylene chloride, isooctane, benzene) require non/intermediate deactivated retention gaps and more polar solvents (methanol) will require polar deactivated retention gaps. If the polarity of the retention gap and solvent do not match, the solvent will form droplets inside the capillary. The carrier gas will "push" this droplet along the retention gap into the analytical column. The result is a broadened injection and possibly even peak splitting.

Retention gaps for large volume injection

Instead of injection of 1µl on a 1-2m retention gap, one can also inject much larger amounts on much longer retention gaps. Here we talk about large volume injection technique where retention gaps of 8-10m are used. Such retention gaps can be loaded with 100-200µl of sample. Injection must be slow to allow the solvent to evaporate while passing through the retention gap. With large volume injection, detection limits can be reduced by a factor of 100. The technique requires some skill to optimize all the injection parameters. Additionally, the large volume retention gaps do pollute relatively quickly due to the large amounts of sample introduced.

Guard columns and retention gaps are useful tools to the practicing chemist and it is important to understand the difference between them. In Part 2 of this article, we will review guard columns and discuss a new segment coating technology that allows retention gaps and guard columns to be built directly into the analytical column tubing. This new technology eliminates column coupling, substantially reducing analytical problems related to leaks and dead volume.

1 Grob, K., Journal of Chromatography 237:15 (1982). 2 Hinshaw J., LC•GC Europe 17(9): 460–466 (2004).

more to come!

See the next issue of *the Restek Advantage* for Part 2 of this article.

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2007.03

Analytical Alternatives

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Fast or faster? Options for chlorinated pesticides.

and much more inside…

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IN THIS ISSUE

Retention Cross-over Phenomenon in Gas Chromatography–Can the Mystery be Revealed? Part 2

By Werner Engewald, Ph.D., Professor Emeritus, University of Leipzig, Institute of Analytical Chemistry, Leipzig, Germany; engewald@uni-leipzig.de

In the last issue of the Restek Advantage (2007.02), I showed some examples of the cross-over phenomenon on polar (polyethyleneglycol) columns. Here in Part 2, we will examine the cross-over phenomenon on nonpolar columns.

It is known to a lesser extent that changes in peak elution order also occur on nonpolar or weakly polar stationary phases for hydrocarbons that differ only in their carbon skeleton, e.g. aliphatic versus cyclic compounds or cyclic compounds differing in their ring number. The terpenes sabinene, β-pinene and myrcene are given as an example in Figure 1. The cross-over effect was observed on a polydimethylsiloxane phase with 5% phenyl (60m, 0.25mm ID, 1µm film thickness) as well as on a 100% polydimethylsiloxane phase (60m, 0.32mm ID, 0.5µm film thickness). The column temperature was increased from 90°C to 160°C using isothermal mode. The elution order changed from sabinene, β-pinene, myrcene at 90°C to myrcene, sabinene, β-pinene at 160°C. What could be the reason for this effect? A closer look at the molecular structure shows that sabinene and β-pinene are double ring systems whereas myrcene is an aliphatic hydrocarbon.

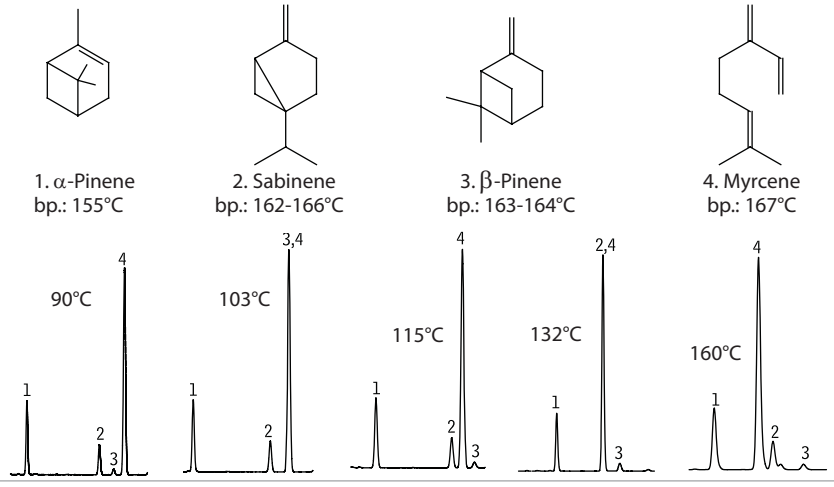
Other interesting analyte pairs prone to cross-over on methylsiloxane phases at different column temperatures are *o*-xylene/*n*-nonane, naphthalene/dodecane, as well as 1,2,3-trimethylbenzene/*n*-decane. In the latter case we also observe coelution and cross-over at different temperature programming rates. At a heating rate of 2°C/min., *n*-decane elutes before 1,2,3-trimethylbenzene, at 5°C/min. coelution occurs, and at 20°C the aromatic hydrocarbon is the first peak (100% PDMS column, 12m, 0.2mm ID, 0.33µm film thickness, starting temperature 35°C). It seems obvious that the geometry of the molecule, e.g. cyclic versus open chain, contributes to the cross-over phenomenon.

Nevertheless, I have this long-standing friendly discussion with a former student of mine, who persistently points out that the examples we have been looking at so far are always pairs of conjugated versus nonconjugated compounds and that π interactions, specifically with phenyl modified phases, should be taken into account.

Let's, therefore, go back to the structure of substances presented in Figure 2: they are exclusively saturated aliphatic and alicyclic hydrocarbons. The data in Figure 2 are from Hively and Hinton (1968) and in that paper the relative retention and retention indices of approxi-

Continued on page 23.

Figure 1 Elution order on a 100% PDMS column at various temperatures (isothermal GC)



New MXT®-Biodiesel TG Column Line

Stable to 430°C, for high temperature analyses.

By Barry L. Burger, Petroleum Innovations Chemist

- Sharp glyceride peaks give more accurate quantitation.
- Stable at 430°C; more robust than fused silica at high temperatures.
- Integra-Gap™ built-in retention gap eliminates manual connection.

Restek has raised the bar with a new high-temperature MXT®-Biodiesel TG column line to complement our fused silica column line for biodiesel analysis. These new MXT®-Biodiesel TG columns are stable to 430°C and offer unique retention gap options that minimize dead volume and leaks. Choose either a 0.32mm column factory-coupled to a 0.53mm retention gap, or select a single unit 0.53mm column featuring Integra-Gap™, a built-in retention gap that eliminates the need for a connector. Both designs are extremely stable at high temperatures and produce fast elution times and sharp peaks for high molecular weight glycerides.

Unsurpassed Stability

The high temperature programs required for analysis of biodiesel oils (B100) by either ASTM D-6584 or EN-14105 methodology present a significant challenge to the analytical column. High-temperature fused silica tubing breaks down under these extreme conditions, but the metal MXT® tubing does not degrade, even at temperatures up to 430°C (Figure 1). This allows analysts to bake out any residue eluting after the triglycerides, preventing carryover without damaging the column.

So how well do the MXT®-Biodiesel TG columns perform? We conducted a benchmarking experiment comparing an MXT®-Biodiesel TG column with Integra-Gap™ to a high-temperature fused silica column coupled to a conventional 0.53mm retention gap. Methodology followed ASTM method D-6584, except the final temperature was modified to 430°C. Both columns were subjected to 100 temperature cycles up to 430°C and derivatized B100 was injected.

This evaluation was performed using a Shimadzu 2010 gas chromatograph equipped with a flame ionization detector, a model AOC 20i + S autosampler with a 10µL SGE syringe and 42mm 26-gauge needle, and a cold on-column programmable injector with a stainless steel injector insert. A Parker hydrogen generator supplied the carrier gas. Peak symmetry and retention time were evaluated as indicators of thermal stability.

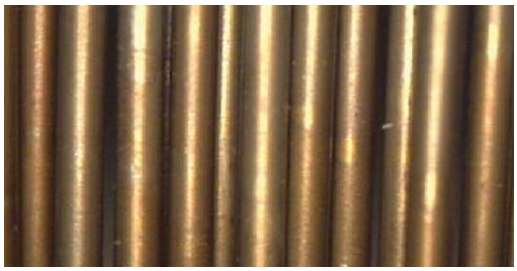
Peak symmetry of butanetriol on a commercial high-temperature fused silica column deteriorates after just 20 injections, compared to the excellent symmetry that is maintained on the MXT®-Biodiesel TG column (Figure 2). In addition to peak shape, retention time stability was used to evaluate column performance. The decrease in retention time seen on the high-temperature fused silica column indicates the liquid phase is being lost (Figure 3). In contrast, the consistent retention times obtained on the MXT®-Biodiesel TG column demonstrate its stability. Practically, this translates into reliable performance and longer column lifetimes.

Analytical Alternatives

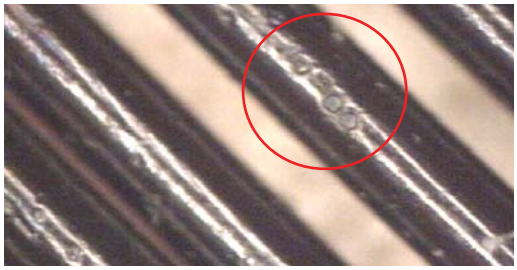
Factory connected 0.32mm MXT®-Biodiesel TG columns & 0.53mm retention gaps

For accurate analysis of heavy triglycerides, on-column injection is required. ASTM D-6584 describes the use of a 0.32mm analytical column coupled with a 0.53mm retention gap. The 0.53mm ID retention gap allows the cool on-column technique to be used, but care must be taken to minimize dead volume and to establish a leak-tight connection. Restek's 0.32mm MXT®-Biodiesel TG columns are factory-coupled to a 0.53mm MXT® retention gap with an Alumaseal™ connector, ensuring a leak-

Figure 1 MXT®-Biodiesel TG columns are undamaged by high thermal cycles compared to high-temperature fused silica columns which breakdown under the same conditions.



MXT®-Biodiesel TG columns are undamaged by high thermal cycles.



HT fused silica columns, labeled as stable to 430°C, show pitting and breakdown.

100 temperature cycles to 430°C totaling 500 minutes at maximum temperature.

Figure 2 Stable and consistent peak shape for the internal standard butanetriol gives you more accurate quantitation.

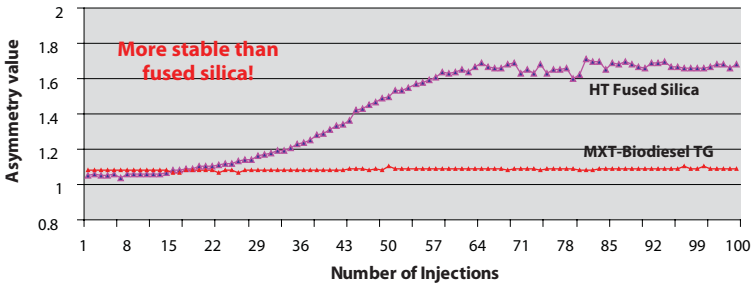


Figure 3 Retention time is stable on a metal MXT®-Biodiesel TG column, even after 100 cycles up to 430°C.

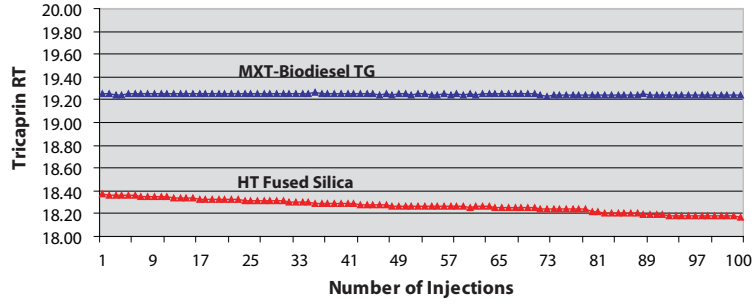
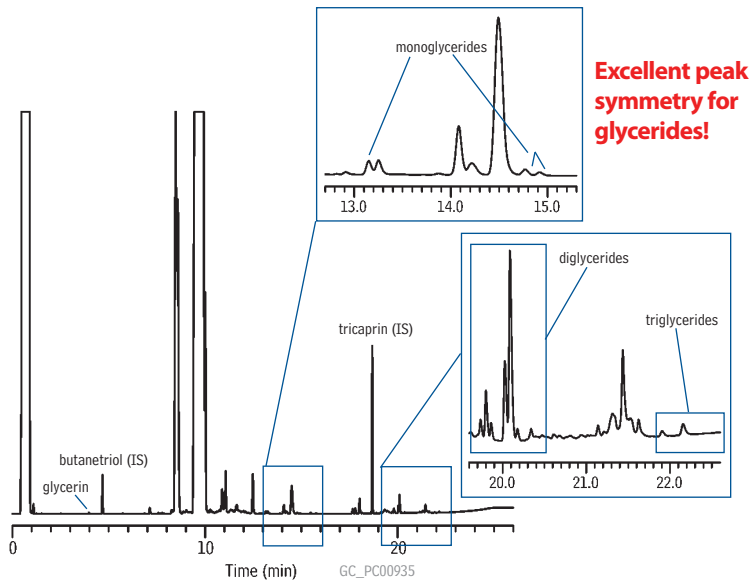
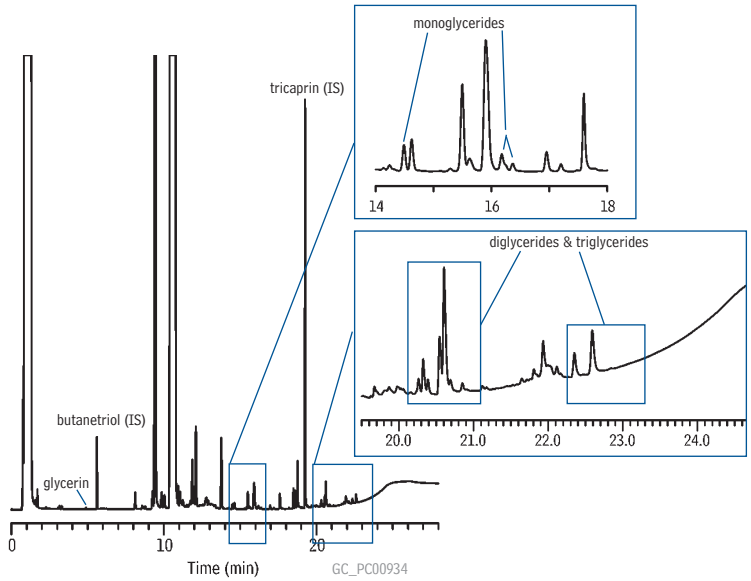


Figure 4 Derivatized B100 samples resolve well on the 0.32mm MXT®-Biodiesel TG column, which is factory-coupled to a 0.53mm MXT® retention gap.



Column: MXT®-Biodiesel TG 10m, 0.32mm ID, 0.1µm with 2m x 0.53mm retention gap
Sample: B100 + IS Butanetriol & Tricaprin derivatized with MSTFA as per ASTM D-6584
Instrument: Shimadzu 2010
Inj.: 1.0µL cool on-column; Inj. temp.: oven track
Carrier gas: hydrogen, constant flow
Flow rate: 4mL/min.
Oven temp.: 50°C (hold 1 min.) to 180°C @ 15°C/min., to 230°C @ 7°C/min., to 430°C @ 30°C/min. (hold 5 min.)
Det.: FID @ 430°C

Figure 5 Equivalent chromatographic quality on the 0.53mm MXT®-Biodiesel TG analytical column with Integra-Gap™



Column: MXT®-Biodiesel TG 13m, 0.53mm ID, 0.16µm with built-in 2m Integra-Gap™ (total column length 15m)
Sample: B100 + IS Butanetriol & Tricaprin derivatized with MSTFA as per ASTM D-6584
Instrument: Shimadzu 2010
Inj.: 1.0µL cool on-column; Inj. temp.: oven track
Carrier gas: hydrogen, constant flow
Flow rate: 4mL/min.
Oven temp.: 50°C (hold 1 min.) to 180°C @ 15°C/min., to 230°C @ 7°C/min., to 430°C @ 30°C/min. (hold 5 min.)
Det.: FID @ 430°C
(Data acquired on prototype column)

tight connection. Target analytes resolve well and the solvent and triglyceride peaks show excellent symmetry (Figure 4).

0.53mm MXT®-Biodiesel TG columns

The 0.53mm MXT®-Biodiesel TG columns are a simpler alternative to using a 0.32mm column coupled to a 0.53mm retention gap. Restek applied Integra-Gap™ technology to the 0.53mm MXT®-Biodiesel TG columns, eliminating the column coupling. These single unit leak-proof columns feature a built-in retention gap, reducing the risk of peak broadening and tailing. Chromatography from the 0.53mm MXT®-Biodiesel TG with Integra-Gap™ technology (Figure 5) is excellent and comparable to that obtained on the 0.32mm ID column in Figure 4.

Conclusion

As demonstrated, for high temperature GC analysis, the metal MXT®-Biodiesel TG column is a rugged column that withstands the harsh temperatures required for total residual glycerin analysis. The column has the resolution needed for accurate, reliable results and is more stable at high temperatures than competitive fused silica columns, leading to longer column lifetimes. To improve the reliability and robustness of your biodiesel analyses, try one of our MXT®-Biodiesel TG columns.

MXT®-Biodiesel TG Column

ID	df (µm)	temp. limits	14-Meter w/2m Integra-Gap™
0.53mm	0.16	-60 to 380/430°C	70289

thank **you**

Instrument provided courtesy of Shimadzu

www.shimadzu.com

Optimize Selectivity & Efficiency in UHPLC Separations

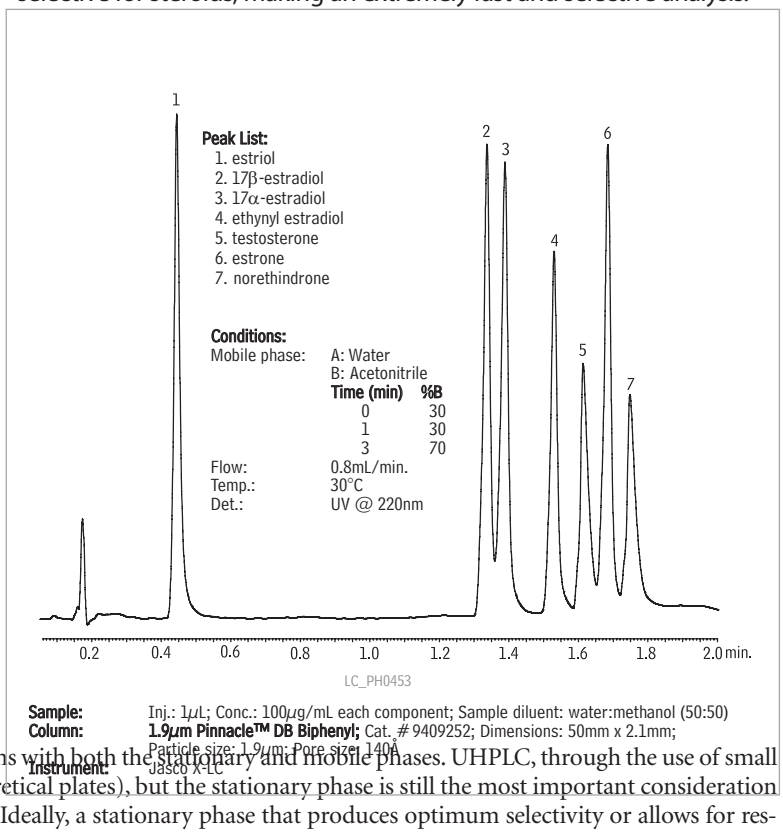
With More Stationary Phase Choices on 1.9µm Pinnacle™ DB HPLC Columns

By Rick Lake, Pharmaceutical Innovations Chemist

- Largest variety of stationary phases for UHPLC.
- Faster analyses, uncompromised chromatography.
- 100% Restek manufactured—from base silica to final packed column.

Since the late 1960s continual advancements have been made in HPLC column technology, and over time the trend has been toward smaller particle sizes. This trend has led us to where we are today—Ultra-High Performance Liquid Chromatography (UHPLC). UHPLC is a milestone in the evolution of LC in that columns packed with <2µm particles, used with instrumentation capable of handling the resulting high back pressures, make possible extremely fast and efficient separations. UHPLC is a very powerful tool for today's practicing chromatographer, as it can significantly increase the efficiency of a chromatographic separation. In addition, the wider range of usable flow rates makes high speed separations possible. However, in light of this new technology, it is important that we do not forget the importance of selectivity. In this article, we will review the significance of selectivity in obtaining acceptable resolution and demonstrate how having choices in stationary phase allows you to maximize the benefits of UHPLC.

Figure 1 Restek's 1.9 µm Pinnacle™ DB Biphenyl columns are highly selective for steroids, making an extremely fast and selective analysis.



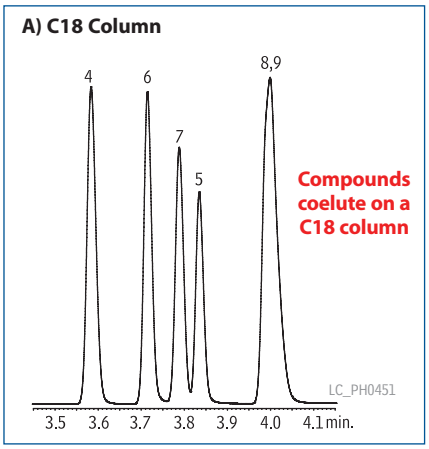
In past articles we have discussed the physical advantages that are driving interest in small particles, mainly the influence of particle size on usable flow rates and peak efficiency. Although small particles have made faster separations possible, selectivity has the greatest effect on resolution. Selectivity, in turn, is governed predominantly by analyte interactions with both the stationary and mobile phases. UHPLC, through the use of small particle columns, does maximize efficiency (e.g. theoretical plates), but the stationary phase is still the most important consideration when attempting to resolve mixtures of compounds. Ideally, a stationary phase that produces optimum selectivity or allows for resolution of compounds in a timely manner should be selected.

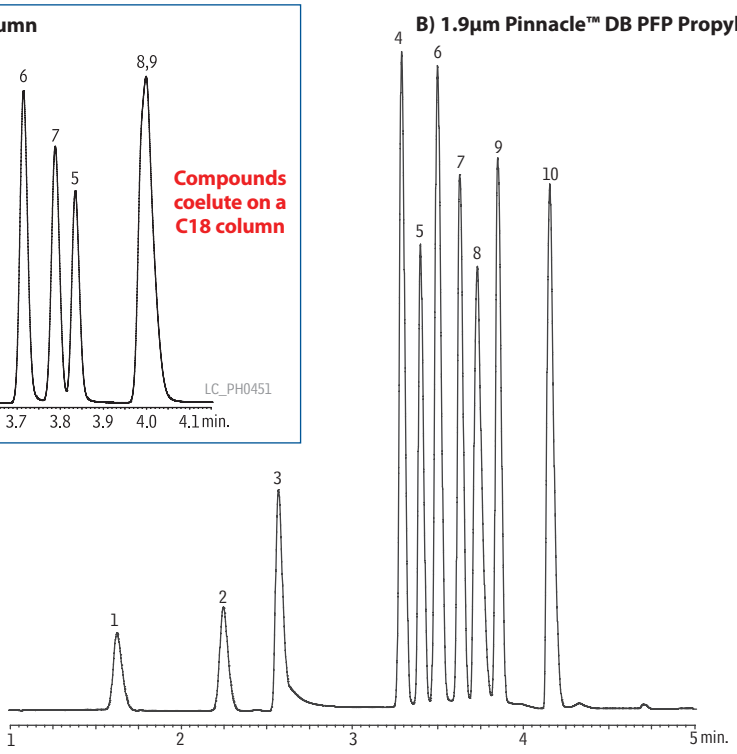
Previously, some advantages of selectivity in specific separations have been noted. For example, the use of a unique Biphenyl stationary phase has shown excellent selectivity for aromatic or fused ring compounds. When using the Biphenyl stationary phase and combining it with the heightened efficiencies of the 1.9µm Pinnacle™ DB column, we can produce highly selective and fast separations of steroids (Figure 1). A Pinnacle™ DB 1.9µm Biphenyl column can separate a test mix of seven hormones in under 2 minutes, a feat not possible through C18 selectivity.

Another example of unique selectivity available on a 1.9µm particle size column is the PFP Propyl (pentafluorphenyl propyl) stationary phase for halogenated drug compounds. This phase is very selective and retentive for organohalogens or other compounds containing basic or electronegative functionalities. To demonstrate heightened selectivity for halogenated drug compounds, we assayed a test mix of eight benzodiazepines and two metabolites, a mix commonly assayed on a C18 colum, in just over 4 minutes with complete resolution (Figure 2). To get the same level of selectivity from a C18 column, a shallower gradient would be needed, prolonging the analysis time. Since the selectivity of the Pinnacle™ DB 1.9µm PFP Propyl column elutes the benzodiazepines in quick succession, a simple gradient still allows for the earlier elution of the more polar metabolites, while maintaining a fast overall run time.

Restek is committed to giving the practicing chromatographer choices, and has therefore sought to deliver the widest selection of stationary phases available with <2µm particle sizes. The goal of chromatography is always to resolve compounds of interest in the fastest time possible. By combining the benefits of UHPLC with Restek's complement of unique stationary phase choices, faster separations become a reality.

Figure 2 Fast, selective analysis of benzodiazepines is made possible by combining the speed of UHPLC with the enhanced selectivity of the 1.9µm Pinnacle™ DB PFP Propyl column.





Peak List:	Conc (µg/ml)
1. 7-amino clonazepam*	20
2. 7-amino flunitrazepam*	20
3. bromazepam	100
4. oxazepam	100
5. lorazepam	100
6. clonazepam	100
7. nitrazepam	100
8. nordiazepam	100
9. flunitrazepam	100
10. diazepam	100

* metabolite

Sample:
Inj.: 1µL
Conc.: as listed
Sample diluent: starting mobile phase (80:20 A:B)

Column: **A. C18 Column**
B. 1.9µm Pinnacle™ DB PFP Propyl
Cat. #: A. 9414212
B. 9419212
Dimensions: 100mm x 2.1mm
Particle size: 1.9µm
Pore size: 140Å

Instrument: Jasco X-LC

Conditions:
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

Time (min.)	%B
0	20
1	20
6	80

Flow: 0.6 mL/min.
Temp.: 40°C
Det.: UV @254 nm

1.9µm Pinnacle™ DB HPLC Columns

Physical Characteristics:

particle size: 1.9µm
pore size: 140Å
endcap: yes
pH range: 2.5 - 7.5
temperature limit: 80°C

1.9µm Pinnacle™ DB C18 column, 2.1mm	cat. #
30mm	9414232
50mm	9414252
100mm	9414212
1.9µm Pinnacle™ DB Silica column, 2.1mm	**cat. #**
30mm	9410232
50mm	9410252
100mm	9410212
1.9µm Pinnacle™ DB PFP Propyl column, 2.1mm	**cat. #**
30mm	9419232
50mm	9419252
100mm	9419212
1.9µm Pinnacle™ DB Biphenyl column, 2.1mm	**cat. #**
30mm	9409232
50mm	9409252
100mm	9409212
1.9µm Pinnacle™ Aqueous C18 column, 2.1mm	**cat. #**
30mm	9418232
50mm	9418252
100mm	9418212

More phases coming soon!

Faster Organochlorine Pesticide Sample Throughput

On New Rtx®-CLPesticides & Rtx®-CLPesticides2 Columns

By Jason Thomas, Environmental Innovations Chemist

- Dramatically improve sample throughput.
- Results in <7min. by conventional analysis, or <5min. using the Gerstel MACH system.
- Outstanding resolution on all columns.

As the environmental testing market continues to be very competitive, laboratory operating costs are a critical concern. Increasing sample throughput is one way to reduce costs, and shortening analytical run time is an effective way to do this. Here we offer methods for reducing run time for the organochlorine pesticides analyzed under US EPA Method 8081. The significant reduction in both analysis time and more significantly, cycle time, offered here is a major benefit for environmental laboratories.

Restek developed the Rtx®-CLPesticides and Rtx®-CLPesticides2 column pair specifically for chlorinated pesticides. These phases were designed to separate the isomers and the structurally similar pairs on the list of target analytes. Here we introduce new film thicknesses with optimized phase ratios for some of the columns in this line. Using these new stationary phase film thicknesses and the optimized run conditions shown, the 20 compounds in US EPA Method 8081 can be separated to baseline in less than 7 minutes (Figure 1). This allows rapid analysis without sacrificing column capacity, which translates, of course, into much improved sample throughput for your laboratory.

An Even Faster Alternative

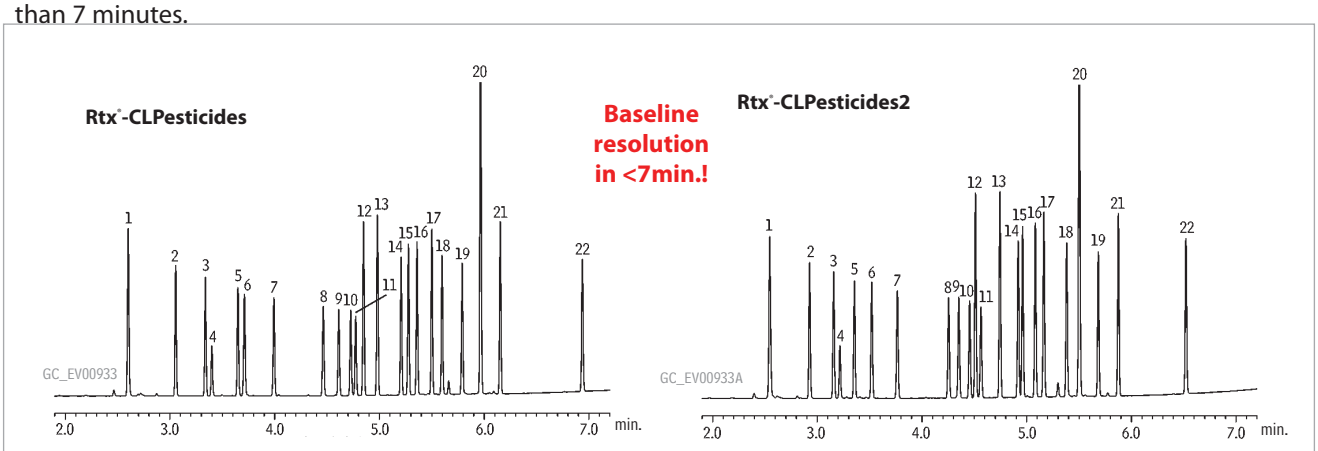
In the attempt to obtain faster analytical run times, several different concepts have been introduced to improve the stock performance of standard GCs. One of the most recent and versatile ideas is the low thermal mass method by Gerstel using an apparatus called the MACH, (Modular Accelerated Column Heater) (Figure 2). This system operates by heating the capillary column outside of the GC oven in a small column module mounted on the oven door.

This apparatus provides several important advantages. First, due to the low thermal mass of the unit, very rapid heating and cooling times can be realized, which significantly shortens cycle times. Second, because of the way the column is wrapped, very uniform heating occurs, which eliminates the eddies and hot spots produced in a conventional GC oven. Finally, since the column modules are independently controlled, two different temperature programs can be run simultaneously, which allows each column to be optimized individually.

Restek applied this novel MACH technology to EPA Method 8081 using an Rtx®-CLPesticides and Rtx®-CLPesticides2 column pair. Almost 100% baseline resolution was obtained for all 22 pesticides and surrogates, on both columns, in under five minutes (Figure 3). This combination of ultra-fast analysis time and outstanding resolution is a result of the unique selectivity and high efficiency of the phases combined with the narrow peaks associated with ultra-rapid ramp rates.

Regardless of whether you choose to embrace the new fast-GC technology, or continue to adhere to more conventional GC, Restek Rtx®-CLPesticides and Rtx®-CLPesticides2 columns can provide exceptional performance and very rapid run times when analyzing chlorinated pesticides.

Figure 1 Baseline resolution of organochlorine pesticides on the 0.18mm ID Rtx®-CLPesticides column pair in less than 7 minutes.



1. 2,4,5,6-tetrachloro-*m*-xylene (SS)
2. α-BHC
3. γ-BHC
4. β-BHC
5. δ-BHC
6. heptachlor
7. aldrin
8. heptachlor epoxide (isomer B)
9. γ-chlordane
10. α-chlordane
11. endosulfan I
12. 4,4'-DDE
13. dieldrin
14. endrin
15. 4,4'-DDD
16. endosulfan II
17. 4,4'-DDT
18. endrin aldehyde
19. endosulfan sulfate
20. methoxychlor
21. endrin ketone
22. decachlorobiphenyl (SS)

Column: Rtx®-CLPesticides 30m, 0.32mm ID, 0.32µm (cat.# 11141) and Rtx®-CLPesticides2 30m, 0.32mm ID, 0.25µm (cat.# 11324) with 5m x 0.32mm ID Rxi™ deactivated guard tubing (cat.# 10039), connected using Universal "Y" Press-Tight® connector (cat.# 20405-261)
Sample: Organochlorine Pesticide Mix AB #2, 8-80µg/mL each component in hexane/toluene (cat.# 32292), Pesticide Surrogate Mix, 200µg/mL each component in acetone (cat.# 32000)
Inj.: 1.0µL splitless (hold 0.3 min.), 4mm single gooseneck inlet liner (cat.# 20799); Inj. temp.: 250°C; Carrier gas: helium, constant flow; Linear velocity: 60cm/sec. @ 120°C; Oven temp.: 120°C to 200°C @45°C/min. to 230°C @15°C/min to 330°C (hold 2 min.) @ 30°C/min.; Det.: ECD @330°C

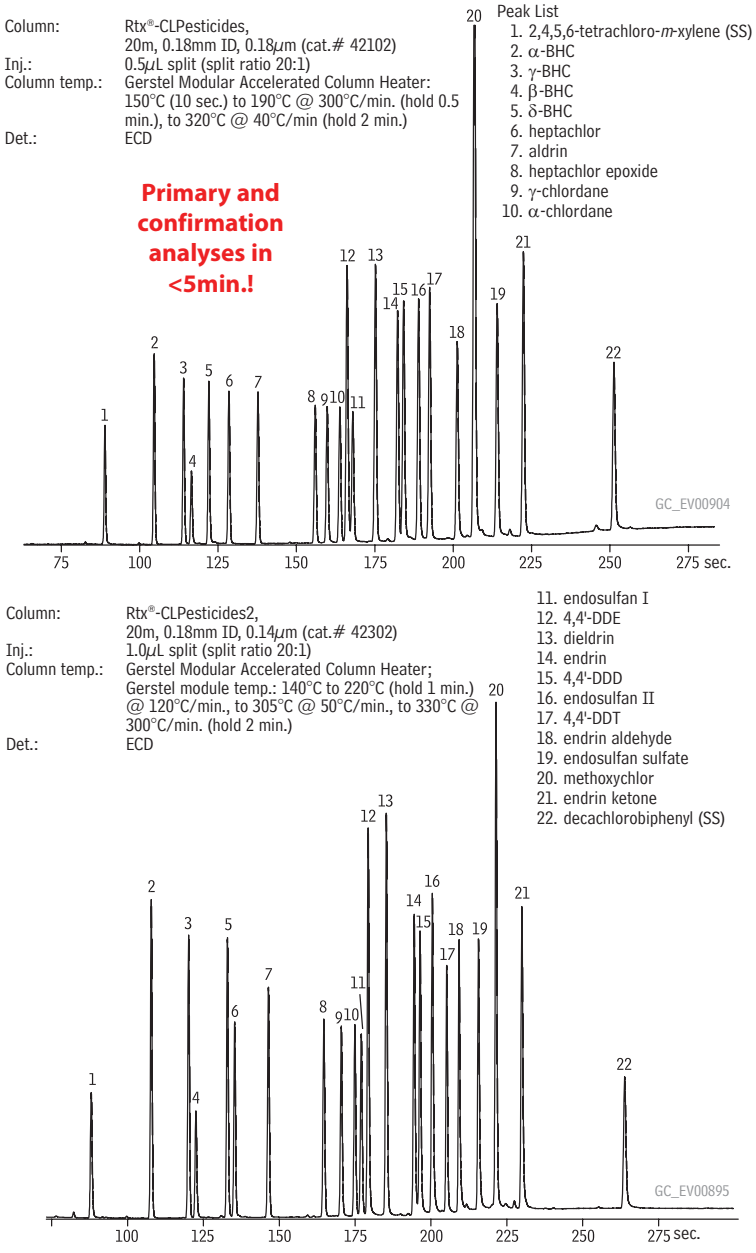
Figure 2 Two column modules in a Gerstel MACH column heating system.

The MACH system allows independent temperature programming of up to two columns, simultaneously.

thank **you**

Instrument provided courtesy of Gerstel USA.
www.gerstelus.com

Figure 3 Resolve organochlorine pesticides in less than 5 minutes using the Rtx®-CLPesticides columns and the MACH system.



Sample: Organochlorine Pesticide Mix AB #2 (8-80µg/mL each component in hexane/toluene 1:1, (cat.# 32292), Pesticide Surrogate Mix (200µg/mL each component in acetone, cat.# 32000)

Rtx®-CLPesticides Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.18	-60 to 310/330°C	20-Meter	42102
0.53mm	0.50	-60 to 300/320°C	30-Meter	11140

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.14	-60 to 310/330°C	20-Meter	42302
0.53mm	0.42	-60 to 300/320°C	30-Meter	11340

Pesticide Surrogate Mix

decachlorobiphenyl 2,4,5,6-tetrachloro-*m*-xylene
200µg/mL each in acetone, 1mL/ampul
cat. # 32000

Organochlorine Pesticide Mix AB #2

(20 components)

aldrin	8µg/mL	dieldrin	16
α-BHC	8	endosulfan I	8
β-BHC	8	endosulfan II	16
δ-BHC	8	endosulfan sulfate	16
γ-BHC (lindane)	8	endrin	16
α-chlordane	8	endrin aldehyde	16
γ-chlordane	8	endrin ketone	16
4,4'-DDD	16	heptachlor	8
4,4'-DDE	16	heptachlor epoxide (B)	8
4,4'-DDT	16	methoxychlor	80

In hexane:toluene (1:1), 1mL/ampul
cat. # 32292

Resprep™ Florisil® SPE Cartridges: Normal Phase

	3mL/500mg (50-pk.)	6mL/500mg (30-pk.)	6mL/1000mg (30-pk.)
Florisil®	24031	—	24034
(EPA SW 846 methods and CLP protocols)	24032*	26086**	26085**

*Teflon® frits **Glass tubes with Teflon® frits

CarboPrep™ SPE Cartridges

	Tube Volume, Bed Weight	qty.	cat#
CarboPrep™ 90	3mL, 250mg	50-pk.	26091

get **connected**

See page 20-21 for our list of connectors and connector kits.

Resolving the Benzo(j)fluoranthene Challenge

Separate New PAHs Quickly Using the Rxi™-17 GC Column

By Robert Freeman, Environmental Innovations Chemist

- Fully resolve benzo(j)fluoranthene from benzo(b) & (k).
- Excellent resolution of 16 priority pollutant PAHs.
- Separate difficult dibenzo pyrene isomers.

New Compounds, New Challenges

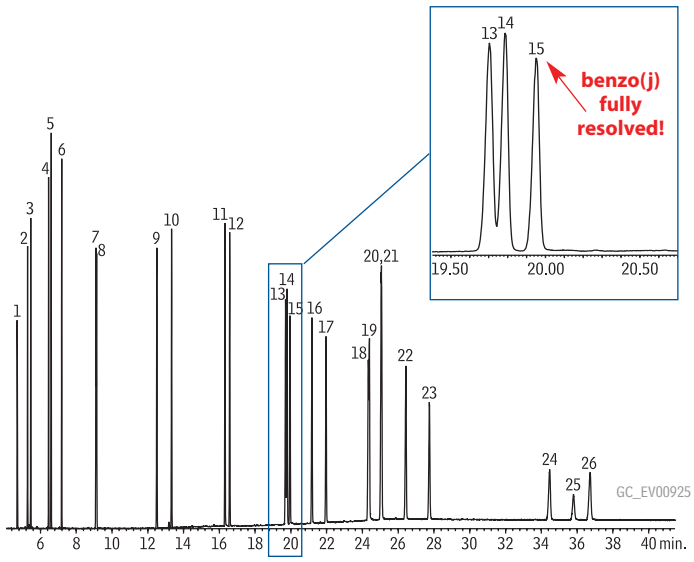
Polynuclear aromatic hydrocarbons (PAHs) are widespread organic pollutants that significantly affect environmental quality and raise human health concerns. The US EPA mandates testing of 16 priority PAH pollutants, while analyte lists in other countries are expanding to include compounds such as benzo(j)fluoranthene, dibenzo(a,h)-acridine, and dibenzo(a,e)pyrene, that are difficult to analyze under conventional test conditions. Benzo(j)fluoranthene and benzo(b)fluoranthene, for example, co-elute on a 5%diphenyl/95%dimethyl polysiloxane stationary phase. When reporting of individual concentrations for each isomer is required, conventional methods are not viable and new solutions must be found.

The Rxi™ Alternative

The Rxi™-17 column contains a 50% diphenyl/50% dimethyl polysiloxane stationary phase. The higher concentration of phenyl groups in this stationary phase increases retention of phenyl-containing compounds, such as PAHs, thus facilitating separation. We also used a Drilled Uniliner® inlet liner since it eliminates sample exposure to cold spots and potentially active metal components in the injection port. Using a pulsed splitless injection, we maximize sample transfer to the column while minimizing high molecular weight discrimination.

The data in Figure 1 demonstrate the excellent resolution of benzo(j)fluoranthene achievable on the Rxi™-17 column. Phenanthrene and anthracene also resolve well on this column under slower run conditions (data not shown). Using the Rxi™-17 column with an optimized temperature program is a practical solution to the challenges posed by expanding PAH analyte lists. If you are struggling to quantify PAHs on conventional columns, try the Rxi™-17 column and the optimized temperature program shown here.

Figure 1 Fast, effective separation of target PAHs using an Rxi™-17 column and an optimized temperature program.



Peak List	Ret. Time (min.)		Ret. Time (min.)
1. naphthalene	4.70	14. benzo(k)fluoranthene	19.78
2. 1-methylnaphthalene	5.28	15. benzo(j)fluoranthene	19.95
3. 2-methylnaphthalene	5.46	16. benzo(a)pyrene	21.17
4. acenaphthylene	6.45	17. 3-methylcholanthrene	21.97
5. acenaphthene	6.60	18. dibenzo(a,h)acridine	24.33
6. fluorene	7.18	19. dibenzo(a,j)acridine	24.39
7. phenanthrene	9.10	20. indeno(1,2,3-cd)pyrene	25.04
8. anthracene	9.14	21. dibenzo(a,h)anthracene	25.07
9. fluoranthene	12.50	22. benzo(ghi)perylene	26.43
10. pyrene	13.33	23. 7H-dibenzo(c,g)carbazole	27.75
11. benzo(a)anthracene	16.32	24. dibenzo(a,e)pyrene	34.46
12. chrysene	16.58	25. dibenzo(a,i)pyrene	35.80
13. benzo(b)fluoranthene	19.70	26. dibenzo(a,h)pyrene	36.73

Column: Rxi™-17, 30m, 0.25mm ID, 0.25µm (cat.# 13523)
Sample: PAH mix, 20µg/mL each component: EPA Method 610 Mix (cat.# 31011), PAH Supplement Mix (cat.# 31857), 1-methylnaphthalene (cat#31283), 2-methylnaphthalene (cat#31285); Inj.: 1.0µL pulsed splitless injection (20ng each component on column), 4mm Drilled Uniliner® inlet liner with hole at top (cat # 21055); pulse: 20psi @ 0.3 min., 40mL/min. @ 0.2 min. Inj. temp.: 300°C; Carrier gas: helium, constant flow; Flow rate: 1.2mL/min.; Oven temp.: 90°C (hold 1.0 min.) to 215°C @ 25°C/min. (hold 0.5 min.) to 235°C @ 4°C/min., to 280°C @ 15°C/min., to 320°C @ 4°C/min. (hold 20 min.); Det.: Agilent 5973 GC/MS; Scan range: 50-550 amu; Solvent delay: 4.0 min.; Tune: DFTPP; Ionization: EI

Rxi™-17 Columns (fused silica)

(Crossbond® 50% diphenyl / 50% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	40 to 280/300°C	30-Meter	13523

Direct Injection Liners for Agilent GCs

ID* x OD & Length (mm)	qty.	cat.#
Drilled Uniliner® (hole on top)		
4.0 ID x 6.3 OD x 78.5	5-pk.	21055

SV Calibration Mix #5 / 610 PAH Mix (16 components)

acenaphthene
acenaphthylene
anthracene
benzo(a)anthracene
benzo(a)pyrene
benzo(b)fluoranthene
benzo(k)fluoranthene
benzo(ghi)perylene
chrysene
dibenzo(a,h)anthracene
fluoranthene
fluorene
indeno(1,2,3-cd)pyrene
naphthalene
phenanthrene
pyrene

2,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 31011

PAH Supplement Mix for Method 8100 (8 components)

benzo(j)fluoranthene
dibenzo(a,h)acridine
dibenzo(a,j)acridine
7H-dibenzo(c,g)carbazole
dibenzo(a,e)pyrene
dibenzo(a,h)pyrene
dibenzo(a,i)pyrene
3-methylcholanthrene

1000µg/mL each in methylene chloride, 1mL/ampul
cat. # 31857

Analysis of Nitrofurans in Honey

Using LC/MS/MS and an Ultra C18 Column

By Eberhardt Kuhn, Ph.D.; International Marketing Specialist; and Becky Wittrig, Ph.D., HPLC Product Marketing Manager

- Sensitive detection of antibiotic metabolites in a complex matrix.
- Ultra C18 column assures the resolution needed for the LC/MS/MS method.
- Excellent peak shape at sub-ppb levels.

Nitrofurans are a class of veterinary antibiotics used to increase growth rate and prevent or treat disease in animals. Animals have been treated with antibiotics since the 1950s and, currently, about 45% of the antibiotics produced each year in the U.S. are administered to livestock. In Europe, this practice is illegal, because the inadvertent consumption of residual antibiotics in animal tissue, such as meat or liver, can lead to increased drug resistance or allergies in humans.

Nitrofurans have been detected not only in treated animals, but also in animal products, including honey. The low levels of these compounds and the complexity of honey as a matrix present challenges for the analysis of nitrofurans. In addition, nitrofurans are unstable and metabolize rapidly *in vivo*. Any analysis method for nitrofurans, therefore, must be able to separate and detect these metabolites. In the analysis of honey, it is of interest to quantify four nitrofurans: furazolidone, furaltadone, nitrofurazone, and nitrofurantoin, through their respective metabolites, 3-amino-2-oxazolidone (AOZ), 5-mofolinomethylmethyl-3-amino-2-oxazolidone (AMOZ), semicarbazide (SC) and 1-aminhydantoin (AHD). The method of choice for the analysis of nitrofuran and nitrofuran metabolites in honey is LC/MS/MS, with separation on a C18 column.

In this study, honey samples treated with the four nitrofuran metabolites were dissolved in water, then extracted with ethyl acetate. After centrifugation, the extract was evaporated and reconstituted in 125mM HCl, then derivatized with 2-nitrobenzaldehyde. After two liquid-liquid extractions with ethyl acetate, the extract was evaporated and reconstituted with mobile phase, filtered, and injected into the LC/MS/MS system. The column used for the analysis was a 100mm x 2.1mm, 3µm Ultra C18 column. For maximum sensitivity and specificity, a triple quadrupole analyzer was used, with electrospray ionization and selected reaction monitoring (SRM).

Results from the analysis of 0.3ppb nitrofuran metabolites in honey are shown in Figure 1. The Ultra C18 HPLC column is an excellent choice for this analysis. As a reliable general purpose column based on a high-purity, base-deactivated silica, its utility extends to other compounds that might be present in animal-derived matrixes, such as steroids and vitamins.

In analyses for nitrofuran antibiotics, an Ultra C18 HPLC column is an excellent choice, especially for analyzing trace levels of these compounds in a complex sample matrix.

Acknowledgement

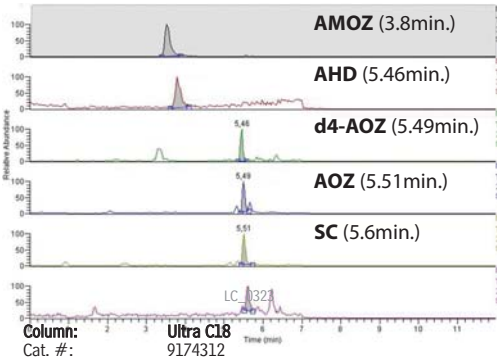
We are grateful to EIDOMET SRL, Restek distributor in Argentina, and application chemist Dr. Alejandro Albornoz, for the analytical work discussed in this article.

Ultra C18 HPLC Column

For many other dimensions, refer to our catalog or visit our website.

3µm Column, 2.1mm	cat. #
100mm	9174312

Figure 1 Nitrofuran metabolites in honey detected at 0.3ppb by LC/MS/MS, using an Ultra C18 column.



Column: Ultra C18
Cat. #: 9174312
Dimensions: 100 x 2.1mm
Particle Size: 3µm
Pore Size: 100Å

Conditions:
Mobile phase: A: 0.05% formic acid in methanol
B: 0.05% formic acid – 5 mM NH_4 formate in water

Time (min)	%B
0	90
2.5	90
5	10
10	10
12	90
15	90

Sample: 0.3ppb each analyte
Flow: 200µL/min.
Temp.: 30°C
Det.: MS/MS triple quadrupoles (Thermo Scientific Discovery)

Analyzer Parameters:
Ion source: ESI (electrospray ionization)
Only segment: 15 min.
Polarity: positive
Data type: centroid
Scan mode: SRM product
Scan width (m/z): 0.7
Scan time (s): 0.25
Peak width: Q1: within 0.7
Q2: 0.7
Collision gas pressure (mTorr): 1.5 (argon)
Divert valve: active, with 3 positions
Positions-1° 2 min., 2° 8 min., 3° 5 min.

Analyte	Prec. Ion	Prod. Ion	Collision E	Tube Lens
AOZ	236	134	12 V	120
AMOZ	335	291	10 V	100
SC	209	166	12 V	80
AHD	249	134	12 V	110

AMOZ = 3-amino-5-morpholinomethyl-2-oxazolidinone
AHD = 1-aminohydantoin hydrochloride
AOZ = 3-amino-2-oxazolidinone
SC = semicarbazide

Data courtesy of Dr. Alejandro Albornoz, EIDOMET SRL, Buenos Aires.

Why Derivatize?

Improve GC Separations with Derivatization

By Kristi Sellers, Innovations Chemist

- Get better separations with increased resolution and response.
- Learn how to choose proper reagents for desired reactions.

Many laboratories include derivatization as part of their sample preparation for gas chromatography (GC) analysis. So, what is derivatization? Why is it important and how do you choose a derivatizing reagent? The discussion below answers these questions. By choosing the right derivatization reagent and procedure you can increase resolution and analyte response, significantly improving your separations.

What is derivatization?

Derivatization is the process by which a compound is chemically changed, producing a new compound that has properties more amenable to a particular analytical method. Some samples analyzed by GC require derivatization in order to make them suitable for analysis. Compounds that have poor volatility, poor thermal stability, or that can be adsorbed in the injector will exhibit nonreproducible peak areas, heights, and shapes. Other compounds that respond poorly on a specific detector may need to be "tagged" with a different functional group to improve detection. For example, tagging with chlorine can improve response on an ECD (electron capture detector). In addition to improving suitability and response, derivatization can improve resolution between coeluting compounds and overlapping peaks.[1]

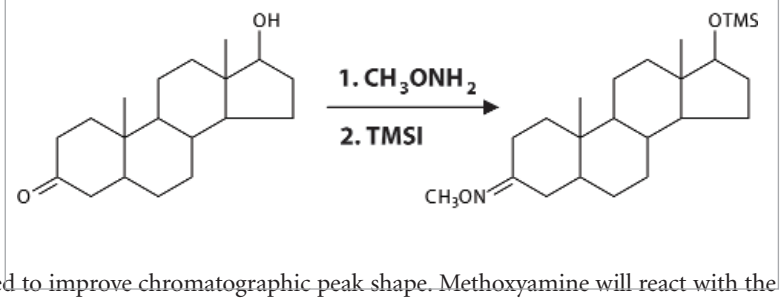
How do I choose a derivatizing reagent?

A good derivatizing reagent and procedure should produce the desired chemical modification of the compound(s) of interest, and be reproducible, efficient, and nonhazardous.[2] For GC, there are three basic types of derivatization reactions: silylation, acylation, and alkylation. Silylating reagents react with compounds containing active hydrogens; these reagents are the most common type used in GC. Acylating reagents react with highly polar functional groups such as amino acids or carbohydrates. Alkylating reagents target active hydrogens on amines and acidic hydroxyl groups.[3] Multiple derivatizing reagents may be necessary for compounds containing several different functional groups such as androsterone (Figure 1). In these multi-step derivatization procedures the use of other types of reagents, such as oxime, hydrazone, methylation, and cyclic derivatives, may be necessary.

A multi-step example

Derivatization can substantially improve chromatographic results, as seen in this example derivatization of androsterone (Figure 1). Androsterone contains a hydroxyl group and a carbonyl group and exhibits poor peak shape and poor separation if analyzed underivatized by GC (Figure 2b). Using silylation, active hydrogens on OH, SH, and NH groups can be replaced.[3] Since *n*-trimethylsilylimidazole (TMSI) is a strong silyl donor, it will react readily with the hydroxyl group on the androsterone molecule creating a trimethylsilyl (TMS) derivative. Because androsterone also contains a carbonyl group, another derivatizing reagent is needed to improve chromatographic peak shape. Methoxyamine will react with the carbonyl group forming an oxime derivative (CH_3ON). Oxime derivatives not only improve chromatographic performance, but also alter GC separations. Figure 2a shows the chromatographic result of derivatizing sex hormones using TMSI and methoxyamine; retention times are increased, separation is increased, and peak shapes and responses are improved.

Figure 1 Derivatization reaction of androsterone using TMSI/methoxyamine.



Conclusion

Derivatizing compounds for GC often is necessary to obtain reproducible chromatographic results. Eliminating this step to save time can be costly and produce inaccurate and unreliable results. A well-chosen derivatization procedure, based on the chemical composition of the target compounds, can significantly improve your chemical separations.

References

1 Knapp D., Handbook of Analytical Derivatization Reactions, Wiley-Interscience, 1979, pp.2-24, 449-453, 482.

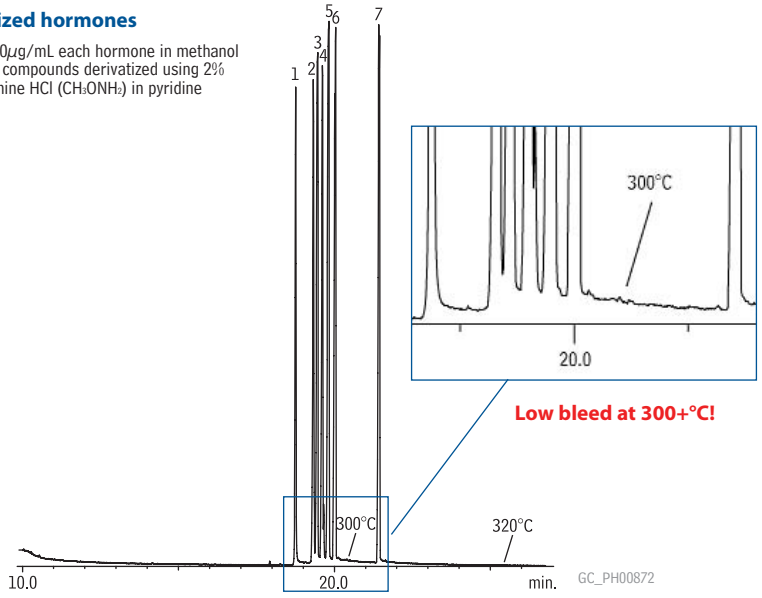
2 www.piercenet.com

3 Grob R., Barry E., Modern Practice of Gas Chromatography, Wiley-Interscience, 2004, pp. 817-818.

Figure 2 Derivatized hormones show excellent resolution and more symmetrical peak shapes than underivatized hormones.

A) Derivatized hormones

Sample: 100µg/mL each hormone in methanol or ethanol; compounds derivatized using 2% methoxylamine HCl (CH_3ONH_2) in pyridine

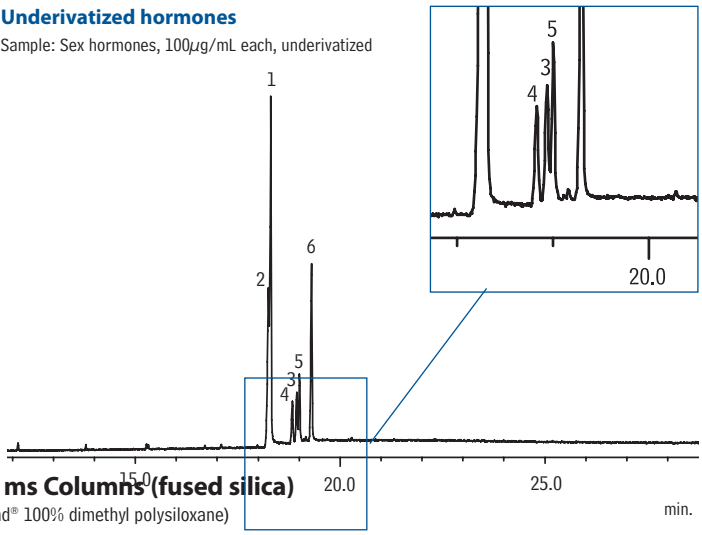


1. androsterone
2. dehydroepiandrosterone (DHEA)
3. 17-α-estradiol
4. estrone
5. 17-β-estradiol
6. testosterone
7. derivatization by-product

For the derivatization procedure used in this analysis, see Knapp's *Handbook of Analytical Derivatization Reactions*, page 482.

B) Underivatized hormones

Sample: Sex hormones, 100µg/mL each, underivatized



Column:	Rxi™-1ms 30m, 0.25mm ID, 0.25µm (cat. # 13323)
Inj.:	1.0µL splitless (hold 0.5min.), 3.5mm single gooseneck inlet liner (cat.# 20961)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	100°C to 320°C @ 10°C/min. (hold for 10 min.)
Det.:	MS: Shimadzu 17A with QP5000
Transfer line temp.:	280°C
Scan range:	40-700amu
Ionization:	EI
Mode:	Scan

Rxi™-1ms Columns (fused silica)
(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	13323

Splitless Liners for Shimadzu GCs

**Nominal ID at syringe needle expulsion point.

Superior Fractionation of Extractable Petroleum Hydrocarbons

Get More Accurate Results Using Restek SPE Tubes

By Lydia Nolan, Innovations Chemist

- Easier quantitation; lower background & less interference.
- Reliable, reproducible results.
- Unique packaging designed for convenience and storage stability.

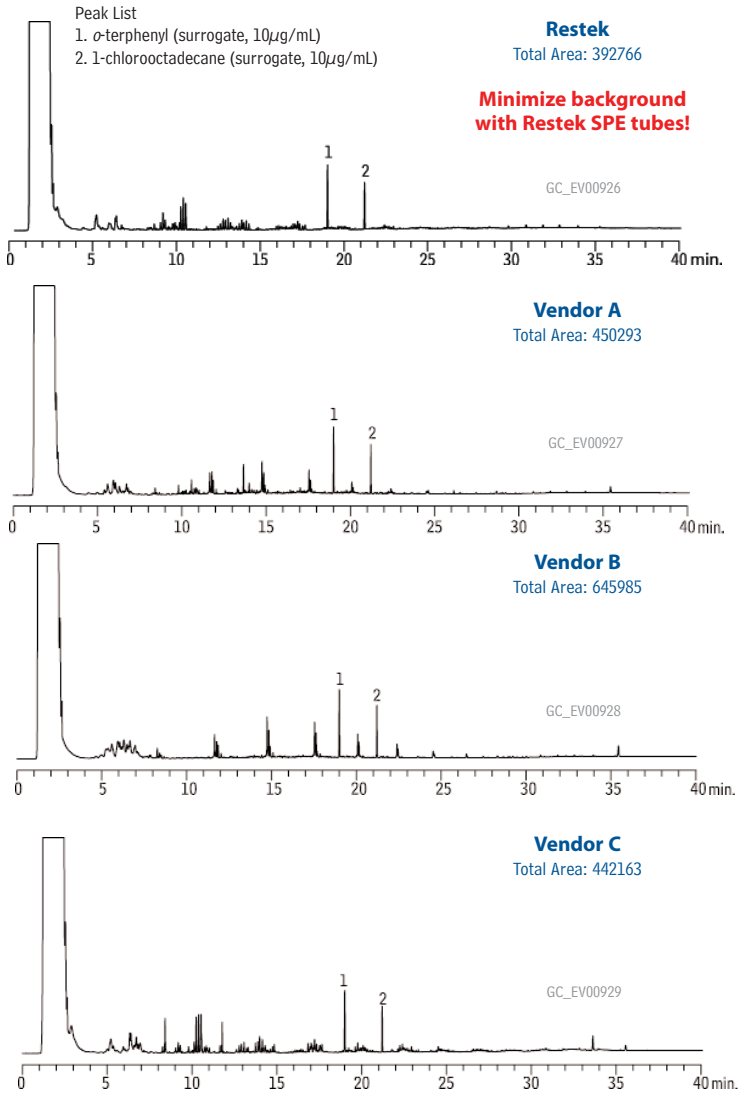
There is an increasing public awareness of the threat to public health from leaking underground storage tanks. Both federal and state agencies have developed methods to address the testing of potential problem sites. The Massachusetts Department of Environmental Protection's "Method for the Determination of Extractable Petroleum Hydrocarbons (EPH)" has recently been updated and is based on solvent extraction of water and soil/sediment matrices, followed by silica gel SPE fractionation of aliphatics and aromatics from C9 through C36 hydrocarbon ranges.

The quality and conformation of the silica SPE clean-up column is essential to acceptable fractionation and recovery results. Commercial silica SPE products streamline this process, but it is important to understand the quality and performance differences among the available products, and the impact they have on your results. The activity level and capacity of the silica, the compression of the bed, and the quality of the constituents and packaging are all critical to getting accurate and reliable results. The data in Table I show how even very minor amounts of excess moisture (known amounts added for experimental purposes during the first conditioning step) or long-term storage without desiccation can produce early breakthrough of the sensitive analytes from the aromatic fraction into the aliphatic fraction.

To ensure maximum shelf-life and minimum environmental exposure after opening these cartridges, Restek packages them into 5 smaller packs of 4 SPE tubes each—the fewest cartridges per pack available. We also provide an additional outer, resealable barrier bag, making successful short- and long-term product storage easier for the user.

Activity level of the silica and consistency of the cartridge packing are essential for reliable fractionation recovery and reproducibility. The recovery and reproducibility of results for the fractionation surrogates (2-fluorobiphenyl, 2-bromonaphthalene and naphthalene) are critical to determining if breakthrough is occurring. Again, in comparing several commercial sources, using optimized conditions for each vendor, results show that the Restek Massachusetts EPH cartridges are capable of quantitative (greater than 97%) and reliable (RSDs less than 7.3) recoveries for these critical markers (Table II).

Figure 1 Restek Massachusetts EPH SPE tubes show the lowest overall level of background response.



Column: Rtx®-5, 30m, 0.32mm ID, 0.25µm (cat.# 10224)
Sample: Massachusetts EPH Surrogate Spike Mix (4000µg/mL each component) diluted to 400µg/mL in hexane.
Spiking standard: 50µL Inj.: 0.5µL, splitless (hold 0.5 min.), PSS split/splitless inlet liner (cat.#21717)
Inj. temp.: 280°C; Carrier gas: helium, constant velocity; Pressure pulse program: 50cm/sec @ -0.71 min., split ratio = 0 @ -0.70 min., split ratio = 20:1 @ 0.75 min., 35cm/sec @ 0.8 min.; Linear velocity: 35cm/sec
Oven temp.: 60°C (hold 1 min.) to 310°C @ 8°C/min. (hold 12 min.), Det.: FID @ 330°C

* Total area counts exclude response for solvent front and surrogate peaks
1. o-terphenyl (surrogate) 10ng on-column
2. 1-chlorooctadecane (surrogate) 10ng on-column
All cartridges were extracted with 15mL hexane, without prior conditioning. Extract blanks were then spiked with the 50µL of MA EPH Surrogate Spike mix, cat#31479 (diluted to 400µg/mL with hexane), and dried down to 1mL with gentle nitrogen purge.

Table I Excess moisture and improper storage compromise results by causing breakthrough into the aromatic fraction.

	% Breakthrough into Hexane (Aliphatic) Fraction			
Analyte	Package intact, no added moisture	200µL water added	Package opened, resealed, stored on shelf, 1 year	Package intact, stored on shelf, 1 year
Naphthalene	0.0	0.0	----	----
2-fluorobiphenyl (surrogate)	0.0	0.0	----	----
2-bromonaphthalene (surrogate)	0.0	4.4	33.3	28.5

Table II Restek Massachusetts EPH SPE tubes provide more accurate and reproducible results for critical marker compounds.

	Restek			Vendor A			Vendor B			Vendor C		
Analyte	percent recovery	STD	%RSD	percent recovery	STD	%RSD	percent recovery	STD	%RSD	percent recovery	STD	%RSD
naphthalene	103.1	7.5	7.2	101.2	10.1	10.0	88.8	2.8	3.1	66.5	2.6	3.9
2-fluorobiphenyl	97.8	6.6	6.7	100.4	13.7	13.6	99.3	5.0	5.0	104.2	6.6	6.4
2-bromonaphthalene	98.6	5.3	5.4	71	7.1	10.0	50.0	8.1	16.1	29.2	1.9	6.6

All tubes were 20 or 25mL with approximately 5g silica packing. Conditioning: 15mL hexane. Sample: 0.5mL of each fractionation check standard and surrogate standard. Elution for fraction #1 (aliphatics): 17-20mL hexane (volume was optimized for each supplier and lot of tubes). Elution for fraction #2 (aromatics): 20mL of CH_2Cl_2. Each fraction was dried to a total volume of 1mL and analyzed by GC.[1]

Coextractables are another major concern with commercial cartridges. The contaminants may be found in the packaging, cartridge materials such as the SPE tube and frits, and the silica itself. The solvent blank extractions shown in Figure 1 were collected from cartridges that were not pre-conditioned. Restek cartridges show the lowest level of background peak area counts, indicating the lowest level of background extractables.

When cartridges start out with low levels of extractables, it may not be necessary to use the methylene chloride pre-treatment allowed in the method. This pre-treatment can easily compromise the fractionation performance of the cartridge beds and should be avoided whenever possible. In addition, fewer product-related contaminants will provide clearer quantitation and require fewer manual reviews of the data generated from the final chromatograms.

In all of the key performance areas, the Restek Massachusetts EPH SPE tubes outperformed other commercially available products. Our cartridges are designed to deliver accurate, reliable, and reproducible results. For high quality separation products developed to prevent breakthrough and minimize background, reach for Restek sample preparation products.

References
1 *Method for the Determination of Extractable Petroleum Hydrocarbons (EPH).* Massachusetts Department of Environmental Protection, Division of Environmental Analysis, Office of Research and Standards, Bureau of Waste Site Cleanup, Revision 1.1, May 2004.

MA Fractionation Check Mix (31 components)

PAHs:	Hydrocarbons:
acenaphthene	*n*-nonane (C9)
acenaphthylene	*n*-decane (C10)
anthracene	*n*-dodecane (C12)
benzo(a)anthracene	*n*-tetradecane (C14)
benzo(a)pyrene	*n*-hexadecane (C16)
benzo(b)fluoranthene	*n*-octadecane (C18)
benzo(k)fluoranthene	*n*-nonadecane (C19)
benzo(ghi)perylene	*n*-eicosane (C20)
chrysene	*n*-docosane (C22)
dibenzo(a,h)anthracene	*n*-tetracosane (C24)
fluoranthene	*n*-hexacosane (C26)
fluorene	*n*-octacosane (C28)
indeno(1,2,3-cd)pyrene	*n*-triacontane (C30)
2-methylnaphthalene	*n*-hexatriacontane (C36)
naphthalene	
phenanthrene	
pyrene	

25µg/mL each in hexane, 1mL/ampul
cat. # 31481

MA Fractionation Surrogate Spike Mix

2-bromonaphthalene 2-fluorobiphenyl
4,000µg/mL each in hexane, 1mL/ampul
cat. # 31480

MA EPH Surrogate Spike Mix

1-chlorooctadecane *o*-terphenyl
4,000µg/mL each in acetone, 1mL/ampul
cat. # 31479

Method Specific SPE Cartridges: Massachusetts EPH

Tube Volume, Bed Weight	qty.	cat.#
20mL, 5g	20-pk.	26065

Rtx®-5 Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	-60 to 330/350°C	30-Meter	10224

Splitless Liners for PerkinElmer GCs

ID* x OD & Length (mm)	qty.	cat.#

for **more** info

For more information on our selection of SPE tubes, visit us online at **www.restek.com**

Prevent Mercury Loss During Transport and Storage

Use Siltek® Surface Treatment on Steel Components

By Gary Barone, Restek Performance Coatings Division

- Rugged—withstands temperatures up to 400°C.
- Meets system inertness requirements.
- Eliminates costly retests.

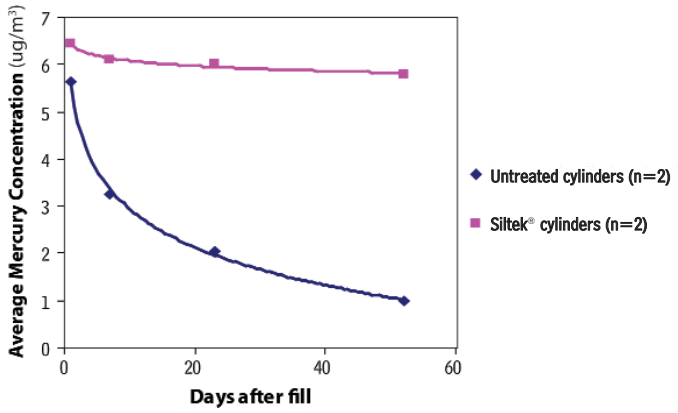
As concerns grow over mercury in the environment, new regulations have been developed to measure, and eventually reduce, mercury emissions from coal-fired electric utilities. For example, the US EPA will require all electric utilities to measure mercury emissions starting on January 1, 2009. The most popular methods of sampling will be based on continuous mercury monitoring systems (CMMS) and sorbent tube samplers. To ensure quantitative storage and transfer, and accurate analysis, of the low levels of mercury in streams sampled from flue stacks, these sampling systems must be inert.

Siltek® surface treatment has been used in a wide variety of applications in which an inert surface is of paramount importance. To measure the impact of Siltek® treatment on adsorption of mercury during storage, we compared the performances of 304 grade stainless steel gas sampling cylinders (Swagelok®, Solon OH) with and without Siltek® treatment.

We filled each cylinder with 8µg/m³ of elemental mercury (approximately 1 part per billion) (Spectra Gases, Alpha NJ) and assessed the mercury concentration in each cylinder over time to determine changes in mercury concentration. Detection was achieved by direct interface gas sampling to an atomic adsorption detector. Sample pathway regulator and tubing were Siltek® treated to ensure accurate transfer.

The data in Figure 1 demonstrate that Siltek® treatment provides a stable surface for elemental mercury, and untreated stainless steel does not. Based on these results, we conclude that Siltek® surface treatment for steel or stainless steel components and tubing in CMMS and sorbent tube mercury sampling systems will improve analytical reliability. For more information about Siltek® surface treatment, visit us at: **www.restekcoatings.com**

Figure 1 Siltek® treated gas sampling cylinders show very good inertness toward mercury.



Siltek®/Sulfinert® Treated Coiled Electropolished 316L Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085" (2.16mm)	1/8" (3.18mm)*	22538				
0.180" (4.57mm)	1/4" (6.35mm)**	22539				

1/8" OD: 5 ft. to 100 ft. in one continuous coil; 1/4" OD: 5 ft. to 300 ft. in one continuous coil. Longer lengths will be more than one coil. Note: required length in meters x 3.2808 = length in feet.

Siltek®/Sulfinert® Treated Coiled 316L Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22508				
0.180" (4.57mm)	1/4" (6.35mm)**	22509				
0.277" (7.04mm)	3/8" (9.52mm)***	22914				

Siltek®/Sulfinert® Treated Straight Seamless 316L Grade Stainless Steel Tubing

6 foot Length

ID	OD	qty.	cat.#
0.055" (1.40mm)	1/8" (3.18mm)**	ea.	22901
0.180" (4.57mm)	1/4" (6.35mm)**	ea.	22902
0.277" (7.04mm)	3/8" (9.52mm)***	ea.	22903

*0.020" wall thickness **0.035" wall thickness ***0.049" wall thickness

Sulfinert® Treated Alta-Robbins Sample Cylinder Valves

Description	qty.	cat.#
1/4" NPT Exit	ea.	21400
1/4" Compression Exit	ea.	21401
1/4" NPT with Dip Tube*	ea.	21402
1/4" NPT with 2850psi Rupture Disc	ea.	21403
1/4" NPT Male Inlet x 1/4" Female Outlet with 2850psi Rupture Disc	ea.	21404

Specify dip tube length or % outage when ordering (maximum length = 5.25"/ 13.3cm)

United States patent 6,444,326 (Siltek®/Sulfinert®)

thank **you**

Ted Neeme and Steve Mandel from Spectra Gases for their contributions to this work.

Sulfinert® Treated Swagelok® Sample Cylinders

Size	qty.	cat.#
75cc	ea.	24130
150cc	ea.	24131
300cc	ea.	24132
500cc	ea.	24133
1000cc	ea.	24134
2250cc	ea.	21394

Protect Sample Integrity and Prolong Sampling System Lifetime

Using Hydroguard™ Deactivated/Silcosteel® Treated Tubing

By Gary Barone, Restek Performance Coatings Division

- Prevents adsorption of sample components to an active surface.
- Long-lasting water resistance, increases instrument up-time.
- Specifically designed and tested for deactivating purge and trap or headspace systems.

Current regulations for drinking water and waste water require quantifying contaminant component concentrations at parts-per-trillion levels. As the demands of analytical methods and the sensitivity of analytical instruments advance, so has the need for improved inertness of the components of the sample pathway. In analyses at parts-per-trillion concentrations, any surface activity in the transfer system can adsorb significant amounts of active analytes and greatly impact the reliability of the data. Furthermore, components of purge and trap or headspace systems often are in contact with steam, which can create activity very quickly—even in coated system components. To address this need, we have created a superior surface for the tubing in purge and trap or headspace systems: Hydroguard™ deactivated/Silcosteel® treated stainless steel tubing.

For more than a decade, Restek's proprietary Silcosteel® and Siltek®/Sulfinert® treatments† have been ideal solutions for creating inert stainless steel pathways. Now, we have developed and rigorously tested Hydroguard™ deactivated/Silcosteel® treated stainless steel tubing specifically to meet the demanding requirements and environments of purge and trap and headspace systems.

Hydroguard™ deactivated/Silcosteel® treated tubing is preferred for situations in which water vaporization is encountered, as in purge and trap systems. Unique deactivation chemistry creates a high-density surface that is not readily attacked by hydrolysis. High-density Hydroguard™ deactivation at the outer surface effectively prevents water vapor from contacting the Silcosteel® treated stainless steel surface below. Thus, an inert surface is maintained in the face of highly aggressive conditions, and active analytes pass through the tubing without adsorbing to the surface.

Regardless of your application, we highly recommend Hydroguard™ deactivated/Silcosteel® treated tubing to improve analytical reliability from your purge and trap or headspace system.

† United States patents 6,511,760 (Silcosteel®) and 6,444,326 (Siltek®/Sulfinert®).

Silcosteel® Treated Hydroguard™ Deactivated Electropolished 316L Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085" (2.16mm)	1/8" (3.18mm)*	22489				
0.180" (4.57mm)	1/4" (6.35mm)**	22488				

Silcosteel® Treated Hydroguard™ Deactivated Seamless 316L Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22491				
0.180" (4.57mm)	1/4" (6.35mm)**	22490				

Silcosteel® Treated Hydroguard™ Deactivated 304 Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.010" (0.25mm)	1/16" (1.59mm)	22497				
0.020" (0.51mm)	1/16" (1.59mm)	22496				
0.030" (0.76mm)	1/16" (1.59mm)	22495				
0.040" (1.02mm)	1/16" (1.59mm)	22494				
0.085" (2.16mm)	1/8" (3.18mm)*	22493				
0.210" (5.33mm)	1/4" (6.35mm)*	22492				

*0.020" wall thickness
**0.035" wall thickness

Hub-Cap Mobile Phase Accessories

Simplify Mobile Phase Delivery with the Hub-Cap Filter Kit

Introducing our new Hub Cap filter kit! The Hub-Cap filter allows you to simultaneously transfer and filter your mobile phases. The bottle tops and adaptors are designed to fit securely on 4-liter solvent bottles and eliminate messy, loose-fitting parafilm or foil wraps. Tidy up your mobile phase delivery—try a Hub-Cap today!

Keep your mobile phase lines under control—use Hub-Cap bottle tops instead of parafilm, aluminum foil, or tape on your mobile phase reservoirs.

Hub-Cap Filter Kit

new!

cat. #26395

Transfer and filter mobile phases in one easy step.
Kit includes: bottle adapter, bottle adapter nut, filter inlet cap, grid support, vacuum hose barb, tube compression fitting, 47mm grid, 47mm .22µm filter membrane, 47mm .45µm filter membrane, 1/4" OD x 1/8" ID ultra chemical resistant, Teflon® FEP lined Tygon® tubing (3'), 6" x 6" box with shrink wrap insert

Description	qty.	cat.#
Hub-Cap Filter Kit	kit	26395
Replacement Membrane Filters	**qty.**	**cat.#**
Polyproylene Membrane Filters, 47mm, 0.45µm	100-pk.	26396
Polyproylene Membrane Filters, 47mm, 0.22µm	100-pk.	26397
Nylon Membrane Filters, 47mm, 0.45µm	100-pk.	26398
Nylon Membrane Filters, 47mm, 0.22µm	100-pk.	26399

Hub-Cap 4 Liter Bottle Tops

cat. #26541

Most bottles use a GL45 cap. New Hub-Cap bottle tops are a great way to neatly keep your mobile phase lines where they belong. Use instead of parafilm, aluminum foil, or tape on your mobile phase reservoirs.

Description	qty.	cat.#
Hub-Cap (assembly of the bottle cap and plug)	kit	26541
Hub-Cap Multi-pack	3-pk.	26542

Hub-Cap Adapters

cat. #26538

cat. #265

Allow the use of the Opti-Cap™ with 4-liter solvent bottles.

Description	qty.	cat.#
Hub-Cap Adapter	ea.	26538
Hub-Cap Adapter Multi-pack	3-pk.	26539
Hub-Cap Adapter and Opti-Cap™	kit	26540

Cool Tools!

Restek Innovations Save You Time and Money

A Clean Square Cut...

The key to obtaining a leak-tight seal in a Press-Tight® connector—or in other connecting devices that make a compression seal with the end of the column—is a clean, right angle cut at the end of the column. If you use an unsuitable device to cut your columns, you run the risk of angled cuts or chipped or jagged edges that will not seal effectively, or even crushing the end of the column. We offer a selection of scoring tools that will help you properly cut your columns.

Make a clean, square cut for optimum connector performance. The cut on the right will produce a poor seal.

Scoring Wafer with Handle

- Ceramic wafer is serrated on one side and straight-edged on the other to cut both fused silica and metal tubing cleanly.
- Unique, ergonomic handle is made of soft, comfortable rubber.

Hold tubing firmly in one hand, allowing about two inches to extend freely. Hold the scoring wafer at a 45° angle to the tubing. Exert just enough pressure to put a slight arc in the tubing. Pull perpendicularly across the tubing.

The tubing should fall off on its own, or it should easily break at the score with a slight tap of the wafer.

Check the cut against the white of the scoring wafer. Look for a clean, square cut.

Description	qty.	cat.#
Scoring Wafer with Handle	2-pk.	23015

Ceramic Scoring Wafers

- Four straight scoring edges for cutting fused silica tubing and four serrated edges for cutting MXT® metal capillary columns.
- Sure-grip handle included.

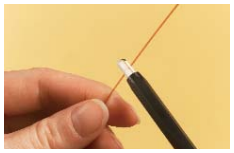
Exert just enough pressure to put a slight arc in the tubing. The tubing should fall off or break with a slight tap of the wafer.

Check the cut against the white of the scoring wafer. Look for a clean, square cut.

Description	qty.	cat.#
Ceramic Scoring Wafers	5-pk.	20116

Sapphire Scribe

- Cuts fused silica tubing.
- Produces a clean, square cut.



One quick stroke...

...and tap leaves a clean, square end.

Description	qty.	cat.#
Sapphire Scribe	ea.	20182

Capillary Column Caps

- Attach to the column in seconds to form an airtight seal.
- Increase column lifetime—prevent moisture and air from entering the column during storage.
- Color-coded for identifying detector and injector ends.
- Not recommended for reuse.

Description	qty.	cat.#
Capillary Column Caps	10-pk.	21044

Peak Performers

Routine Connections Made Simple

By Donna Lidgett, GC Accessories Product Marketing Manager

restek
innovation!

SeCure™ "Y" Connector Kits

- Connect two analytical columns to a transfer line or guard column.
- Use standard "Y" Press-Tight® connectors and 1/16" graphite ferrules.
- Reliable seal integrity, will not unexpectedly disconnect during temperature-programmed analyses.
- Open design allows visual confirmation of the seal for added confidence in the connection.

Combine the simplicity of a "Y" Press-Tight® connector with the strength of a metal union. The ferrules and knurled nuts hold the fused silica tubing in place, which prevents the tubing from unexpectedly disconnecting, even at temperatures as high as 400°C.

Kits include: SeCure™ "Y" connector body, 3 knurled nuts, "Y" Universal Press-Tight® union, 3 ferrules.

Description	Ferrules Fit Column ID	qty.	cat.#
SeCure™ "Y" Connector Kit	0.18/0.25/0.28mm	kit	20276
SeCure™ "Y" Connector Kit	0.32mm	kit	20277
SeCure™ "Y" Connector Kit	0.45/0.53mm	kit	20278
Knurled nut		3-pk.	20279

Make secure, reliable column-to-column connections with SeCure™ "Y" connectors.

Graphite Ferrules for SeCure™ "Y" Connectors

- Preconditioned to minimize out-gassing.
- High-purity, high-density graphite.
- Stable to 450°C.
- No binders that can off-gas or adsorb analytes.
- Smooth surface and clean edges.

Ferrule ID	Fits Column ID	Graphite 10-pk.	Graphite 50-pk.
0.4mm	0.18/0.25/0.28mm	20200	20227
0.5mm	0.32mm	20201	20228
0.8mm	0.45/0.53mm	20202	20224

Vu2 Union™ Connectors

- Connect a guard column to an analytical column.
- Connect a column to a transfer line.
- Connect two columns in series.
- Repair a broken column.

Kits include: Vu2 Union™ body, 2 knurled nuts, 2 Press-Tight® unions, and 4 ferrules

Description	Ferrules Fit Column ID	qty.	cat.#
Vu2 Union™ Connector Kit	0.10/0.15mm	kit	22220
Vu2 Union™ Connector Kit	0.18/0.28mm	kit	21105
Vu2 Union™ Connector Kit	0.32mm	kit	21106
Vu2 Union™ Connector Kit	0.45/0.53mm	kit	21107
Knurled nut		2-pk.	21108

NOTE: Not recommended for GC column-to-MS connections—use the Vacuum Vu-Union® available at www.restek.com.

The Vu2 Union™ conector's open design allows visual confirmation of the seal; secondary seals ensure a leak-tight connection.

Graphite Ferrules for Vu2 Union™ Connectors

- High-purity, high-density graphite.
- Stable to 450°C.
- No binders that can off-gas or adsorb analytes.
- Smooth surface and clean edges.

Ferrule ID	Fits Column ID	Graphite 2-pk.	Graphite 10-pk.
0.3mm	0.10/0.15mm	22221	22222
0.4mm	0.18/0.28mm	20280	20281
0.5mm	0.32mm	20282	20283
0.8mm	0.45/0.53mm	20284	20285

Universal Press-Tight® Connectors

- Connect a guard column to an analytical column.
- Repair a broken column.
- Connect a column outlet to a transfer line.
- Deactivated Press-Tight® connectors assure better recovery of polar and non-polar compounds.
- Siltek® treated connectors are ideal for organochlorine pesticides analysis.
- Fit column ODs from 0.33–0.74mm (Restek 0.1mm–0.53mm ID).

Description	5-pk.	25-pk.	100-pk.
Universal Press-Tight® Connectors	20400	20401	20402
Deactivated, Universal Press-Tight® Connectors	20429	20430	20431
Siltek® Treated Universal Press-Tight® Connectors	20480	20449	20481

Universal Angled Press-Tight® Connectors

- Angle approximates the curvature of a capillary column, reduces strain on column-end connections.

Description	5-pk.	25-pk.	100-pk.
Universal Angled Press-Tight® Connectors	20446	20447	20448
Deactivated Universal Angled Press-Tight® Connectors	20446-261	20447-261	20448-261
Siltek® Treated Universal Angled Press-Tight® Connectors	20482	20483	20484

Universal "Y" Press-Tight® Connectors

- Split sample flow onto two columns.
- Split a single column flow to two detectors—perform confirmation analysis with a single injection.
- Deactivated Press-Tight® connectors assure better recovery of polar and non-polar compounds.
- Siltek® treated connectors are ideal for organochlorine pesticides analysis.
- Fit column ODs from 0.33–0.74mm (Restek 0.1mm–0.53mm ID).

An alternative method of performing dual-column confirmational analyses!

Description	ea.	3-pk.
Universal "Y" Press-Tight® Connector	20405	20406
Deactivated Universal "Y" Press-Tight® Connector	20405-261	20406-261
Siltek® Treated Universal "Y" Press-Tight® Connector	20485	20486

Universal Angled "Y" Press-Tight® Connectors

- Inlet and outlet ends conform to the column curvature—alleviates column-end connection strain.

Description	ea.	3-pk.
Universal Angled "Y" Press-Tight® Connector	20403	20404
Deactivated Universal Angled "Y" Press-Tight® Connector	20403-261	20404-261
Siltek® Treated Universal Angled "Y" Press-Tight® Connector	20487	20469

MXT™-Union Connector Kits for Fused Silica Columns

- Low-dead-volume, leak-tight connection.
- Reusable.
- Siltek® treatment ensures maximum inertness.
- Ideal for connecting a guard column or transfer line to an analytical column.
- Use to oven temperatures of 350°C.
- Available in union and "Y" configurations.

SILTEK® treated

These MXT™ connectors can be used with fused silica tubing, because of a Valcon polyimide 1/32-inch one-piece fused silica adaptor. This unique graphite-reinforced composite allows a capillary column to slide into the adaptor and be locked in place simply by loosening and tightening the fitting. Each kit contains the MXT™ union, two 1/32-inch nuts and two one-piece fused silica adaptors.

MXT™-Union Connector Kits for Fused Silica Columns

Description	qty.	cat.#
For 0.25mm ID Fused Silica Columns	kit	21386
For 0.32mm ID Fused Silica Columns	kit	21385
For 0.53mm ID Fused Silica Columns	kit	21384

MXT™ "Y"-Union Connector Kits for Fused Silica Columns

Description	qty.	cat.#
For 0.25mm ID Fused Silica Columns	kit	21389
For 0.32mm ID Fused Silica Columns	kit	21388
For 0.53mm ID Fused Silica Columns	kit	21387

Get Connected!

By Al Carusone, Technical Service

Have you ever had to connect a GC analytical column to a guard column or transfer line? Or repair a broken column? How about connecting two columns in series or performing confirmation analysis with a single injection? All of these connections are possible with Restek's extensive selection of GC connectors. In most situations, connector choice is a personal preference and Restek offers several options. Here we review differences among our connectors and answer some frequently asked questions about our popular Press-Tight® connectors.

The **Press-Tight® connector**, a glass connector with a tapered internal diameter at each end, is the quickest and least expensive option. Straight or angled Press-Tight® connectors are effective for fused silica-to-fused silica connections for standard applications at temperatures below 325°C. The resulting connections are inert and have low dead volume.

The **MXT™-Union connectors** are unbreakable metal connectors that are reusable and ensure a low dead volume. They are designed for metal-to-metal connections, but also can make metal-to-fused silica unions using a Valcon polyimide adaptor. This unique graphite-reinforced composite allows a capillary column to slide into the adaptor and be locked in place simply by loosening and tightening the nuts.

If you require a fused silica-to-fused silica connector for high temperature applications, try Restek's **Vu2 Union™ connector** or **SeCure™ "Y" connector**. They combine the simplicity of a glass connector with the strength of a metal connector. Both connectors feature an open design that allows visual confirmation of the seal, and also have secondary seals to help maintain a leak-tight connection. These ultra-strong connections will not disconnect unexpectedly under temperature changes, vibrations, or other stresses normally encountered in GC analysis.

Restek also offers a **Vacuum Vu-Union® connector** for connecting a fused silica column to a mass spec transfer line. The Vacuum Vu-Union® connector utilizes Vespel® ferrules for nonpermeable vacuum connections. A specifically designed Vu-Union® glass insert permits more torque to be applied to the ferrules without fear of cracking the insert. As with the Vu2 Union™, you can confirm the seal through the window of the connector.

get the connection

see page 20-21 for a sampling of our connectors, or visit us online at www.restek.com

What is the difference between angled and regular Press-Tight® connectors?

The only difference between these connectors is their shape. A Press-Tight® connector is a straight tube; an angled Press-Tight® connector has a slight angle in the middle which reduces the strain on the fused silica tubing. This is of particular use in making a connection in a broken column, when you must make the connection within the column coils.

How can I obtain a leak-tight seal using a Press-Tight® connector?

Press-Tight® connectors are easy to use, but if they are not properly sealed, they can loosen due to thermal expansion during temperature-programmed runs. The keys to successful sealing are: 1) making a clean, square cut on the column and 2) moistening the end of the column with methanol before seating it into the connector. A small amount of polyimide resin also helps prevent the seal from separating during temperature cycling.

A brown ring indicates a proper seal.

Can Press-Tight® connectors be used with MXT® columns?

No. To achieve a leak-tight metal-to-metal connection, we recommend the **MXT™ Low Dead Volume connector** for metal columns. These low dead volume connectors are Siltek® treated to make them inert to active compounds, and they can be used up to 400°C without degrading the deactivation layer. MXT™ tubing can even be connected to fused silica tubing using an MXT™ connector with a Valcon Polyimide ferrule instead of a stainless steel ferrule.

Retention Cross-over Phenomenon in Gas Chromatography—Can the Mystery be Revealed? Part 2

Continued from page 2

mately 250 compounds were measured on a squalane stationary phase at four temperatures.[1] From these data one can identify numerous reversals in elution order of aliphatic and cyclic hydrocarbons. The solute interactions with a squalane stationary phase, the most nonpolar stationary phase one can use, are largely a result of dispersion interactions. The authors stated that the magnitude of temperature variation is a function of the size of the molecule expressed by the cross-sectional area of the molecules, which should also prove my point in my next discussion over coffee with my former student.

Finally, coming back to our first example in Part 1, both components not only show different functional groups, they also differ in their carbon skeleton (Figure 3). Linalool is an aliphatic alcohol and camphor is a bi-cyclic ketone, which means that not only the functional groups but also the difference in molecular geometry will contribute to the cross-over phenomenon.

What can we learn from this discussion? Peak overlapping and cross-over in peak elution order caused by variation of column temperature or temperature programming rate can occur not only on polar stationary phases for compounds with different functional groups but also on nonpolar or weak polar stationary phases for compounds that differ in their carbon skeleton. The analyst should, therefore, carefully examine the structure of the compounds to be separated if the information is available. Furthermore, it is recommended to study analyte retention carefully at various temperatures for difficult separations as an important aspect of method optimization.

References:
1 Hively, R.A. and R.E. Hinton, J. Gas Chromatogr. 6 (1968) 203 – 217.

Figure 2 Retention indices on squalane (IS) as a function of Tc for isothermal GC at 27°C, 49°C, 67°C, and 86°C.[1]

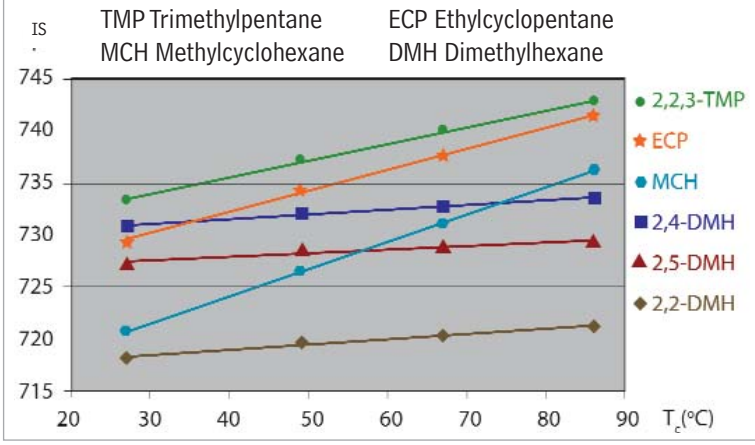
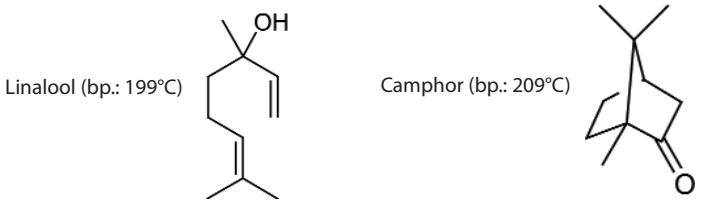


Figure 3 Functional groups influence elution order.



Tradeshow Schedule

We'd be happy to talk with you at any of the following meetings or shows. We'll post our booth numbers as they become available to us.

September, 2007

Date September 2-7
Show Dioxin 2007
Location Hotel Okura, Tokyo

Date September 13
Show New Jersey Mass Spectrometry Discussion Group Annual Vendor Show
Location DoubleTree Hotel, Somerset, NJ

Date September 16-20
Show AOAC International 121st Annual Meeting & Expo
Location Hyatt Regency Orange County, Anaheim, CA

Date September 25-28
Show Midwestern Association of Forensic Scientists (MAFS)
Location Park Place Hotel, Traverse City, MI

Date September 26-28
Show Vapor Intrusion Conference
Location Providence, RI

October, 2007

Date October 2-4
Show ISA Expo 2007
Location Reliant Center, Houston, TX

Date October 10-12
Show ACIL National Meeting
Location InterContinental Hotel Buckhead, Atlanta, GA

Date October 13-20
Show Society of Forensic Toxicology (SOFT)
Location Chapel Hill, NC

Date October 16-17
Show Gulf Coast Conference
Location Moody Garden Convention Center, Galveston, TX

Date October 18-21
Show Beijing Conference & Exhibition on Instrument Analysis
Location Beijing Exhibition Center, Beijing, China, Booth #00

Date October 30-November 1
Show Chem Show
Location Javits Convention Center, New York, NY

Date October 30-November 2
Show 2007 SEMA Show
Location Las Vegas Convention Center, Las Vegas, NV

Date October 31-November 3
Show 33rd Annual NEAFS Meeting
Location The Sagamore Resort, Bolton Landing, NY

November, 2007

Date November 1
Show 2007 ANACHEM Symposium
Location Burton Manor, 27777 Schoolcraft Road, Livonia, MI

Date November 7-9
Show 3rd International Symposium on Recent Advances in Food Analysis
Location Diplomat Hotel–Conference Center, Prague, Czech Republic

Date November 11-15
Show Eastern Analytical Symposium (EAS)
Location Garden State Convention & Exhibit Center, Somerset, NJ

Date November 11-15
Show 2007 AAPS Annual Meeting and Exposition
Location San Diego Convention Center, San Diego, CA

Date November 28-30
Show 31st Int'l Symposium on Capillary Chromatography & Electrophoresis
Location Hotel Albuquerque, Albuquerque, NM

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IN THIS ISSUE

Retention Cross-over Phenomenon in Gas Chromatography–Can the Mystery be Revealed? Part 1

By Werner Engewald, Ph.D., Professor Emeritus, University of Leipzig, Institute of Analytical Chemistry, Leipzig, Germany; engewald@uni-leipzig.de

Have you ever faced changes in elution order after modifying the column temperature or the heating rate in the temperature program of the GC analysis of complex samples? This so-called cross-over phenomenon, which can lead to problems in peak identification, has been a well-known mystery in GC for decades.[1] But, so far, the physico-chemical background is still not well understood.

The cross-over phenomenon is very common when separating compounds with different functional groups on polar stationary phases. For example, we observed a reversal in the elution order for components like linalool and camphor on a polyethylene glycol column (Carbowax 20M) after changing the column temperature programming rate: at 5°C/min. linalool elutes before camphor but at 3°C/min. camphor will elute first. Effects like this are often observed when essential oils are analyzed or, to be more precise, when the GC methods are optimized. The reversal of the elution order is mainly explained as a result of the different temperature-dependencies of the intermolecular interactions, which are responsible for the retention: London-type dispersion forces and induction forces are independent of temperature, whereas the orientation forces and hydrogen bridge bonds depend strongly on the temperature (Figure 1).

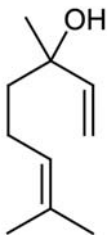
However, this explanation is only half the truth and we should examine the influence of column temperature on retention in some more detail. It is generally known that the column temperature is one of the two most important variables in GC (the other being of course the nature of the stationary phase). In partition GC, the effect of temperature on the solute partition coefficient K is given by the van´t Hoff relationship ln K = HS/RTc + C (with HS being the molar heat of solution of solute). From this follows the fundamental correlation between column temperature Tc and retention factors:

$$\ln k' = HS/RTc + C' - \ln ß$$

where k´ is the retention or capacity factor (k´ = t´R/t M) and ß the column phase ratio. This equation indicates that the retention decreases logarithmically as the column temperature increases.

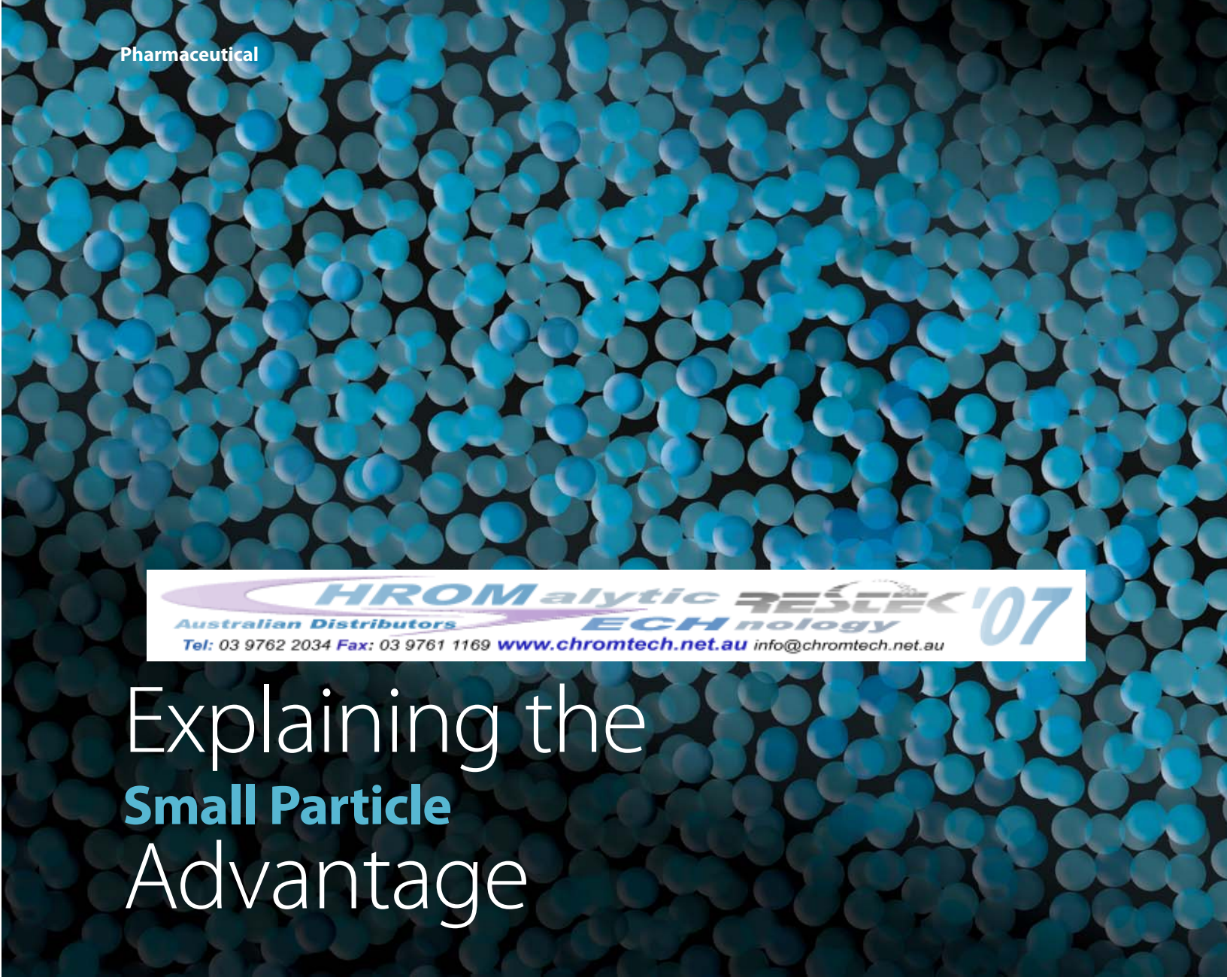
Figure 1 Functional groups influence elution order.

Linalool (bp.: 199°C)



Camphor (bp.: 209°C)

Continued on page 23.



Explaining the Small Particle Advantage

Faster Sample Throughput on a 1.9µm Pinnacle™ DB HPLC Column

By Rick Lake, Pharmaceutical Innovations Chemist, Randy Romesberg, HPLC Innovations Chemist, and Becky Wittrig, Ph.D., HPLC Product Marketing Manager

- Faster analyses, uncompromised chromatography using a 1.9µm Pinnacle™ DB column.
- Narrow particle size distribution ensures consistent, high efficiencies and longer column lifetimes.
- 100% Restek manufactured–from base silica to final packed column–assures quality and reliability.

Restek is pleased to introduce an exciting new addition to our family of HPLC columns–the 1.9µm Pinnacle™ DB small particle column. Intended for use in ultra-high pressure liquid separations, the 1.9µm Pinnacle™ DB column combines the benefits of a popular technique with the unmatched quality you expect from Restek. From the manufacturing of the base silica through the packing of the column, Restek performs and tightly controls every step in the manufacturing process, guaranteeing ruggedness and reliability. Here we discuss how and why small particle HPLC columns provide faster separations, and demonstrate the high efficiency, excellent peak symmetry, and rapid analysis times that can be achieved on the 1.9µm Pinnacle™ DB column. *Continued on page 4.*

Explaining the Small Particle Advantage *(continued from page 3)*

In HPLC column terminology, particle size refers to the mean diameter of the silica spheres used as the support material to which the stationary phase is bonded. Until recently, the practical particle size limit was around 3µm; smaller particles created backpressures above the limit of conventional LC systems. The advent of LC systems capable of handling higher backpressures (>10000psi) now allows chromatographers to realize the benefits of sub-2µm particle size columns. Smaller particles give rise to greater column efficiencies and a wider range of usable flow rates, resulting in better resolution and higher sensitivity with a significantly faster overall analysis time. Figure 1 and Table 1 illustrate the excellent peak shape and higher efficiency characteristic of a 1.9µm Pinnacle™ DB C18 column, compared to competitive columns.

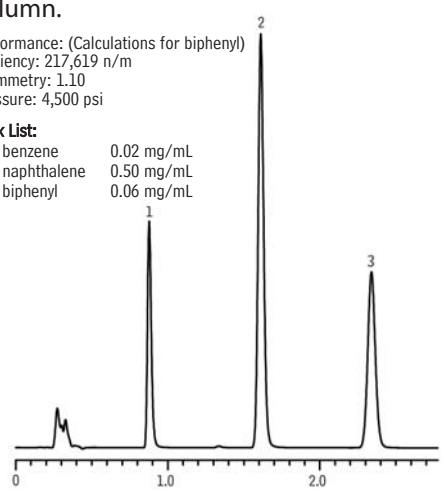
To demonstrate the substantial gain in sample throughput that is possible on a small particle column, we assayed a series of parabens under conditions that give comparable linear velocities on both a C18 column with conventional dimensions and on a 1.9µm Pinnacle™ DB C18 column (Figure 2B & C). Similar resolution was achieved in a much shorter analysis time on the 1.9µm Pinnacle™ DB C18 column. We also doubled the flow rate on the 1.9µm Pinnacle™ DB C18 column: the resolution and peak efficiencies again were comparable, but the analysis time was cut in half (Figure 2A). This illustrates the considerable effect that small particles can have on chromatographic separations; a much wider range of usable flow rates translates into significantly faster analysis times–in this case 10-fold faster, with no loss in chromatographic quality.

Figure 1 Excellent peak symmetry and efficiency on a 1.9µm Pinnacle™ DB C18 column.

Performance: (Calculations for biphenyl)
Efficiency: 217,619 n/m
Asymmetry: 1.10
Pressure: 4,500 psi

Peak List:

1. benzene	0.02 mg/mL
2. naphthalene	0.50 mg/mL
3. biphenyl	0.06 mg/mL



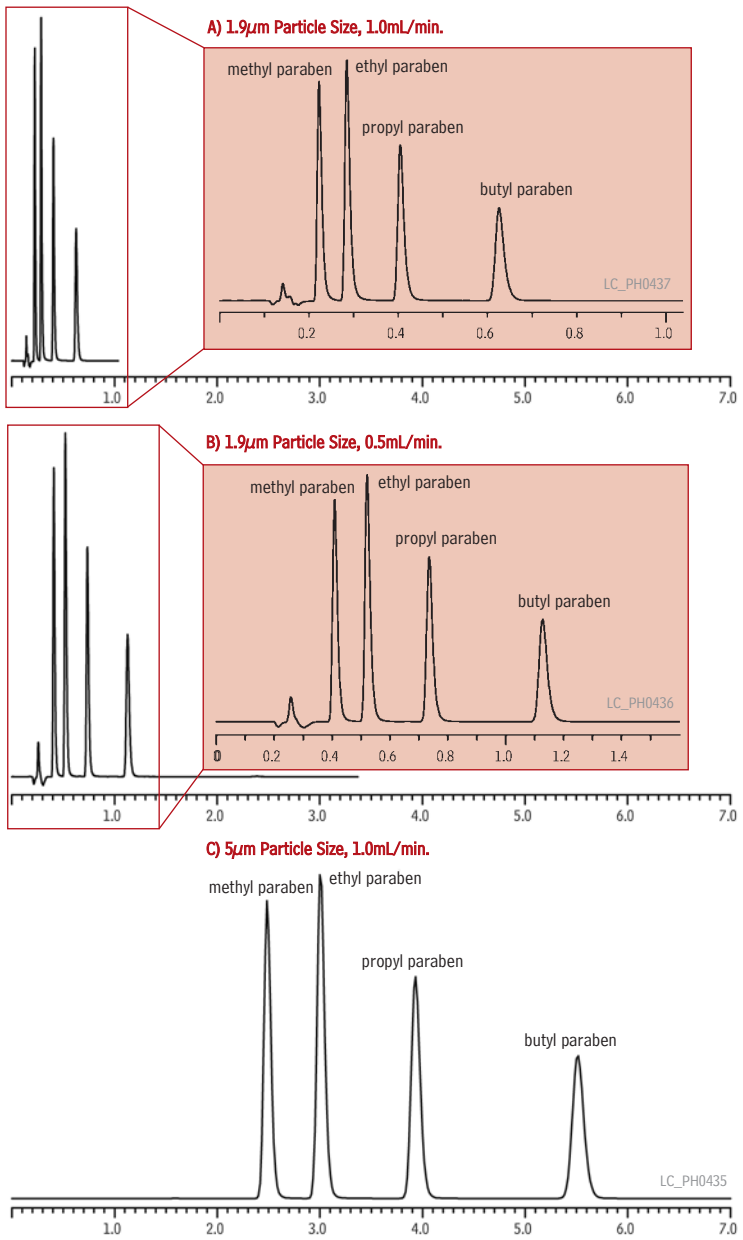
Sample: Inj.: 2µL, HPLC Reversed Phase Test Mix #1 (cat.# 35005), Sample diluent: water:methanol (25:75), Sample temp.: ambient, Column: Pinnacle™ DB C18, Cat. #: 9414252, Dimensions: 50 x 2.1mm, Particle size: 1.9µm, Pore size: 140Å, Mobile phase: water:acetonitrile (45:55), Flow: 0.4 mL/min., Temp.: 25°C, Det.: UV @ 254nm LC_EX0427

Table 1 1.9µm Pinnacle™ DB C18 column offers the highest efficiency of all columns tested.

Column	Efficiency (n/m)	Pressure (psi)	Asymmetry
1.9µm Pinnacle™ DB	217,619	4,500	1.10
Competitor A	177,999	4,400	1.13
Competitor B	188,508	4,300	1.09

Data from the biphenyl peak of a reversed phase test mix.

Figure 2 1.9µm Pinnacle™ DB columns offer a wider range of usable flow rates, dramatically increasing sample throughput–with no loss in resolution.



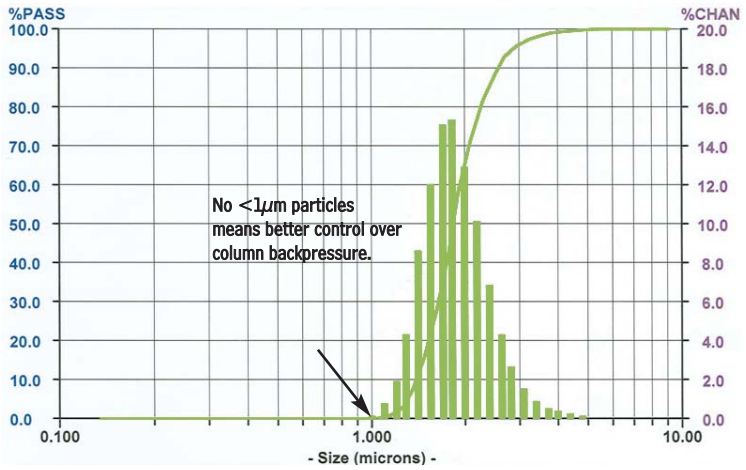
All chromatograms; Column: Pinnacle™ DB C18, Pore size: 140Å; Sample Conc.: ~100 µg/mL each component in mobile phase (50:50 0.1% acetic acid:acetonitrile), Temp.: Ambient, Det.: UV @ 254nm

A) Inj.: 2µL, Cat. #: 9414252; Dimensions: 50 x 2.1 mm; Particle size: 1.9µm

B) Inj.: 2µL, Cat. #: 9414252; Dimensions: 50 x 2.1 mm; Particle size: 1.9µm

C) Inj.: 10µL, Cat. #: 9414565; Dimensions: 150 x 4.6 mm; Particle size: 5µm

Figure 3 Pinnacle™ DB silica particle size distribution shows an exceptionally tight, symmetrical distribution around 1.9µm.



Column	Target Particle Size (µm)	Actual Mean Particle Size (µm)	Standard Deviation	Particles present <1µm
1.9µm Pinnacle™ DB	1.9	1.952	0.437	No
Competitor A	1.7	1.993	0.529	No
Competitor B	1.8	1.832	0.468	Yes

The stated particle size of an HPLC column is actually the mean of the distribution of all particles used in manufacturing the column. In practice, the smaller the particle size distribution, the more uniformly packed the column will be, resulting in higher efficiencies. This distribution is even more critical when manufacturing columns with particle sizes less than 2µm. If the distribution contains many larger particles and is not tightly controlled, the efficiency of the column and column-to-column reproducibility will suffer. More importantly, if the column contains particles less than 1µm (termed "fines"), clogging of the column frit and excessively high column backpressure can result. 1.9µm Pinnacle™ DB columns have a narrow, symmetric particle size distribution; they contain no particles less than 1µm in diameter. Figure 3 illustrates this exceptional distribution, which is tighter and more accurate than competitive sub-2µm columns.

1.9µm Pinnacle™ DB columns offer practical advantages for today's chemist across a wide range of analytes, from acidic to basic. For higher sample throughput, matched with the reliability and ruggedness of a column made entirely by chromatographers for chromatographers, reach for Restek small particle HPLC columns.

1.9µm Pinnacle™ DB C18 HPLC Columns

Physical Characteristics:

particle size: 1.9µm
pore size: 140Å
carbon load: 11%
endcap: yes
pH range: 2.5 - 10
temperature limit: 80°C

1.9µm Column, 2.1mm	cat. #
30mm	9414232
50mm	9414252
100mm	9414212

Revised USP 467 Residual Solvent Method

Satisfy New Method Requirements with Restek Columns and Standards

By Rick Lake, Pharmaceutical Innovations Chemist

- Overview of the new USP 30/NF 25 procedure.
- New reference standards - stock mixes, custom preparations.
- Optimize your testing within the constraints of the method.

Organic volatile impurities (OVIs), commonly referred to as residual solvents, are trace level chemical residues in drug substances and drug products that are byproducts of manufacturing, or that form during packaging and storage. The United States Pharmacopeia recently revised the general chapter on residual solvent analysis, USP 467, to mirror the International Conference on Harmonization (ICH) guidelines. This revision, effective July 1, 2007, replaces previous methods that were not consistent with the ICH guidelines.

The revised procedure consists of a static headspace extraction coupled with a gas chromatographic separation and flame ionization detection (GC/FID), and is divided into two sections based on sample solubility – water soluble and water insoluble articles. Altogether, the test method consists of three separate procedures – A, B and C–that are designed to identify, confirm and quantify residual solvents in pharmaceuticals.

Procedure A is the first step in the identification process and is performed to screen samples for residual solvents. A series of residual solvent mixes, consisting of Class 1 and Class 2 mixes A and B, are analyzed along with the system suitability and test solutions on an Rtx®-624 column – equivalent to an Rtx®-1301 (G43) column (Figures 1-3). If a peak in the sample matches a retention time, and exceeds the response of the corresponding standard, the analyst proceeds to Procedure B for verification of the analyte.

Once a residual solvent is identified, Procedure B is performed to confirm analyte identity. We recommend a Stabilwax® (G16) capillary column as a confirmation column because it yields an alternate selectivity compared to an Rtx®-624 column or an Rtx®-1301 (G43) column. (See our OVI retention time index at www.restek.com/ovi). The same reference mixes are analyzed with an acetonitrile/trichloroethylene system suitability solution. If a residual solvent is verified, Procedure C is used to quantify the analyte by comparison to a specific, individual standard for the analyte identified. For water-insoluble articles, the procedure is the same, except dimethylformamide and 1,3-dimethyl-2-imidazolidinone are used as the diluent and Class 2 Mix C (higher boiling point solvents mix) is analyzed as a reference solution.

Figure 1 USP Residual Solvent Class 1 standard solution on an Rtx®-624 (G43) column.

Column:	**Rtx®-624 30m, 0.32 ID, 1.8µm (cat. # 10970)**
Inj.:	Headspace injection (split ratio 1:5),1mm Split liner Siltek® deactivated (cat. # 20972-214.1)
Inj. temp.:	140°C
Carrier gas:	helium, constant flow
Flow rate:	2.16 mL/min., 35.3 cm/sec.
Oven temp.:	40°C for 20 min., to 240°C @ 10°C/min., hold for 20 min.
Det.:	FID @ 240°C

Headspace Conditions	
Instrument:	Tekmar HT3
Transfer Line Temp.:	105°C
Valve Oven Temp.:	105°C
Sample Temp.:	80°C
Sample Equil. Time:	45 min.
Vial Pressure:	10psi
Pressurize Time:	0.5 min.
Loop Fill Pressure:	5psi
Loop Fill Time:	2.00 min.
Inject Time:	1.00 min.

Sample:
USP 467 Class 1 Standard Solution (cat.# 36279) in 20mL headspace vial

1. 1,1-dichloroethene
2. 1,1,1-trichloroethane
3. carbon tetrachloride
4. benzene
5. 1,2-dichloroethane

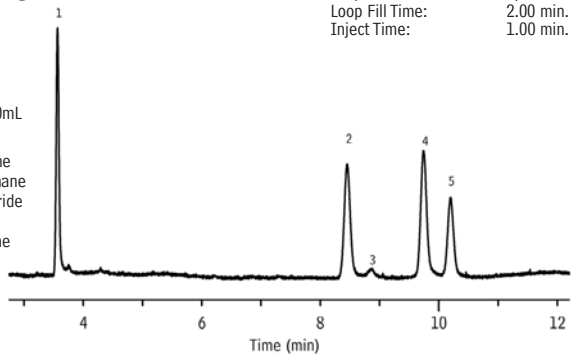
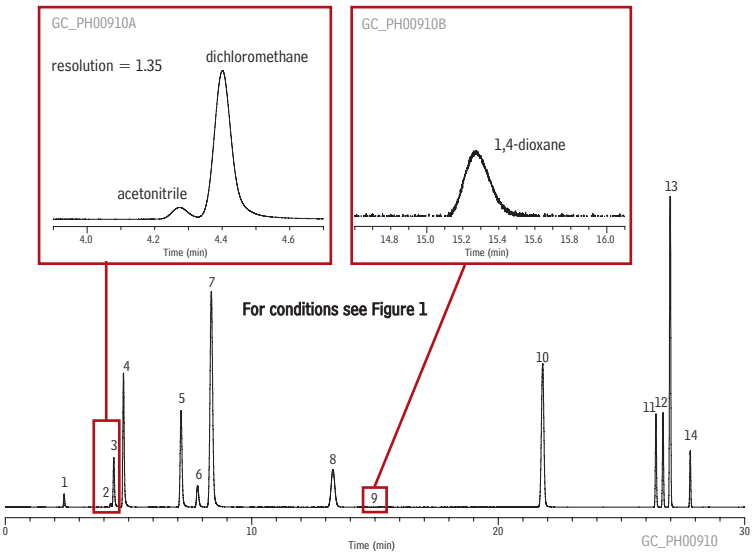


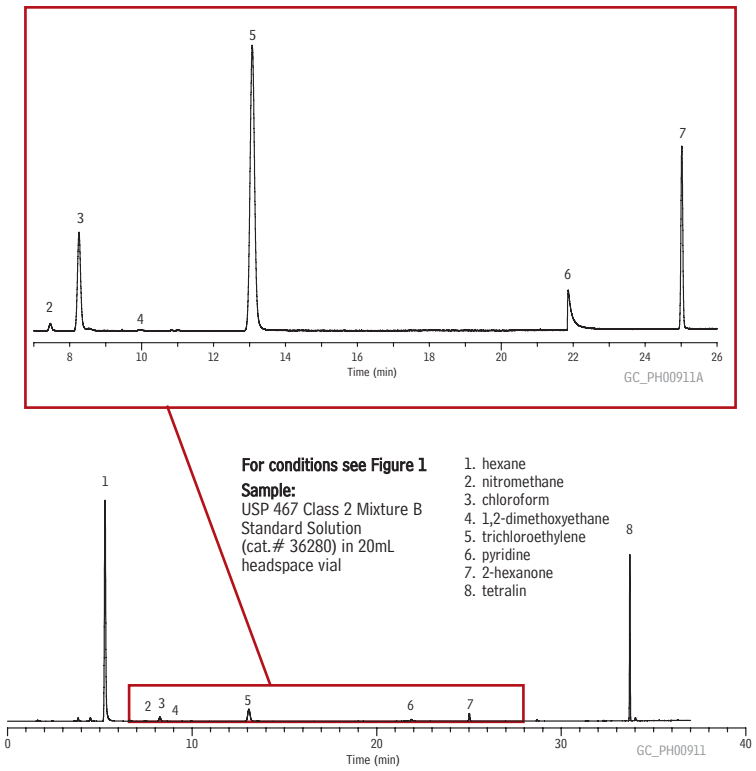
Figure 2 USP Residual Solvent Class 2 Mixture A standard solution on an Rtx®-624 (G43) column.



Sample: USP 467 Class 2 Mixture A Standard Solution (cat.# 36271) in 20mL headspace vial

1. methanol
2. acetonitrile
3. dichloromethane
4. *trans*-1,2-dichloroethene
5. *cis*-1,2-dichloroethene
6. tetrahydrofuran
7. cyclohexane
8. methylcyclohexane
9. 1,4-dioxane
10. toluene
11. chlorobenzene
12. ethyl benzene
13. *m*-xylene / *p*-xylene
14. *o*-xylene

Figure 3 USP Residual Solvent Class 2 Mixture B standard solution on an Rtx®-624 (G43) column.



Restek can supply all your USP 467 materials and can help you optimize your testing within the constraints of the method. Visit us on the web at www.restek.com or contact our Technical Support team at 800-356-1688, ext.4, for solutions to your residual solvent testing needs and tips on optimizing your analysis.

Residual Solvents - Class 1

benzene	10mg/mL	1,1-dichloroethene	40
carbon tetrachloride	20	1,1,1-trichloroethylene	50
1,2-dichloroethane	25		

In dimethyl sulfoxide, 1mL/ampul
cat. # 36279 (ea.)

Residual Solvents Class 2 - Mix A (15 components)

acetonitrile	2.05mg/mL	methylcyclohexane	5.90
chlorobenzene	1.80	methylene chloride	3.00
cyclohexane	19.40	tetrahydrofuran	3.45
cis-1,2-dichloroethylene	4.70	toluene	4.45
trans-1,2-dichloroethylene	4.70	*m*-xylene	6.51
1,4-dioxane	1.90	*o*-xylene	0.98
ethylbenzene	1.84	*p*-xylene	1.52
methanol	15.00		

In dimethyl sulfoxide, 1mL/ampul
cat. # 36271 (ea.)

Residual Solvents Class 2 - Mix B (8 components)

chloroform	60µg/mL	nitromethane	50
1,2-dimethoxyethane	100	pyridine	200
n-hexane (C6)	290	tetralin	100
2-hexanone	50	trichloroethylene	80

In dimethyl sulfoxide, 1mL/ampul
cat. # 36280 (ea.)

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Restek offers a full day seminar on headspace analysis. Join us September 26, in Edison, NJ for a day of learning focused exclusively on headspace principles, techniques, and applications (cat.# 65563). To register, visit us online at **www.restek.com/seminar**

for **more** info

- Technical poster: *Comprehensive Dual-Column Analysis of Residual Solvents in Water-soluble Articles Using Dynamic Headspace and Modular Accelerated Column Heating.* **www.restek.com/usp467**
- *A Technical Guide for Static Headspace Analysis Using GC*, cat.# 59895A.
- OVI retention time index **www.restek.com/ovi**

Rtx®-624 (G43) Columns (fused silica)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.80	-20 to 240°C	30-Meter	10970
0.53mm	3.00	-20 to 240°C	30-Meter	10971

Stabilwax® (G16) Columns (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	40 to 250°C	30-Meter	10624
0.53mm	0.25	40 to 250°C	30-Meter	10625

Siltek® 1mm Split Liners for Agilent GCs

Use this liner for increased sensitivity. Exclusive Siltek® deactivation makes liner inert to active sample components.

Benefits/Uses:	ID*/OD & Length (mm)	cat.# ea.	cat.# 5-pk.
for purge & trap inlet splitting or sample <1µL	1.0 ID 6.3 OD x78.5	20972-214.1	20973-214.5

*Nominal ID at syringe needle expulsion point.

GC Inlet Liner Deactivations for Basic Drug Analysis

By Kristi Sellers, Clinical/Forensic Innovations Chemist, and Lydia Nolan, Innovations Chemist

- Base-deactivated inlet liners are inert to basic drugs, for greater responses.
- Inertness of Rtx®-5 Amine column is enhanced for basic compounds.
- Use this liner / column combination for the lowest %RSDs for basic drugs.

Clinical and forensic toxicologists are required to detect low levels of abused drugs in body fluids and confirm their presence by GC/MS. Typical limits of detection are 1-15ng/mL, depending on the sample matrix. For basic drugs (e.g., Figure 1), selecting the proper surface treatment for the GC inlet liner is important, because this parameter can affect responses. The surface of a glass inlet liner contains active silanol groups (Si-OH) that can act as electron pair acceptors, and react with nitrogen or oxygen electron pair donors in basic drug molecules (Figure 2).[1] These reactions usually are rapid and reversible, but they are expressed chromatographically as broad, tailing peaks and/or reduced responses. To eliminate these acid-base reactions, make chromatographic peaks sharp, Gaussian, and easy to integrate, and thereby help ensure reproducible and accurate responses, the -OH groups on the glass surface must be deactivated.

We evaluated several alternatives for deactivating inlet liners to determine the best deactivation chemistry for the analysis of basic drugs. Standards composed of the free base forms of the drugs shown in Figure 1 were prepared at concentrations of 5, 10, 25, 50, and 100 ng/mL for analysis on a 15m, 0.25mm ID, 0.25µm Rtx®-5 Amine column (5% diphenyl/95% dimethyl polysiloxane stationary phase). The analysis of these drug standards was repeated on a series of 4mm ID single gooseneck liners that had been treated with different deactivation techniques, as well as an untreated liner. Three replicate analyses were performed on each liner to determine which deactivation treatment offered the highest and most consistent response for these basic drugs.

We used these results to generate box plots that display the range of data distribution, or variation – an indication of the reproducibility of the performance. We chose phencyclidine (PCP) and cocaine plots to represent the nitrogen-containing and nitrogen/oxygen-containing drugs, respectively (Figure 2). The line in each box indicates the mean response.

The data show that undeactivated liners and liners that received intermediate polarity treatment provided poorer responses or reproducibility, com-

Figure 1 Basic compounds can react with silanol groups on glass liner surfaces, causing poor chromatography.

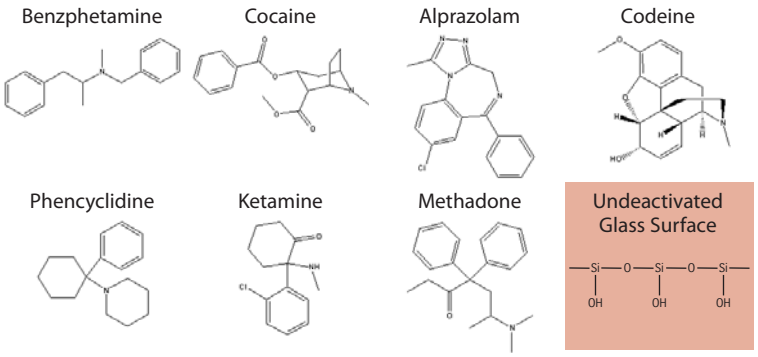


Figure 2 A base-deactivated inlet liner provides highest mean responses for PCP.

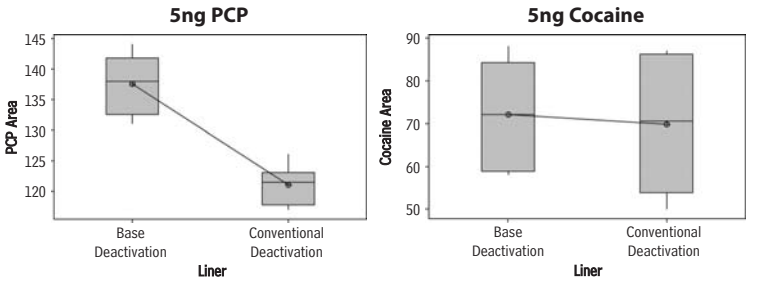
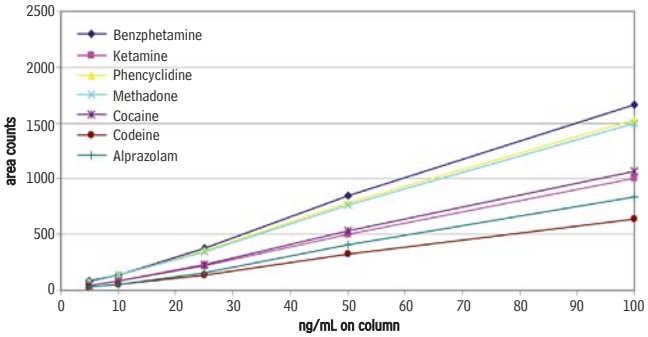


Figure 3 Linearity plots for all drugs, analyzed using a base-deactivated inlet liner and an Rtx®-5 Amine column.



Base Deactivated Inlet Liners for Basic Drug Analysis

For Agilent GCs	cat.# ea.	5-pk.	25-pk.
Gooseneck Splitless, Base Deactivated (4.0mm ID* x 6.5mm OD x 78.5mm) 	20798-210.1	20799-210.5	20800-210.25
Gooseneck Splitless, Base Deactivated w/ Base Deactivated Wool (4.0mm ID* x 6.5mm OD x 78.5mm) 	20798-211.1	20799-211.5	20800-211.25
Split Straight, Base Deactivated w/ Base Deactivated Wool (4.0mm ID* x 6.3mm OD x 78.5mm) 	20781-211.1	20782-211.5	20783-211.25
Cyclosplitter®, Base Deactivated (4.0mm ID* x 6.3mm OD x 78.5mm) 	20706-210.1	20707-210.5	20708-210.25

*Nominal ID at syringe needle expulsion point.
For liners for other instruments, refer to our catalog or website.

Base-Deactivated Inlet Liners

qty.	Base-Deactivated Liner		Base-Deactivated Liner w/ Base-Deactivated Wool	
each	-210.1	addl. cost	-211.1	addl. cost
5-pk.	-210.5	addl. cost	-211.5	addl. cost
25-pk.	-210.25	addl. cost	-211.25	addl. cost

For base-deactivated inlet liners, add the corresponding suffix number to the liner catalog number.

Base-Deactivated Wool

Ideal for amines and other basic compounds.

Description	qty.	cat.#
Base-Deactivated Wool	10 grams	20999

Mini Wool Puller/Inserter

Insert and remove wool plugs easily.

Description	qty.	cat.#
Mini Wool Puller/Inserter	2-pk.	20114

Inlet Liner Removal Tool

- Easily remove liner from injector—no more burned fingers.
- Made from high-temperature silicone.
- Won't chip or crack the liner.

Description	qty.	cat.#
Inlet Liner Removal Tool	3-pk.	20181

Rtx®-5 Amine Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 300/315°C	15-Meter	12320
0.25mm	0.25	-60 to 300/315°C	30-Meter	12323

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pared to base-deactivated or Siltek® treated liners, due to the acidic nature of the undeactivated glass surface or to a small but influential number of residual acidic sites remaining on the intermediate polarity deactivated surface.

Because the undeactivated liners and intermediate polarity treated liners exhibited either low mean response or high variation, we reanalyzed the data, excluding these treatments and comparing the remaining data (for base-deactivated liners and Siltek® treated liners) for responses and reproducibility. As shown by the examples in Figure 2, base-deactivated liners and Siltek® treated liners performed equally well for cocaine, but the base-deactivated liners yielded the best responses and reproducibility for PCP. Ultimately, a base-deactivated liner would give the best overall performance. Figure 3 shows the linearity plots for all analyzed drugs, obtained using a base-deactivated liner and an Rtx®-5Amine column. Low %RSD values for ketamine (3%), phencyclidine (2%), methadone (2%), cocaine (3%), codeine (5%), and alprazolam (12%) confirm the reproducibility of data obtained from this combination.

Because nitrogen- and oxygen-containing drugs react with silanol groups on glass surfaces, it is important to use properly deactivated glass inlet liners when analyzing these compounds by GC. This work demonstrates that a base-deactivated inlet liner, used in combination with a base-deactivated column, produces high and reproducible responses for basic drugs.

Reference

1. Seyhan N. and D.C. Ege, *Organic Chemistry* Health and Company, 1984, pp.124-136.

Simple, Reliable HPLC Analyses of Organic Acids

Using Water-Compatible Allure® or Ultra C18 Columns

Julie Kowalski, Ph.D., Innovations Chemist, and Becky Wittrig, Ph.D., HPLC Product Marketing Manager

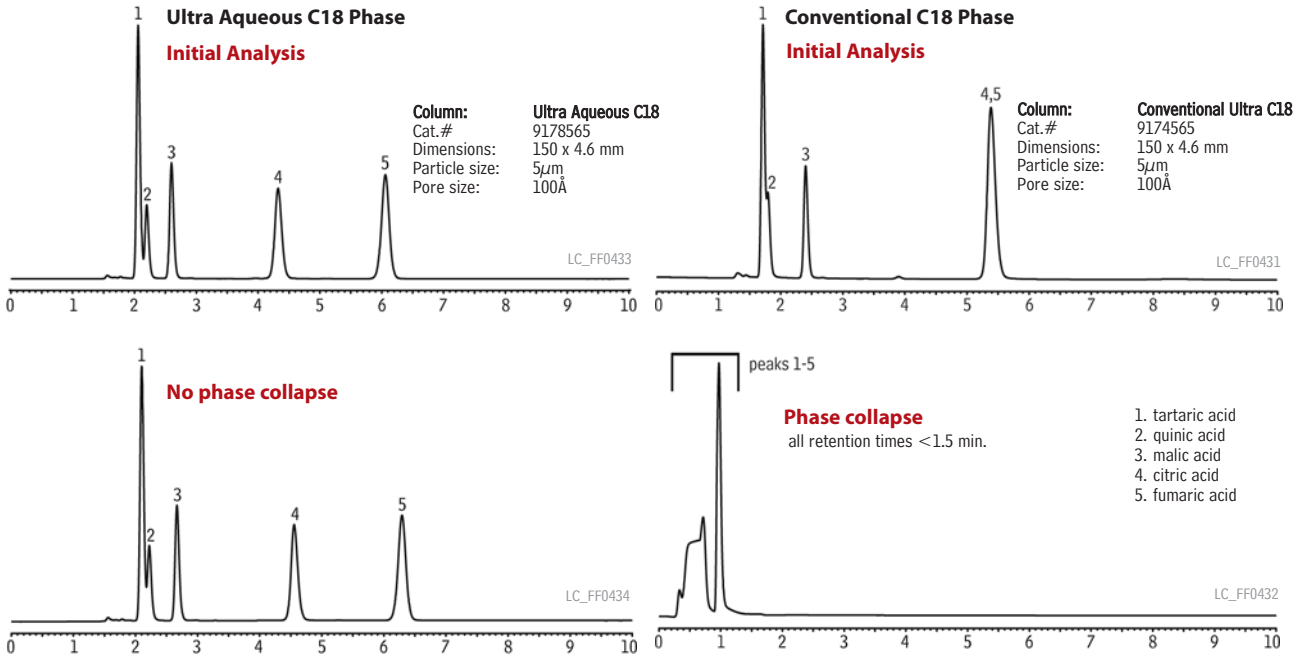
- Use 100% aqueous mobile phases without losing retention.
- Simple, isocratic method.
- Complete resolution of critical fruit juice organic acids, including quinic and tartaric acids.

Organic acids are common components in foods and beverages, and play a critical role in product characteristics like taste and aroma. They can be tested for in many food products including fruits, cheeses, and various beverages such as juices and wines. Organic acids can originate in the foods themselves (e.g. cranberries) or can be produced by food processing (e.g. alcoholic fermentation). A method that allows resolution of organic acids, as well as their quantification, can help determine product quality and authenticity.

Reversed phase HPLC coupled with UV-Vis detection is a popular technique for organic acid analysis. One common method, AOAC method 986.13, stipulates reversed phase HPLC using two C18 stationary phase columns in series. Because organic acids are low in molecular weight, and have polar functionalities, 100% aqueous buffer is needed for adequate retention. A low pH buffer is used to ensure that the organic acids remain protonated or neutral, thus allowing the best interaction between the organic acids and the C18 stationary phase. However, using a 100% aqueous mobile phase can cause the C18 chain in conventional C18 columns to collapse. Phase collapse results in loss of retention, and the column must be flushed with organic mobile phase, a time consuming step, to restore chain structure and column performance.

Three Restek columns – the Ultra Aqueous C18 column, the Allure® Aqueous C18 column, and the Allure® Organic Acids column – use aqueous-compatible C18 phases that do not exhibit phase collapse, even with 100% aqueous mobile phases. The advantage of using these columns is demonstrated in Figure 1 by the fast analysis of organic acids on a Shimadzu Prominence 20A system. Here, we compared the ability of the Ultra Aqueous C18 phase and a conventional C18 phase to withstand phase collapse. Figures 1A and 1B show that the Ultra Aqueous C18 phase resolves organic acids in a 100% aqueous mobile phase without loss of retention. In comparison, the conventional C18 phase shown in Figure 1C and 1D suffers a complete loss of retention following phase collapse when used under the same conditions. Thus, in an analysis that requires, or is improved by, a mobile phase with a high aqueous content, an Ultra Aqueous C18 column is the superior choice.

Figure 1 Restek's water-compatible C18 phase does not collapse in a 100% aqueous mobile phase, compared to a conventional C18 column which shows a complete loss of retention.



Instrument: Shimadzu Prominence 20A
Sample: Inj.: 10µL; Conc.: 2000µg/mL each component except fumaric acid (10µg/mL) (Organic Acids Reference Mixture cat.# 35080); Sample diluent: deionized water
Conditions: Mobile phase: 20mM potassium phosphate (pH 2.5); Flow: 1.0mL/min.; Temp.: 30°C; Det.: UV @ 226nm
Phase collapse caused for experimental purposes by releasing column pressure

Figure 2 Excellent resolution of organic acids, including tartaric and quinic acids, on an Allure® Organic Acids column.

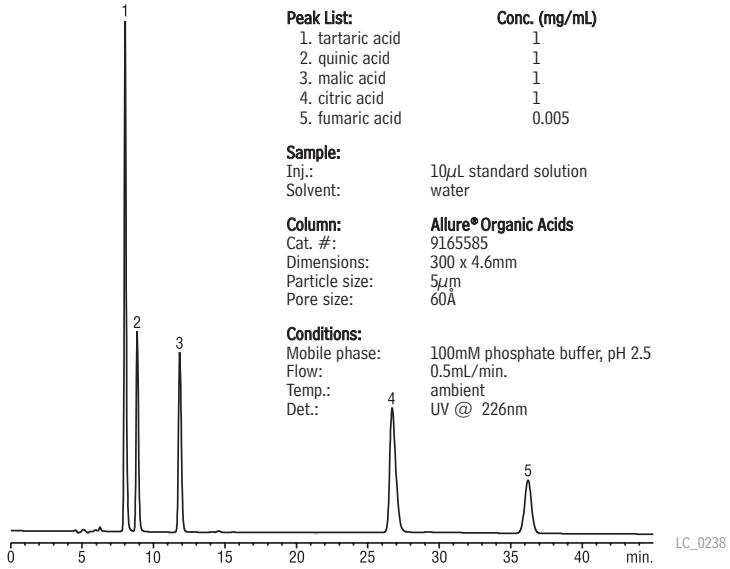
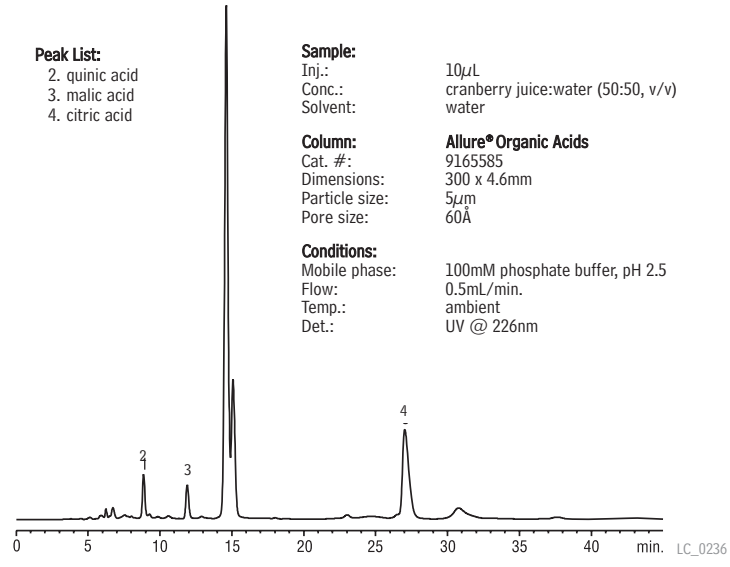


Figure 3 Sharp, easily differentiated organic acid profiles for cranberry juice cocktail on an Allure® Organic Acids column.



In analyses of organic acids, specifically, under high aqueous mobile phase conditions, the Allure® Organic Acids column is the column of choice. We have developed a method using a 300mm Allure® Organic Acids column to separate critical organic acids: tartaric, quinic, malic, citric and fumaric acids. This method calls for 100% aqueous mobile phase as recommended by AOAC method 986.13. The Allure® Organic Acids column is tested specifically for resolving critical organic acids. Figure 2 shows that tartaric and quinic acids are resolved to baseline; Figure 3 shows typical analyses under the conditions we recommend.

References

1. http://www.restek.com/advantage/adv_2003_03_02a.pdf.
2. Official Methods of Analysis (2000). AOAC International, 17th edition, method # 986.13.
3. Manolaki, P. et al., Food Chemistry, 98 (2006), page 658-663.
4. Kafkas, E. et al., Food Chemistry, 97 (2006), page 732-736.

Fruit Juice Organic Acid Standard

citric acid	2000µg/ml	quinic acid	2000
fumaric acid	10*	tartaric acid	2000
malic acid	2000		

In water, 1mL/ampul
cat. # 35080 (ea.)

In water, 5mL/ampul
cat. # 35081 (ea.)

*Fumaric acid is a trace impurity in malic acid, as well as an added component of the mix. The amount of fumaric acid in malic acid will not affect the stated concentration of malic acid, but can represent a significant and variable deviation from the low concentration of fumaric acid stated to be in the mix. All other components of the mix are at the specified concentration.

Allure® Organic Acids Column

5µm Column, 4.6mm	cat. #
150mm	9165565

Allure® Aqueous C18 Column

5µm Column, 4.6mm	cat. #
150mm	9168565

Ultra Aqueous C18 Column (USP L1)

5µm Column, 4.6mm	cat. #
150mm	9178565

for **more** info

For more information on our Allure® Aqueous C18, Ultra Aqueous C18 and Allure® Organic Acid columns, visit us online at **www.restek.com**.

ordering **note**

For guard cartridges for these columns, visit our website at **www.restek.com**.

Separate Explosives and Propellant Residues

Using Ultra C18 and Pinnacle™ II Biphenyl Columns

by Robert Freeman, Environmental Innovations Chemist

- Easily quantify and confirm new US EPA Method 8330B target analytes.
- Excellent resolution, improved accuracy.
- Simple, easy to use, isocratic method.

US EPA 8330, a test method for determining trace amounts of 14 nitramines and nitrate esters, was recently revised to include three new target analytes. The new method, EPA 8330B, includes nitroglycerin (NG), pentaerythritol tetranitrate, (PETN), and 3,5-dinitroaniline (3,5-DNA) and now covers 17 analytes that are commonly found in explosives and propellants residues. This method uses reversed phase HPLC and dual wavelength UV detection (210 & 254nm) in conjunction with a primary and a confirmation column.

We recently assessed the performance of our current column offerings relative to the elution order and retention times of the new analytes in the revised method. Separations on all columns were accomplished using a simple, isocratic water:methanol mobile phase (Table 1). The primary and confirmation columns that we recommend for the EPA 8330 analysis are the Ultra C18 and Pinnacle™ II Biphenyl columns, respectively. Based on this work, we conclude this combination will work well for the revised method, EPA 8330B, as shown by the chromatograms in Figures 1 and 2. Both columns provide excellent resolution of the EPA 8330B analytes and their differing selectivity provides a true confirmation analysis.

As an alternative to the Ultra C18/Pinnacle™ II Biphenyl combination, a Pinnacle™ II C18 column and a Pinnacle™ II Cyano column work well together as a primary-confirmation column set. Another column of interest is the Allure® Biphenyl column. A high organic mobile phase was required on this column but the analysis was completed in approximately six minutes (Table 1).

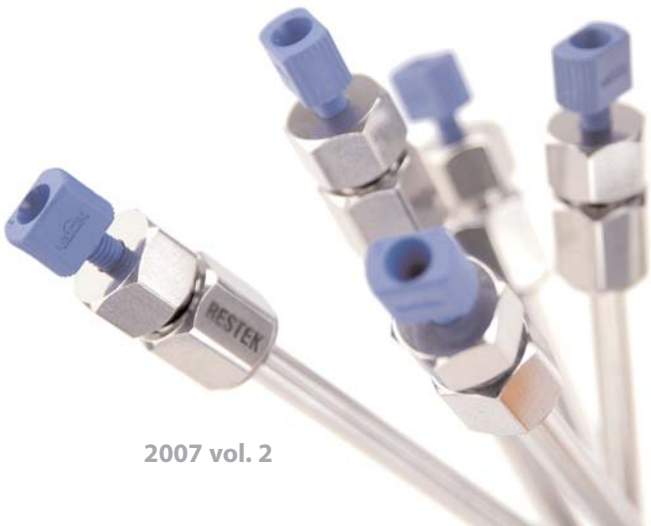
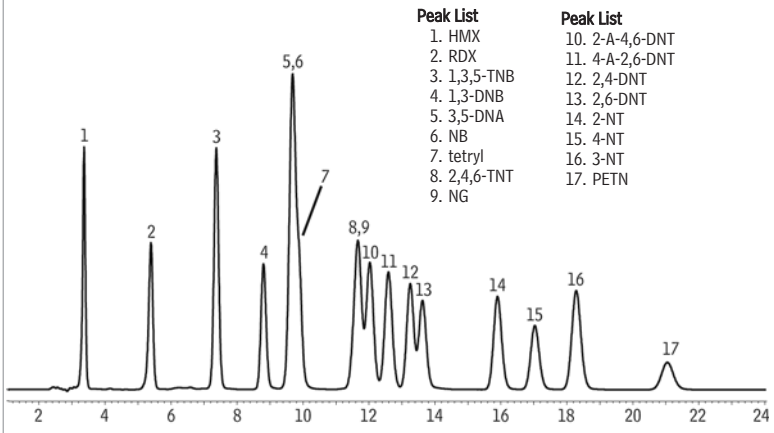


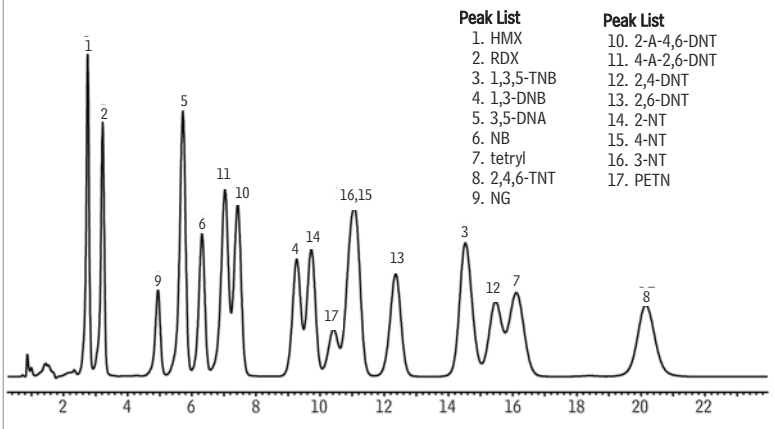
Figure 1 Excellent resolution of EPA 8330B target analytes on the Ultra C18 column.



Sample:	50µg/mL each compound diluted in acetonitrile 8330 Calibration Mix #1 (cat.# 31450) 8330 Calibration Mix #2 (cat.# 31451) PETN Standard(cat.# 31600) 3,5-dinitroaniline Reference Mix (cat.# 31661) Nitroglycerin Reference Mix (cat.# 31498)	**Conditions:** Mobile phase: Flow: Temp.: Det.:	 water:methanol (44:56 v/v) 1.0mL/min. 30°C UV detection @ 210nm
Inj.:	10µL		
Column:	**Ultra C18**		
Cat.#:	9174575		
Dimensions:	250mm x 4.6mm		
Particle size:	5µm		
Pore size:	100Å		

LC_EV0428

Figure 2 Alternate selectivity of EPA 8330B analytes on the Pinnacle™ II Biphenyl column confirms compound identity.



Sample:	50µg/mL each compound diluted in acetonitrile 8330 Calibration Mix #1 (cat.# 31450) 8330 Calibration Mix #2 (cat.# 31451) PETN Standard (cat.# 31600) 3,5-dinitroaniline Reference Mix (cat.# 31661) Nitroglycerin Reference Mix (cat.# 31498)	**Conditions:** Mobile phase: Flow: Temp.: Det.:	 water:methanol (44:55 v/v) 1.2mL/min. 30°C UV detection @ 210nm
Inj.:	10µL		
Column:	**Pinnacle™ II Biphenyl**		
Cat.#:	9209565-700		
Dimensions:	250mm x 4.6mm		
Particle size:	5µm		
Pore size:	110Å		

LC_EV0429

Table 1 Retention times for EPA 8330B analytes on various Restek columns.

- new target analytes are shown in red
- highlighted cells indicate coelution.

	Primary Columns		Confirmation Columns		
H_2O:MeOH	**50:50**	**44:56**	**45:55**	**50:50**	**20:80**
Flow	1.5 mL/min	1.0 mL/min	1.2 mL/min	1.5 mL/min	1.5 mL/min
Analytes	**Pinnacle™ II C18**	**Ultra C18**	**Pinnacle™ II Biphenyl**	**Pinnacle™ II Cyano**	**Allure® Biphenyl**
HMX	2.29	3.38	2.76	18.65	1.61
RDX	3.63	5.41	3.22	9.38	1.75
1,3,5-TNB	4.89	7.39	14.54	4.78	5.69
1,3-DNB	5.94	8.82	9.26	4.59	3.92
3,5-DNA	6.63	9.71	5.73	6.34	2.30
tetryl	6.97	9.71	16.12	11.47	4.42
NB	6.97	9.88	6.31	3.80	2.79
2,4,6-TNT	8.23	11.69	20.17	5.94	6.22
NG	8.23	11.69	4.94	8.52	1.98
2-A-4,6-DNT	8.94	12.05	7.43	7.24	2.50
4-A-2,6-DNT	8.94	12.61	7.02	6.34	2.41
2,6-DNT	9.73	13.27	12.36	5.10	4.09
2,4-DNT	9.73	13.64	15.46	5.58	5.14
2-NT	11.92	15.92	9.73	4.38	3.40
4-NT	12.76	17.05	11.07	4.38	3.72
3-NT	13.74	18.32	11.07	4.38	3.73
PETN	16.13	21.08	10.43	17.24	2.67

Ultra C18 Column (USP L1)

5µm Column, 4.6mm	cat. #
250mm	9174575
250mm (with Trident™ Inlet Fitting)	9174575-700

Pinnacle™ II Biphenyl Column (USP L11)

5µm Column, 4.6mm	cat. #
150mm	9209565
150mm (with Trident™ Inlet Fitting)	9209565-700

ordering note

For guard cartridges for these columns, visit our website at **www.restek.com**.

8330 Calibration Mix #1 (7 components)

1,3-dinitrobenzene
2,4-dinitrotoluene
HMX
nitrobenzene
RDX
1,3,5-trinitrobenzene
2,4,6-trinitrotoluene

1,000µg/mL each in acetonitrile, 1mL/ampul

cat. # 31450 (ea.)

8330 Calibration Mix #2 (7 components)

2-amino-4,6-dinitrotoluene
4-amino-2,6-dinitrotoluene
2,6-dinitrotoluene
2-nitrotoluene
3-nitrotoluene
4-nitrotoluene
tetryl

1,000µg/mL each in acetonitrile, 1mL/ampul

cat. # 31451 (ea.)

Single-Component Explosives Reference Mixes

Volume is 1mL/ampul unless otherwise noted. Concentration is µg/mL unless otherwise noted.

3,5-dinitroaniline	ACN	1,000	31661
nitroglycerin	M	1,000	31498
PETN (pentaerythritol tetranitrate)	M	1,000	31600

ACN=acetonitrile
M = methanol

Fast, Accurate FAMEs Analyses of Biodiesel Fuel

Using a Stabilwax® Capillary GC Column

By Barry L. Burger, Petroleum Innovations Chemist

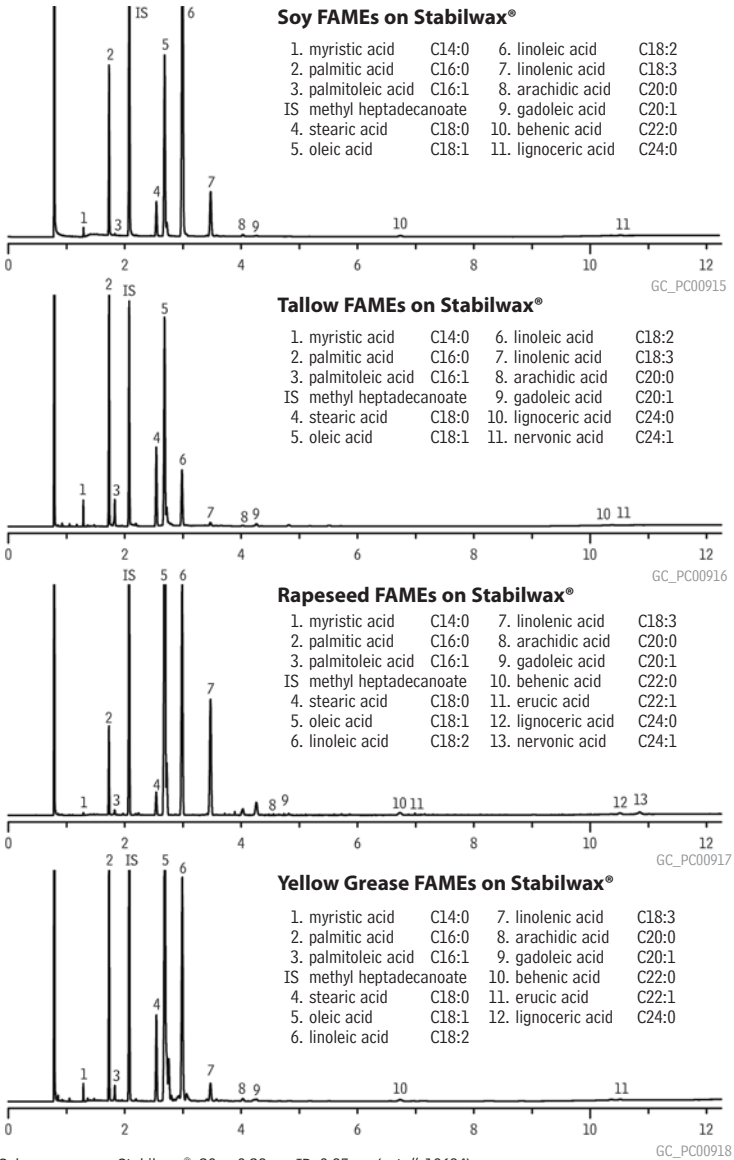
- Stable baselines, excellent peak symmetry, baseline resolution of all compounds.
- Analysis complete in less than 11 minutes using hydrogen.
- All RSD% values less than 1%.

A Stabilwax® fused silica GC column affords excellent peak symmetry, resolution, and reproducibility for determining the fatty acid methyl ester (FAME) and linolenic acid methyl ester content in B100 biodiesel fuel, using European standard method EN 14103. The chromatograms and quantified data shown here were generated from four different sources of biodiesel fuel, and meet or exceed the method criteria.

As biodiesel fuel continues to stimulate interest worldwide as an energy source, several gas chromatographic methods have been developed to determine the quality of B100 fuel. European standard method EN 14103 is used for determining the FAME and linolenic acid methyl ester content, European standard method EN 14105 and ASTM standard method D-6584-00e1 are used for determining free and total glycerin, and European standard method EN 14110 is used for determining residual methanol. Method EN 14103 permits the analyst to assure the B100 product is greater than 90% fatty acid methyl esters (m/m) and the linolenic acid content is between 1% and 15% (m/m). The analysis is appropriate for FAME compositions between C14:0 and C24:1.

In evaluating the suitability of the Stabilwax® column for quantifying FAMEs and linolenic acid methyl ester by method EN 14103, we prepared reference standards from each of the four B100 fuel sources – soy, tallow, rapeseed, and yellow grease (Table 1) – by weighing 250mg of the source material into a 10mL vial, then adding 5mL of a 10mg/mL solution of internal standard methyl heptadecanoate. (Avoid allowing the samples to stand longer than 12 hours, or quantification will be inaccurate.) We installed the 30m x 0.32mm ID x 0.25µm Stabilwax® column (cat.# 10624) in an Agilent 6890 instrument equipped with a split/splitless injector, a flame ionization detector, and ChemStation software. To obtain the fastest analysis, without sacrificing resolution, we selected hydrogen as the carrier gas, supplied from a Parker Balston hydrogen generator.

Figure 1 Stable baselines, excellent peak symmetry, and rapid, baseline resolution of all compounds characterize FAMEs analyses on a Stabilwax® column.



Column:	Stabilwax®, 30m, 0.32mm ID, 0.25µm (cat.# 10624)
Sample:	various sources of biodiesel (B100), prepared according to European Method EN 14103
Inj.:	1.0µL split (split ratio 100:1), Cyclosplitter® inlet liner (cat.# 20706)
Inj. temp.:	250°C
Carrier gas:	hydrogen, constant flow, 3mL/min.
Linear velocity:	60cm/sec.
Oven temp.:	210°C (hold 5 min.) to 230°C @ 20°C/min. (hold 5 min.)
Det.:	FID
Det. temp.:	250°C

Table 1 Sources of FAMEs in B100 biodiesel fuel (% m/m).

		Soy	Tallow	Rapeseed	Yellow Grease
Myristic acid	C14:0	0.21	1.7	0.11	0.68
Palmitic acid	C16:0	11.24	25.5	4.1	16.35
Palmitoleic acid	C16:1	0.2	3.27	0.27	1.23
Stearic acid	C18:0	4.04	14.41	1.8	9.32
Oleic acid	C18:1	21.93	40.34	58.57	47.8
Linoleic acid	C18:2	53.84	12.02	22.2	20.01
Linolenic acid	C18:3	7.29	0.99	13.26	2.93
Arachidic acid	C20:0	0.36	0.4	0.79	0.46
Gadoleic acid	C20:1	0.26	1.03	1.79	0.39
Behenic acid	C22:0	0.45		0.57	0.44
Erucic acid	C22:1			0.13	0.23
Lignoceric acid	C24:0	0.16	0.34	0.3	0.24
Nervonic acid	C24:1		0.17	0.54	

Table 2 Relative standard deviations for FAMEs do not exceed 1% in analyses on a Stabilwax® column (n = 3).

		Soy	Tallow	Rapeseed	Yellow Grease
Myristic acid	C14:0	0.33	0.42	0.24	0.36
Palmitic acid	C16:0	0.04	0.06	0.02	0.04
Palmitoleic acid	C16:1	0.23	0.17	0.19	0.09
Stearic acid	C18:0	0.05	0.02	0.13	0.19
Oleic acid	C18:1	0.02	0.3	0.2	0.25
Linoleic acid	C18:2	0.25	0.41	0.11	0.22
Linolenic acid	C18:3	0.13	0.16	0.07	0.14
Arachidic acid	C20:0	0.3	0.37	0.23	0.31
Gadoleic acid	C20:1	0.33	0.28	0.37	0.41
Behenic acid	C22:0	0.28		0.29	0.17
Erucic acid	C22:1			0.21	0.26
Lignoceric acid	C24:0	0.53	0.14	0.1	0.33
Nervonic acid	C24:1		0.55	0.83	

Parker Hydrogen Generators

- Selectable delivery pressure: 0–100psig.
- High hydrogen purity—99.9995%—for better chromatography.
- No high-pressure cylinders—greater convenience and improved lab safety.

Description	Capacity	qty.	cat.#
Hydrogen Generator A9090	90cc/min.	ea.	22033
Hydrogen Generator A9090 with European Power Cord	90cc/min.	ea.	22033-551
Hydrogen Generator A9150	160cc/min.	ea.	22034
Hydrogen Generator A9150 with United Kingdom Power Cord	160cc/min.	ea.	22034-550
Hydrogen Generator B9200	250cc/min.	ea.	22035
Hydrogen Generator B9400	500cc/min.	ea.	22036
Replacement Components for Hydrogen Generators (for all models listed above)			
Replacement Deionizer Bag		2-pk.	21670
Replacement Desiccant Cartridge		ea.	21671

Figure 1 shows, for each source material, the analysis to FAME C24:1 is completed in less than 11 minutes. Particularly notable are the stability of the baselines, the excellent peak symmetry, and baseline resolution of all compounds of interest. Table 2 summarizes the RSD% values for the FAMEs measurements, all of which are less than 1%.

A 30m x 0.32mm ID x 0.25µm Stabilwax® column, used with hydrogen carrier gas, permits high speed analysis and ensures precise data acquisition for accurate quantification of C14:0-C24:1 FAMEs and linolenic acid methyl ester.

Stabilwax® Column (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	40 to 250°C	30-Meter	10624

did **you** know?

Restek offers FAME standards for a wide range of oils. See our general catalog or visit us online **www.restek.com**.

for **more** info

For information about Parker Balston hydrogen generators, refer to our general catalog or visit us online at **www.restek.com/hydrogen-generator**

Assure Accurate Sampling and Reliable Sample Purity

Restek Sampling System Treatments Prevent Adsorption, Protect Components

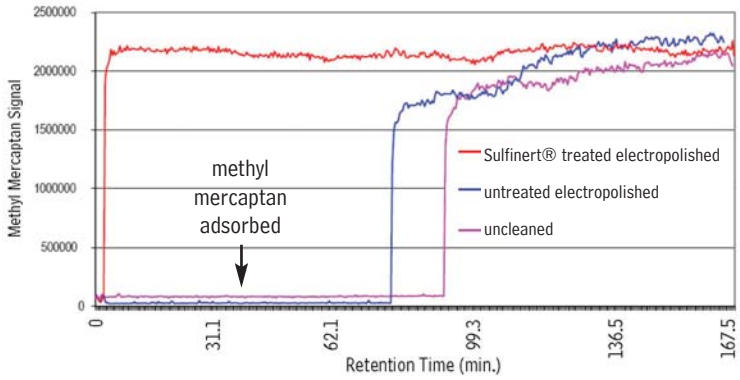
By Gary Barone, Manager, Restek Performance Coatings

- Quantify active compounds (e.g., sulfur, mercury, NOx) at parts-per-billion levels.
- Corrosion protection equal to specialty alloys – at lower cost.
- Assemble a new system from treated stock, or treat an existing system.

When surface activity or corrosion are a concern, solutions must be engineered. Restek Performance Coatings offers a family of surface treatments that address reactivity and corrosion over a wide spectrum of applications. These treatments reduce process upsets, reduce capital costs, and reduce maintenance costs.

Accurate sampling with Siltek®/Sulfinert® tubing and fittings.
Adsorption problems in sample pathways often can be traced to the tubing and fittings used to transfer the sample to the analytical instrument. Always use treated tubing and fittings for applications involving active compounds. To ensure maximum inertness and minimal surface area, use Siltek®/Sulfinert® treated electropolished tubing. Figure 1 shows uptake and release curves for 500ppbv of methyl mercaptan, an active sulfur compound, in a gas stream passing through a variety of tubing substrates. Siltek®/Sulfinert® treated tubing reduces uptake by orders of magnitude, relative to untreated stainless steel tubing.

Figure 1 Sulfinert® treated electropolished seamless stainless steel tubing does not adsorb methyl mercaptan (500ppbv).



Reduce maintenance cost, extend system life with Silcosteel®-CR tubing and fittings.
In corrosive environments, Silcosteel®-CR treatment is an excellent alternative to expensive alloys. Silcosteel®-CR treatment extends component life while reducing the frequency of preventive maintenance and ensuring the purity of the process or sample stream. Silcosteel®-CR improves corrosion resistance by up to 10X over untreated 316 stainless steel (Figure 2).

Figure 3 shows the results of a 4000-hour salt spray test on Silcosteel®-CR treated 316L stainless steel and untreated 316L stainless steel. The Silcosteel®-CR treated material exhibited virtually no change. Silcosteel®-CR treatment has extended the life of process systems in oil and gas production, oil refining, petrochemical processing, aerospace equipment, food and beverage processing, and laboratory testing. Figure 4 shows Silcosteel®-CR treatment can reduce the overall lifetime cost of a typical process system by hundreds of thousands of dollars. While the initial cost of an unprotected stainless steel system is lower than that of a comparable Silcosteel®-CR system, the overall lifetime cost, considering replacement cost due to corrosion, is nearly double that of a Silcosteel®-CR treated system. High performance alloy systems offer superlative corrosion performance, but the initial material cost can be up to six times that of a stainless steel system.

simply **the best**

Restek-treated electropolished tubing is the best tubing choice when purity, inertness, or reproducibility are concerns.

Top: electropolished finish, surface roughness average number: 5-10. Bottom: conventional finish, surface roughness average number: 23-27.

Treat the entire sample pathway for maximum benefit.

Fittings

Connections can be a source of adsorption and sample loss, and there is benefit to employing Restek surface treatment on many of these components. In corrosive environments, Silcosteel®-CR treatment will extend the useful life of system fittings, as well as tubing. We offer extensive lines of treated Swagelok® and Parker fittings, in sizes from 1/16" to 3/8".

Valves

The sample flow path through a valve can prolong contact between the sample stream and the valve components. Restek surface treatments have been applied to many valve geometries, to eliminate adsorption to bodies, stems, diaphragms, or other components.

Filters

Frits and other filtering devices trap particles and prevent them from entering the analytical instrument, but they also very effectively adsorb active components in sample streams. Their large surface areas can increase sample/system contact by orders of magnitude. Siltek®/Sulfinert® treatment of frits and filters creates an inert flowpath. Our chemical vapor deposition technology ensures the treatment penetrates even the smallest pores in sintered metal frits.

Sample Vessel Equipment

Restek treated sampling containers prevent active components from adsorbing to vessel, valve, or outage tube surfaces. We offer a complete line of high pressure sampling equipment for applications involving liquefied petroleum gases, ethylene, natural gas, or propylene.

Sampling Probes

An untreated probe contributes to the active surface area in the system, and this should be considered when identifying potential adsorption sites during active stream transfer.

Heated Transport Lines

Active compounds in the sample quickly can be adsorbed onto the hot tubing in a heated "trace line". Restek surface treatment prevents adsorption of active compounds.

Summary

Surface treatments from the Restek Performance Coatings group prevent adsorption of active compounds or corrosion in process systems, and always should be considered in applications in which active or corrosive streams are to be sampled, transferred, or analyzed.

Siltek®/Sulfinert® Treated Coiled 316L Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22508				
0.180" (4.57mm)	1/4" (6.35mm)**	22509				
0.277" (7.04mm)	3/8" (9.52mm)***	22914				

Silcosteel®-CR Treated Coiled 316L Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	>400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22896				
0.180" (4.57mm)	1/4" (6.35mm)**	22897				
0.277" (7.04mm)	3/8" (9.52mm)***	22915				

Siltek®/Sulfinert® Treated Coiled Electropolished 316L Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085" (2.16mm)	1/8" (3.18mm)*	22538				
0.180" (4.57mm)	1/4" (6.35mm)**	22539				

*0.020" wall thickness **0.035" wall thickness ***0.049" wall thickness

Figure 2 Silcosteel®-CR resists pitting and crevice corrosion when exposed to ferric chloride, per ASTM G48, B.

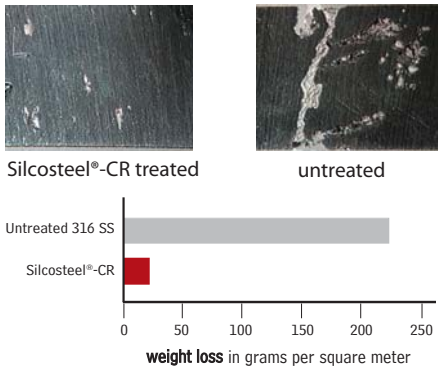


Figure 3 Silcosteel®-CR treated stainless steel shows no sign of attack after 4000-hour salt spray exposure, per ASTM B117.

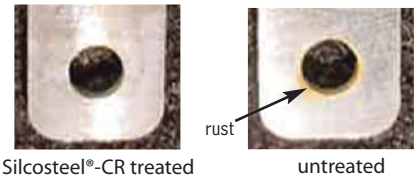
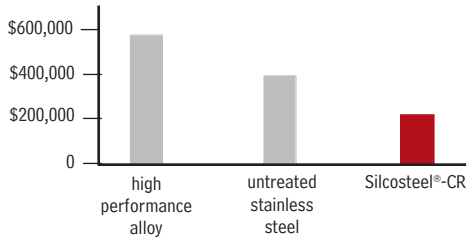


Figure 4 Silcosteel®-CR demonstrates significant cost savings, compared to untreated stainless steel or alloys (US dollars).



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- Siltek® treated in-line filters
- Sulfinert® treated Swagelok® sample cylinders
- Sulfinert® treated Alta-Robbins sample cylinder valves
- Additional treated stainless steel tubing
  - Siltek®/Sulfinert® treated, electropolished, 316L grade
  - Siltek®/Sulfinert® treated, 316L grade
  - Siltek®/Sulfinert® treated, 304 grade
  - Silcosteel®-CR treated, electropolished, 316L grade
  - Silcosteel®-CR treated, 316L grade

Peak Performers

Introduction to Pressure Regulators

by Donna Lidgett, GC Accessories Product Manager

General Purpose or Analytical?

General-purpose regulators usually are best suited for applications involving gases that are less than 99.995% pure: pneumatically-actuated valves and autosamplers, blanketing, inert atmospheres, and any other application not directly integrated with analytical data production. General purpose regulators have nylon-reinforced neoprene diaphragms that provide very good pressure control but are prone to air and moisture diffusion and hydrocarbon off-gassing.

Analytical regulators are recommended for applications in which maintaining the purity of a gas or mixture is the overriding concern, i.e., for applications requiring gases that are greater than 99.995% pure. They are commonly used in analytical labs. Analytical regulators have stainless steel diaphragms for pressure control. Stainless steel is not subject to the diffusion and off-gassing associated with neoprene diaphragms, and is easily purged of atmospheric contaminants when put into service.

Dual- or Single-Stage?

Dual-stage regulators reduce the source pressure to outlet pressure in two steps. The first stage reduces the inlet pressure to about three times the maximum working pressure. Outlet pressure regulation is controlled by the second stage and is set through an adjusting knob. This two-step regulation is highly recommended for services requiring a near constant delivery pressure as the source pressure decays, including chromatographic analyses.

Single-stage regulators perform the same function as dual-stage regulators, but in a single step down from source pressure to outlet pressure. For this reason, the outlet pressure cannot be as accurately maintained as the source pressure decays. We highly recommend that single-stage regulators be used only in circumstances in which the operator can monitor and adjust the regulator as needed, when the regulator is supplied with a nearly constant source pressure, or when additional pressure regulation is supplied downstream.

Brass or Stainless Steel?

Analytical regulators made from brass bar stock provide optimum performance for most analytical applications. Brass provides excellent strength and cleanliness and the machined bar stock design has less dead volume than forged-body regulators, making purging of atmospheric contaminants faster and more assured.

Regulators with stainless steel bodies were designed for delivering corrosive gases that would be incompatible with brass. With the advent of semiconductor manufacturing and high sensitivity analytical techniques, stainless steel also has proven to be a better surface for removing "sticky" atmospheric contaminants that interfere with detectors downstream. Unless these regulators are used in an all-stainless-steel system that incorporates welded tubing and special fittings, and in which rigorous cleaning and proper gas management are practiced, the extra expense relative to brass is not justified.

Overview of Restek's Brass and Stainless Steel Body Ultra-High-Purity Regulators

These regulators feature metal-to-metal seals throughout for long-term leak-tightness, and a metal diaphragm outlet valve ensures gas purity. Each regulator is helium leak-test-certifiable to $1x10^{-8}$scc/sec. and is fully assembled and tested for your convenience. 100psig maximum delivery pressure supports pressure controlled operation. Maximum inlet pressure is 3000psig. Brass bar stock construction minimizes dead volume. Stainless steel construction is more easily purged of atmospheric contaminants, and is more resistant to attack from dry corrosive gases.

Ultra-High-Purity Stainless Steel Body Regulators

These regulators are the standard for ultra-high-purity and corrosion-resistant pressure regulation. They are more easily purged of atmospheric components, compared to brass regulators, making them ideal for the most demanding applications. Regulation performance is equal to our brass body regulators. For use in all-stainless steel systems where welded tubing and special fittings are used, and rigorous cleaning and proper gas management are practiced.

Dual-Stage Ultra-High-Purity Stainless Steel Regulators

- Most stable outlet pressure control throughout the life of a high-pressure gas cylinder.
- Secondary pressure regulation not needed.

Outlet pressure: 0 to 100psig
Outlet gauge: 30" – 0 to 200psig
Inlet gauge: 0 to 4000psig
Outlet assembly: diaphragm valve, $^1/_4$" tube fitting

Fitting	qty.	cat.#
CGA 580 (N_2, He, Ar)	ea.	20662
CGA 350 (H_2, P_5)	ea.	20663
CGA 590 (Air)	ea.	20664

Single-Stage Ultra-High-Purity Stainless Steel Regulators

- Use when there is secondary pressure regulation downstream.
- Identical gas purity protection as with our dual-stage regulators.

Outlet pressure: 0 to 100psig
Outlet gauge: 30" – 0 to 200psig
Inlet gauge: 0 to 4000psig
Outlet assembly: diaphragm valve, 1/4" tube fitting

Fitting	qty.	cat.#
CGA 580 (N_2, He, Ar)	ea.	20665
CGA 350 (H_2, P_5)	ea.	20666
CGA 590 (Air)	ea.	20667

Dual-Stage Ultra-High-Purity Chrome-Plated Brass Regulators

- Oxidation-resistant, chrome-plated.
- Most stable outlet pressure control throughout the life of a high-pressure gas cylinder.
- Secondary pressure regulation not needed.
- Most widely used regulator.
- Less internal volume than stainless steel regulators.

Outlet pressure: 0 to 100psig
Outlet gauge: 30" – 0 to 200psig
Inlet gauge: 0 to 4000psig
Outlet assembly: diaphragm valve, 1/4" tube fitting

Fitting	qty.	cat.#
CGA 580 (N_2 He, Ar)	ea.	21667
CGA 350 (H_2, P_5)	ea.	21668
CGA 590 (Air)	ea.	21669

also **available**

Intrument-Grade Tubing and Tubing Tools
For more information see our general catalog or visit us online at **www.restek.com**

Single-Stage Ultra-High-Purity Chrome-Plated Brass Regulators

- Oxidation-resistant, chrome-plated.
- Use when there is secondary pressure regulation downstream.
- Identical gas purity protection as with our dual-stage regulators.

Outlet pressure: 0 to 100psig
Outlet gauge: 30" – 0 to 200psig
Inlet gauge: 0 to 4000psig
Outlet assembly: diaphragm valve, 1/4" tube fitting

Fitting	qty.	cat.#
CGA 580 (N_2, He, Ar)	ea.	20646
CGA 350 (H_2, P_5)	ea.	20647
CGA 590 (Air)	ea.	20648

Ultra-High-Purity Chrome-Plated Brass Line Regulator

- Oxidation-resistant, chrome-plated.
- Use where you need to reduce the line pressure by 20psi or more.
- Same purity protection as high-pressure cylinder regulators.

Inlet connections: 1/4" FPT
Outlet assembly: 1/4" FPT port

Fitting	Outlet Gauge	Outlet Pressure	qty.	cat.#
1/4" female NPT ports*	30" - 0 to 100psig	0-50psig	ea.	21666
1/4" female NPT ports*	30" - 0 to 200psig	0-100psig	ea.	22452

*Order appropriate male connector, pipe-to-tube fittings.

male connector

tube end reducer

Male Connector, Pipe-to-Tube Fittings

Fitting Type	Size (inches)	Parker #	Similar to Swagelok®	Brass qty.	Brass cat.#	Stainless Steel qty.	Stainless Steel cat.#
Male Connector	1/4" to 1/4" NPT	4 MSC 4N	400-1-4	10-pk.	21842	2-pk.	21942
Male Connector	1/8" to 1/4" NPT	2 MSC 4N	200-1-4	10-pk.	21844	2-pk.	21944
Tube End Reducer	1/4" tube to 1/8"	4 TUR 2	200-R-4	5-pk.	21834	2-pk.	21934

Preventing Septum Problems

By Donna Lidgett, GC Accessories Product Marketing Manager and Scott Grossman, GC Accessories Chemist

- Avoid extraneous peaks with proper septum handling & maintenance.
- Handy size chart & septum choice guidelines.
- Optimize performance by choosing the right septum for the job.

All septa, regardless of their composition, puncturability, or resistance to thermal degradation, will be a source of problems if they are mishandled or used inappropriately. Poor septum choice and improper treatment can significantly compromise both qualitative and quantitative analytical results. Proper septum choice and careful handling can minimize septum bleed and septum coring, two of the most common septum problems that affect chromatography.

Septum bleed occurs when volatiles from the septum (e.g., silicone oils, phthalates) enter the column and then elute, creating elevated baselines (for isothermal analyses), baseline disturbances, or extraneous (but consistent) peaks in the chromatogram. Either baseline rise or extraneous peaks can interfere with identification and quantification of target analytes. This problem is prevalent in temperature-programmed analyses, because the septum volatiles collect on the column during the oven cool-down and initial hold periods.

To avoid septum bleed, either condition your septum prior to running your analysis, or use a pre-conditioned septum that is ready for immediate use. All Restek septa are preconditioned and ready to use. Allowing the septum to condition at operating temperatures for a few hours is an excellent way to assure optimum performance. Also always use clean forceps or wear clean powderless latex gloves, or cotton gloves when handling septa. Do not handle them with bare fingers or with powdered latex gloves since contaminants such as finger oils, perfumes, make-up, fingernail polish, skin creams, hand soaps, and talcum can be absorbed into the septum and bleed out during analysis.

Septum coring is another common problem that can diminish chromatographic performance. Coring occurs when the septum has been punctured too many times, the needle is damaged, or the wrong needle tip type is used. In these cases, small particles may be cored from the body of the septum and fall into the inlet liner. Once in the liner, they are subjected to higher temperatures, causing the release of septum volatiles which are swept into the column and can appear on the chromatogram (see "How Hot is Your Septum?" on page 22).

To prevent septum coring, always follow the septum and instrument manufacturers' installation recommendations and take care not to over-tighten the septum nut. Over-tightening the septum nut invariably reduces septum lifetime by increasing coring and splitting. Routinely replacing your septum and inspecting your syringe needle (manual or autosampler) for tip damage also help prevent septum damage.

Softer septa, such as Ice-Blue™ septa, are less likely to core than firmer septa. However, softer septa usually have a lower maximum operating temperature than firmer septa, so consider your method requirements carefully before deciding to switch. Changing syringe needle styles also can help reduce coring. For example, a point-style #2 needle (beveled point) is much more likely to cause coring (especially when the tip has become bent or dull) than a point-style #5 needle (conical needle with side-port).

A septum that can be penetrated cleanly and easily by the needle is less prone to coring and has a longer life. Moreover, consistent injections made through such a septum help ensure accurate results. The soft silicone rubber from which all Restek septa are manufactured is specially formulated for chromatographic performance, which ensures our septa are easy to puncture. However, in cases in which a small degree of pliability is sacrificed for high-temperature optimization, the CenterGuide™ dimple will help guide the syringe, for clean, consistent injections, minimizing septum coring.

Careful consideration of instrument and method requirements should dictate your septum choice, but proper handling and maintenance are the keys to minimizing septum damage and maximizing the accuracy of your analyses. Restek offers septa for all major brands of gas chromatographs and injectors. Use our handy septum size chart to determine the septum diameter for your instrument or contact us at **1-800-356-1688 (ext. 4)** to discuss your application.

Restek Septa

- Precision molding assures consistent, accurate fit.
- Ready to use
- Do not adhere to hot metal surfaces.
- Packaged in non-contaminating glass jars.

Septum Diameter	25-pk.	50-pk.	100-pk.
Thermolite® Septa			
5mm (3/16")	27120	27121	27122
6mm (1/4")	27123	27124	27125
7mm	27126	27127	27128
8mm	27129	27130	27131
9mm	27132	27133	27134
9.5mm (3/8")	27135	27136	27137
10mm	27138	27139	27140
11mm (7/16")	27141	27142	27143
11.5mm	27144	27145	27146
12.5mm (1/2")	27147	27148	27149
17mm	27150	27151	27152
Shimadzu Plug	27153	27154	27155
IceBlue™ Septa			
9mm		27156	27157
9.5mm (3/8")		27158	27159
10mm		27160	27161
11mm (7/16")		27162	27163
11.5mm		27164	27165
12.5mm (1/2")		27166	27167
17mm		27168	27169
Shimadzu Plug		27170	27171
BTO® Septa			
5mm CenterGuide™		27100	27101
6mm (1/4")		27102	27103
9mm CenterGuide™		27104	27105
9.5mm (3/8")		27106	27107
10mm		27108	27109
11mm (7/16") CenterGuide™		27110	27111
11.5mm CenterGuide™		27112	27113
12.5mm (1/2") CenterGuide™		27114	27115
17mm CenterGuide™		27116	27117
Shimadzu Plug		27118	27119

HANDY septum size chart

Instrument	Septum Diameter (mm)
Agilent (HP)	
5880A, 5890, 6890, 6850, PTV	11
5700, 5880	9.5/10
On-Column Injection	5
Thermo Scientific	
TRACE™ GC	17
GCQ w/TRACE™, PTV	17
8000 series	17
Finnigan (TMQ)	
GC 9001	9.5
GCQ	9.5
QCQ™	9.5
TRACE™ 2000	9.5
Gow-Mac	
6890 series	11
All other models	9.5
PerkinElmer	
Sigma series	11
900,990	11
8000 series	11
Auto SYS™	11
Auto SYS™ XL	11

Instrument	Septum Diameter (mm)
Pye/Unicam	
All models	7
Shimadzu	
All models	Plug
SRI	
All models	Plug
Tracor	
540	11.5
550,560	9.5
220,222	12.5
Varian	
Injector type:	
Packed column	9.5/10
Split/splitless 1078/1079	10/11
1177	9
1075/1077	11

How Hot Is Your Septum?

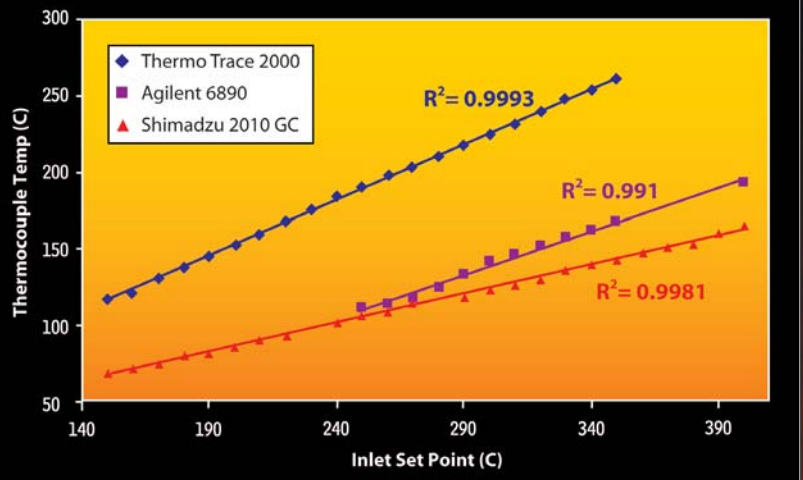
By Scott Grossman, GC Accessories Chemist

Different septa brands are given a single, maximum operating temperature based on their performance in a specific instrument inlet, not the actual temperature that the septum can withstand and still function properly. Understanding how different inlets influence the actual temperature at the septum can help prevent problems such as sticking. The temperature at the septum is affected by the heating element and the overall inlet design, which varies significantly among manufacturers. To illustrate this, we placed a thermocouple at the bottom of the septum in several instruments and compared the actual temperature to the inlet set point. The resulting data demonstrate that for any given setting the temperature at the septum is lower than the set point, but the degree of difference, or gradient, varies among instruments (Figure 1).

There are distinct advantages and disadvantages associated with different temperature gradients that should be considered. Inlets with a larger gradient (cooler septum compartment) typically experience fewer problems with septa sticking. In contrast, inlets with a smaller gradient (hotter septum compartment) are more prone to septa sticking, but have the advantage of a more evenly heated inlet and thus more uniform sample vaporization. Uniform vaporization reduces analyte discrimination, the bias against higher boiling point (i.e. higher molecular weight) compounds in favor of lower boiling point compounds that occurs when compounds are not vaporized with equal efficiency.

Operators of instruments that have a smaller temperature gradient should consider using septa that are rated for the highest possible temperature and setting the inlet at the lowest permissible temperature. Low bleed BTO® septa are one of the best choices for temperature resistance, and have the added benefit of a needle guide, which increases septum lifetime (see "Preventing Septum Problems" on page 20 for more information on septum selection and care). Understanding how your inlet temperature setting relates to the actual temperature at the septum allows you to control bias, avoid septum problems, and better understand your results.

Figure 1 Septum temperature differs from inlet temperature set point; the degree of difference varies by manufacturer.



See page 21 for a list of septa we offer or visit us online at www.restek.com

Retention Cross-over Phenomenon in Gas Chromatography- Can the Mystery be Revealed? Part 1

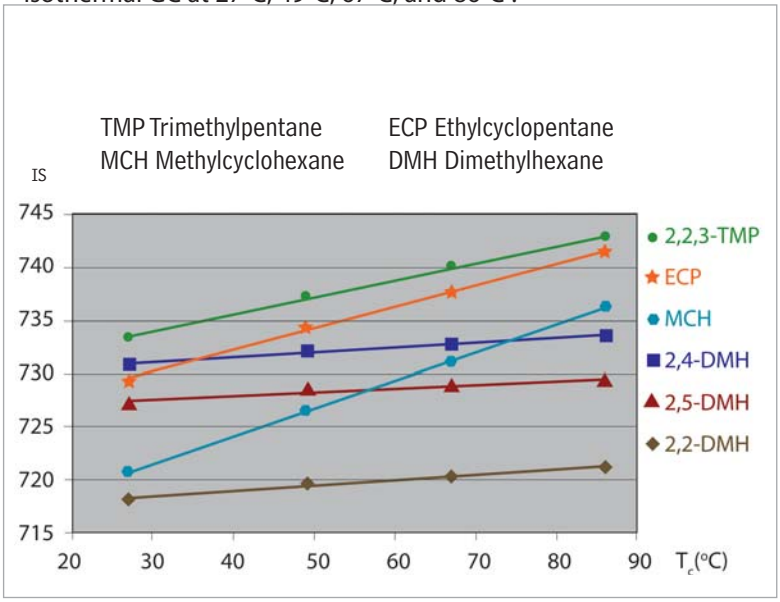
Continued from page 2

Therefore, the dependence of the retention time upon column temperature is usually expressed graphically as the log of the retention parameter (net retention time t´R or retention factor k´ or retention index I) vs. Tc or 1/Tc, where Tc is the absolute column temperature. In many cases, the plots are linear over the temperature range employed and, furthermore, the lines are approximately parallel to each other indicating that there is little change in selectivity by changing the column temperature in isothermal mode. This is valid for chemically similar compounds. But closer inspection reveals that some lines diverge slightly in their slope and even cross each other (Figure 2).[2] The practical implication is coelution of the two compounds at the temperature where the lines intersect. By further changing the column temperature the compounds are again separated but in reverse elution order. As mentioned above, this kind of behavior is often experienced when compounds of different chemical nature are analyzed on moderate to highly polar stationary phases.

But not only compounds with different functional groups will behave this way! In the next issue of the Restek Advantage, you will see examples of aliphatic versus cyclic compounds or cyclic compounds differing in their ring number, and the cross-over effect on non-polar columns.

References:
1 Mehran M. et al., HRC, 14 (1991) 745 – 750.
2 Hively, R.A. and R.E. Hinton, J. Gas Chromatogr. 6 (1968) 203 – 217.

Figure 2 Retention indices on squalane (IS) as a function of Tc for isothermal GC at 27°C, 49°C, 67°C, and 86°C[2].



more to reveal!

See the next issue of *the Restek Advantage* for Part 2 of *Retention Cross-over Phenomenon in Gas Chromatography– Can the Mystery be revealed?*

Tradeshow Schedule

We'd be happy to talk with you at any of the following meetings or shows. We'll post our booth numbers as they become available to us.

June, 2007

Date June 3-7
Show 55th ASMS Conference on Mass Spectrometry
Location Indiana Convention Center, Indianapolis, IN

Date June 4-7
Show 30th International Symposium on Capillary Chromatography (ISCC)
Location Dalian World Expo Centre, Dalian, P.R. China (Booth 80)

Date June 11-14
Show Metabolomics Society 3rd Annual Conference
Location Renold Building, The University of Manchester, Manchester, UK

Date June 17-21
Show HPLC 2007
Location International Convention Centre, Ghent, Belgium

July, 2007

Date July 17-19
Show Semicon West 2007
Location Moscone Center, San Francisco, CA

Date July 22-25
Show Florida Pesticide Residue Workshop (FPRW)
Location TradeWinds Island Grand, St. Pete Beach, FL

August, 2007

Date August 19-23
Show ACS 234th National Meeting & Exposition
Location Boston, MA

Date August 20-24
Show National Environmental Monitoring Conference (NEMC)
Location Hyatt Regency, Cambridge, MA

Date August 26-31
Show T2007 - TIAFT (International Conference (NEMC)
Location Seattle Sheraton, Seattle, WA

For latest updates, see our Tradeshow Calendar at **www.restek.com/ontheroad**.

Seminar Schedule

Date	Cat. #	City	State
GC Hands-On Maintenance and Troubleshooting			
6/13	65552	Lafayette Hill	PA
Comprehensive Capillary GC			
6/12	65551	Lafayette Hill	PA
6/15	65553	Wilmington	DE
6/19	65554	Cleveland	OH
6/20	65555	Buffalo	NY
6/22	65556	Pittsburgh	PA
7/23	65560	Idaho Falls	ID
7/25	65561	Boise	ID
7/26	65562	Spokane	WA
Comprehensive HPLC			
7/9	65557	Chicago	IL
7/11	65558	Madison	WI
7/13	65559	Kansas City	MO

Register at www.restek.com/seminar

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Hub-Cap Multi-pack	3-pk.	26542

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the RESTEK ADVANTAGE

2007.01

New pHidelity™ HPLC Columns

For Analyses at Extreme pH Conditions

See **page 3**.

RESTEK

the Restek Advantage
2007.01

IN THIS ISSUE

Restek: A Company of Owners

by Paul Silvis, Restek Founder & former Head Coach

Twenty-one years ago, I had a vision of creating a company where employees would enjoy coming to work as much as going home. Everyone kept telling me that we couldn't keep alive the Restek vision of being a great place to work as we got bigger, but I've always been too stubborn to agree. Today, more than 250 employee-owners and our families work, play, and celebrate milestones in our state-of-the-art facility in Bellefonte, in our new research facility in California, and in our subsidiary locations in England, France, Germany, and Ireland. And, in keeping with my vision, Restek is celebrating its twenty-first year in business by being selected as one of Pennsylvania's Top 100 Places To Work – for the third time!! We understand that our customers' happiness and our own are tightly intertwined – we care about the products we make, and the Plus 1 service we provide, because your best interests also are ours.

Plus 1 Service, Innovation, and Execution (PIE®) have been, and continue to be, the keys to our success, but another vital component to our success is that Restek employees have a positive vision of their future. Why? Because as the founder and controlling shareholder, I have set in motion a plan to sell the company to the employees, providing them the opportunity to chart their own future and continue the tradition of our customer-first culture.

Friends of mine who started companies, including Walt Jennings of J&W, and Nick Pelick and Walt Supina of Supelco, had a significant impact on my vision for Restek. These individuals all expressed regret once their exciting, entrepreneurial companies were sold. Each watched as the cultures they so carefully assembled began to change, and employees lost that "enjoy coming to work" feeling. I have come to believe that success is not measured by the price for which you can sell a company, but by the way the company prospers under the next generation of leadership. In 2005, I turned over the reins of Restek to Don McCandless, who took over as Head Coach and in the supporting role of mentoring and teaching the next generation of leadership. Now, we are fully engaged in the process of executing an employee stock ownership plan for selling the company to those whose labors have had a major role in building it. As we advance toward total employee ownership, our people and our products are positioning Restek to meet new challenges and opportunities, with even better service to our customers and community, and it is no exaggeration to say that Restekians truly do enjoy their work.

Restek will continue to be successful because our employees are excited that their future is in their own hands. Customers will continue to benefit because Restek will remain independent – able to work with all of the instrument companies – and will continue to create top quality products for all of them. We will be able to continue responding to all the ideas that pour in from customers around the world, telling us what products and services they need to make their lives easier in the lab.

Our company of owners will control their own destiny. I will be smiling from ear to ear when I see how employee ownership works to create a company in which employees still enjoy coming to work as much as going home! Isn't it fun to do business with employee-owners who love coming to work every day?

New pHidelity™ pH-Stable HPLC Columns

For Analyses at Extreme pH Conditions

By Becky Wittrig, Ph.D., HPLC Product Marketing Manager, Frank Dorman Ph.D., HPLC Innovations Manager, Rick Lake, Pharmaceutical Innovations Chemist, Vernon Bartlett, HPLC Innovations Scientist, Bruce Albright, HPLC Innovations Chemist, and Randy Romesberg, HPLC Innovations Chemist

We are pleased to introduce pHidelity™ pH-stable HPLC columns, designed for analyses that require, or benefit from, extreme pH conditions. pHidelity™ columns incorporate a proprietary barrier layer that protects the base silica particle, and a secondary layer that provides the functional stationary phase ligand. pHidelity™ columns can be used routinely up to pH 12 – a significant improvement over the typical pH 2.5 to 7.5 range for silica-based materials. pHidelity™ columns give you more control over analyte retention and resolution; mobile phase pH can be increased to enhance retention of basic analytes – without sacrificing column lifetime.

Continued on page 4.

New pHidelity pH-Stable HPLC Columns *(continued from page 3)*

- Stable pH 12 – superior chromatography for basic compounds.
- Patented barrier technology protects silica particles.
- True C18 selectivity, for simpler and more reproducible analyses.

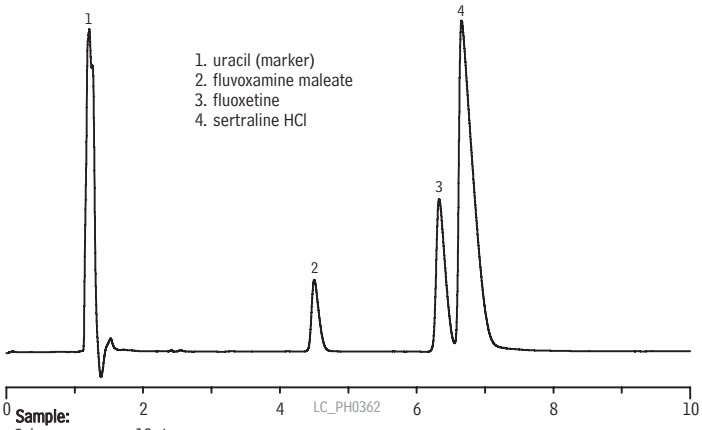
Practically, the useable pH range for conventional silica-based HPLC columns is pH 2.5 to 7.5. Columns are used outside of this range only when there is an extreme need for a separation, and the inevitable price – a very short column lifetime – must be accepted. pHidelity™ pH-stable HPLC columns can be used far above the typical pH range for silica-based stationary phases, with mobile phases up to pH 12, giving more control over analyte retention and resolution.

To illustrate the advantages of using high pH mobile phases for assaying basic analytes, we first analyzed selective serotonin reuptake inhibitors (SSRIs). SSRIs are basic compounds with high pKa values. Ideally, a high pH mobile phase would be used for this analysis. A high pH mobile phase will keep the analytes in their neutral forms and allow better retention and resolution on an alkyl C18 column. However, if using a mobile phase pH appropriately above compound pKa values (approximately 1.5-2 pH units) on a column with a limited alkaline range, the caustic mobile phase would rapidly degrade the silica particles – significantly shortening column lifetime. Therefore, with a conventional C18 column, an acidic or neutral mobile phase pH must be used. As result, when the compounds are assayed at a mobile phase pH below their pKas, they are in their ionized forms and their retention, peak shape, and resolution is limited on a conventional C18 column (Figure 1A). An extended range pHidelity C18 column allows the use of high pH mobile phases, above the analytes pKa, without deleterious effects to the column. Under these conditions, basic analytes are neutral, more hydrophobic and better retained (Figure 1B). By using a pHidelity™ column mobile phase pH can be optimized, improving retention, peak shape, and resolution on a C18 column.

Another advantage to extending the pH range of silica based columns, is improved analysis of multi-component test mixes with high pH mobile phases. When faced with a mixture of basic analytes, choosing the appropriate mobile phase pH can be problematic. An example of this is the mixture of bases which vary in pKa value, as shown in Figure 2. If a conventional C18 column was employed to assay this ionic mixture, a pH approximately 1.5 - 2 units below the lowest pKa would need to be used (a pH above the highest pKa would be above the operating range of conventional silica columns). This would result in protonation of the basic analytes, making them more hydrophilic, and less

Figure 1 Improve SSRI retention, peak shape, and resolution using an extended pH range pHidelity column.

A) Conventional C18 columns require mobile phases below the SSRI's pKa, limiting chromatography

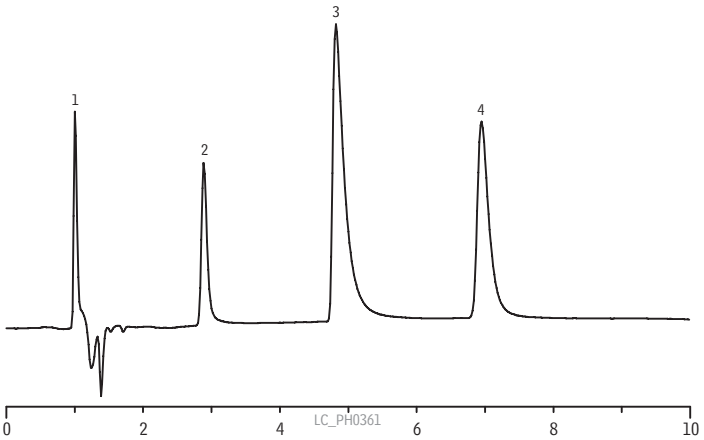


Sample:
Inj.: 10µL
Conc.: 100µg/mL each component
Sample diluent: acetonitrile

Column: C18
Dimensions: 150 x 4.6 mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 20mM potassium phosphate, monobasic **(pH 3)**:acetonitrile, 60:40
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 230 nm

B) pHidelity columns allow use of mobile phases above the SSRI's pKa – for improved chromatography

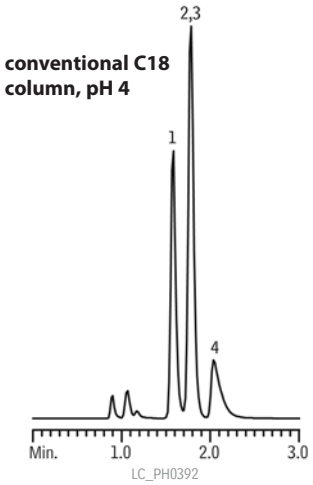


Column: pHidelity™ C18 (cat.# 9579365)
Dimensions: 150 x 4.6 mm
Particle size: 3µm
Pore size: 200Å

Conditions:
Mobile phase: 10mM ammonium bicarbonate **(pH 11)**:acetonitrile:tetrahydrofuran, 45:45:10
Flow: 1.5mL/min.
Temp.: ambient
Det.: UV @ 230 nm

Figure 2 Using a high pH mobile phase is an easy way to improve peak retention, resolution, and symmetry in a test mix of compounds varying in pKa values.

A) Poor separation of bases under typical conditions



conventional C18 column, pH 4

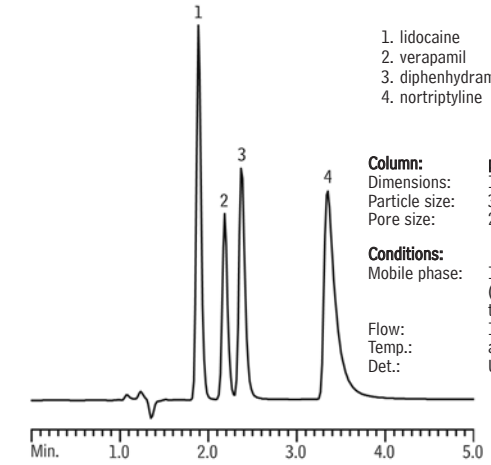
1. lidocaine
2/3. verapamil/diphenhydramine
4. nortriptyline

Sample:
Inj.: 10µL
Conc.: 100µg/mL each component
Sample diluent: acetonitrile

Column: C18
Dimensions: 150 x 4.6 mm
Particle size: 3µm
Pore size: 200Å

Conditions:
Mobile phase: 20mM ammonium acetate (pH 4):acetonitrile 50:50
Flow: 0.75mL/min.
Temp.: ambient
Det.: UV @ 254 nm

B) Complete separation of bases on a pHidelity™ column at pH 11



1. lidocaine
2. verapamil
3. diphenhydramine
4. nortriptyline

Column: pHidelity™ C18 (cat.# 9579365)
Dimensions: 150 x 4.6 mm
Particle size: 3µm
Pore size: 200Å

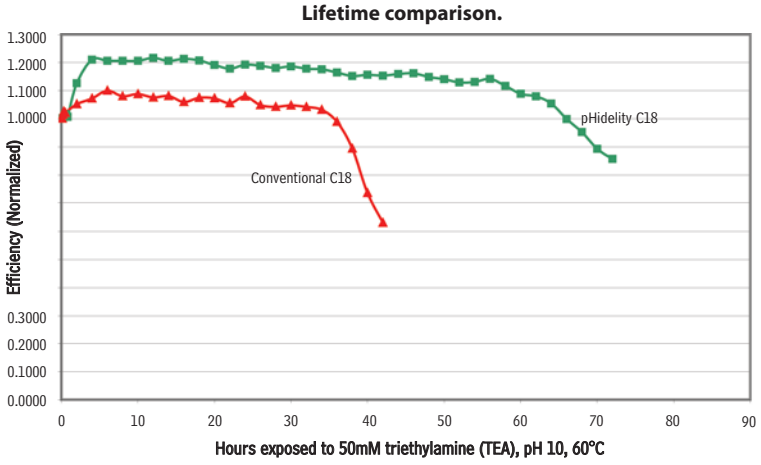
Conditions:
Mobile phase: 10mM ammonium bicarbonate (pH 11): acetonitrile: tetrahydrofuran, 35:55:10 (v/v/v)
Flow: 1.5mL/min.
Temp.: ambient
Det.: UV @ 254 nm

retained (Figure 2A). In contrast, using an extended range pHidelity™ C18 column and a mobile phase pH above the highest pKa of the analytes, the compounds will be uncharged and more hydrophobic, resulting in greater retention (Figure 2B). The analysis of basic compounds using a high pH mobile phase is an easy way to increase retention and to enhance resolution and peak shape. This makes a simpler task of method development, especially for complex test mixtures.

Non-silica-based chemistries have been developed in attempts to overcome the pH constraints of conventional silica-based HPLC columns, but silica-based phases offer a number of advantages, including high efficiencies, consistent lot-to-lot reproducibility, and predictable selectivities. Silica-based pHidelity™ pH-stable columns offer selectivity similar to conventional materials, but with dramatically increased column lifetime, even under the most harsh conditions. Figure 3 shows equivalent comparisons of a pHidelity™ C18 column and a conventional C18 column in an accelerated lifetime test under high pH conditions, at pH 10 and 60°C. This test demonstrates that pHidelity™ columns have a much greater lifetime when used in caustic environments than conventional C18 columns. Additionally, the pHidelity™ packing material is based on a true silica particle, ensuring a more C18-like selectivity than any competitive column based on non-silica or hybrid materials.

If your separation would benefit from extended pH conditions, we recommend you take advantage of pHidelity™ column for extreme-pH stability, C18-like selectivity, and long lifetimes. To discuss your separation, or for more information, please contact Restek's HPLC technical service group, and we will be happy to discuss how you can improve your analysis, and make fewer column [illegible] pHidelity™ column.

Figure 3 pHidelity™ C18 columns - exceptional performance under accelerated high pH stability testing conditions.



pHidelity™ C18 Columns

Physical Characteristics:

particle size: 3µm
pore size: 200Å

pH range: 1 to 12
temperature limit: 80°C

3µm Column, 4.6mm		cat. #
30mm		9579335
50mm		9579355
100mm		9579315
150mm		9579365
pHidelity™ C18 Guard Cartridges	**qty.**	**cat. #**
10 x 2.1mm	3-pk.	957930212
10 x 4.0mm	3-pk.	957930210
20 x 2.1mm	2-pk.	957930222
20 x 4.0mm	2-pk.	957930220

ordering note

For guard cartridges for these columns, visit our website at **www.restek.com**.

To order a column with a Trident™ inlet fitting, add -700 to the column's catalog number.

Simplified LC/MS/MS Analysis of Fluoroquinolones

Using An Allure® PFP Propyl Column

By Rick Lake, Pharmaceutical Innovations Chemist, and Benjamin Smith, Applications Technician

- Increase retention without ion-pairing.
- Better selectivity than C18 or cyano phases.
- Use desirable high-organic mobile phases for better ESI LC/MS sensitivity.

Fluoroquinolones are broad-spectrum antibiotics, used in both human and veterinary medicine. Because they are widely used, fluoroquinolones are target compounds in many analysis sectors, from research and clinical testing to environmental impact and residues in food. We have determined that an Allure® PFP Propyl column offers good retention capacity, and better selectivity than a C18 column, allowing simple method development strategies for fluoroquinolones.

Parent compound nalidixic acid is the structural basis for all quinolones, and fluoroquinolones are a fluorine-containing subset of this group (Figure 1). Chemically, fluoroquinolones exhibit amphoteric behavior: the nalidixic acid portion of the molecule has acidic functionality (carboxylic acid), while the compound as a whole also expresses a basic functionality. These characteristics, and the typical presence of polar functional groups, make chromatographic retention of the compounds difficult when using an alkyl phase and a simple (two-component) mobile phase. Polar groups reduce retention on alkyl phases, making a highly aqueous mobile phase, or ion-pairing, necessary for acceptable retention.

For non-selective, non-MS analyses, like potency assays, fluoroquinolones traditionally have been analyzed by reversed phase HPLC, on a C18 phase and in a highly aqueous mobile phase, as described in the USP monograph for ciprofloxacin.[1] When mass spectrometry is dictated, and a highly aqueous mobile phase is undesirable, ion-pairing with a volatile "MS friendly" reagent, like nonafluoropentanoic acid, has been used to increase retention. Although these mechanisms are sufficient, we sought to determine if, with a simple mobile phase, an Allure® PFP Propyl column would offer better retention, and possibly better selectivity, than a C18 phase.

Initially, we assayed the analytes on a C18 column, in an aqueous buffer and acetonitrile, to evaluate the retention and selectivity that could be achieved with a conventional stationary phase and isocratic mobile phase. As expected, retention was poor: an acceptable retention capacity value (roughly 2-5) required an aqueous concentration of 80% (Figure 2). Next, to see if we could improve retention through ionic

Figure 1 The polarity of fluoroquinolones make them a challenge to retain on C18 phases.

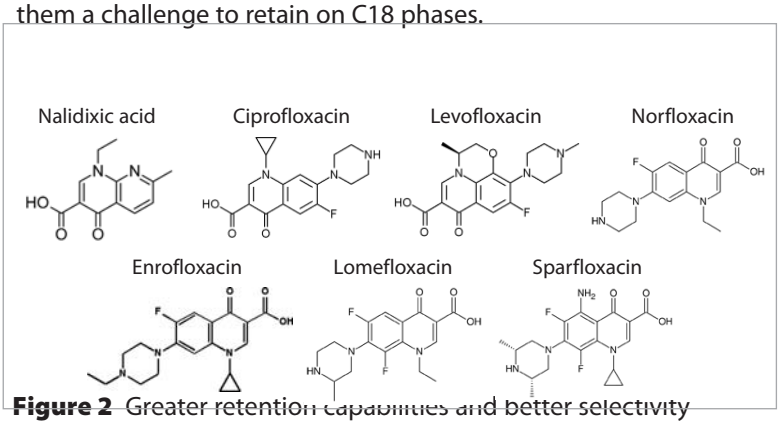


Figure 2 Greater retention capabilities and better selectivity enable you to use simple two-component mobile phases with an Allure® PFP Propyl column.

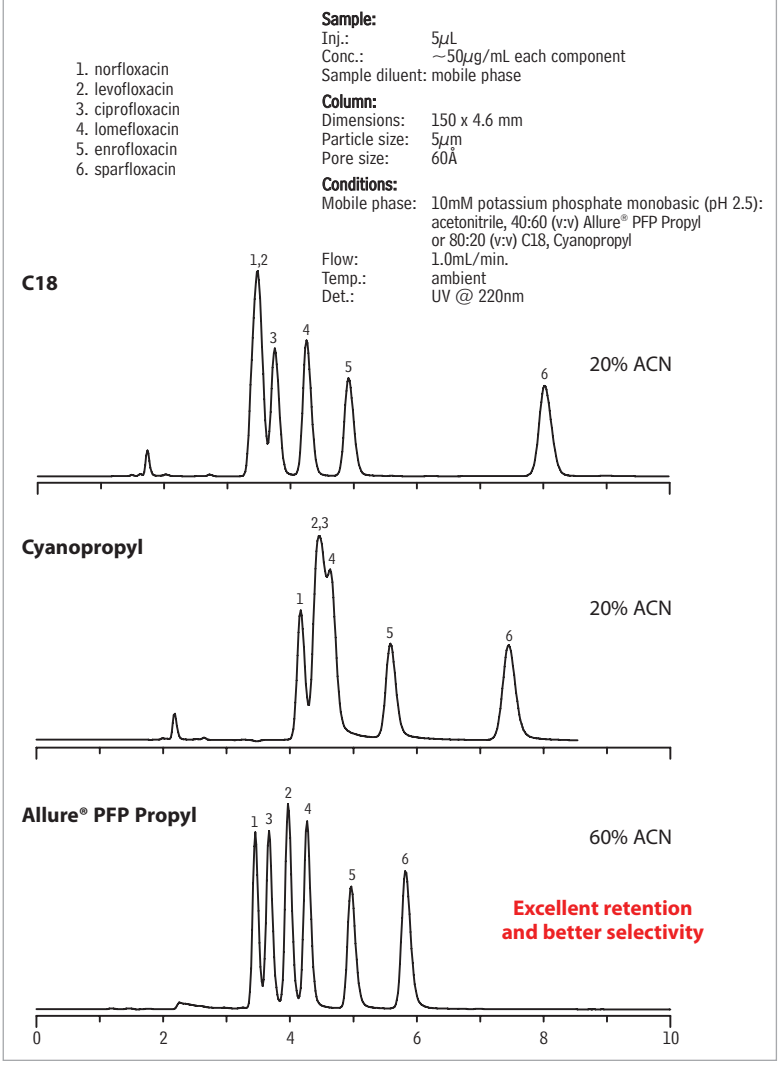
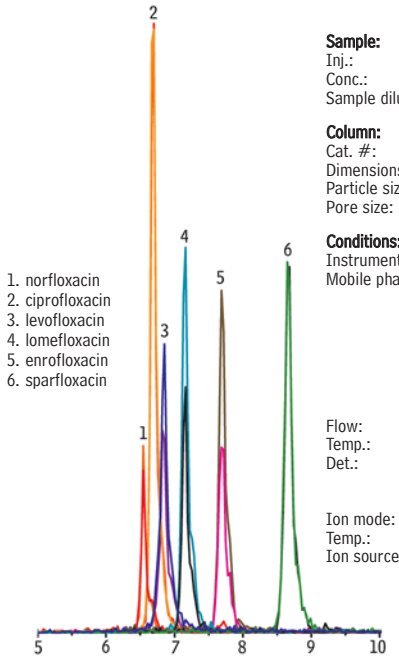


Figure 3 Optimizing retention with the Allure PFP Propyl gives high sensitivity and low matrix interference when analyzing fluoroquinolones by LC/MS/MS.



Sample:
Inj.: 5µL
Conc.: 50ng/mL
Sample diluent: mobile phase

Column: **Allure® PFP Propyl**
Cat. #: 9169352
Dimensions: 50 x 2.1 mm
Particle size: 3µm
Pore size: 60Å

Conditions:
Instrument: Shimadzu Prominence HPLC
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

Time (min.)	%B
0.00	10
10.00	90
10.10	10
15.00	10

Flow: 300µL/min.
Temp.: 30°C
Det.: Applied Biosystems API 3200 Triple Quadrupole LC/MS/MS Mass Spectrometer
Ion mode: positive
Temp.: 600°C
Ion source: TurboIonSpray®, Electrospray 4000V

LC_PH0426

Compound	Precursor Ion	Fragment Ion	Declustering Potential (V)	Collision Energy (V)
1. Norfloxacin	319.9	276.0	36.00	23.00
		233.1	36.00	35.00
2. Ciprofloxacin	332.1	288.2	41.00	23.00
		244.9	41.00	31.00
3. Levofloxacin	362.1	318.1	31.00	25.00
		261.0	31.00	41.00
4. Lomefloxacin	351.9	265.1	41.00	29.00
		308.0	41.00	23.00
5. Enrofloxacin	360.2	316.1	36.00	25.00
		245.3	36.00	37.00
6. Sparfloxacin	393.1	349.4	36.00	25.00
		292.2	36.00	29.00

interactions, we evaluated a cyanopropyl phase under the same conditions. This combination produced similar retention, but less selectivity (Figure 2). In contrast, an Allure® PFP Propyl column (pentafluorophenyl propyl phase), used under the same conditions, enabled us to achieve comparable retention capacities with the water content of the mobile phase reduced to 40% (Figure 2). In addition to greater retention capacity than the other phases, the Allure® PFP Propyl stationary phase has better selectivity – unlike with the C18 and cyano phases, there are no coelutions.

Another advantage to the Allure® PFP Propyl column's high retention capacity for fluoroquinolones is in LC/MS analysis. Maximizing retention causes the analytes to elute in mobile phases having higher percentages of the organic component. This can increase desolvation efficiency in electrospray ionization (ESI), and can eliminate unwanted adduct formation or charge competition from matrix interferences that are less retained by the column. The result is a potential for increasing sensitivity, while using simple analytical conditions. A simple mobile phase gradient, starting with a highly aqueous content and moving to a highly organic content, can be employed to elute salts and low molecular weight sample matrix interferences ahead of the compounds of interest. We observed the same improved retention when we assayed our fluoroquinolone test mix through positive ESI LC/MS/MS on an Applied Biosystems/MDS SCIEX API 3200 triple quadrupole LC/MS/MS mass spectrometer equipped with a Shimadzu Prominence binary pump LC system (Figure 3).

The Allure® PFP Propyl phase will retain polar analytes much more effectively than a C18 phase. When greater retention is needed to give the desired selectivity, or when LC/MS analysis is desired or required, simplify your method – use an Allure® PFP Propyl column and a simple mobile phase rather than a C18 column and an ion-pairing technique.

Reference
1. United States Pharmacopoeia, 28th revision; National Formulary, 23rd edition.

thank **you**

Instrument provided courtesy of Applied Biosystems

www.appliedbiosystems.com

Allure® PFP Propyl Columns (USP L43) Excellent Columns for LC/MS and ELSD

5µm Column, 4.6mm	cat. #
150mm	9169565
150mm (with Trident™ Inlet Fitting)	9169565-700

ordering **note**

For guard cartridges for these columns, visit our website at **www.restek.com**.

Monitor Antioxidants in Tea Extract

Using an Ultra Aqueous C18 HPLC Column and Unique® TOFMS

by Julie Kowalski, Ph.D., Innovations Chemist

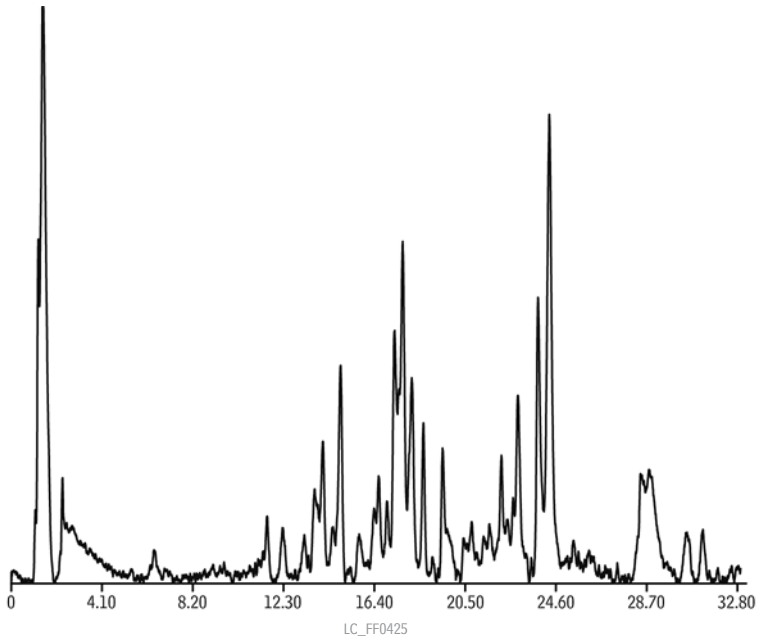
- Use highly aqueous mobile phases without collapsing the stationary phase.
- Extract data for specific compounds and manually inspect spectra for other compounds.
- Simple sample preparation.

Much focus has been given to the health benefits of foods and beverages that contain antioxidant compounds. By reacting with free radical-forming compounds before they can cause cell damage, antioxidants protect the body against oxidative stress.[1] Some foods and beverages naturally contain antioxidants, but supplementing foodstuffs has been on the rise due to demands by health conscious consumers. Recently, green tea has been successfully promoted as a health drink because it contains antioxidant phenolic compounds.

Using LC/TOFMS, we show a straightforward method for determining the presence of antioxidant compounds in commercial tea formulations. Samples were prepared by adding approximately 15g of dry tea product to 200mL of methanol which was cooled to approximately 20°C. The mixture was stirred for 5 minutes and decanted. The tea product was rinsed with an additional 20mL of cooled methanol. The 200mL and 20mL solutions were combined, then filtered through a 0.45µm syringe filter to capture particles. The filtered solution was used directly for analysis.

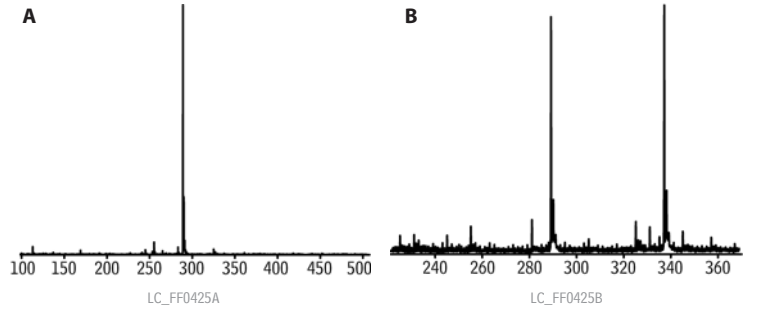
We used a 150 x 2.1mm Ultra Aqueous C18 HPLC column for the analysis and, because a tea extract is a complex matrix, we used a gradient elution and mobile phases with a high water content. The Ultra Aqueous C18 stationary phase is ideal for such an application: the phase is specifically designed to prevent collapse of the C18 alkyl chains in highly aqueous mobile phases.[2]

Figure 1 A complex mix of tea extract components is best separated on an Ultra Aqueous C18 column with a highly aqueous mobile phase (total ion chromatograms of Table 1 compounds).



For conditions see Figure 3.

Figure 2 (-)-Epicatechin produced by infusion of a standard (A) and spectrum of (-)-epicatechin created from an extracted ion chromatogram (B).



For conditions see Figure 3.

Figure 3 Spectra of phenolic compounds identified in the tea extract.

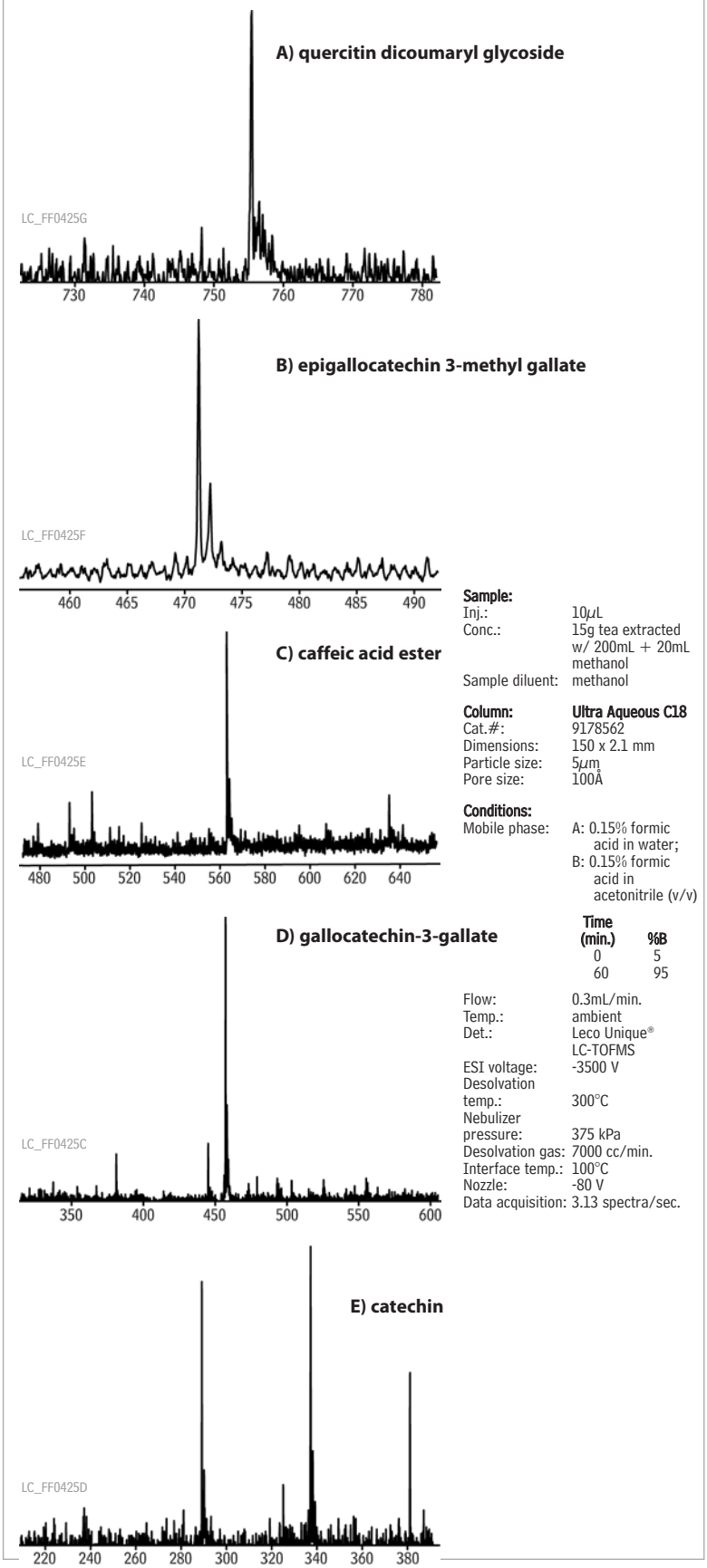


Table 1 Phenolic compounds of interest.

Compound	[M-H]-
gallocatechin-3-gallate	457.206
catechin	289.154
epigallocatechin-3-methyl gallate	471.208
epicatechin di-gallate	609.318
epicatechin-3-gallate	441.208
catechin gallate	

Note: m/z 441.2 can be either epicatechin-3-gallate or catechin gallate.

The Ultra Aqueous C18 phase proved ideal for resolving the complex tea matrix, as shown by the large number of peaks in Figure 1. The resolving power of this chromatographic system, in combination with the LECO Unique® TOF Mass Spectrometer, allow the analyst to both extract data for specific compounds of interest and manually inspect spectra for other compounds, including phenolic glycosides and esters of phenolic acid.

If you are analyzing antioxidants in tea, or other complex mixtures of compounds, an Ultra Aqueous C18 column gives you the reliable results you need, without restricting your ability to use the mobile phase composition that works best for your application.

For information about the LECO Unique® TOFMS, please visit the LECO website: **www.leco.com**

1 Free radical damage is implemented in many disease models, including cancer, in many degenerative illnesses, and in the aging process.

2 When the long, hydrophobic alkyl chain of a conventional C18 stationary phase is exposed to a highly aqueous mobile phase it folds down on itself, causing loss of retention. A prolonged equilibration time in a high organic solution is needed to restore the phase. The Ultra Aqueous C18 stationary phase is not susceptible to phase collapse – not even in mobile phases with very highly aqueous content.

Ultra Aqueous C18 Columns (USP L1)

5µm Column, 2.1mm	cat. #
150mm	9178562
150mm (with Trident™ Inlet Fitting)	9178562-700

ordering note

For guard cartridges for these columns, visit our website at **www.restek.com**.

Syringe Filters

- Color coded for easy identification.
- Reusable storage container.

Size	Porosity	Color	qty.	cat.#
Nylon				
25mm	0.45µm	pink	100-pk.	26149
25mm	0.45µm	pink	500-pk.	26203

See our catalog or website for other sizes and materials.

thank you

Instrument provided courtesy of LECO Corporation.

www.leco.com

Superior Chromatography for Semivolatile Organics

Using the Rtx®-5Sil MS Capillary GC Column

by Robert Freeman, Environmental Innovations Chemist

- Superior resolution of benzo(b)- and benzo(k)fluoranthene.
- Symmetric peaks and excellent responses for phenols.
- Excellent thermal stability and exceptionally low bleed.

GC/MS analytical methods for semivolatile compounds, such as U.S. Environmental Protection Agency Method 8270D and equivalent methods in other countries, cover a broad range of environmental pollutants. The target lists often include complex mixtures of acidic, basic, and neutral analytes. Further, the sample extracts often contain problematic matrix interferences. These factors, coupled with the increasing need for lower detection limits, place significant demand on the thermal stability, inertness, and efficiency of the analytical column.

Restek chemists designed the Rtx®-5Sil MS capillary column to address the challenging demands of semivolatiles analysis. Phenyl rings in the polymer backbone of the stationary phase stiffen the siloxane chain, preventing thermal breakdown and reducing bleed. The content of this aryl functionality has been adjusted so that selectivity is similar, but improved, compared to that of conventional 5% diphenyl/95%dimethyl phases. The silarylene polymer not only exhibits improved thermal stability and reduced bleed, it has increased separation for aromatic isomers benzo(b)- and benzo(k)fluoranthene – as shown in Figure 1.

Surface activity in a column is revealed by the response factors for active analytes, such as 2,4-dinitrophenol (acidic) and pyridine (basic). Most column manufacturers struggle to attain adequate responses and good peak shapes for such analytes. Our unique deactivation process for the Rtx®-5Sil MS silarylene phase assures unsurpassed inertness and excellent responses for these active analytes – note the response for 2,4-dinitrophenol in Figure 2, and for many other semivolatiles in Figure 3.

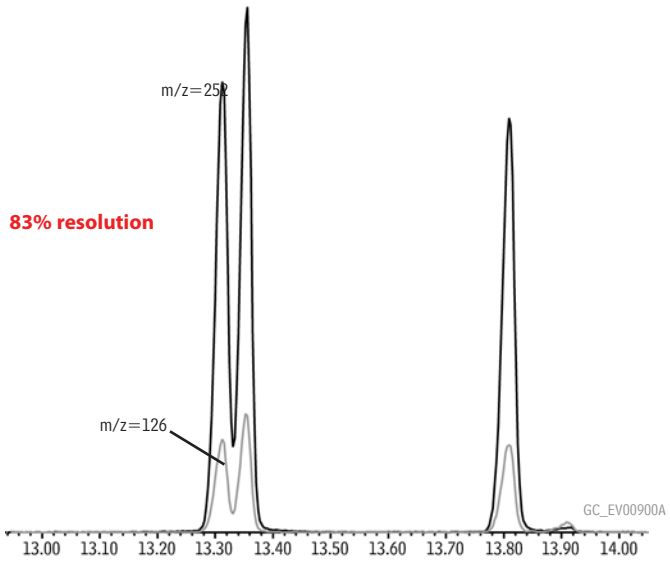
Featuring an optimized stationary phase, inherently low bleed, and proprietary deactivation, Rtx®-5Sil MS columns overcome the inherent problems associated with semivolatiles analyses. If you are performing these analyses, you can simplify life in your laboratory – rely on these new columns to help you obtain consistent results.

Rtx®-5Sil MS Column (fused silica)

(Crossbond®, selectivity similar to 5% diphenyl/95% dimethyl polysiloxane)

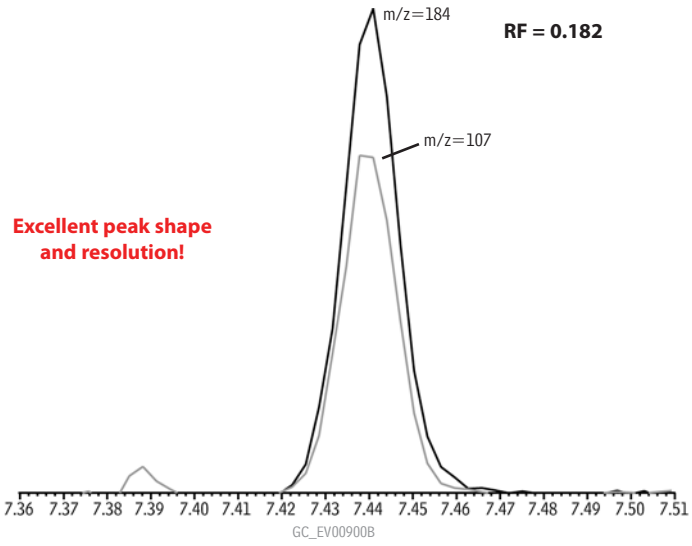
ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	12723

Figure 1 Superior resolution of benzo(b)fluoranthene, benzo(k)fluoranthene, and benzo(a)pyrene (10µg/mL).



For conditions see Figure 3.

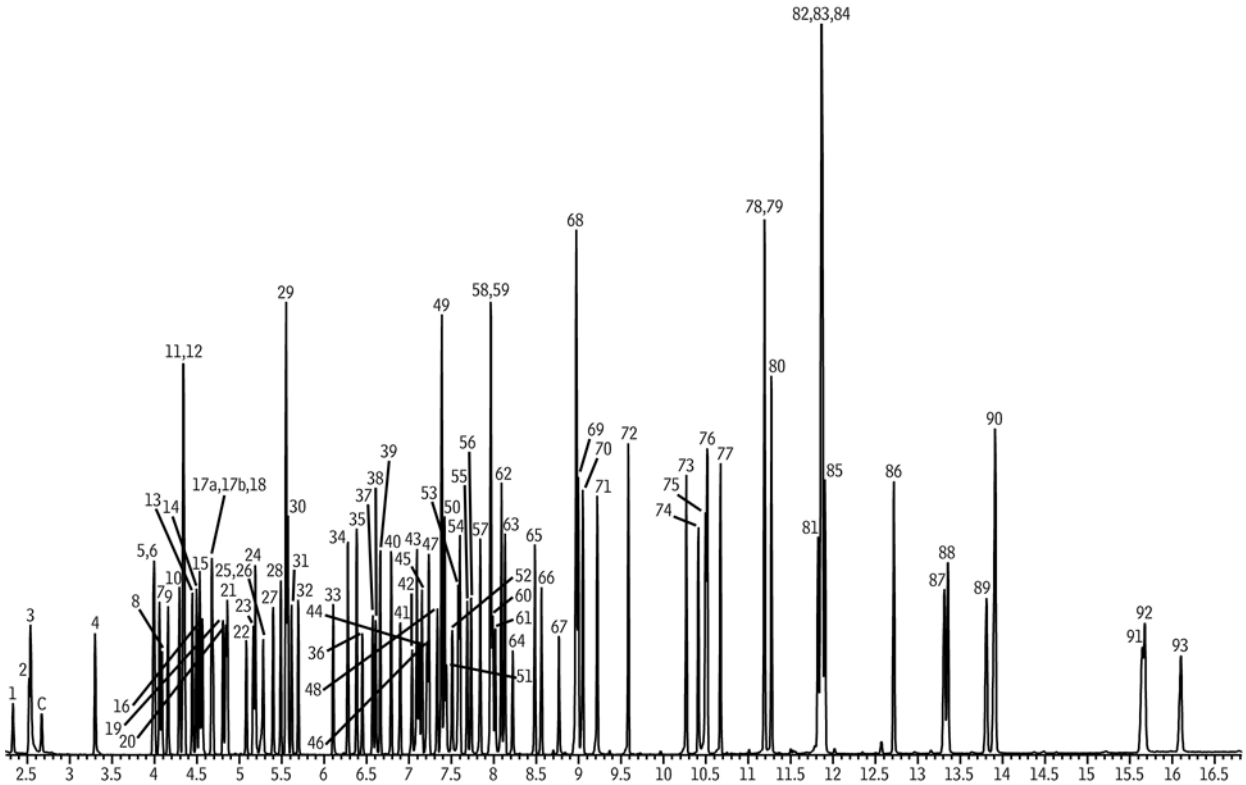
Figure 2 Excellent response for 2,4-dinitrophenol (10µg/mL).



For conditions see Figure 3.

Figure 3 Total ion chromatogram for 94 semivolatile analytes (10µg/mL).

	Peak	RT		Peak	RT		Peak	RT		Peak	RT
1.	1,4-dioxane	2.33	24.	2,4-dimethylphenol	5.19	49.	acenaphthene-d10 (IS)	7.39	72.	di-n-butyl phthalate	9.59
2.	N-nitrosodimethylamine	2.52	25.	benzoic acid	5.27	50.	acenaphthene	7.42	73.	fluoranthene	10.27
3.	pyridine	2.54	26.	bis(2-chloroethoxy)methane	5.28	51.	2,4-dinitrophenol	7.44	74.	benzidine	10.41
C	toluene	2.67	27.	2,4-dichlorophenol	5.40	52.	4-nitrophenol	7.51	75.	pyrene-d10 (Surr.)	10.50
4.	2-fluorophenol (Surr.)	3.30	28.	1,2,4-trichlorobenzene	5.49	53.	2,4-dinitrotoluene	7.58	76.	pyrene	10.52
5.	phenol-d6 (Surr.)	3.99	29.	naphthalene-d8 (IS)	5.55	54.	dibenzofuran	7.60	77.	p-terphenyl-d14 (Surr.)	10.67
6.	phenol	4.00	30.	naphthalene	5.57	55.	2,3,5,6-tetrachlorophenol	7.69	78.	3,3'-dimethylbenzidine	11.19
7.	aniline	4.06	31.	4-chloroaniline	5.62	56.	2,3,4,6-tetrachlorophenol	7.73	79.	butyl benzyl phthalate	11.20
8.	bis(2-chloroethyl) ether	4.09	32.	hexachlorobutadiene	5.70	57.	diethyl phthalate	7.84	80.	bis(2-ethylhexyl) adipate	11.27
9.	2-chlorophenol	4.16	33.	4-chloro-3-methylphenol	6.11	58.	4-chlorophenyl phenyl ether	7.96	81.	3,3'-dichlorobenzidine	11.82
10.	1,3-dichlorobenzene	4.29	34.	2-methylnaphthalene	6.28	59.	fluorene	7.97	82.	bis(2-ethylhexyl) phthalate	11.86
11.	1,4-dichlorobenzene-d4 (IS)	4.34	35.	1-methylnaphthalene	6.38	60.	4-nitroanaline	7.99	83.	benzo(a)anthracene	11.86
12.	1,4-dichlorobenzene	4.35	36.	hexachlorocyclopentadiene	6.45	61.	4,6-dinitro-2-methylphenol	8.02	84.	chrysene-d12 (IS)	11.88
13.	benzyl alcohol	4.45	37.	2,4,6-trichlorophenol	6.57	62.	N-nitrosodiphenylamine		85.	chrysene	11.91
14.	1,2-dichlorobenzene	4.50	38.	2,4,5-trichlorophenol	6.61		(diphenylamine)	8.09	86.	di-n-octyl phthalate	12.72
15.	2-methylphenol	4.53	39.	2-fluorobiphenyl (Surr.)	6.66	63.	1,2-diphenylhydrazine		87.	benzo(b)fluoranthene	13.31
16.	bis(2-chloroisopropyl) ether	4.56	40.	2-chloronaphthalene	6.79		(as azobenzene)	8.13	88.	benzo(k)fluoranthene	13.36
17a.	4-methylphenol	4.67	41.	2-nitroaniline	6.90	64.	2,4,6-tribromophenol (Surr.)	8.22	89.	benzo(a)pyrene	13.81
17b.	3-methylphenol	4.67	42.	1,4-dinitrobenzene	7.04	65.	4-bromophenyl phenyl ether	8.49	90.	perylene-d12 (IS)	13.91
18.	N-nitroso-di-n-propylamine	4.69	43.	dimethyl phthalate	7.09	66.	hexachlorobenzene	8.56	91.	dibenzo(a,h)anthracene	15.65
19.	hexachloroethane	4.81	44.	1,3-dinitrobenzene	7.12	67.	pentachlorophenol	8.77	92.	indeno(1,2,3-cd)pyrene	15.68
20.	nitrobenzene-d5 (Surr.)	4.84	45.	2,6-dinitrotoluene	7.15	68.	phenanthrene-d10 (IS)	8.97	93.	benzo(ghi)perylene	16.10
21.	nitrobenzene	4.86	46.	1,2-dinitrobenzene	7.21	69.	phenanthrene	9.00			
22.	isophorone	5.08	47.	acenaphthylene	7.23	70.	anthracene	9.05			
23.	2-nitrophenol	5.16	48.	3-nitroaniline	7.34	71.	carbazole	9.22			



GC_EV00900

Column: Rtx®-5Sil MS, 30m, 0.25mm ID, 0.25µm (cat.# 12723)
Sample: US EPA Method 8270D mix: 8270 MegaMix® (cat.# 31850), Benzoic Acid (cat.# 31879), 8270 Benzidines Mix (cat.# 31852), Acid Surrogate Mix (4/89 SOW) (cat.# 31025), Revised B/N Surrogate Mix (cat.# 31887), 1,4-Dioxane (cat.# 31853), SV Internal Standard Mix (cat.# 31206)
Inj.: 1.0µL, pulsed splitless, 10µg/mL each component, int. stds. 20µg/mL (10ng or 20ng on column), 4mm Drilled Uniliner® inlet liner, hole on bottom (cat.# 20756), pulse: 30psi @ 0.4 min.; 60mL/min. @ 0.3 min.
GC: Agilent 6890
Inj. temp.: 250°C
Carrier gas: helium
Flow rate: 1.2mL/min., constant flow
Oven temp.: 50°C (hold 0.5 min.) to 290°C @ 23°C/min., to 325°C @ 6°C/min. (hold 0.5 min.)
Det.: Agilent 5973 GC/MS
Transfer temp.: 280°C
Scan range: 35-550 amu
Solvent delay: 2.20 min.
Tune: DFTPP
Ionization: EI

8-Minute Dual Column Analysis of Organochlorine Pesticides

Using Rtx®-CLPesticides / Rtx®-CLPesticides2 Columns

By Jason Thomas, Environmental Chemist

- Analysis and confirmation of 20 pesticides in 8 minutes.
- Baseline resolution of all compounds, for improved accuracy.
- Low-bleed columns, for reliable data.

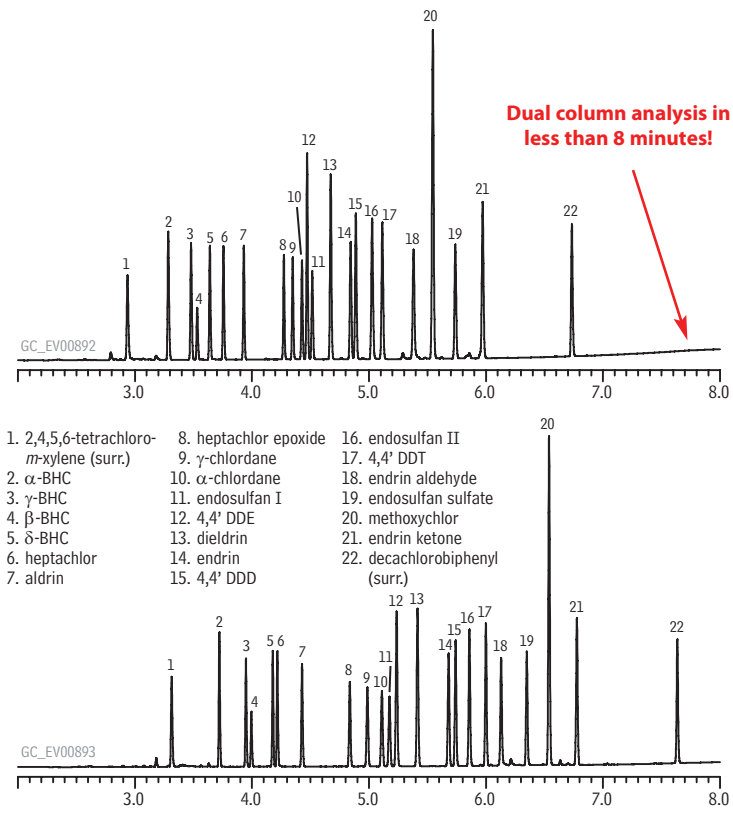
Analyses for organochlorine pesticides are among the most common pesticide methodologies in use today. US EPA Method 8081, for example, requires separation of 20 organochlorine pesticides, some of which are isomers or are otherwise structurally similar and, therefore, are difficult to separate. Restek introduced two proprietary phases to address this issue, the Rtx®-CLPesticides phase and the Rtx®-CLPesticides2 phase, which have proven very popular within the environmental community. The unique selectivities of this column pair allow laboratories to significantly reduce analysis times for Method 8081.

There is a constant need for faster analyses, to help increase sample throughput and, thereby, increase productivity. Fast GC is a good solution, but the reduced column internal diameters and thinner phase film coatings associated with fast GC have been deterrents, due to concerns about the columns' ability to cope with the harsh sample matrices often encountered in environmental samples, and shortened column lifetimes have not been acceptable.

Using a 0.53mm ID guard column at the inlet end of a dual column configuration protects the analytical columns downstream. This configuration allows 20m x 0.18mm ID thin film columns to be used, with their associated high efficiency, to greatly reduce analysis time without the reduction of column lifetime usually associated with introducing "dirty" samples into small bore columns.

Figure 1 shows separation of the 20 target pesticides in EPA Method 8081 in 8 minutes using 20m x 0.18mm ID Rtx®-CLPesticides/Rtx®-CLPesticides2 columns with a 0.53 ID guard column. In addition to rapid, baseline resolution, the pesticides are eluted as sharp, symmetric peaks. This, in turn, helps assure reliable quantification data for these analytes. Clearly the Rtx®-CLPesticides/Rtx®-CLPesticides2 columns, in conjunction with a 0.53mm ID guard column, are an excellent choice for analyzing EPA Method 8081 pesticides, or equivalent target lists of these pesticides.

Figure 1 20 organochlorine pesticides resolved and confirmed in 8 minutes, using Rtx®-CLPesticides/Rtx®-CLPesticides2 columns.



Column: Rtx®-CLPesticides, 20m, 0.18mm ID, 0.18µm (cat.# 42102) (top) and Rtx®-CLPesticides2, 20m, 0.18mm ID, 0.14µm (cat.# 42302) with 5m x 0.53mm ID intermediate-polarity deactivated guard tubing (cat.# 10045), connected using SeCure™ "Y" Connector Kit (cat.# 20276) with Universal "Y" Press-Tight® Connector
Sample: Organochlorine Pesticide Mix AB #2 (cat.# 32292), 8-80µg/mL each component in hexane/toluene, Pesticide Surrogate Mix (cat.# 32000), 200µg/mL each component in acetone
Inj.: 0.5µL splitless (hold 0.75 min.), 2mm single gooseneck inlet liner (cat.# 20795)
Inj. temp.: 250°C
Carrier gas: helium, constant flow
Linear velocity: 20cm/sec. @ 140°C
Oven temp.: 140°C (hold 1 min.) to 250°C @ 35°C/min. (hold 1 min.) to 330°C @ 35°C/min. (hold 3 min.)
Det.: ECD @ 350°C

Rtx®-CLPesticides Column (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.18	-60 to 310/330°C	20-Meter	42102

Rtx®-CLPesticides2 Column (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.14	-60 to 310/330°C	20-Meter	42302

IP Deactivated Guard Column

length, ID	cat. #
5m, 0.53mm ID	10045

SeCure™ "Y" Connector Kits

Ferrules Fit Column ID	qty.	cat.#
0.25/0.28mm	kit	20276

Organochlorine Pesticide Reference Mixes

Popular Restek Analytical Standards

By Ken Herwehe, Analytical Reference Materials Product Marketing Manager

Organochlorine Pesticide Mix AB #2

(20 components)

aldrin	8µg/mL	dieldrin	16
α-BHC	8	endosulfan I	8
β-BHC	8	endosulfan II	16
δ-BHC	8	endosulfan sulfate	16
γ-BHC (lindane)	8	endrin	16
α-chlordane	8	endrin aldehyde	16
γ-chlordane	8	endrin ketone	16
4,4'-DDD	16	heptachlor	8
4,4'-DDE	16	heptachlor epoxide (B)	8
4,4'-DDT	16	methoxychlor	80

In hexane:toluene (1:1), 1mL/ampul

cat. # 32292 (ea.)

Organochlorine Pesticide Mix AB #1

(20 components)

same components as Organochlorine Pesticide Mix AB #2, listed above

200µg/mL each in hexane:toluene (1:1), 1mL/ampul

cat. # 32291 (ea.)

Organochlorine Pesticide Mix AB # 3

(20 components)

same components as Organochlorine Pesticide Mix AB #2, listed above

2,000µg/mL each in hexane:toluene (1:1), 1mL/ampul

cat. # 32415 (ea.)

Pesticide Surrogate Mix

decachlorobiphenyl	2,4,5,6-tetrachloro-*m*-xylene

200µg/mL each in acetone, 1mL/ampul

cat. # 32000 (ea.)

Pesticide Surrogate Mix

decachlorobiphenyl	200µg/mL
2,4,5,6-tetrachloro-*m*-xylene	100

In P&T methanol, 1mL/ampul

cat. # 32453 (ea.)

did you **know**?

Restek offers the ChemService product line of neat pesticides and metabolites.

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ChemService

Resprep™ Florosil® SPE Cartridges

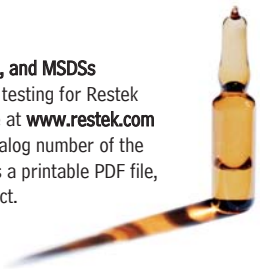
(EPA SW 846 methods and CLP protocols)

3mL/500mg (50-pk.)	6mL/500mg (30-pk.)	6mL/1000mg (30-pk.)
24031	—	24034
24032*	26086**	26085**

*Teflon® frits

**Glass tubes with Teflon® frits

free data



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Restek, "the company chromatographers trust™", should be your first choice for custom-made reference materials. Maximum convenience, maximum value, minimum time spent blending calibration mixtures in your laboratory.

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Analyze and Confirm Cannabinoids by LC/MS/MS

Using an Allure® Biphenyl Column

by Kristi Sellers, Clinical/Forensic Innovations Chemist, Becky Wittrig, Ph.D., HPLC Product Marketing Manager, and André Schreiber, Ph.D., Application Chemist, Applied Biosystems

- Faster sample throughput (short analysis time, no derivatization)
- Reliable response at 1ng on-column
- Undisputable identification, using two +MRM transitions

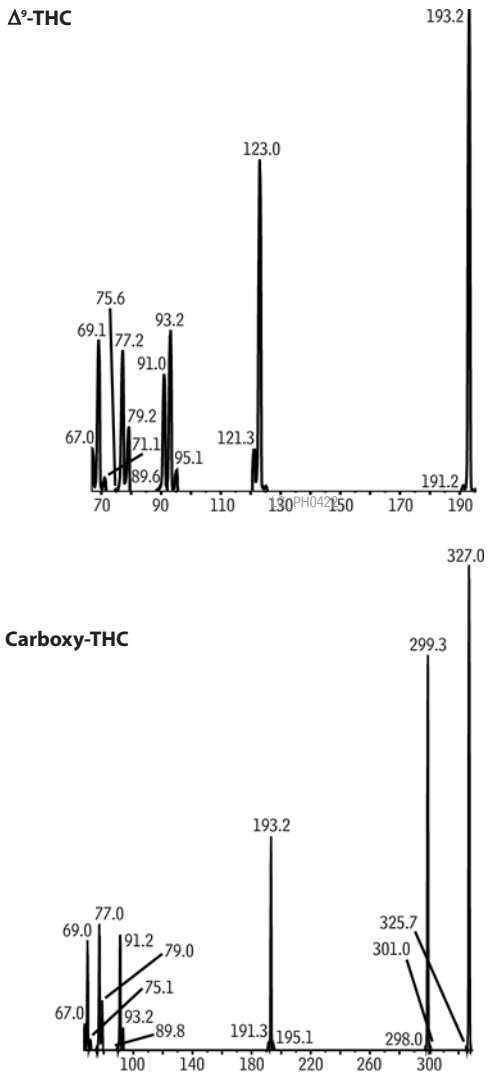
As marijuana is smoked, the main psychoactive component, Δ^9-tetrahydrocannabinol (Δ^9-THC), is quickly absorbed and metabolized to 11-hydroxy-Δ^9-tetrahydrocannabinol (hydroxy-THC), an active metabolite. Hydroxy-THC is further metabolized, rapidly, to 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (carboxy-THC), an inactive metabolite commonly found in urine, blood, hair, and tissues.[1] GC/MS often is used for confirming and quantifying Δ^9-THC and carboxy-THC[2]; however, GC/MS methods require time-consuming steps, like derivatization, to obtain acceptable chromatography. By using HPLC, derivatization can be eliminated, saving time without sacrificing sensitivity.

We developed a quantitative method for analyzing underivatized cannabinoids by HPLC/tandem mass spectrometry. Our goals were threefold; 1) to optimize column selection, 2) to provide a short analysis time, and 3) to obtain reliable confirmation and quantification data in the low nanogram range (< 10ng). We used an Applied Biosystems API 3200 MS/MS detector coupled to a Shimadzu LC20AD Prominence Series chromatograph for optimum chromatographic and detection capabilities.

Figure 1 shows the final product spectra for Δ^9-THC and carboxy-THC used to develop the +MRM (multiple reaction monitoring) method.[3] We determined the 30mm, 2.1mmID, 3µm Allure® Biphenyl HPLC column to be the best column for this analysis. This column employs a unique separation mechanism, π-π interaction, which greatly improves selectivity and retention, relative to conventional C18 phases. In addition, with the increased retention of the biphenyl phase, higher amounts of methanol can be used in the mobile phase. This noticeably increases sensitivity when using an electrospray interface.

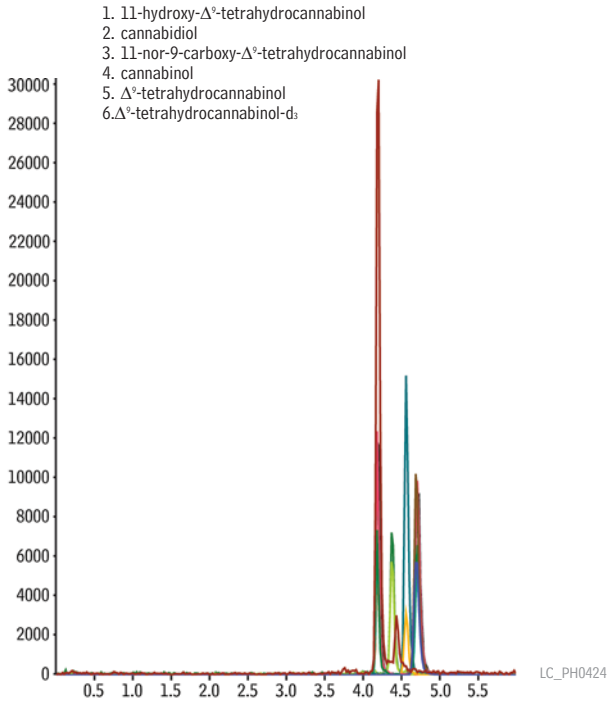
The Allure® Biphenyl column provides good resolution of all compounds in less than 5 minutes – including baseline resolution of Δ^9-THC and cannabidiol, which have very similar product ion spectra and +MRM transitions (Figure 2). By using MS/MS detection, we were able to target two

Figure 1 Final product spectra used in developing MRM transitions for compound identification and optimized sensitivity.



For conditions see Figure 2.

Figure 2 Fast, selective separation of Δ⁹-THC and its metabolites, using an Allure® Biphenyl HPLC column (extracted ion chromatography).



Sample:
Inj.: 10µL
Conc.: 100ng/mL each component
Sample diluent: mobile phase

Column: Allure® Biphenyl
Cat. #: 9166332
Dimensions: 30 x 2.1 mm
Particle size: 3µm
Pore size: 60Å

Conditions:
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in methanol

Time (min.)	%B
0.5	50
2.5	90
5.0	90
6.0	50
6.1	—

Flow: 0.5mL/min.
Temp.: ambient
Det.: Spectrum Model (Sciex API 3200 Triple Quadrupole)
Interface: ESI
Ion mode: positive
Temp.: 500°C
Ion source: 5500V

Table 1 MRM transitions for THC and metabolites: multiple transitions are monitored for each compound for definitive identifications.

Analyte	Q1 Mass	Q3 Mass	Time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
Hydroxy-THC (MRM1)	331.2	313.1	100	36	5	21	10
Hydroxy-THC (MRM2)	331.2	193.1	100	36	5	35	6
Carboxy-THC (MRM1)	345.2	327.0	100	41	4.5	21	10
Carboxy-THC (MRM2)	345.2	299.3	100	41	4.5	25	6
Cannabidiol (MRM1)*	315.2	193.2	100	36	4.5	31	6
Cannabidiol (MRM2)*	315.2	123.2	100	36	4.5	43	6
Cannabinol (MRM1)	311.2	223.0	100	46	8.5	27	8
Cannabinol (MRM2)	311.2	222.5	100	46	8.5	37	10
Δ⁹-THC (MRM1)*	315.2	193.2	100	41	4.5	33	6
Δ⁹-THC (MRM2)*	315.2	123.1	100	41	4.5	43	6
Δ⁹-THC-d3 (MRM1)	318.3	196.3	100	36	4.5	31	6
Δ⁹-THC-d3 (MRM2)	318.3	123.2	100	36	4.5	43	6

*Note, cannabidiol and Δ⁹-THC share the same transitions, but are separated chromatographically.
DP – declustering potential, EP – entrance potential, CE – collision energy, CXP – collision cell exit potential

+MRM transitions per compound to verify compound identity at approximately 1ng on-column. Table 1 shows the +MRM transitions and the source conditions for approximately 1ng each of several cannabinoid metabolites.

Based on this work, we conclude an Allure® Biphenyl column, coupled with an API MS/MS 3200 detector and a Shimadzu LC20AD Prominence, can be used to quantify low levels of cannabinoid analytes from underivatized sample, and can achieve baseline separation of Δ⁹-THC and cannabidiol, in less than 5 minutes.

References:
1 Abbara, C., R. Galy, A. Benyamina, M. Reynaud and L. Bonhomme-Faivre, *Development and validation of a method for quantitation of Δ⁹-tetrahydrocanabinol in human plasma by high performance liquid chromatography after solid phase extraction* J. Pharma. Biomed. Anal. 41 (2006) 1011-1016.
2 Sellers, K. *Reliably Confirm Cannabinoids by GC/MS* Restek Advantage 2006.04 (2006) 16-17.
3 Weinmann, W., S. Vogt, R. Goerke, C. Muller and A. Bromberger, *Simultaneous determination of THC-COOH and THC-COOH-glucuronide in urine samples by LS/MS/MS* Forens. Sci. Intl. 113 (2000) 381-387.

Reference 2 available from Restek – request lit. cat.# 580120.

Allure® Biphenyl Columns (USP L11)

3µm Column, 2.1mm	cat. #
30mm	9166332
50mm	9166352
100mm	9166312
30mm (with Trident™ Inlet Fitting)	9166332-700
50mm (with Trident™ Inlet Fitting)	9166352-700
100mm (with Trident™ Inlet Fitting)	9166312-700

ordering note

For guard cartridges for these columns, visit our website at **www.restek.com**.

Exempted Drug of Abuse Reference Materials

1,000µg/mL in P&T methanol, 1mL/ampul

Compound	CAS#	Individual cat.#
Cannabinoid & Metabolites		
cannabidiol	13956-24-1	34011
cannabinol	521-35-7	34010
Δ⁹-THC	1972-08-3	34067
±11-nor-9-carboxy-Δ⁹-THC	104874-50-2	34068

No datapacks available.

thank you

Instrument provided courtesy of Applied Biosystems

www.appliedbiosystems.com

Sampling Volatile Organic Compounds in Air

Restek Sampling Equipment Helps Assure Accurate Data

By Irene DeGraff, Air Monitoring Product Marketing Manager

One of the most widely used methods for ambient air monitoring, USEPA TO-15, specifies sample collection with a specially prepared stainless steel canister, followed by GC/MS analysis. Restek can support all facets of this or other air monitoring programs – from state-of-the-art sampling equipment to high quality analytical reference standards.

An inert canister surface is critical to obtaining accurate sample results. Restek offers a complete line of TO-Cans™ (Summa® equivalent canisters) which are electropolished and extensively cleaned prior to shipping to ensure a high-quality passivated surface for improved analyte stability. No weld marks on the spheres further reduce the occurrence of active sites. For reactive compounds, such as sulfur-containing components, a SilcoCan™ is your best canister choice. SilcoCan™ canisters are deactivated with Siltek® surface treatment ensuring exceptional inertness and maximum sample stability, even for low level sulfur compounds.

Optional gauge
- Quickly confirm vacuum or pressure inside canister.
- Monitor pressure changes.
- Fully protected by canister frame.
- Can be heated to 90°C during cleaning.

High-quality vacuum gauge

2 or 3 Port high quality valve
Metal-to-metal seal, 2/3 turn with stainless steel diaphragm.

All configurations also available in 1L, 3L, and 15L volumes: please see our website.

free literature

A Guide to Passive Air Sampling
request lit. cat. # 59977B

TO-Can™ Air Monitoring Canisters

Optimized for US EPA Methods TO-14 and TO-15, and ASTM D5466

Description	qty.	cat.#
6L Volume		
TO-Can™ Canister, 1/4" Valve	ea.	24174
TO-Can™ Canister with Gauge, 1/4" Valve	ea.	24178
TO-Can™ Canister with No Valve	ea.	22096

SilcoCan™ Air Monitoring Canisters

Ideal for low-level reactive sulfur (1-20ppb), TO-14, or TO-15 compounds

Description	qty.	cat.#
6L Volume		
SilcoCan™ Canister, 1/4" Valve	ea.	24182
SilcoCan™ Canister, Siltek® Treated 1/4" Valve	ea.	24182-650
SilcoCan™ Canister with Gauge, 1/4" Valve	ea.	24142
SilcoCan™ Canister with Gauge, Siltek® Treated 1/4" Valve	ea.	24142-650
SilcoCan™ Canister with No Valve	ea.	22092
Replacement 1/4" Valves for Air Monitoring Canisters		
1/4" Replacement Valve (2-port)	ea.	24145
1/4" Siltek® Replacement Valve (2-port)	ea.	24144
1/4" Replacement Valve (3-port)	ea.	24147
1/4" Siltek® Replacement Valve (3-port)	ea.	24146

Restek canisters are originally equipped with high-quality Parker Hannifin diaphragm valves. Each valve is helium leak-tested to 4 x 10^{-9}cc/sec. The all-stainless steel construction eliminates contamination and withstands temperatures from -100°C to 250°C. Compression outlet fitting, indicator plate to display open or closed position, 1/4" inlet and outlet.

Rxi™-1ms Column (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.00	-60 to 330/350°C	60-Meter	13357

TO-15 62 Component Mix (62 components)

Cylinder Construction:	aluminum
Cylinder Size:	8 x 24 cm.
Volume/Pressure:	104 liters of gas @ 1800psig
Cylinder Fitting:	CGA-180 outlet
Weight:	1.5 lbs./0.7 kg

acetone
benzene
benzyl chloride*
bromodichloromethane
bromoform
bromomethane
1,3-butadiene
2-butanone (MEK)
carbon disulfide*
carbon tetrachloride
chlorobenzene
chloroethane
chloroform
chloromethane
cyclohexane
dibromochloromethane
1,2-dichlorobenzene
1,3-dichlorobenzene
1,4-dichlorobenzene
1,1-dichloroethane
1,2-dichloroethane
1,1-dichloroethene
cis-1,2-dichloroethene
trans-1,2-dichloroethene
1,2-dichloropropane
cis-1,3-dichloropropene
trans-1,3-dichloropropene
1,4-dioxane
ethanol*
ethyl acetate
ethyl benzene
ethylene dibromide (1,2-dibromoethane)
4-ethyltoluene
trichlorofluoromethane (Freon® 11)
dichlorodifluoromethane (Freon® 12)
1,1,2-trichloro-1,2,2-trifluoroethane (Freon® 113)
1,2-dichlorotetrafluoroethane (Freon® 114)
heptane
hexachloro-1,3-butadiene
hexane
2-hexanone (MBK)
4-methyl-2-pentanone (MIBK)
methylene chloride
methyl *tert*-butyl ether (MTBE)
2-propanol
propylene
styrene
1,1,2,2-tetrachloroethane
tetrachloroethene
tetrahydrofuran
toluene
1,2,4-trichlorobenzene
1,1,1-trichloroethane
1,1,2-trichloroethane
trichloroethene
1,2,4-trimethylbenzene
1,3,5-trimethylbenzene
vinyl acetate
vinyl chloride
m-xylene
o-xylene
p-xylene

In nitrogen, 104 liters @ 1800psig

1ppm cat. # 34436 (ea.)

100ppb cat. # 34437 (ea.)

*Stability of this compound cannot be guaranteed.

TO-14A Internal Standard/Tuning Mix

Cylinder Construction:	aluminum
Cylinder Size:	8 x 24 cm.
Volume/Pressure:	104 liters of gas @ 1800psig
Cylinder Fitting:	CGA-180 outlet
Weight:	1.5 lbs./0.7 kg

bromochloromethane
1-bromo-4-fluorobenzene (4-bromofluorobenzene)
chlorobenzene-d5
1,4-difluorobenzene

In nitrogen, 104 liters @ 1800psig

1ppm cat. # 34408 (ea.)

Additional TO-14 and TO-15 Analytical Reference Materials are also available. Please see our catalog or website.

2007.01

Increase Accuracy & Efficiency

Air Canister Heating Jacket

Our heating jacket can help you prepare your canisters for sampling faster and more efficiently. The jacket's novel design ensures complete cleaning by heating the canister and valve together. When used during the analysis, it prevents condensation, ensuring more accurate results. Two temperature settings, 75ºC and 150ºC. Fits all canisters up to 6L in size.

Description	qty.	cat.#
Air Canister Heating Jacket	ea.	24123

*Not CE certified.

The ultimate in controlled heating, for reliably cleaning your air canisters!

Passive Air Sampling Kits

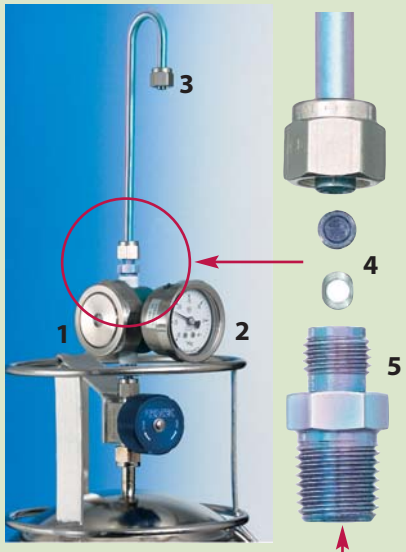
Our easy-to-assemble passive sampling kits include all hardware required for field sampling (except the canister). Our kits were designed to reduce the number of potential leak sites and are available in seven flow ranges, and in stainless steel or with Siltek® surface treatment. Individual parts also are available.

1. Veriflo™ SC423XL flow controller
This flow controller is a high-quality device designed to maintain a constant mass flow as the pressure changes from 30" Hg to 5" Hg (we recommend you stop sampling at or before 5" Hg of vacuum). All wetted parts of the flow controller can be Siltek® treated.

2. Stainless steel vacuum gauge
Fitted to the flow controller, the gauge monitors canister vacuum change during sampling.

3. ¼-inch Siltek® sample inlet
The 0.3m x ¼-inch tubing includes a stainless steel nut on the inlet end, to prevent water droplets from accumulating at the edge of the tubing, where they could be pulled into the sampling train.

4. 2-micron frit filter and washer
Located prior to the critical orifice to prevent airborne particles from clogging the critical orifice. Replaceable. Available in stainless steel, or Siltek® treated for optimum inertness.



All fitting connections are ¼" tube, except where noted.

¼" NPT

5. Interchangeable critical orifice
An interchangeable ruby critical orifice allows you to control the flow with very high precision. To select the correct critical orifice for your sample, see the table below. Available in stainless steel, or Siltek® treated for optimum inertness.

Sampling Time 6 Liter	Flow (sccm)	Orifice size	Siltek® Treated Sampling Kits*	Stainless Steel Sampling Kits*
125 hour	0.5–2	0.0008"	24217	24216
24 hour	2–4	0.0012"	24160	24165
12 hour	4–8	0.0016"	24161	24166
8 hour	8–20	0.0020"	24162	24167
3 hour	20–40	0.0030"	24163	24168
1.5 hour	40–80	0.0060"	24164	24169
0.5 hour	80–350	0.0090"	22101	22100

*Air sampling canisters sold separately. Available in 400cc, 1L, 3L, 6L, and 15L volumes.

See our catalog or website for other canister volumes and sampling times.

Faster Extraction and Cleanup of Pesticide Residue Samples

With QuEChERS Products

By Lydia Nolan, Innovations Chemist

cat. # 26123

cat. # 26124

cat. # 26125

cat. # 26126

- Fast, simple sample cleanup.
- Variety of formats, to meet all needs.
- Custom products prepared on request.

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe, the QuEChERS ("catchers") method is based on work done and published by the US Department of Agriculture Eastern Regional Research Center in Wyndmoor, PA.[1] Researchers there were looking for a simple, effective, and inexpensive way to extract and clean pesticide residues from the many varied sample matrices with which they routinely worked. They had been using the Modified Luke Extraction Method, which is highly effective and rugged, but is both labor and glassware intensive, leading to a relatively high cost per sample. Solid phase extraction also had been effective, but the complex matrices the investigators were dealing with required multiple individual cartridges and packings to remove the many classes of interferences, adding costs and complexity to the process. A new method would have to remove sugars, lipids, organic acids, sterols, proteins, pigments and excess water, any of which often are present, but still be easy to use and inexpensive.

The researchers developed a simple two-step procedure. First, the homogenized samples are extracted and partitioned, using an organic solvent and salt solution. Then, the supernatant is further extracted and cleaned, using a dispersive SPE technique. Multiple adsorbents are placed in a centrifuge tube, along with the 1mL of organic solvent and the extracted residues partitioned from step 1. The contents are thoroughly mixed, then centrifuged, producing a clean extract ready for a variety of GC or HPLC analytical techniques.[2] Validation and proficiency data for the QuEChERS method are available for a wide variety of pesticides in several common food matrices at **www.quechers.com**

Using the dispersive SPE approach, the quantity and type of adsorbents, as well as the pH and polarity of the solvent, can be easily adjusted for differing matrix interferences and "difficult" analytes. Results from this approach have been verified and modified at several USDA and Food and Drug Administration labs, and the method now is widely accepted for many types of pesticide residue samples.

Commercially available products make this approach even simpler. We offer QuEChERS extraction products in a variety of standard sizes and formats. The centrifuge tube format, available in 2mL and 15mL sizes, contains magnesium sulfate (to partition water from organic solvent) and PSA* adsorbent (to remove sugars and fatty acids), with or without graphitized carbon (to remove pigments and sterols) or C18 packing (to remove nonpolar interferences). The PSA and graphitized carbon packings also are available in a 6mL packed bed SPE cartridge, with Teflon® frits, for whenever a standard SPE format is preferred. Custom products are available by quote request. If you are frustrated by the time and cost involved with your current approach to pesticide sample cleanup, we suggest you try this simple and economical new method.

*PSA – primary and secondary amine exchange material

QuEChERS SPE Cartridges

SPE Cartridge	qty.	cat#
QuEChERS SPE 2mL Micro-Centrifuge Cartridge Packed with 150mg Magnesium Sulfate and 50mg PSA	100-pk.	26124
QuEChERS SPE 2mL Micro-Centrifuge Cartridge Packed with 150mg Magnesium Sulfate, 50mg PSA, and 50mg Graphitized Carbon	100-pk.	26123
QuEChERS SPE 2mL Micro-Centrifuge Cartridge Packed with 150mg Magnesium Sulfate, 50mg PSA, and 50mg C18	100-pk.	26125
QuEChERS SPE 15mL Centrifuge Cartridge Packed with 900mg Magnesium Sulfate, 300mg PSA, and 150mg Graphitized Carbon	50-pk.	26126
QuEChERS SPE 6mL SPE Cartridge Packed with 200mg Graphitized Carbon and 400mg PSA, Teflon® Frits	30-pk.	26127
QuEChERS SPE 6mL SPE Cartridge Packed with 250mg Graphitized Carbon and 500mg PSA, Teflon® Frits	30-pk.	26128
QuEChERS SPE 6mL SPE Cartridge Packed with 500mg Graphitized Carbon and 500mg PSA, Teflon® Frits	30-pk.	26129

References

1. Anastassiades, M., S.J. Lehotay, D. Stajnbaher, F.J. Schenck, *Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce*, J AOAC International, 2003, vol 86 no 22, pp 412-431.
2. Schenck, F.J., *SPE Cleanup and the Analysis of PPB Levels of Pesticides in Fruits and Vegetables*. Florida Pesticide Residue Workshop, 2002.

References not available from Restek.

Resprep™ Cell Parts and Tools for ASE® Extraction Units

Enhanced Design For Faster Installation and Easier Cleaning

By Irene DeGraff, Sample Preparation Product Marketing Manager

Resprep™ Extraction Cell Parts for ASE® 200 Systems, Restek Enhanced Design

- Choose original equipment-equivalent stainless steel, or Siltek® deactivation for improved inertness.
- Inner surfaces polished, for easier cleaning.
- Caps include frit, washer, PTFE O-ring, and threaded insert.

	Stainless Steel		Siltek® Treated	
Description	**qty.**	**cat.#**	**qty.**	**cat.#**
Extraction Cell Kit, Resprep™ for ASE® 200, 1mL	kit	25980	kit	25981
Extraction Cell Kit, Resprep™ for ASE® 200, 5mL	kit	25982	kit	25983
Extraction Cell Kit, Resprep™ for ASE® 200, 11mL	kit	25984	kit	25985
Extraction Cell Kit, Resprep™ for ASE® 200, 33mL	kit	25986	kit	25987
Extraction Cell Body, Resprep™ for ASE® 200, 1mL	ea.	25960	ea.	25961
Extraction Cell Body, Resprep™ for ASE® 200, 5mL	ea.	25962	ea.	25963
Extraction Cell Body, Resprep™ for ASE® 200, 11mL	ea.	25964	ea.	25965
Extraction Cell Body, Resprep™ for ASE® 200, 33mL	ea.	25966	ea.	25967
Extraction Cell Caps, Resprep™ for ASE® 200	2-pk.	25968	2-pk.	25969
PEEK® Seal/Frit Assembly, Resprep™ for ASE® 200	2-pk.	25970	2-pk.	25971
Frit, Resprep™ for ASE® 200	12-pk.	25972	12-pk.	25973

Description	**qty.**	**cat.#**
PEEK® Seal, Resprep™ for ASE® 200	12-pk.	25974
PEEK® Seal, Resprep™ for ASE® 200	48-pk.	25975
PTFE O-Rings for ASE® 200 & ASE® 300 Caps	100-pk.	26187
Viton® O-Rings for ASE® 200 & ASE® 300 Caps	50-pk.	26188

- Simpler design with fewer parts.
- Faster installation.
- Easier cleaning.

20mm Filters for ASE® 200 Extraction Cells

- Cellulose or glass fiber construction.
- Cellulose filters available in economical 1000-packs.

Description	**Similar to Dionex part #**	**qty.**	**cat.#**
Cellulose Filters for ASE® 200	049458	100-pk.	26118
Cellulose Filters for ASE® 200	049458	1000-pk.	26190
Glass Fiber Filters for ASE® 200	047017	100-pk.	26119

Resprep™ Tools for ASE® Systems

- Specialized tools that simplify routine chores.
- Use with ASE® 100, ASE® 200, or ASE® 300 systems.

New 2-in-1 Filter/O-Ring Insertion Tool Kit for ASE® 100/200/300

Inserting a filter, using the Filter Insertion Attachments and the Resprep™ Tool Handle.

Screw the appropriate attachment onto the end of the tool.

Place a filter at the top of the extraction cell.

Push the filter to the bottom of the extraction cell.

Inserting an O-ring, using the Resprep™ Tool Handle.

Place the O-ring over the tip of the tool.

Insert the tool into the center hole of the extraction cell cap.

Press the tool firmly until the O-ring snaps into place.

Resprep™ Tool Handle

Filter Insertion Attachments

Description	**qty.**	**cat.#**
2-in-1 Filter/O-Ring Insertion Tool Kit for ASE® 100/200/300 (includes Resprep™ Tool Handle and Filter Insertion Attachments)	kit	26181
Resprep™ Tool Handle for ASE® 100/200/300	ea.	26182
Filter Insertion Attachments for ASE® 100/200/300 (1mL, 5mL, 11mL, 33mL)	4-piece set	26183

Extend Process Component Lifetime and Enhance Durability

Restek Surface Treatments Improve Sampling and Transfer Component Performance

by Marty Higgins and Carrie Sprout, Restek Performance Coatings Division

- Economical—lower cost than specialty alloys, more durable than traditional stainless steels.
- Versatile—suitable in a variety of environments and temperature ranges.
- Simple—can be applied to existing equipment; stock tubing and fittings also available.

When surface activity or corrosion are a concern, solutions must be engineered. The Restek Performance Coatings group offers a family of surface treatments that address activity and corrosion concerns over a wide spectrum of applications. Table 1 lists applications in which a Restek Performance Coating treatment of sample pathway components prevents adsorption of active compounds, thereby contributing toward reliable and accurate information, or greatly reduces corrosion.

Adsorption problems in sample pathways often can be traced to the tubing and fittings used to transfer the sample to the analytical instrument. Always use deactivated tubing and fittings for applications involving active compounds. For special requirements, ensure maximum inertness and minimal surface area by applying the deactivating treatment to electropolished tubing. Figure 1 shows uptake and release curves for 500ppbv of methyl mercaptan, an active sulfur compound, in a gas stream passing through a variety of tubing substrates.[1] Siltek®/Sulfinert® treated tubing reduces uptake by orders of magnitude, relative to untreated stainless steel tubing.

In corrosive environments, Silcosteel®-CR treated tubing is an excellent alternative to expensive alloys. Silcosteel®-CR treatment extends the lifetime of the tubing, reducing the frequency of preventive maintenance and helping to ensure the purity of the process or sample stream.†

Silcosteel®-CR improves corrosion resistance by up to 10X over untreated 316 stainless steel (per ASTM G48 Method B, see graph below).

Figure 1 Sulfinert® treated electropolished seamless stainless steel tubing (red) does not adsorb methyl mercaptan (500ppbv).
Blue-untreated electropolished tubing; violet-raw tubing.

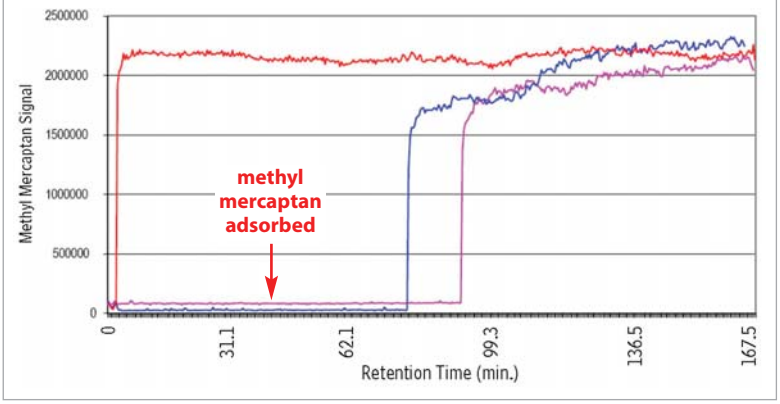


Table I Applications in which Restek treated sample pathway components minimize corrosion** or prevent adsorption of active compounds*.

Sulfur compounds in:*
- automotive exhaust
- beverage grade CO_2
- diesel fuels
- environmental samples
- ethylene
- gasoline
- liquefied petroleum gas
- natural gas (odorants)
- propylene
- stack gas emissions
- wines and beers

Nitric oxide (NOx) compounds in:*
- automotive exhaust
- stack gas emissions

Mercury compounds in:*
- crude oil
- environmental samples
- exhaust
- stack gas emissions from coal fired electric power plants

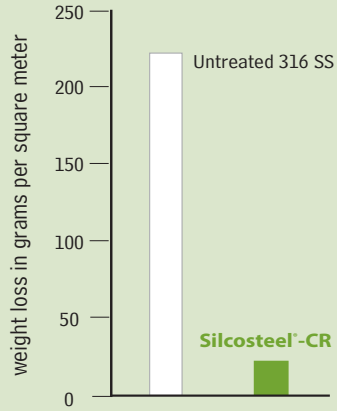
Corrosive environments:**
- hydrochloric acid
- hydrogen peroxide
- seawater

Moisture hold-up in high purity sampling lines**
- sample systems
- gas delivery systems
- process systems

*Siltek®/Sulfinert® treatment.
**Silcosteel®-CR treatment.

†Note that with any corrosive stream, regular inspections are needed to confirm there are no leaks or breakthroughs.

Silcosteel®-CR treated stainless steel outperforms uncoated metal by an order of magnitude!
(ASTM G 48, Method B).



Siltek®/Sulfinert® Treated and Silcosteel®-CR Treated Swagelok® Fittings

- Wide selection of treated 1/16", 1/8", 1/4", and 3/8" fittings.
- Siltek®/Sulfinert® treatment ensures ultimate inertness.
- Silcosteel®-CR treatment enhances corrosion resistance by 10X, or more.
- Custom treatment available for any Swagelok® fittings, or other system parts.

Fitting Type	Size	Siltek®/Sulfinert® treated cat.#	Silcosteel®-CR Treated cat.#
Union	1/16"	22540	22575
	1/8"	22541	22576
	1/4"	22542	22577
	3/8"	22909	22904
Tee	1/16"	22543	22578
	1/8"	22544	22579
	1/4"	22545	22580
	3/8"	22910	22905
Reducing Union	1/8" to 1/16"	22546	22581
	1/4" to 1/16"	22547	22582
	1/4" to 1/8"	22548	22583
	3/8" to 1/4"	22911	22906
Elbow	1/8"	22549	22584
	1/4"	22550	22585
Plug	1/16"	22572	22619
	1/8"	22573	22620
	1/4"	22574	22597
Cross	1/8"	22551	22586
	1/4"	22552	22587
Tube End Reducer	1/8" tube to 1/16"	22553	22588
	1/4" tube to 1/16"	22554	22589
	1/8" tube to 1/4"	22555	22590
	1/4" tube to 1/8"	22556	22591
Port Connector	1/8"	22557	22592
	1/4"	22558	22593
	1/8" tube to 1/4"	22559	22594
Male Connector	1/8" to 1/8" NPT	22561	22595
	1/4" to 1/4" NPT	22562	22596
	1/16" to 1/8" NPT	22563	22610
	1/8" to 1/4" NPT	22564	22611
	1/4" to 1/8" NPT	22565	22612
	3/8" to 3/8" NPT	22912	22907
	3/8" to 1/4" NPT	22913	22908
Female Connector	1/8" to 1/8" NPT	22566	22613
	1/4" to 1/4" NPT	22567	22614
	1/4" to 1/8" NPT	22568	22615
	1/8" to 1/4" NPT	22569	22616
Bulkhead Union	1/8"	22570	22617
	1/4"	22571	22618

Silcosteel®-CR Treated Coiled Stainless Steel Tubing

Electropolished 316L Grade, Coiled

ID	OD	cat.#	5-24 ft.	25-99 ft.	100-299 ft.	>300 ft.
0.085" (2.16mm)	1/8" (3.18mm)*	22536				
0.180" (4.57mm)	1/4" (6.35mm)**	22537				
316L Grade, Coiled						
0.055" (1.40mm)	1/8" (3.18mm)**	22896				
0.180" (4.57mm)	1/4" (6.35mm)**	22897				
0.277" (7.04mm)	3/8" (9.52mm)***	22915				

Straight Seamless 316L Grade, 6 foot Length

ID	OD	qty.	cat.#
0.055" (1.40mm)	1/8" (3.18mm)**	ea.	22898
0.180" (4.57mm)	1/4" (6.35mm)**	ea.	22899
0.277" (7.04mm)	3/8" (9.52mm)***	ea.	22900

*0.020" wall thickness **0.035" wall thickness ***0.049" wall thickness

Summary

Surface treatments from the Restek Performance Coatings group prevent corrosion or adsorption of active compounds in delivery systems, and always should be considered in applications in which corrosive or active streams are to be sampled, transferred, or analyzed.

References

1 *Relative Response Time of True Tube™ when Measuring Moisture Content in a Sample Stream* Test Report, Haritec Scientific & Engineering Support, Calgary, Alberta, Canada, May 2004. Reference courtesy of O'Brien Canada, available on request from Restek.

Economical solutions for varied sample stream challenges

Restek surface treatments are:

Silcosteel®—A general-purpose passivation layer for steel and stainless steel. U.S. patent 6,511,760.

Silcosteel®-AC—Dramatically reduces carbon buildup on stainless steel components. U.S. patent 6,444,326.

Silcosteel®-CR—A corrosion resistant layer that increases the lifetime of system components in acidic environments containing hydrochloric acid, nitric acid, or seawater. U.S. patent 7,070,833.

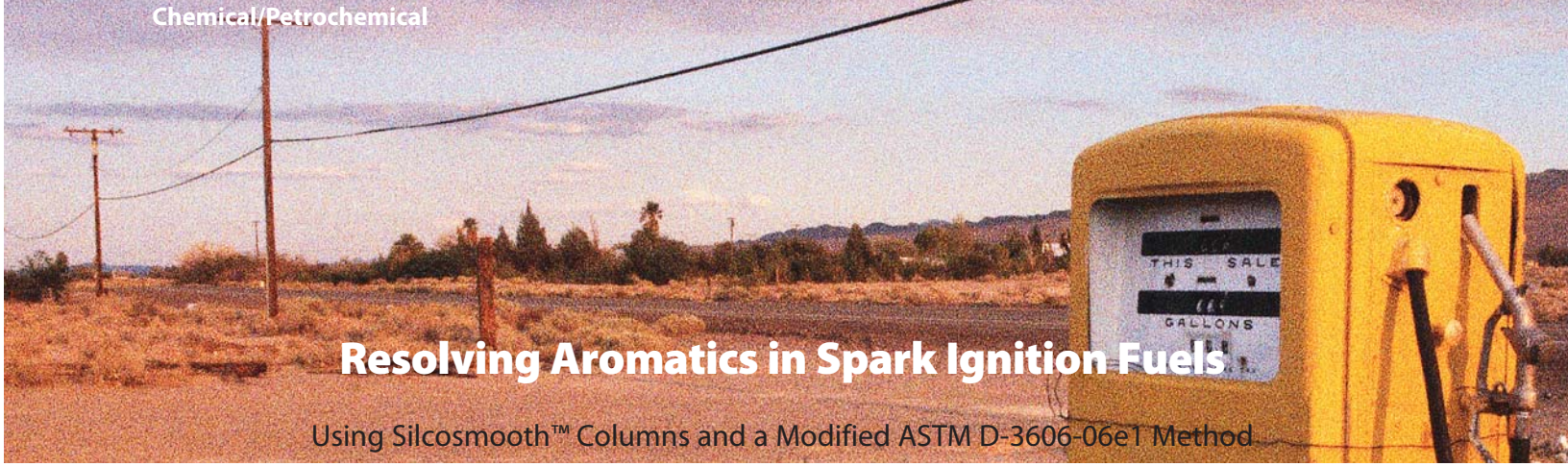
Silcosteel®-UHV—Greatly reduces outgassing from components of ultra-high vacuum systems. U.S. patent 7,070,833.

Siltek®—The ultimate passivation for treated components, from glass to high nickel alloys of steel. U.S. patent 6,444,326.

Sulfinert®—A required treatment for metal components when analyzing for parts-per-billion levels of organo-sulfur compounds. U.S. patent 6,444,326.

for **more** info

For more information about Restek performance coatings, request lit. cat.# 59493, or visit us online at **www.restekcoatings.com**.



Resolving Aromatics in Spark Ignition Fuels

Using Silcosmooth™ Columns and a Modified ASTM D-3606-06e1 Method

By Barry L. Burger, Petroleum Chemist

- Easy quantification of aromatics, using 2-butanol as an internal standard.
- Complete resolution of benzene from ethanol.
- Fully conditioned column set – ready to use out of the box.

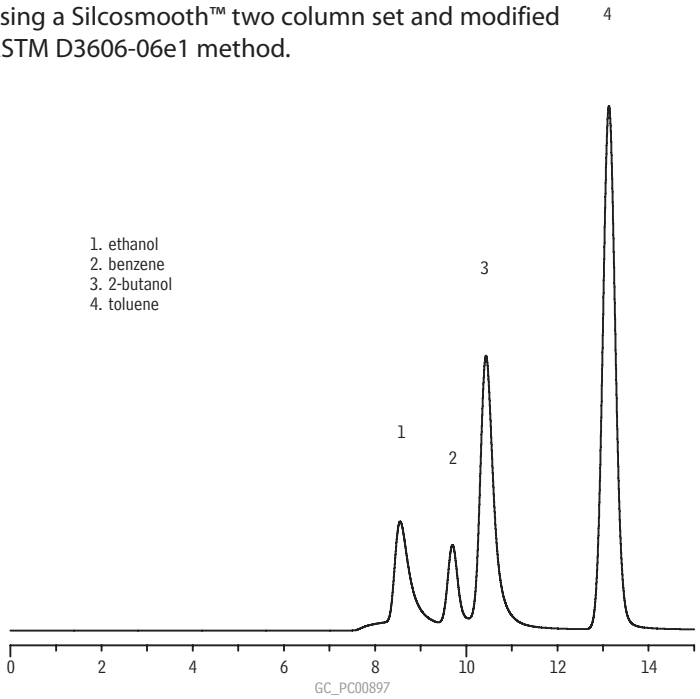
Laboratories analyzing benzene and toluene in spark ignition fuels reformulated to contain ethanol must use a modified ASTM D-3606-06e1 method to prevent the co-elution of ethanol and benzene. This method modification also is a requirement of the US EPA. The benzene range of determination is between 0.1 and 5 volume percent, and the toluene range is between 2 and 20 volume percent.

Our robust two column set for this modified D-3606-06e1 application completely resolves benzene from ethanol. Column A is a 2.46m x 1/8" OD x 2mm ID Silcosmooth™ (Silcosteel® treated) stainless steel column packed with 10% Rtx®-1 on 80/100 Silcoport™ W, which separates the components by boiling point. After the elution of n-octane (C8) from Column A, the column is backflushed to prevent the heavier compounds from entering Column B, the main analytical column. Column B is a unidirectional 6.15m x 1/8" OD x 2mm ID Silcosmooth™ stainless steel column packed with separate beds of 15% Carbowax® 1540 on 80/100 Chromosorb® WAW and 20% TCEP on 80/100 Chromosorb® PAW. To demonstrate the performance of the column set, we installed it in an Agilent 6890 GC equipped with a flame ionization detector (FID). Helium was used as the carrier gas at a flow rate of 25mL/min. in the constant flow mode. Figure 1 shows the aromatic compounds are fully resolved, and can easily be quantified, using 2-butanol as an internal standard.

This column set is fully conditioned, and is ready to use right out of the box. Only a brief (10 min.) carrier gas purge at ambient temperature, followed by a 30 min. hold at 165°C, is required.

If your laboratory has been struggling with ASTM method D-3606-06e1 for reformulated fuels containing ethanol, Restek's new column set is the solution.

Figure 1 Complete resolution of benzene from ethanol, using a Silcosmooth™ two column set and modified ASTM D3606-06e1 method.



Column:	Column A: 10% Rtx® 1 on 80/100 Silcoport™ W, 2.46m x 1/8" OD x 2mm ID Silcosteel® treated stainless steel Column B: 20% TCEP on 80/100 Chromosorb® PAW / 15% Carbowax® 1540 on 80/100 Chromosorb® WAW, 6.15m x 1/8" OD x 2mm ID Silcosteel® treated stainless steel
Sample:	ethanol (10%), benzene (1.5%) toluene, (10%), and 2-butanol (10%) in carbon disulfide
Inj.:	1.0µL on-column
Inj. temp.:	200°C
Carrier gas:	helium, constant flow
Flow rate:	25.0mL/min.
Oven temp.:	135°C
Det.:	FID @ 200°C

D3606 Application Columns (2 column set)

OD	ID	9.2-Meter cat. #*
1/8" Silcosmooth™	2.0mm	80487

*Please add column configuration suffix number from our catalog to cat.# when ordering–see our catalog or website.

Separate Argon from Oxygen Above Ambient Temperatures

Using an Rt-Msieve™ 5A PLOT Column

By Gary Stidsen, GC Columns Product Marketing Manager, and Barry L. Burger, Petroleum Chemist

- Fast, efficient separations at above ambient temperatures.
- High permeability and narrow column diameter mean sharper peaks.
- 100% bonding process eliminates the need for particle traps.

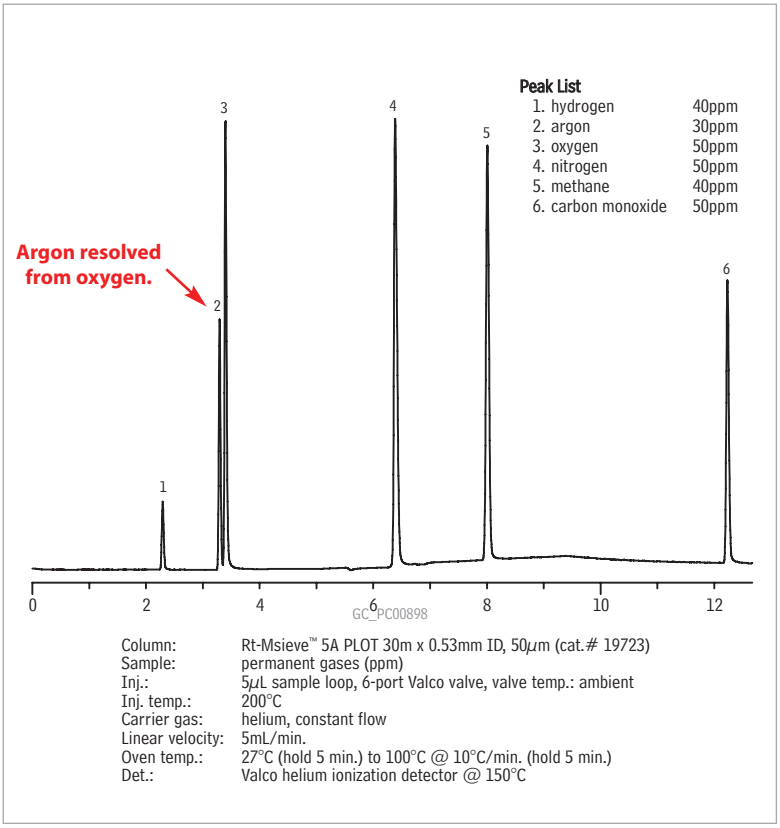
Porous layer open tubular columns—PLOT columns—offer significant advantages over packed gas-solid chromatography (GSC) columns. The open tubular design gives PLOT columns greater permeability, and their narrow diameter ensures sharper peaks. The open construction affords a smaller pressure drop per unit length, so longer columns can be used. This means much higher column efficiency and, therefore, superior resolution. In brief, PLOT columns provide faster and more sensitive analyses than packed GSC columns.

Restek PLOT columns are especially effective for separating mixtures of gaseous analytes. Rt-Msieve™ 5A PLOT columns contain molecular sieve 5A particles that are bonded to prevent particle dislocation, thus protecting valves and detection systems from damage. They are designed for fast, efficient separation of argon and oxygen, hydrogen and helium, and other permanent gases, including permanent gases admixed in refinery or natural gas. Finely controlled pore size allows selective adsorption of specific target compounds, ensuring that difficult separations can be made without subambient temperatures.

Figure 1 shows a 30m x 0.53mm ID Rt-Msieve™ 5A PLOT column can separate oxygen from argon to baseline, at above ambient temperature, in approximately 4 min. Also, the permanent gases are resolved from methane in the same analysis. Carbon dioxide does not elute from a molecular sieve 5A column, but can be chromatographed on an Rt-QPLOT™ porous polymer column. For more information, and additional example analyses on Restek PLOT columns, refer to our current chromatography products catalog or our website.

If your analyses call for difficult separations of gaseous analytes, and neither conventional packed GC columns nor WCOT capillary columns are providing the separations you want, or if your analyses depend on costly or time-consuming conditions, a Restek PLOT column may be your solution.

Figure 1 Excellent resolution at above ambient temperatures on an Rt-Msieve™ 5A-PLOT column.



Rt-Msieve™ 5A Columns (fused silica PLOT)

ID	df (µm)	temp. limits	length	cat. #
0.53mm	50	to 300°C	30-Meter	19723
0.32mm	30	to 300°C	15-Meter	19720
0.32mm	30	to 300°C	30-Meter	19722
0.53mm	50	to 300°C	15-Meter	19721

Plot Column **Advantages**

Gas-liquid chromatography (GLC), the most common mode of gas chromatography, has limited application in analyses of gases. Subambient temperatures often are required to achieve a separation, and cryogenic cooling systems are costly and inconvenient.

Gas-solid chromatography (GSC), in which gaseous analytes are adsorbed onto the packing particles, rather than into a surface coating, is far more effective for separating gases. Difficult-to-separate small molecules, such as argon and oxygen, ethane isomers, and many others, can be separated by GSC at above ambient temperatures.

When analyzing gases, PLOT columns offer significant advantages over both GLC and GSC packed columns, including:

- Excellent separations at above ambient temperature; no costly cooling systems required.
- Sharper peaks, due to smaller tubing internal diameters.
- Higher efficiency and greater sensitivity.

Biodiesel Analysis by European Methodology

Exceptional Peak Symmetry, Using an Rtx®-Biodiesel GC Column

By Barry L. Burger, Petroleum Chemist

- Excellent peak shape, even for free glycerin.
- Low column bleed at >350°C.
- Quantify oil components more easily and more reliably.

In less than a decade biodiesel oil has become a significant fuel source, especially in European countries, where current usage has soared to 1,800,000 tons annually.[1] Transesterification of the rapeseed oil or other fats from which biodiesel oil is prepared yields two products: methyl esters – biodiesel oil – and glycerin. Glycerin is extremely challenging to analyze by GC, but because excessive amounts in biodiesel products can cause problems during storage or in the engine it is necessary to monitor glycerin levels. In the US, American Society for Testing and Materials (ASTM) Method D6584-00e1 is an accepted GC procedure for biodiesel oil analysis; the standard European method is Deutsches Institut fur Normung (DIN) EN14105. Both methods set limits on free glycerin and glycerides in biodiesel oil product. While these methods differ in GC column specifications and chromatographic conditions, both require a column that can perform reliably at elevated temperatures, with minimal bleed.

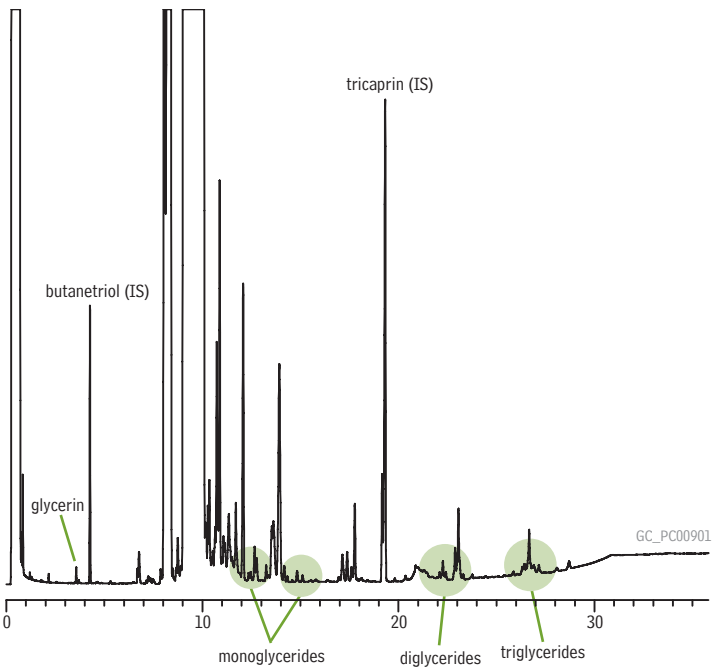
Figure 1 shows the chromatography for the DIN analysis, using an Rtx®-Biodiesel column. Peaks for glycerin and the glycerides exhibit minimal tailing, and bleed is low, even at 370°C. Thus, components of the oil can be more easily and more reliably quantified. These results confirm the Rtx®-Biodiesel column is a wise choice for biodiesel oil analysis according to DIN EN14105 conditions. The Rtx®-Biodiesel column also has proven well suited for analyzing biodiesel oil according to the ASTM method.[2]

To obtain Figure 1, we spiked a soybean oil-based sample of B100 biodiesel oil with internal standards butanetriol and tricaprin, silylated the mixture with MSTFA and, using simple on-column injection mode, injected a 1µL aliquot into a low dead volume direct injection liner in a Shimadzu 2010 GC equipped with an on-column injector (OCI). The liner has a 1mm internal diameter and a Press-Tight® constriction one-third of its length from the outlet end. The Rtx®-Biodiesel column forms a seal with the liner at the Press-Tight® constriction; the sample is injected into, and vaporizes in, the top two-thirds of the liner.

Glycerin is a notoriously difficult challenge in GC, particularly at the levels involved in biodiesel oil analysis, yet an Rtx®-Biodiesel column provides a symmetric peak that makes quantification easier and more reliable. Restek chromatographers always are happy to help you with your toughest analytical problems. If you have questions regarding biodiesel oil analysis, please call our technical service team, or contact your Restek distributor, for fast and reliable assistance.

References
1. www.ufop.de/publikationen_english.php
2. Restek Advantage 2006.04, pp 3-5 (2006).
Reference 2 available from Restek – request lit. cat.# 580120.

Figure 1 Biodiesel oil analysis using an Rtx®-Biodiesel column and DIN EN14105 conditions: peaks for glycerin and glycerides are symmetric, and bleed is low, even at 370°C.



Column:	Rtx®-Biodiesel, 10m, 0.32mm ID, 0.10µm (cat.# 10292)
Sample:	B100 biodiesel oil plus butanetriol and tricaprin, in heptane, derivatized with MSTFA
Inj.:	1µL onto Shimadzu on-column injector (OCI) equipped with low dead volume Shimadzu direct injection liner
Inj. temp.:	oven track
Carrier gas:	hydrogen, constant flow
Flow rate:	4mL/min.
Oven temp.:	50°C (hold 1 min.) to 180°C @ 15°C/min., to 230°C @ 7°C/min., to 370°C @ 10°C/min. (hold 5 min.)
Det.:	FID
Det. temp.:	390°C

Rtx®-Biodiesel Column (fused silica)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.10	330°/380°C	10-Meter	10292

did you know?

We also offer biodiesel calibration standards.
For more information visit us online at **www.restek.com**

COOL TOOLS!

Restek Innovations Save You Time and Money

MLE Capillary Tool Kits

All kits include these components:

- 1/8" nylon brush
- 3/16" nylon brush
- 1/4" nylon brush
- 1/4" stainless steel wire tube brush
- 3/8" stainless steel wire tube brush
- 3/16" stainless steel wire tube brush
- stainless steel surface brush
- 6 stainless steel jet reamers (0.25–0.65mm OD)
- 1/4" x 5/16" open end wrench
- 3/8" x 7/16" open end wrench
- rubber-tipped slide-lock tweezers
- scoring wafers with handles
- inlet liner removal tool
- septum puller
- mini wool puller/inserter tool
- 4-inch tapered needle file
- swivel head flashlight
- mini hand drill set
- 15cm compact steel ruler
- pocket magnifier
- high temperature string (1 meter)
- pipe cleaner (12-inch)
- cotton tip swabs (pk. of 25)

MLE Capillary Tool Kit for Agilent GCs (cat.# 22186) also includes:

- capillary installation gauge for Agilent GCs
- injector wrench for Agilent GCs
- septum nut removal tool
- 7/16" x 1/2" open end wrench
- 1/2" x 9/16" open end wrench

MLE Capillary Tool Kit for PerkinElmer GCs (cat.# 22185) also includes:

- 7/16" x 1/2" open end wrench
- 1/2" x 9/16" open end wrench

MLE Capillary Tool Kit for Shimadzu GCs (cat.# 22182) also includes:

- capillary installation gauge for Shimadzu GCs
- injector wrench for Shimadzu GCs
- 6mm x 7mm open end wrench
- 8mm x 10mm open end wrench
- 16mm x 17mm open end wrench

MLE Capillary Tool Kit for Thermo Scientific GCs (cat.# 22183) also includes:

- capillary installation gauge for Thermo Fisher GCs
- liner cap removing tool for Thermo Fisher GCs
- 6mm x 7mm open end wrench
- 8mm x 10mm open end wrench
- 16mm x 17mm open end wrench

MLE Capillary Tool Kit for Varian GCs (cat.# 22184) also includes:

- capillary installation gauge for Varian GCs
- 7/16" x 1/2" open end wrench
- 1/2" x 9/16" open end wrench

For Agilent GCs
(cat.# 22186)

new!

For PerkinElmer GCs
(cat.# 22185)

For Shimadzu GCs
(cat.# 22182)

For Thermo Scientific GCs
(cat.# 22183)

For Varian GCs
(cat.# 22184)

Description	qty.	cat.#
MLE Capillary Tool Kit for Agilent GCs	kit	22186
MLE Capillary Tool Kit for PerkinElmer GCs	kit	22185
MLE Capillary Tool Kit for Shimadzu GCs	kit	22182
MLE Capillary Tool Kit for Thermo Scientific GCs	kit	22183
MLE Capillary Tool Kit for Varian GCs	kit	22184

did you **know**?

Make Life Easier!

MLE Tool Kits provide the tools necessary for easier installation and maintenenace of capillary columns!

The essential tool kits for capillary chromatographers!

Super-Clean™ Click-On Traps

Click-On Inline Super-Clean™ Traps

by Donna Lidgett, GC Accessories Product Manager

- High-purity output ensures 99.9999% pure gas.
- Click-On fittings for easy, leak-tight cartridge changes; no tools required!
- Helium-Specific Triple Trap is packaged and purged under helium; ideal for GC/MS.

Using the same features and benefits as the Super-Clean™ base-plates and filters, SGT designed an inline trap. Click-On adaptor connectors allow cartridges to be exchanged without introducing oxygen. Spring-loaded check valves seal when a filter is removed and open only when a new filter has been locked in place. There is no need for loosening and tightening fittings every time a trap is changed, and your system will not become contaminated during the process.

The Triple Trap is ideal for purifying carrier gas—it contains oxygen, moisture, and hydrocarbon scrubbers in one cartridge.

The Fuel Gas Trap is ideal for purifying flame ionization detector (FID) fuel gases, removing both moisture and hydrocarbons.

The Helium-Specific Triple Trap is ideal for purifying helium in GC/MS systems. This trap is packed and purged under helium and contains oxygen, moisture, and hydrocarbon scrubbers in one cartridge.

please **note**

Super-Clean™ Gas Filters are recommended for purifying non corrosive gases with low concentrations of contaminants. The maximum concentration of oxygen in the incoming gas stream for oxygen purifiers is 0.5%.

Trap replacement depends on the quality of the incoming gas. Use the double connector and install an indicating cartridge after a trap to indicate when the trap should be replaced.

Filter Type	Gas Quality at Outlet	Maximum Pressure	Maximum Flow (L/min.)	Use For	H_2O (g)	Capacity O_2 (mL)	Hydrocarbons (g)	Estimated Lifetime (years)
Moisture cat.#22467	>99.9999	11 bar 160psi	25	Inert carrier gas, helium, air, H_2	21	NA	NA	>3
Oxygen cat.#22468	>99.9999	11 bar 160psi	25	Inert carrier gas	NA	3000	NA	>3
Hydrocarbon cat.#22466	>99.9999	11 bar 160psi	25	Inert carrier gas, helium, air, H_2	NA	NA	36[3]	>3
Fuel Gas[1] cat.#22465	>99.9999	11 bar 160psi	25	Inert carrier gas, helium, air, H_2	10	NA	18[3]	>2
Triple cat.#22464	>99.9999	11 bar 160psi	25	Inert carrier gas	6	1000	12[3]	>2

[1]Removes hydrocarbons, moisture. [2]Removes hydrocarbons, moisture, oxygen. [3]As *n*-butane.

Click-On Inline Super-Clean™ Traps and Connector Kits

Brass or stainless steel 1/4" or 1/8" fittings available.

Description	qty.	cat.#
Carrier Gas Purification Kit, 1/8" Stainless Steel Includes (2) 1/8" SS connectors and (1) oxygen/moisture/hydrocarbon triple trap	kit	22456
Carrier Gas Purification Kit, 1/8" Brass Includes (2) 1/8" brass connectors and (1) oxygen/moisture/hydrocarbon triple trap	kit	22457
Carrier Gas Purification Kit, 1/4" Stainless Steel Includes (2) 1/4" SS connectors and (1) oxygen/moisture/hydrocarbon triple trap	kit	22458
Carrier Gas Purification Kit, 1/4" Brass Includes (2) 1/4" brass connectors and (1) oxygen/moisture/hydrocarbon triple trap	kit	22459
Fuel Gas Purification Kit, 1/8" Stainless Steel Includes (4) 1/8" SS connectors and (2) hydrocarbon/moisture traps	kit	22460
Fuel Gas Purification Kit, 1/8" Brass Includes (4) 1/8" brass connectors and (2) hydrocarbon/moisture traps	kit	22461
Fuel Gas Purification Kit, 1/4" Stainless Steel Includes (4) 1/4" SS connectors and (2) hydrocarbon/moisture traps	kit	22462
Fuel Gas Purification Kit, 1/4" Brass Includes (4) 1/4" brass connectors and (2) hydrocarbon/moisture traps	kit	22463

Click-On Inline Super-Clean™ Replacement Traps

Description	qty.	cat.#
Click-On Super-Clean™ Replacement Triple Trap (removes oxygen, moisture and hydrocarbons)	ea.	22464
Click-On Super-Clean™ Replacement Fuel Gas Trap (removes moisture and hydrocarbons)	ea.	22465

Click-On Inline Super-Clean™ Ultra-High Capacity Traps

Description	qty.	cat.#
Ultra-High Capacity Hydrocarbon Trap	ea.	22466
Ultra-High Capacity Moisture Trap	ea.	22467
Ultra-High Capacity Oxygen Trap	ea.	22468

Helium-Specific Click-On Inline Super-Clean™ Trap and Connector Kits

Description	qty.	cat.#
Kits		
Helium-Specific Carrier Gas Cleaning Kit, 1/8" Stainless Steel Includes (2) 1/8" SS connectors and (1) oxygen/moisture/hydrocarbon helium-specific triple trap	kit	22469
Helium-Specific Carrier Gas Cleaning Kit, 1/8" Brass Includes (2) 1/8" brass connectors and (1) oxygen/moisture/hydrocarbon helium-specific triple trap	kit	22470
Helium-Specific Carrier Gas Cleaning Kit, 1/4" Stainless Steel Includes (2) 1/4" SS connectors and (1) oxygen/moisture/hydrocarbon helium-specific triple trap	kit	22471
Helium-Specific Carrier Gas Cleaning Kit, 1/4" Brass Includes (2) 1/4" brass connectors and (1) oxygen/moisture/hydrocarbon helium-specific triple trap	kit	22472
Replacement Trap		
Helium-Specific Replacement Triple Trap (removes oxygen, moisture and hydrocarbons)	ea.	22473

did you **know**?

Helium-Specific Click-On Inline Super-Clean™ Trap and Kits are designed specifically for purification of helium in GC/MS systems!

tech tip

Install an indicator after the Click-On inline trap so there is no confusion about when to replace the trap.

Click-On Inline Super-Clean™ Indicator

- Oxygen: Green to Grey
- Moisture: Beige to Clear

Description	qty.	cat.#
Click-On Inline Super-Clean™ Indicator (oxygen, moisture)	ea.	22474

Click-On Inline Super-Clean™ Connectors

- Click-On connectors allow you to change traps quickly, without introducing oxygen into your system.

Description	qty.	cat.#
1/8" Brass Click-On Inline Super-Clean™ Connectors	2-pk.	22475
1/8" Stainless Steel Click-On Inline Super-Clean™ Connectors	2-pk.	22476
1/4" Brass Click-On Inline Super-Clean™ Connectors	2-pk.	22477
1/4" Stainless Steel Click-On Inline Super-Clean™ Connectors	2-pk.	22478

Click-On Inline Super-Clean™ Double Connector

- Connects any Click-On trap to a Click-On indicator.

Description	qty.	cat.#
Click-On Inline Super-Clean™ Double Connector, stainless steel	ea.	22479

Wall-Mounting Clamps for Click-On Inline Super-Clean™ Traps

Description	qty.	cat.#
Wall-Mounting Clamps for Click-On Inline Super-Clean™ Traps	4-pk.	22480

Replacement O-Rings for Click-On Inline Super-Clean™ Connectors

Description	qty.	cat.#
Replacement O-Rings for Click-On Inline Super-Clean™ Connectors	20-pk.	22481

- Pack includes 10 large O-rings and 10 small O-rings.

Genuine Restek Replacement Parts

For Agilent HPLC Systems

By Becky Wittrig, Ph.D., HPLC Product Marketing Manager

new!

Outlet Cap and Gold Seal Assembly Tool for Agilent 1100 HPLC Systems

Easily install the gold seal into the outlet cap.

Put Outlet Cap on male part of Assembly Tool.

Push the Assembly Tool together.

Hold onto Outlet Cap and pull Assembly Tool apart.

Put Gold Seal over pin on male part of Assembly Tool.

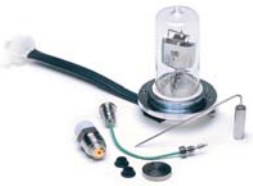
Push the Assembly Tool together and press the Gold Seal in the Outlet Cap.

Pull the Assembly Tool apart and remove assembled Outlet Cap and Gold Seal.

Description	qty.	cat.#
Outlet Cap and Gold Seal Assembly Tool for Agilent 1100 HPLC Systems	ea.	24989

Restek Replacement Parts for Agilent HPLC Systems

• Meet or exceed OEM performance.



Outlet Ball Valve

Needle Seat Assembly

Lamp, VWD G1314A

Description	Model #	Similar to Agilent part #	qty.	cat.#
Preventive Maintenance Kit (Includes: rotor seal, needle seat, needle assembly, seat cap)	1050	01078-68721	kit	25259
Autosampler Preventive Maintenance Kit (Includes: rotor seal, needle assembly, needle seat)	1100	G1313-68709	kit	25271
Pump Maintenance Kit (Includes: PTFE frit, outlet cap, active inlet cartridge, gold disk seal, 2 piston seals, glass solvent filter)	1050 & 1100	G1311-68710	kit	25270
Outlet Ball Valve, Binary Pump	1100	G1312-60012	ea.	25267
Outlet Ball Valve	1050 & 1100	G1311-60012	ea.	25276
Sieves for Outlet Valve	1050 & 1100	5063-6505	10-pk.	25266
Check Valve Cartridge Assembly	1090	79835-67101	ea.	25344
Piston Seals, Teflon® w/Graphite	1050 & 1100	5063-6589	2-pk.	22482
Piston Seals, Teflon® w/Graphite	1050 & 1100	5063-6589	10-pk.	22483
Piston Seals (Black)	1090	5062-2494	4-pk.	25347
Seal Wash Kit, Binary Pump (4 seals, 4 gaskets)	1100	—	kit	25268
Seal Wash Kit (2 seals, 2 gaskets)	1100	—	kit	25269
Wash Seal	1050 & 1100	0905-1175	ea.	25277
Sapphire Piston	1050 & 1100	5063-6586	ea.	25273
Sapphire Piston	1090	6980-0672	ea.	25345
Needle Seat	1050	79846-67101	ea.	25258
Needle Seat	1090	79846-67101	ea.	25348
Needle Seat Assembly	1100	G1313-87101	ea.	25265
Needle Assembly	1100	G1313-87201	ea.	25278
Rotor Seal (not for use with 7125 injection valve)	1050	0101-0626	ea.	25272
Rotor Seal	1100	0100-1853	ea.	25275
Rotor Seal (Rheodyne®-style)	1090	0101-0623	ea.	25349
Frits, PTFE	1050 & 1100	01018-22707	5-pk.	25466
Seal, Gold Disk (outlet)	1050 & 1100	5001-3707	ea.	25467
Outlet Cap	1050 & 1100	5062-2485	4-pk.	25139
Outlet Cap & Gold Seal Assembly	1050 & 1100	—	2-pk.	25140
Connecting Tube	1050 & 1100	G1311-67304	ea.	25058
Detector Lamp, 1090 DA, 1050 VW/DA/MWD	1090, 1050	79883-60002	ea.	25260
Lamp, DAD G1315A, G1365A	1100	2140-0590	ea.	25261
Lamp, VWD G1314A	1100	G1314-60100	ea.	25262
8453 Deuterium Lamp	—	2140-0605	ea.	25263
G1321 Fluorescence Detector Flash Lamp	—	2140-0600	ea.	25264
Lamp, DAD Long Life Deuterium (2000 hours)	1100	5181-1530	ea.	25399

HPLC Mobile Phase Accessories

An economical way to store and deliver your mobile phases.

By Becky Wittrig, Ph.D., HPLC Product Marketing Manager

Hub-Cap Bottle Tops and Adapters

new!

cat. # 26541

cat. # 26540 cat. # 26538

Allows the use of the Opti-Cap™ with 4-liter solvent bottles.

Description	qty.	cat.#
Adapters		
Hub-Cap Adapter	ea.	26538
Hub-Cap Adapter Multi-pack	3-pk.	26539
Hub-Cap Adapter and Opti-Cap™	kit	26540
4 Liter Bottle Tops		
Hub-Cap (assembly of the bottle cap and plug)	kit	26541
Hub-Cap Multi-pack	3-pk.	26542

Opti-Cap™ Bottle Top

The most economical way to helium-sparge and deliver HPLC mobile phases. The Opti-Cap™ top fits all standard GL-45 bottles and has two $^1/_8$-inch holes and one $^1/_{16}$-inch hole for tubing.

Description	qty.	cat.#
Opti-Cap™ (Cap and PEEK® Plug)	ea.	25300
Opti-Cap™ Kit (Opti-Cap™, 3 meters of tubing, sparging filters)	kit	25301
Opti-Cap™ Kit with 1L Bottle	kit	25302
Opti-Cap™ Kit with 2L Bottle	kit	25303
Related items and replacement parts	**qty.**	**cat.#**
Mobile Phase Mobile Phase Sparge Filter: 2µm, stainless steel	ea.	25311
Mobile Phase Inlet Filter: 10µm	ea.	25312
Teflon® Tubing, $^1/_8$" OD x 0.094" ID x 3m (2.4mm ID)	3m	25307
Teflon® Tubing, $^1/_8$" OD x 0.063" ID x 3m (1.6mm ID)	3m	25306
PEEK® Plug, $^1/_4$"-28 threads	3-pk.	25319
1L Graduated Safety-Coated Bottle – GL-45 threads	ea.	25304
2L Graduated Safety-Coated Bottle – GL-45 threads	ea.	25305

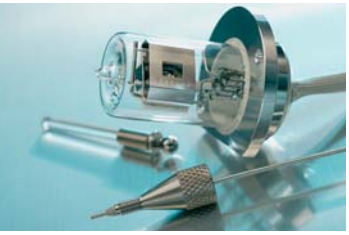
Opti-Cap™ Kit with bottle

Solvent Debubbler

Bubbles in an HPLC system can cause check valve malfunctions and pump cavitation, seriously affecting pump performance. The debubbler removes bubbles from the fluid stream before it enters the pump.

Special geometry at the base of the housing allows bubbles entrained in the inlet fluid stream to rise and be trapped in the reservoir. The gas/liquid interface is easily visible through the translucent wall of the device. Loosening the airtight cap releases the trapped gas. The debubbler is fitted with a bracket and universal connecting tips.

Description	qty.	cat.#
Solvent Debubbler with Bracket	ea.	25014



Using Micropacked Columns

By Alan Sensue, Technical Service Specialist

Most analysts are familiar with capillary gas chromatography columns and packed GC columns, but many are not familiar with micropacked columns. Here, we briefly discuss these useful columns, instrument requirements, and applications.

What Are Micropacked Columns?

Micropacked columns are short, narrow bore stainless steel columns packed with diatomaceous earth solid support, porous polymer, molecular sieve, or other particles. Standard Restek micropacked columns are 1 meter or 2 meters long and 0.75mm ID x 0.95mm OD or 1mm ID x 1/16 inch OD. Like most micropacked columns, ours have a larger internal diameter than mega-bore (wide-bore) capillary columns (0.53mm ID) and a smaller outside diameter than traditional packed columns (1/8 inch or 3/16 inch).

As you might suspect from this description, performance characteristics of micropacked columns are intermediate between those of packed columns and those of capillary columns: they offer higher efficiency than traditional packed columns, and higher capacity than wall coated open tubular (WCOT) capillary columns or porous layer open tubular (PLOT) columns. They are inexpensive, very durable, and easy to install and operate.

Instrument Requirements

To use micropacked columns, a high carrier gas head pressure is needed to overcome the large pressure drop created in the narrow, densely packed bore. For helium, typical column head pressures for 1-2 meter micropacked columns range from 30-45psi for 1mm ID columns to 50-65psi for 0.75mm ID columns.

Installation of micropacked columns will vary according to instrument make and model. The injection port nuts in many capillary column injection ports will accommodate 0.95mm OD micropacked columns, but not 1/16 inch OD columns. If the injection port nut will not accommodate the column, you can attach a short piece of 0.53mm ID fused silica tubing to each end of the column, using 1/16 inch compression fitting unions and appropriate ferrules. Alternatively, Restek sells inlet conversion kits which contain appropriate selections of injection port accessories.

For GCs with packed column injection ports, a reducing ferrule or a tube-end reducer fitting, and appropriate ferrules, usually are all that are needed for installing a micropacked column.

Applications

Micropacked columns have a wide range of applications, from analyses of the lightest gases (permanent gases) to simulated column distillation (Sim-Dist). They are especially useful for analyses of gas mixes, including sulfur compounds or light hydrocarbons, in which the use of a packed column is necessary to obtain baseline separations of the gaseous components.

Typically, chromatogram peaks are sharper than from traditional packed columns, and micropacked columns are less likely to be overloaded by concentrated samples than are capillary columns. Micropacked columns do have limitations, however: like packed columns, they do not have the efficiency of capillary columns. Therefore, they typically are not adequate for baseline separations of complex multi-component mixtures. Also like packed columns, they require a carrier gas flow rate that is higher than most mass spectrometer pumping systems can accept.

When choosing a micropacked column, consider that, as with any column, internal diameter affects column capacity. If you intend to use a sensitive detector, such as a helium ionization detector (HID), flame ionization detector (FID), nitrogen-phosphorus detector (NPD), or flame photometric detector (FPD), typically you can use a smaller ID column – either a conventional capillary column or a micropacked column. If you intend to use a thermal conductivity detector (TCD), however, consider using a 1/8 inch OD packed column, rather than either a capillary column or a micropacked column.

For a complete listing of micropacked columns and installation kits offered by Restek, please visit our website, www.restek.com and enter "micropacked columns" in the search feature. For typical applications, see web page: **www.restek.com/micropacked**

You also will find these items in the Restek Chromatography Products Catalog. For additional information concerning micropacked columns, please contact Restek Technical Service at **800-356-1688, ext 4**.

Custom **Micropacked** Columns

To Order:

Contact your Restek representative and specify the following:

1) dimensions (length, OD, ID, and tubing material)

2) packing description (percent coating and phase, support mesh size, and treatment)

3) installation kit

Ordering Example:
(2m x 1/16" OD x 1.00mm ID)
(Silcosteel® tubing) (5%)
(Carbowax® 20M)
(CarboBlack™ B) (80/120)
(installation kit for valve applications, cat. #21065)

To Obtain a Quote:

See our catalog or website, or contact technical service at 800-356-1688, ext. 4.

Maximum length for custom micropacked columns is 25ft./8m.

Pittcon Presentations by Restek Personnel

Sunday Feb. 25

Analysis of EPA Method 527 Using New Capillary Column Technology

JASON THOMAS, Gary Stidsen, Neil Mosesman, William Goodman (PerkinElmer Co.)

Poster Session 220: New Developments in Analytical Instrumentation and Software

Posters on display from 3:30 pm - 7:30 pm, authors present from 5:30 pm - 7:30 pm. Location: S100A (poster 220-41P)

Monday Feb. 26

Enhancing Resolution of Unsaturated Compounds Using a Unique Biphenyl Stationary Phase

RICHARD LAKE, Rebecca Wittrig, Frank Dorman

Oral Session 420: New Developments in Pharmaceutical Separations Room 501BC (420-8 / 11:05 am)

Forensic Applications Using a New 5% Diphenylpolysiloxane Stationary Phase for Gas Chromatography

KRISTI SELLERS, Richard Lake, Gary Stidsen, Neil Mosesman

Poster Session 820: Homeland Security/Forensics

Posters on display from 9:00 am - 4:30 pm, authors present from 2:30 pm - 4:30 pm. Location: Hall A1-A2 (poster 820-14P)

Tuesday Feb. 27

GCxGC-TOFMS of Volatile Organic Compounds in Urban and Rural Air

JACK COCHRAN, Mark Libardoni (LECO Corporation), Frank Dorman, David M. Shelow

Oral Session 1340: GC-MS Methodology II Room 501A (1340-1 / 1:30 pm)

Thursday Mar. 1

An Innovative Approach to Low Mass, Zero Dead Volume Connection of Fused Silica Columns

MICHAEL GOSS, William Grove, Brad Rightnour, Matt Lininger, Paul Silvis, Gary Stidsen

Poster Session 2330: Gas Chromatography: Development and Applications

Posters on display from 9:00 am - 2:00 pm, authors present from 9:30 am - 11:30 am. Location: Hall A1-A2 (poster 2330-24P)

New, In-Situ Cross-Linkable Wax Phase for Gas Chromatography

JULIE KOWALSKI, Shawn Reese, Roy Lautamo, Gianna Barlupi, Rick Morehead, Don Rhodes, Frank Dorman, Chris Cox, Jennifer Weston, Gary Stidsen

Oral Session 2500: Gas Chromatography: Method Development Room 501D (2500-5 / 3:05 pm)

If you're attending Pittcon 2007, please stop by and visit us at **Booth 1313**!

Leading Chromatographers to Join Restek

New expertise available to help solve your technical challenges

Restek celebrates continued growth in 2007 with the addition of two key chromatographers: Jack Cochran and Jaap de Zeeuw.

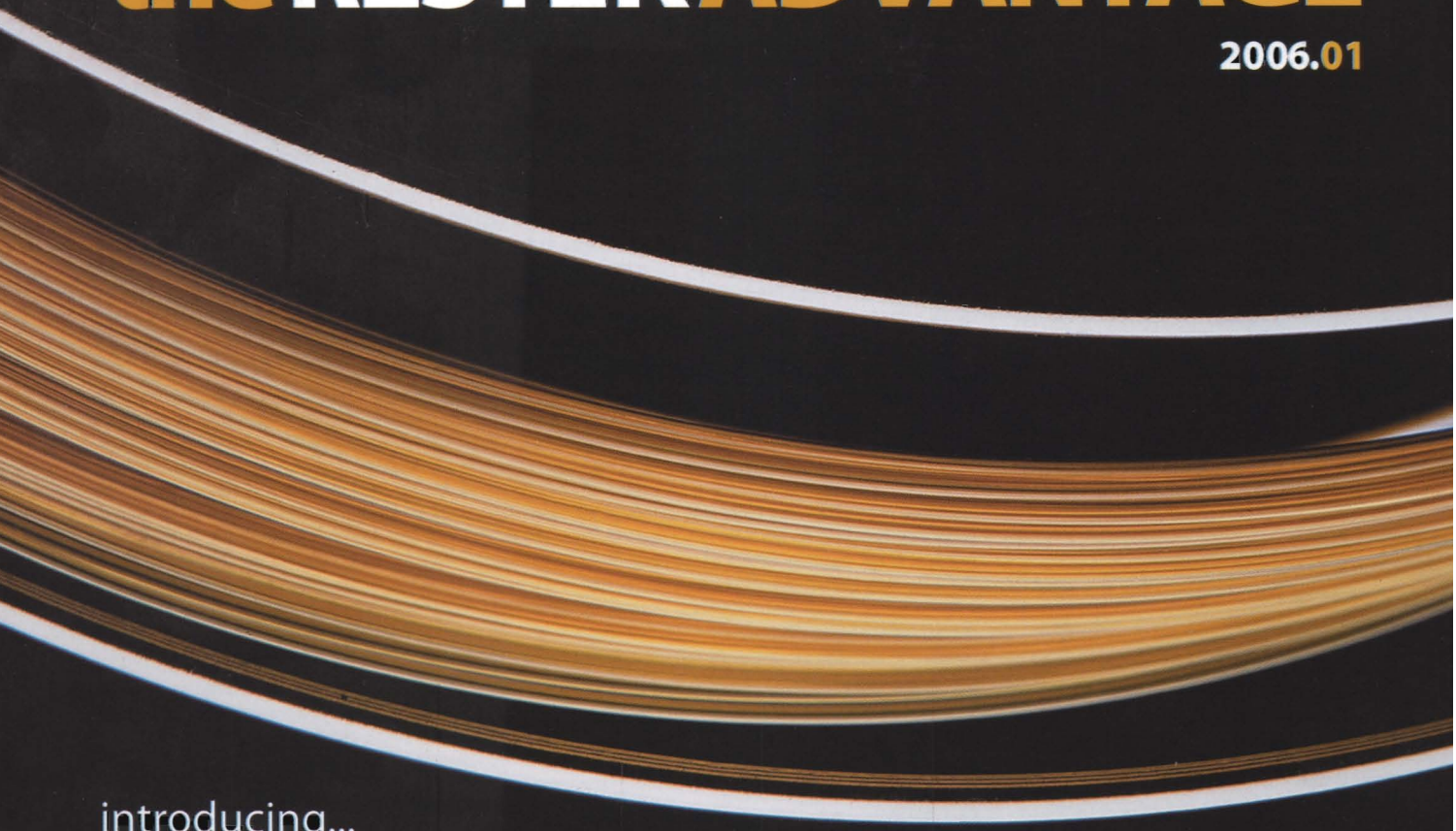
Jack Cochran comes to Restek with extensive experience at LECO Corporation, where he was most recently the International Director of Separation Science. Jack is a recognized expert in GC-TOFMS and GCxGC-TOFMS, as well as in the analysis of pesticides, PCBs, explosives, PAHs, and other priority pollutants in soils, sediments, air, and waters. His many years of employment at the Waste Management and Research Center in Champaign, IL and with the US EPA in Ada, OK provide real-world experience in methods development, sample preparation, and analysis that he can share with chromatographers world-wide, in order to help them optimize their separations. Jack will be based at our headquarters in Bellefonte, Pennsylvania.

Jaap de Zeeuw spent 27 years with Varian/Chrompack, and has distinguished himself as an authority on every aspect of capillary column technology. After working as an R&D scientist, product specialist, and international product manager for GC and LC columns, he has most recently focused on industrial analysis issues in the USA, Europe, and the Far East. Jaap is widely published, and he travels extensively, giving seminars, workshops, and presentations at international symposia. In 1999 he received the first "Presenter of the Year" award at the Gulf Coast Conference in Galveston, Texas. Jaap will be based in Middleburg, the Netherlands; his main focus will be supporting Restek's European activities in the form of training, seminars, and participation in professional meetings and trade shows.

We welcome Jack & Jaap into the Restek family!

the RESTEK ADVANTAGE

2006.01



introducing...

New Rxi™ GC Column Series

The Ultimate High Performance Fused Silica Capillary Column

While GC analytical methods have continued to evolve, capillary column technology has been largely unchanged over the last several years. Using new techniques for deactivation, stationary phase synthesis, and coating, and tight controls over column manufacturing, Restek has set the benchmark for column performance by introducing the Rxi series. Inside you will find more information about this revolutionary new family of GC columns which demonstrate...

- unsurpassed inertness
- ultra low bleed
- guaranteed column to column reproducibility

Exclusively from Restek!

See **pages 3 & 8**.

RESTEK 20 YEARS

Turning Visions into Reality™

www.restek.com

the Restek Advantage
2006.01

IN THIS ISSUE

Erratum
The transfer line used in the methyl *tert*-butyl ether / *tert*-butyl alcohol analysis reported in Advantage 2005v4 (Figure 1, page 4) was the factory-installed Eclipse transfer line.

We thank Laura Chambers at O.I. Analytical, College Station, Texas, for reviewing the analysis with us, and we are very grateful to O.I. Analytical for their generous loan of the O.I. 4660 Eclipse purge and trap system.

By Professor Walter Jennings ("Walt")
Professor Emeritus, University of California, Davis; Co-Founder, J&W Scientific, Inc.; Co-Founder, AirToxics, Ltd.; waltj@pacbell.net

For the past few years, the aging process has been catching up with the Jennings family. While I still enjoy participating in seminars and lectures, I now find seven to ten events on a two week trip is more tiring than it was just a few years ago. In addition, my wife has been successfully battling Parkinson's disease for almost twenty years, but we realize that it is now inexorably advancing. Hence, she needs more of my time, and I must limit myself to shorter absences. Because of these developments, I permitted my Agilent contract to expire when it ran out on June 30, 2005. Agilent was sympathetic and understanding, our parting was amicable, and I still value my contacts with them. But after two months in my home office, I sometimes feel a need for the challenge of discussion and argument, and when friends at Restek asked if I would be interested in writing a short paper that was purely educational and pushing no particular product line, it sounded appealing. Here it is.

The "Replacement" Column, A Recurring Problem in Gas Chromatography

One of the problems that gas chromatographers frequently ask concerns the behavior of a "replacement" column. Even skilled practitioners have been known to protest when they install a replacement column, use the same operational parameters, and find that not only have solute retention times shifted, but peak 15 now elutes prior to peak 13. In most such cases, they blame the column manufacturer. There are programs available to correct this problem, but some of those solutions have been so simplified that the user still has no comprehension of the causative factors, a state of blissful ignorance which should be corrected.

Columns are produced, bought, and sold using nominal measurements, e.g., "30 meters x 0.25mm, film thickness 0.25µm". As a specification, this is not equivalent to "30.0 meters x 250µm". Depending on the manufacturer's specifications, the actual column dimensions may be "30 +/- 1 meter x 250 +/- 6µm". Some manufacturers now give better attention to the length tolerance, but the diametric variation will continue to be a problem. Fused silica draw towers are often computer controlled, with the feed rate of the silica blank, the draw rate of the capillary tube, and the temperature of the softening oven controlled by a computer whose input comes from a laser micrometer that monitors the tubing diameter during the drawing process. In our hands, a blank could be drawn to approximately 14 kilometers of 0.25mm capillary tubing. The two ends of that tube may show a significant variation in diameter, but those changes occur so slowly that over lengths of a few hundred meters the diameter is reasonably constant. It is rare to find a column where the diameters at the two ends are significantly different, but it is not unusual to find that columns from the two ends of that draw, or from different draws, do exhibit significant differences in diameter, e.g., 244µm vs. 256µm[i].

An analyst whose original 29.9m x 256µm column is replaced by one measuring 30.1m x 244µm will likely experience difficulties if he or she uses the same operational parameters, i.e., same temperature program, same carrier gas, same inlet and outlet pressures. Because of the geometric differences, the columns possess different pressure drops and under the same operational parameters, the carrier gas velocities would be different in the two columns. This will affect solute retention times, and this introduces the major problem.

Gas chromatography is a volatility phenomenon, and solutes elute in a sequence mandated by what I prefer to call their "net vapor pressures". The net vapor pressure is a function of the intrinsic vapor pressure of that solute, increased by the temperature at that point in the program, and *further decreased by the sum of all interactions between that solute and that stationary phase.*[ii] The strengths of these various interactive forces usually vary inversely with temperature in a non-linear manner, and for a given increase in temperature both the rate of change and the degree of change are unique functions of that solute in that stationary phase under these particular conditions. As a result, the molecules of a chromatographing solute experience a specific temperature-sensitive "selectivity profile" in their passage through the column. These interactions are rendered moot while those molecules are in the mobile phase, and endure only while they are in contact with the stationary phase. Hence we are interested in keeping retention times, and particularly t'_R (time in stationary phase) constant from column to column and run to run. From the two relationships of $K_c = ßk$ and $\mu = L/t_M$ we can establish that $t'_R = c_S/c_M \times d_f/d_c \times L/u$. The three terms of course are the distribution constant, the reciprocal of the phase ratio, ß, and column length divided by the average linear gas velocity. K_c is a function of the solute, the stationary phase, and the temperature. While, by definition, the temperature changes in program mode, the rate of change is constant, run to run and column to column, under the same program parameters, and one can usually ignore this term if the two stationary phases are indeed identical[iii]. The second term can also be ignored, provided the ratio of d_f/d_c is constant. Column diameter, d_c and column length are both nominal values and usually differ from column to column. We can compensate for either or both of these by varying the gas velocity, u. This is most easily accomplished in constant pressure mode. In constant flow mode it is more complicated and beyond the scope of this paper.

In constant pressure mode, the solution is quite simple, assuming that the replacement column has the same stationary phase and the same phase ratio as the original column. 1) Using the original operational parameters (initial temperature and program parameters, column inlet and outlet pressures, same carrier) install the new column and inject the same mixture. 2) Determine the retention time of an easily identifiable peak, and compare this to the retention time of that peak on the original column. 3) Adjust the column inlet pressure to make the retention time of the target peak the same as it was on the original column. Retention times on the replacement column should now agree closely with the values observed on the original column, each solute will now experience its original temperature-sensitive "selectivity profile", and chromatograms generated on the replacement column should essentially duplicate those from the original column.

i Fortunately, the column phase ratio (ß) is usually unaffected by these changes in diameter because almost all manufacturers currently employ static coating methods. Provided the concentration of the stationary phase in the coating solution remains constant, the ratio of the film thickness (d_f) to column diameter (d_c) will remain constant.

ii These interactions include (but are not limited to) dispersive interactions, hydrogen bonding and other proton forms of proton sharing, dipole interactions, and in some cases, molecular size and shape.

iii In some cases, surface preparation and deactivation treatments can also affect retentions. These treatments are generally proprietary and vary from manufacturer to manufacturer. With complex mixtures, the separations achieved on columns coated with the same stationary phase but from different suppliers may yield slightly different results.

New Rxi™ Fused Silica Columns

Ultimate High-Performance Fused Silica Capillary Columns

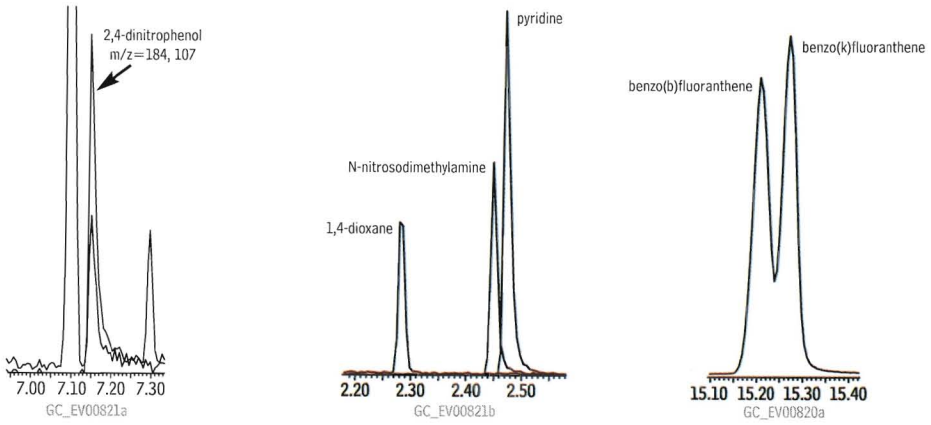
- Unsurpassed inertness for low-level acidic and basic compounds.
- Ultra-low bleed.
- Reliable performance, guaranteed column to column reproducibility.
- Guaranteed to work perfectly with retention time-locking software.

In recent years there have been few advances in capillary GC column technology. Through new, innovative technology, Restek has developed and optimized a column-making procedure that assures low bleed, unsurpassed inertness, and exceptionally reproducible columns, batch to batch.

Unsurpassed Inertness

Rxi™ columns improve chromatography for many acidic or basic compounds. Surface activity in a column is revealed by the sensitivity and peak shapes for analytes such as 2,4-dinitrophenol (acidic) and pyridine (basic). Sub-nanogram quantities of these compounds are a stringent test of inertness. Rxi™ columns' unsurpassed inertness allows analysis of acidic or basic compounds under the same conditions, as shown here.

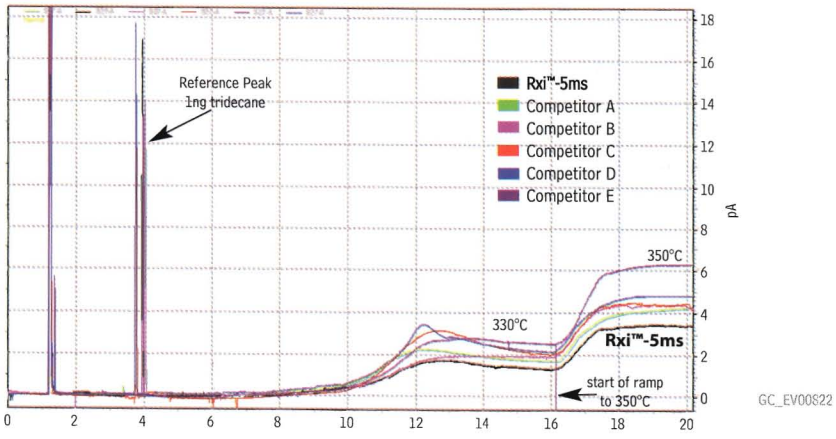
Figure 1 Rxi™-5ms columns have excellent sensitivity and symmetry for difficult compounds.



Ultra-Low Bleed

Bleed from Rxi™ columns is the lowest in the industry, simplifying trace-level analysis with mass spectrometric detectors (MSD, ion trap, etc.), electron capture detection (ECD), nitrogen-phosphorus detection (NPD), or other sensitive detection methods.

Figure 2 Profiles for widely used columns prove Rxi™-5ms has the lowest bleed!



Introducing...

Restek's exceptionally inert (Rxi) fused silica capillary columns:
In addition to bleed, column-to-column uniformity has been elusive - see editorial *The "Replacement" Column A Recurring Problem in Gas Chromatography*, by guest editor Professor Walter Jennings, on the facing page.

The processes we use to make new Rxi™ columns enable us to **guarantee** highly uniform performance, column to column and lot to lot, including perfect match-up with retention time-locking software. It is our promise and commitment to you that every Rxi™ column you receive will be **exactly** as good as the one it replaces.

Continued on the outside back cover.

Improved SPE Cartridges for Massachusetts EPH Analysis

Monitoring Environmental Petroleum Hydrocarbons by SPE/GC

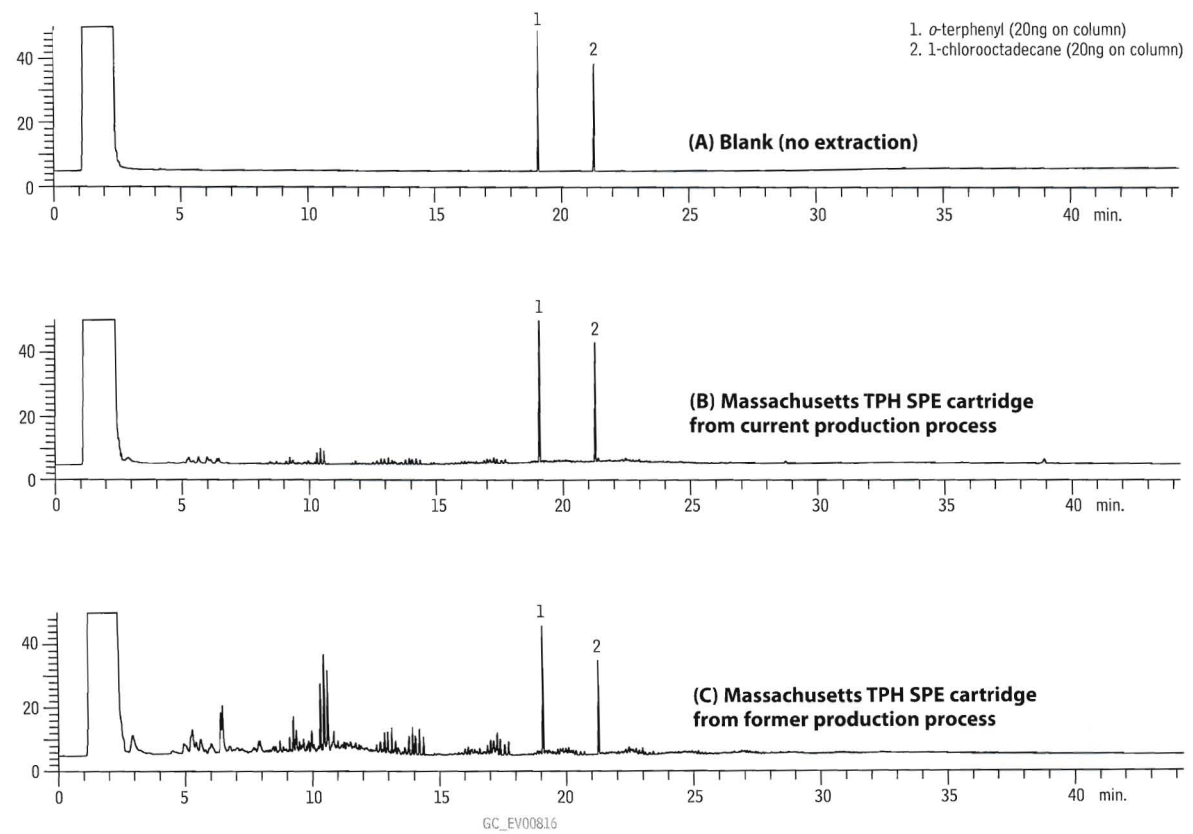
By Lydia Nolan, Innovations Chemist

- New Massachusetts TPH SPE cartridges reduce extractable contaminants and assure more reliable fractionation.
- Large uniform lots of silica reduce frequency of verifying fractionation results.
- New packaging reduces coextractables, provides better protection from humidity.

Concern about the effects of materials from leaking underground petroleum storage tanks has grown over the last ten years. In addition to the US Environmental Protection Agency and international groups, several US states, including Massachusetts[1] and Texas[2], have developed methods for analyzing samples from their geographical areas. Because of the broad and thorough nature of the quantitative information generated by the Massachusetts Department of Environmental Protection method[1], many site managers and engineering firms outside of Massachusetts request this method for their samples.

Most frustrating for labs using this method has been the uniformity of commercial silica gel-containing solid phase extraction (SPE) cartridges used to prepare the samples for analysis. The activity level and capacity of the silica, the uniformity of the bed, and the quality of the cartridge components and packaging are critical toward good results. Slight variations in the silica material, or in humidity levels during manufacture of the cartridges, can have a dramatic effect on the hexane fractionation results. The volume of hexane required to fractionate the aliphatic portion of the sample, without allowing any aromatic analytes to break through into the hexane fraction, can vary, typically from 17mL to 20mL ± 0.5mL, and must be determined for every lot of cartridges. Trace levels of phthalates and other contaminants from cartridges, frits, and packaging are easily extracted with the desired analytes, complicating

Figure 1 Low levels of background extractables in new Restek Massachusetts TPH SPE cartridges.



Column:	Rtx®-5 30m, 0.32mm ID, 0.25µm (cat. #10224)
Sample:	50µL diluted MA EPH Surrogate Spike Mix (cat.# 31479) (diluted to 400µg/mL)
Inj.:	0.5µL splitless (hold 0.75 min.), Precision™ split inlet liner with wool (cat.# 21027)
Inj. temp.:	290°C
Inj. press. prog.:	pressure pulse to 50cm/sec. @ -0.71min. pressure to 35cm/sec. @ 0.8 min.
Carrier gas:	helium
Linear velocity:	35cm/sec., constant velocity
Oven temp.:	40°C (hold 1 min.) to 310°C @ 15°C/min.
Det.:	PerkinElmer AutoSYS™ GC-FID @ 330°C

Table I Recovery and reproducibility for aliphatics and aromatics via Massachusetts TPH SPE fractionation, using new Restek SPE cartridges.

	Aliphatic Fraction			Aromatic Fraction		
	% Rec.ov.	Std. Dev.	RSD	% Recov.	Std. Dev.	RSD
1. nonane (C9)	86.4	9.11	10.5			
2. decane (C10)	84.7	7.17	8.5			
3. naphthalene				82.3	6.09	7.4
4. dodecane (C12)	83.8	8.33	9.9			
5. 2-methylnaphthalene				89.1	5.50	6.2
2-fluorobiphenyl (surrog.)				92.1	6.70	7.3
6. tetradecane (C14)	90.7	6.29	6.9			
7. acenaphthylene				91.6	7.63	8.3
2-bromonaphthalene (surrog.)				84.9	6.82	8.0
8. acenaphthene				93.4	6.32	6.8
9. fluorene				92.4	6.19	6.7
10. hexadecane (C16)	90.9	4.37	4.8			
11. phenanthrene				90.4	5.55	6.1
12. octadecane (C18)	94.9	3.45	3.6			
13. anthracene				91.5	5.29	5.8
14. nonadecane (C19)	91.1	3.63	4.0			
o-terphenyl (int. std.)				96.4	3.43	3.6
15. eicosane (C20)	89.8	2.64	2.9			
16. fluoranthene				93.4	3.16	3.4
1-chlorooctadecane (int. std.)	83.1	5.02	6.0			
17. pyrene				95.1	3.84	4.0
18. docosane (C22)	85.2	3.97	4.7			
19. tetracosane (C24)	85.0	3.23	3.8			
20. benzo(a)anthracene				91.2	2.38	2.6
21. chrysene				90.9	2.56	2.8
22. hexacosane (C26)	85.8	2.97	3.5			
23. octacosane (C28)	85.7	2.51	2.9			
24. benzo(b)fluoranthene				91.3	2.23	2.4
25. benzo(k)fluoranthene				90.8	2.10	2.3
26. benzo(a)pyrene				91.0	2.67	2.9
27. triacontane (C30)	86.0	2.49	2.9			
28. dibenzo(a,h)anthracene				90.9	1.78	2.0
29. indeno(1,2,3-cd)pyrene				91.4	1.48	1.6
30. benzo(ghi)perylene				90.7	2.21	2.4
31. hexatriacontane (C36)	78.6	3.95	5.0			

n=4 (2 analyses on each of 2 lots of SPE cartridges)

Analytical Conditions

Column:	Rtx®-5 30m, 0.32mm ID, 0.25µm (cat. #10224)
Sample:	50µL Mass EPH Surrogate Spike Mix (cat.# 31479) diluted to 400µg/mL 1mL MA Fractionation Check Mix (cat.# 31481), 25µg/mL in hexane 1mL MA Fractionation Surrogate Spike Mix (cat.# 31480), diluted to 40µg/mL in hexane
Inj.:	0.5µL splitless (hold 0.75 min.), Precision™ split inlet liner with wool (cat.# 21027)
Inj. temp.:	290°C
Inj. press. prog.:	pressure pulse to 50cm/sec. @ -0.71min. pressure to 35cm/sec. @ 0.8 min.
Carrier gas:	helium
Linear velocity:	35cm/sec., constant velocity
Oven temp.:	40°C (hold 1 min.) to 310°C @ 15°C/min.
Det.:	PerkinElmer AutoSYS™ GC-FID @ 330°C

SPE Method

Tube:	Massachusetts TPH 20mL/5g, cat.# 26065
Tube conditioning:	30mL hexane; do not allow top frit or bed to dry.
Sample:	Add 1mL EPH sample in hexane.
Elution #1:	Using gravity or very low vacuum, pass 18mL hexane through tube.* Do not allow top frit or bed to dry; collect this aliphatic fraction in a clean sample container. Reduce eluate to 1mL under gentle nitrogen purge or other concentration technique. Do not concentrate to less than 1mL or allow eluate to dry before analysis.
Elution #2:	Using gravity or low vacuum, pass 20mL methylene chloride through tube. Do not allow top frit or bed to dry; collect this aromatic fraction in a clean sample container. Reduce to 1mL (see above) and analyze.

*Note that the volume of hexane will vary, and should be verified in each laboratory.
For details concerning the SPE method, refer to the original method in Reference 1.

References

1 Method for the Determination of Extractable Petroleum Hydrocarbons (EPH) Massachusetts Department of Environmental Protection, Division of Environmental Analysis, Office of Research and Standards, Bureau of Waste Site Cleanup, Revision 1.1, May 2004.

2 Total Petroleum Hydrocarbons, TNRCC Method 1005, Revision 03 (June 1, 2001); Draft TNRCC Method 1006 (May 2000) Texas Natural Resource Conservation Commission.

low level quantification. Consequently, quality must be assured for each lot of cartridges and, sometimes, even within lots.

We have always specially treated our Massachusetts TPH SPE cartridges (cat.# 26065) to ensure minimum background extractables and maximum silica activity. Now, a new process has allowed us to reduce extractables even further, and assure greater reliability of fractionation. Larger uniform lots of silica will reduce the frequency with which a lab will need to verify fractionation results. New packaging ensures reduced levels of coextractables and better protection from environmental humidity.

Figure 1C shows the background of a typical previous lot of cartridges, compared to the significantly lower background from the new product, in Figure 1B. All cartridges were extracted with 15mL of hexane, with no prior conditioning. The hexane was evaporated, o-terphenyl and 1-chlorooctadecane were added, and samples were reconstituted to 1mL for analysis by GC-FID. Fractionation, extraction efficiency, and reproducibility also are excellent, as shown by the summary in Table I. Details of the extraction method, based on the Massachusetts procedure, also are presented in Table I.

If you are conducting Massachusetts EPH analyses, or similar analyses, and have been concerned about the quality and uniformity of the SPE cartridges you have been using, we think you will be as impressed as we are with the quality of our new product.

Massachusetts TPH SPE Cartridges

20mL, 5g, 20-pk. 26065

Rtx®-5 Column (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	-60 to 330/350°C	30-Meter	10224

MA EPH Surrogate Spike Mix

1-chlorooctadecane *o*-terphenyl
4,000µg/mL each in acetone, 1mL/ampul
cat. # 31479 (ea.)

MA Fractionation Check Mix

(31 components)
25µg/mL each in hexane, 1mL/ampul
cat. # 31481 (ea.)

MA Fractionation Surrogate Spike Mix

2-bromonaphthalene 2-fluorobiphenyl
4,000µg/mL each in hexane, 1mL/ampul
cat. # 31480 (ea.)

New Reference Mix of Canadian Drinking Water Volatiles

By Jason Thomas, Environmental Innovations Chemist

- New, complete mix includes 19 volatiles on Canadian Drinking Water List.
- Simple purge and trap GC/MS analysis.
- Rtx®-VMS column provides sharp peaks for early eluters, resolves heavier compounds.

Much like the US Environmental Protection Agency's regulation of environmental contaminants in drinking water through the Safe Drinking Water Act, Canada has its own stipulations regarding drinking water. These mandates are laid out in the *Guidelines for Canadian Drinking Water Quality* published by Health Canada's Water Quality and Health Bureau. Regulation falls under the jurisdiction of the individual provinces and territories, which use these guidelines to establish water quality requirements for municipal water sources.[1]

Here, we illustrate the analysis of the volatiles portion of the Canadian contaminants list, now available from Restek as Canadian Drinking Water Volatiles Mix (cat.# 30610). We analyzed a 25mL water sample containing 50ppb each analyte, using an OI Analytical 4660 purge and trap system, with autosampler, and an HP 5890/5971 GC/MS system. A 30m x 0.25mm ID x 1.4µm Rtx®-VMS column (cat.# 19915), in conjunction with a Siltek® deactivated 1mm ID split inlet liner and a 35:1 split, affords good peak shape for the early-eluting components, as well as good resolution for the heavier compounds. The Rtx®-VMS column is an excellent choice for many other volatiles applications as well.

Reference

[1]http://www.hc-sc.gc.ca/ewh-semt/water-eau/drink-potab/index_e.html

Rtx®-VMS Column (fused silica)

(proprietary Crossbond® phase)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	1.40	-40 to 240/260°C	30-Meter	19915

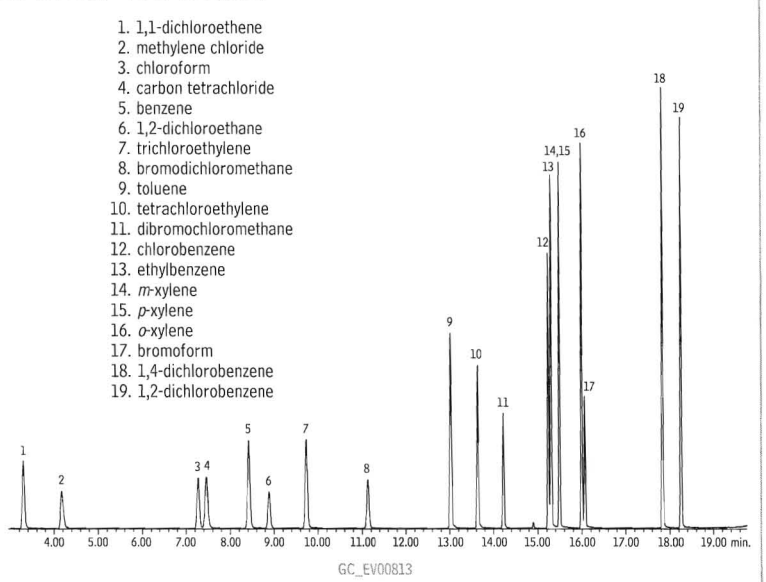
Canadian Drinking Water Volatiles Mix new!

(19 components)

benzene
bromodichloromethane
bromoform
carbon tetrachloride
chlorobenzene
chloroform
dibromochloromethane
1,2-dichlorobenzene
1,4-dichlorobenzene
1,2-dichloroethane
1,1-dichloroethylene
ethylbenzene
methylene chloride
tetrachloroethylene
toluene
trichloroethylene
m-xylene
o-xylene
p-xylene

2,000µg/mL each in P&T methanol, 1mL/ampul
cat. # 30610 (ea.)

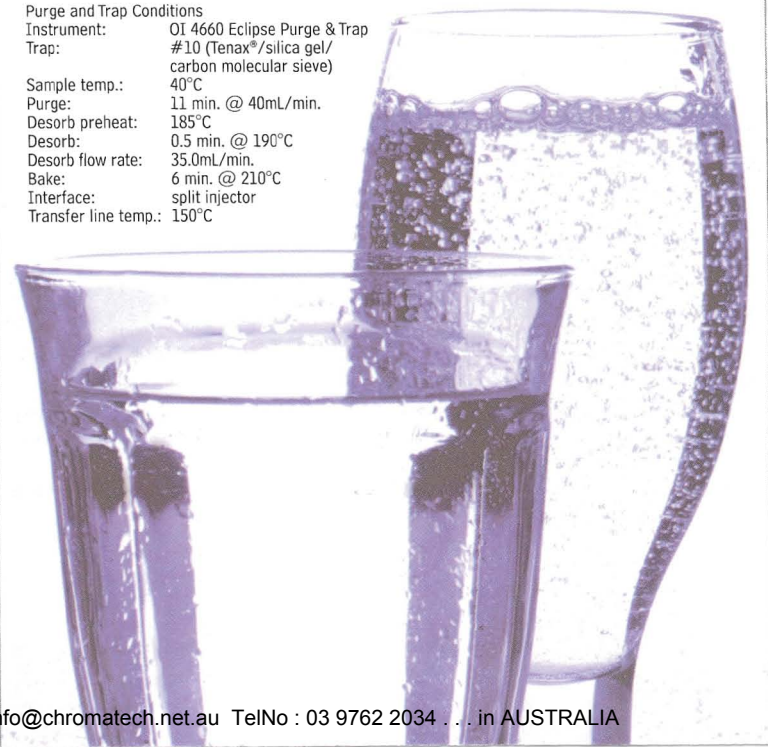
Figure 1 Canadian Drinking Water Volatiles Mix analyzed on an Rtx®-VMS column.



Column:	Rtx®-VMS 30m, 0.25mm ID, 1.4µm (cat.# 19915)
Sample:	50 ppb each analyte in 25mL water, prepared from Canadian Drinking Water Volatiles Mix (cat.# 30610), 2000µg/mL each component in purge & trap methanol
Inj.:	split, 35:1, 1mm ID Siltek®-treated split inlet liner (cat.# 20972-214.1)
Inj. temp.:	200°C
Carrier gas:	helium
Linear velocity:	30cm/sec. @ 35°C, constant pressure
Oven temp.:	35°C (5 min.), to 70°C @ 5°C/min., to 220°C @ 20°C/min. (hold 3 min.)
Det.:	MS
Transfer line temp.:	150°C
Scan range:	35-250 amu
Ionization:	EI
Mode:	scan

Purge and Trap Conditions

Instrument:	OI 4660 Eclipse Purge & Trap
Trap:	#10 (Tenax®/silica gel/carbon molecular sieve)
Sample temp.:	40°C
Purge:	11 min. @ 40mL/min.
Desorb preheat:	185°C
Desorb:	0.5 min. @ 190°C
Desorb flow rate:	35.0mL/min.
Bake:	6 min. @ 210°C
Interface:	split injector
Transfer line temp.:	150°C



Analyze Hydrocarbons on OPN/Res-Sil™ C Bonded GC Packing

Superior Replacement for Porasil® Packings

By Barry Burger, Petroleum Chemist

- Unique separations of saturated and unsaturated hydrocarbons.
- Innovative bonding chemistry for batch-to-batch reproducibility, excellent thermal stability, and long life.
- Other bonded phases available.

For years, Porasil® C and Porasil® B, modified with covalently attached liquid phases such as OPN (cyanopropyl) or *n*-octane functional groups, offered important advantages, relative to conventional GC packings, in analyses of C1-C4 hydrocarbons: faster separations, higher thermal stability, shorter conditioning times, and longer lifetimes. Porasil® C / Porasil® B products were discontinued in the 1980s, however, and inventories have been depleted, forcing those that use these packings to search for comparable materials.

Restek chemists solved the problem by developing Res-Sil™ C and Res-Sil™ B bonded packings. These packings afford all of the advantages of the Porasil® C and Porasil® B materials, with the added advantage of consistent batch-to-batch performance - and they are readily available for immediate delivery. Compared to diatomaceous earth media, Res-Sil™ C has a small surface area, good inertness, low friability, and less reactivity.

Unique Selectivity for Process GC and High-Speed Analysis

Speed of analysis is crucial in process GC, and in laboratory gas analyzers in which multiple columns and valve switching are used to separate complex gas mixtures. Res-Sil™ C bonded packings are ideal for resolving the difficult-to-separate saturated and unsaturated C4 hydrocarbons under these demanding conditions. Figure 1 illustrates the unique selectivity of OPN on Res-Sil™ C packing for eluting *cis*-2-butene before 1,3-butadiene. When used in series with other columns, this unique material provides petroleum and petrochemical method developers with a powerful tool for fast determination of C1-C4 hydrocarbons.[1]

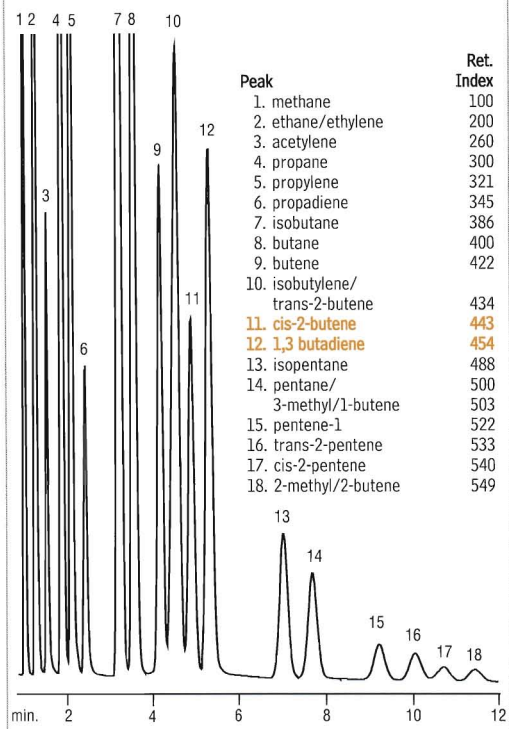
Stringent QA Assures Batch-to-Batch Consistency

Historically, one of the problems with bonded phases on Porasil® media has been batch-to-batch variations in the amount of liquid stationary phase incorporated on the silica support. Through our new synthesis pathways, we precisely control the amount of bonded liquid phase on Res-Sil™ C in every batch of packing, assuring reproducible retention times and separations. Each batch of packing is tested with a complex mixture of hydrocarbons, to confirm it meets demanding retention time and retention index specifications. We evaluate column bleed at the recommended maximum temperature, 150°C, to ensure that there are no retention shifts or high baselines.

In addition to OPN on Res-Sil™ C packing, we bond *n*-octane and Carbowax® 1540 phases to Res-Sil™ C. Each of these packings offers a conditioning time of less than 30 minutes, low bleed, long lifetime, and consistent batch-to-batch reproducibility. For details about *n*-octane or Carbowax® on Res-Sil™ C, and for bonded phase packings on Silcoport™ deactivated diatomaceous earth, refer to our current chromatography supplies catalog. We test every batch of every Restek bonded phase packing for bleed, efficiency, retention index, and retention time reproducibility. In addition, we make a broad range of packed and micropacked columns in specially-deactivated Silcosteel® tubing, for superior inertness and efficiency.

If you have been looking for a replacement for a Porasil® C or Porasil® B packing, we invite you to contact us. Your search should end here.

Figure 1 OPN on Res-Sil™ C packing has unique selectivity for *cis*-2-butene and 1,3-butadiene.



Peak	Ret. Index
1. methane	100
2. ethane/ethylene	200
3. acetylene	260
4. propane	300
5. propylene	321
6. propadiene	345
7. isobutane	386
8. butane	400
9. butene	422
10. isobutylene/ trans-2-butene	434
11. cis-2-butene	443
12. 1,3 butadiene	454
13. isopentane	488
14. pentane/ 3-methyl/1-butene	500 503
15. pentene-1	522
16. trans-2-pentene	533
17. cis-2-pentene	540
18. 2-methyl/2-butene	549

Reference standard courtesy of DCG Partnerships, Ltd., Pearland, TX.

12' x 2mm ID x 1/8" OD Silcosteel® column packed with OPN on Res-Sil™ C 80/100 mesh.
20µL on-column injection of refinery gas

Oven Temp.:	50°C
Inj. temp.:	200°C
Det. temp.:	200°C
Flow rate:	30mL/min., He

Res-Sil™ C Packings

Description	cat.#
Res-Sil™ C, 80/100 mesh, 10g*	25028
OPN on Res-Sil™ C, 80/100 mesh, 10g*	25042
n-Octane on Res-Sil™ C, 80/100 mesh, 10g*	25030
2% Carbowax® 1540 on Res-Sil™ C, 80/100 mesh, 10g*	25044

Reference

1 Saha, N.C., S.K. Jain, and R.K. Dua. *J. Chromatogr. Sci.* 16: 323-328 (1978).
Reference not available from Restek.

Sensitive GC/MS Analysis for Drugs of Abuse

Rxi™-5ms Column Resolves Acidic/Neutral or Basic Drugs

By Kristi Sellers, Clinical/Forensic Innovations Chemist

- New stationary phase, inert to acidic or basic drugs.
- Unique deactivation for low column bleed at 330°C.
- Column technology specially developed for GC/MS.

new **column** technology!

GC/MS is considered the standard for confirming the presence of abused drugs in body fluids, including acidic drugs (e.g., methaqualone), neutral drugs (e.g., phenobarbital), and basic drugs (e.g., methamphetamine). These methods are well established, and the positive identifications mass spectral data generate are accepted as confirming evidence in courts of law. The accepted stationary phase for these analyses is a 5% phenyl / 95% methyl polysiloxane phase, because it provides the best selectivity for separating the drugs and their metabolites. Unfortunately, not all 5% phenyl columns provide the inertness needed to accurately quantify low concentrations of reactive acidic or basic drugs.

Now, Restek's R&D chemists have developed a new 5% phenyl stationary phase and a unique column deactivation technology specifically for GC/MS. The product of this combination - the Rxi™-5ms column - ensures enhanced inertness for acidic or basic compounds, while maintaining the selectivity of a conventional 5% phenyl column.

Using mixtures of acidic/neutral drugs and basic drugs in their free base form, at an on-column concentration of 50ng for each drug, we evaluated a 30m, 0.25mm ID, 0.25µm Rxi™-5ms column for resolution, inertness, and bleed. Figure 1 shows chromatography for acidic/neutral drugs and Figure 2 shows basic drugs. In either analysis, all compounds are resolved to baseline and exhibit Gaussian peak shapes. Furthermore, there is no interference from column bleed - not even at 330°C. Note that a Siltek® treated inlet liner contributes to these results: our unique Siltek® surface passivation process assures the liner will have the inertness needed for accurate low-level analyses of reactive acids or bases.

In combination, an Rxi™-5ms column and a Siltek® treated inlet liner represent a complete solution for analyzing acidic, neutral, and basic drugs by GC/MS. For additional dimensions of Rxi™-5ms columns, and for Siltek® treated inlet liners for your chromatograph, please refer to the 2006 Restek catalog - or visit our website.

Rxi™ Columns, Ultimate High Performance Capillary GC Columns

Rxi™ columns were created at Restek's cutting-edge research facility, Restek West, in California. Our senior polymer chemists developed new column technology, based on our Crossbond® chemistry, to create this new column line. The columns we produce as a result of their work exhibit exceptional inertness and unsurpassed reproducibility, from column to column and lot to lot. Acidic or basic compounds chromatograph beautifully, at sub-nanogram on-column levels, with no peak tailing. Ultra-low bleed assures compatibility with sensitive detectors or in trace-level GC/MS analysis. We tuned this unique chemistry until polymer selectivity was locked in, to allow install-and-run use of Rxi™ columns with retention time-locking software.

Restek West
Shawn Reese, Gianna Barlupi, Roy Lautamo

What makes Rxi™ columns different from other columns?

First, and foremost, unique deactivation and our modified Crossbond® chemistry create columns with superior performance. The raw materials we use in the manufacturing process - both tubing and chemicals - are strictly controlled. Cleanliness and precision are critical to every step in the process. In addition, we looked in-depth at all other aspects of the column manufacturing process, to establish a highly reproducible process. In both performance and column-to-column consistency, Rxi™ columns are surpassed by no other columns.

In developing Rxi™ columns, our first step was to work with our fused silica tubing supplier to establish rigorous controls on internal diameter, outer diameter, ovality, and surface activity. These controls guarantee our tubing is a known starting point. Then, we treat this highly uniform tubing with our unique deactivation chemistry, producing a consistent, inert surface on which to apply the polymer.

Next, we reformulated our polymers, taking steps to ensure neutrality and to fine tune selectivity for retention time locking. A neutral polymer and a neutral tubing surface are important contributors toward excellent peak shape for both acidic and basic compounds.

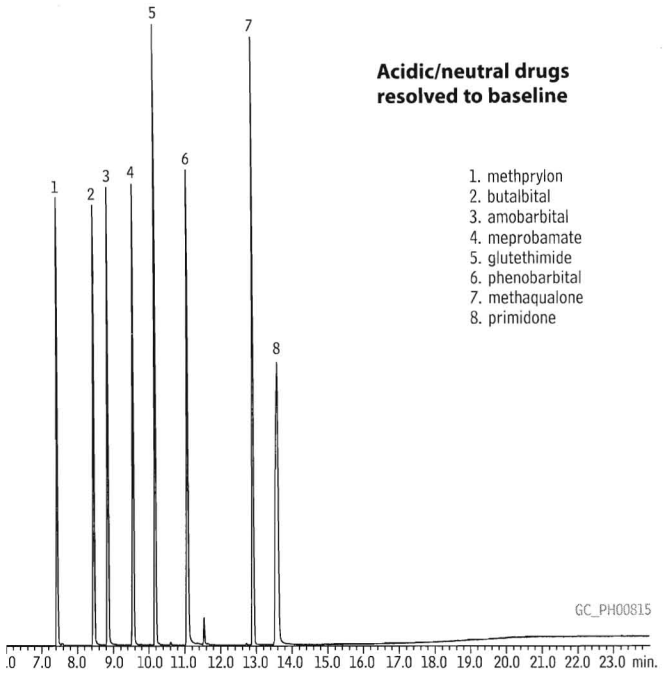
To complement these efforts, we developed a new column manufacturing process that creates a very reproducible product. This is critical, because our customers' workdays are simplified when every new column they purchase performs exactly as its predecessor.

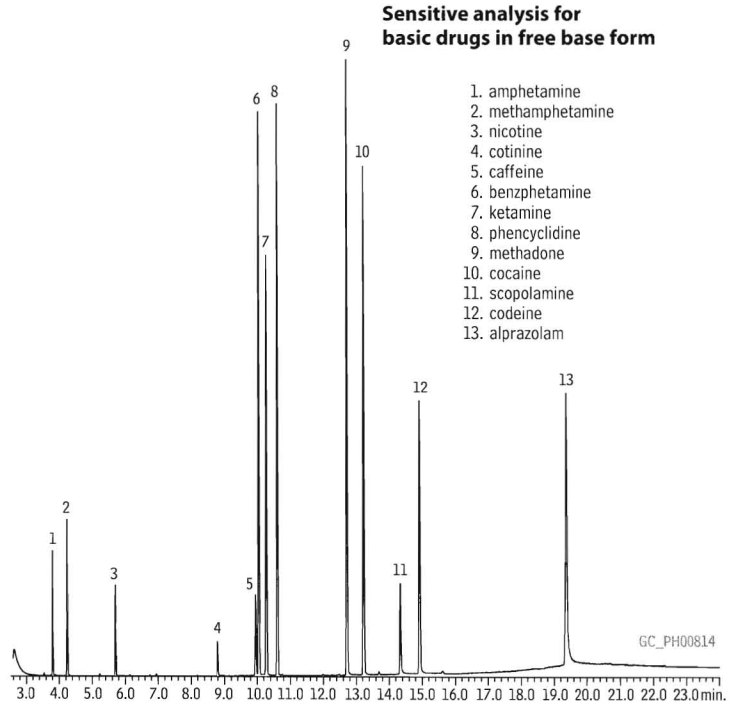
Overall, the results of these efforts are columns that define *unsurpassed inertness*, *ultra low bleed*, and *totally reliable column-to-column performance*.

Guaranteed Quality and Reliability

Restek is committed to supplying the most reliable GC columns in the industry. Every Rxi™ column is individually challenged to pass our stringent requirements for film thickness, coating efficiency, selectivity, inertness, and bleed. We believe Rxi™ column technology produces the most reliable columns available, anywhere, and we promise that every Rxi™ column you receive will be exactly as good as the one it replaces.

Figure 1 Analyze acidic drugs or basic drugs under the same conditions, using an Rxi™-5ms column.





Column:	Rxi™-5ms 30m, 0.25mm ID, 0.25µm (cat.# 13423)
Sample:	1000µg/mL each in methanol
Inj.:	1.0µL split (50:1), 20ng each compound on column; Siltek® treated 4mm gooseneck splitless inlet liner (cat # 20799-214.5)
Inj. temp.:	250°C
Carrier gas:	helium, constant pressure
Linear velocity:	30cm/sec.
Oven temp.:	100°C to 220°C @ 15°C/min., to 330°C @ 10°C/min. (hold 5 min.)
Det.:	Agilent 5973 MSD
Transfer line temp.:	300°C
Scan range:	35-550
Tune:	PFTBA
Ionization:	EI

Exempted Drug of Abuse Reference Materials

new!

1,000µg/mL in P&T methanol (*except where noted), 1mL/ampul

Compound	Individual cat.#
Benzodiazepines	
alprazolam	34042
bromazepam	34043
chlordiazepoxide HCL	34044
clobazam	34045
clonazepam	34046
diazepam	34047
flunitrazepam	34049
flurazepam di-HCL	34050
lorazepam	34051
nitrazepam	34053
oxazepam	34054
prazepam	34055
temazepam	34056
triazolam	34057
Cocaine & Metabolites	
cocaine HCL	34015
benzoylecgonine	34016
ecgonine	34017
ecgonine methyl ester	34018
Methadone & Metabolites	
methadone HCL	34005
Amphetamines & Metabolites	
d-amphetamine	34020
(+)methamphetamine	34021
Opiates & Metabolites	
codeine	34000
hydrocodone	34002
hydromorphone	34063
morphine	34006
oxycodone	34007
oxymorphone	34065
Cannabinoid & Metabolites	
cannabidiol	34011
cannabinol	34010
Barbiturates	
amobarbital	34028
aprobarbital	34029
barbital	34030
butabarbital	34031
butalbital	34032
DL-glutethimide	34058
hexobarbital	34033
mephobarbital	34034
methohexital	34035
pentobarbital	34036
phenobarbital	34037
secobarbital	34038
talbutal	34039
thiamylal	34040
thiopental	34041
Other	
benzphetamine	34022
cocaethylene*	34066
fenfluamine	34023
levorphanol	34003
meperidine	34004
meprobamate	34059
methaqualone	34064
methyprylon	34060
pentazocine	34062
phencyclidine	34027
phendimetrazine	34025
phenmetrazine	34026
phentermine	34024
dextro-propoxyphene	34008
thebaine	34009

Rxi™-5ms Column (fused silica)

new!

(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	13423

RP-HPLC Analysis of Selective Serotonin Reuptake Inhibitors

Using Allure™ Basix and Ultra PFP Polar Stationary Phases

By Rick Lake, Pharmaceutical Innovations Chemist

- Good retention and selectivity without ion-pairing chromatography.
- Practical at acidic pH (Ultra PFP phase) or neutral pH (Allure™ Basix phase).
- Improved peak shape for basic compounds, compared to alkyl phases.

Selective serotonin reuptake inhibitors (SSRIs) are a novel class of antidepressants that have gained much acceptance in the medical community. Although they have been found to be no more effective than the "older" tricyclic antidepressants, they produce fewer side effects. Historically, SSRIs have been analyzed using ion-pairing chromatography (IPC) on alkyl stationary phases (e.g., C18). IPC is a good alternative when reversed phase chromatography (RPC) on hydrophobic alkyl phases cannot provide adequate separation. IPC has disadvantages, however, including artifactual peaks, slow column equilibrium, poor peak shape, and incompatibility with MS detection. Because of these downsides to IPC, we evaluated the use of polar stationary phases, including the Allure™ Basix and Ultra PFP phases, for analysis of SSRIs.

The chemical structures of SSRIs (Figure 1) reveals that these compounds are polar bases capable of ionic separations. To ensure complete ionization, a pH value approximately 2 units from an analyte's pKa should be used. SSRIs have high pKa values (fluvoxamine maleate: 8.7, fluoxetine: 9.1, sertraline HCl: 9.5), however, and two pH units above these analytes' pKa values will be outside the acidic to neutral operating range for silica-based columns. Because SSRIs are basic, their retention can be increased by increasing the mobile phase pH. According to acid-base equilibria, as pH decreases, bases gain a proton (ionize), making them more hydrophilic and less retained by RPC. Thus, the greatest retention of SSRIs would occur at neutral pH, rather than at an acidic pH.

At neutral pH, an Allure™ Basix column shows good retention, selectivity, and peak shape for SSRIs (Figure 2). This stationary phase and pH are a good choice if optimum retention and selectivity are desired. Adding an amine modifier can alter selectivity and improve peak shape (Figure 3). As the concentration of amine modifier is increased, the retention of basic analytes decreases, and the peaks sharpen. This could be an effective way to produce alternate selectivity and enhance peak

Figure 1 Selective serotonin reuptake inhibitors (SSRIs) are a chromatographic challenge.

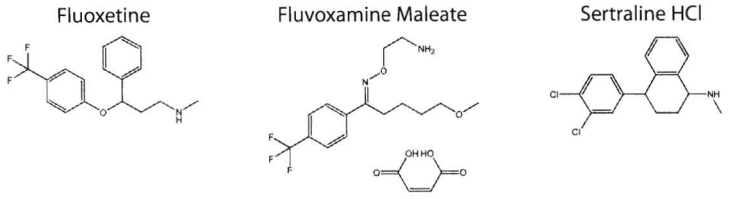
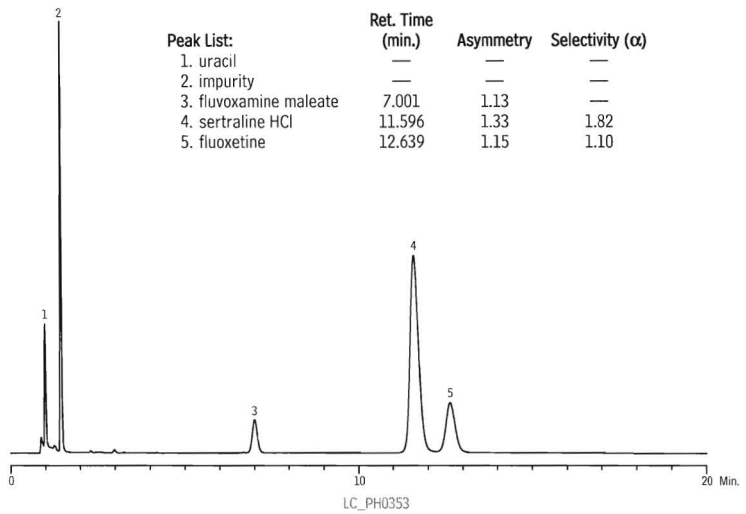


Figure 2 At neutral pH, an Allure™ Basix column provides good retention, selectivity, and peak shape for SSRIs.



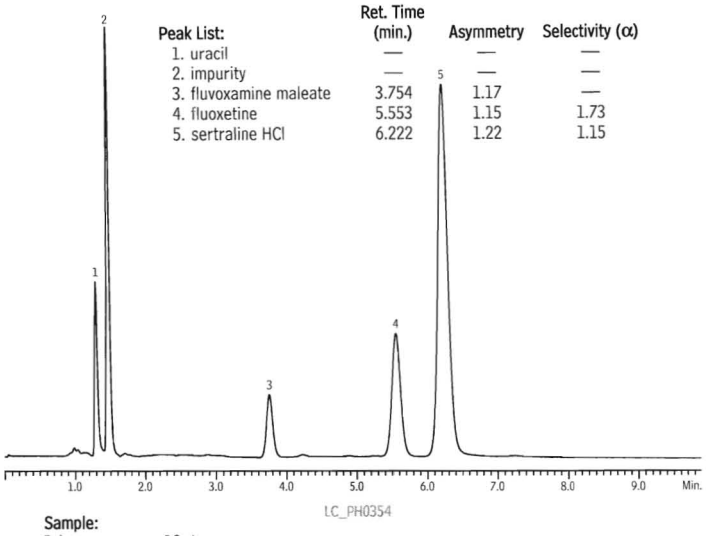
Peak List:	Ret. Time (min.)	Asymmetry	Selectivity (α)
1. uracil	—	—	—
2. impurity	—	—	—
3. fluvoxamine maleate	7.001	1.13	—
4. sertraline HCl	11.596	1.33	1.82
5. fluoxetine	12.639	1.15	1.10

Sample:
Inj.: 10µL
Conc.: 100µg/mL each component
Sample diluent: water:acetonitrile, 50:50

Column: **Allure™ Basix** (cat.# 9161565)
Dimensions: 150 x 4.6 mm
Particle size: 5µm
Pore size: 60Å

Conditions:
Mobile phase: 20mM potassium phosphate dibasic in water (pH 7):acetonitrile, 40:60
Flow: 1mL/min.
Temp.: 30°C
Det.: UV @ 230 nm

Figure 3 An amine modifier improves peak shape and changes selectivity for SSRIs on an Allure™ Basix column.

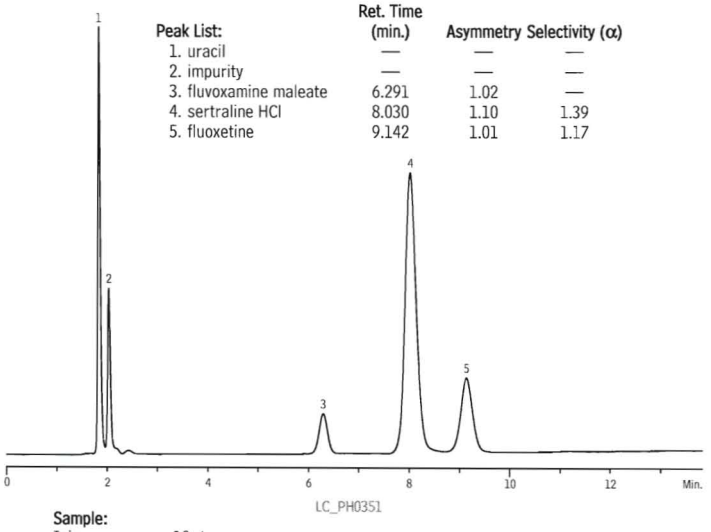


Sample:
Inj.: 10µL
Conc.: 100µg/mL each component
Sample diluent: water:acetonitrile, 50:50

Column: Allure™ Basix (cat.# 9161565)
Dimensions: 150 x 4.6 mm
Particle size: 5µm
Pore size: 60Å

Conditions:
Mobile phase: 1% triethylamine in water (pH 6):acetonitrile, 50:50
Flow: 1mL/min.
Temp.: 30°C
Det.: UV @ 230 nm

Figure 4 An Ultra PFP column shows good performance at acidic pH.



Sample:
Inj.: 10µL
Conc.: 100µg/mL each component
Sample diluent: water:acetonitrile, 50:50

Column: Ultra PFP (cat.# 9176565)
Dimensions: 150 x 4.6 mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 20mM potassium phosphate monobasic in water (pH 3):acetonitrile, 70:30
Flow: 1mL/min.
Temp.: 30°C
Det.: UV @ 230 nm

shape in reversed phase mode. However, amine additives work by blocking ionizable silanols, which can vary from column to column, so be sure the amine concentration is high enough to suppress all potential silanol effects.

The Ultra PFP phase shows the best performance at an acidic pH (Figure 4).

These analyses reveal that polar stationary phases can effectively replace IPC in analyses of SSRIs. Overall, in RPC, polar stationary phases provide better peak shape than alkyl phases for basic analytes. When analyzing SSRIs at neutral pH, the Allure™ Basix phase is a good choice. When analyzing SSRIs at an acidic pH, the Ultra PFP phase is the better candidate.

Allure™ Basix Column

5µm Column, 4.6mm	cat. #
150mm	9161565

Ultra PFP Column

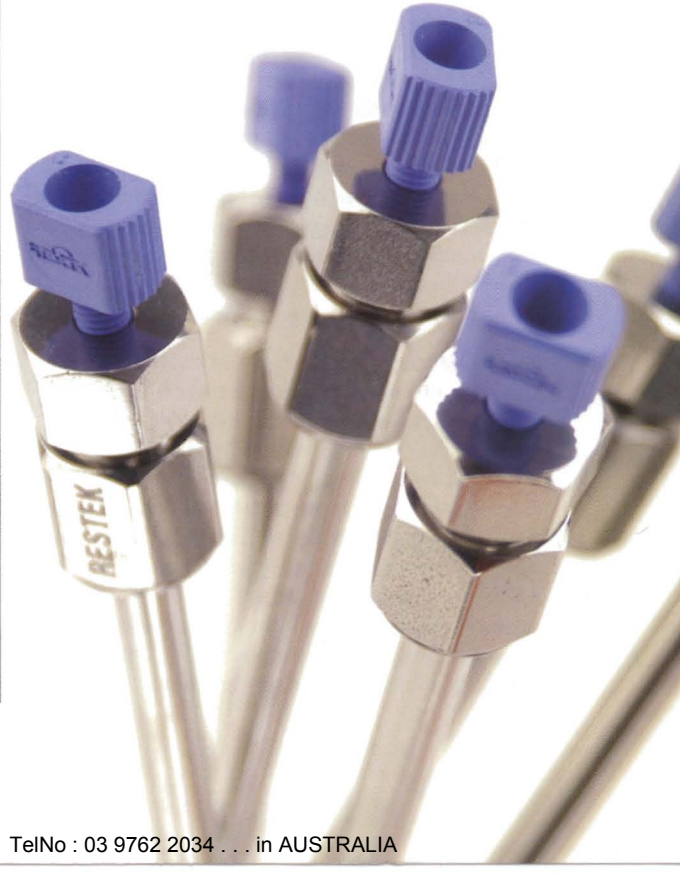
5µm Column, 4.6mm	cat. #
150mm	9176565

ordering note

To order a 2.1mm, 3.2mm, or 4.6mm ID column with a Trident™ Integral Inlet Fitting, add "-700" to the catalog number for the column.

Nominal additional charge

For guard cartridges and XG-XF guard cartridge fittings for these columns, visit our website at **www.restek.com**.



Assaying Tetracyclines by HPLC

Using the Allure™ Biphenyl Stationary Phase

By Rick Lake, Pharmaceutical Innovations Chemist, and Sherry Moyer, Innovations Chemist

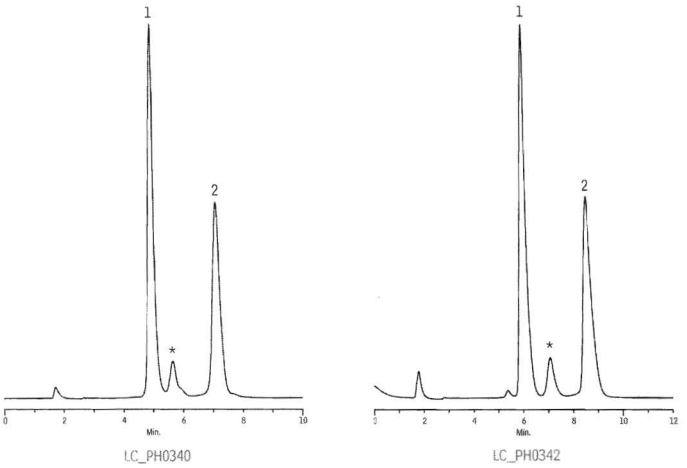
- Superior selectivity and efficiency, using an Allure™ Biphenyl column.
- Simplified analysis for high-throughput potency and stability-indicating assays.
- More easily achievable system suitability criteria.

Tetracyclines are a widely used class of antibiotics whose applications range from topical acne medications for humans to premix feed additives for livestock. Because of their widespread and liberal use, tetracyclines are manufactured in large quantities, which generates the need for a large number of potency and stability-indicating assays. These assays must be completed at regular intervals, in a timely manner, over extended periods of time. Consequently, it is critical that simple, rugged, and selective methods be developed. By selecting a stationary phase that produces optimum selectivity, less demand to produce selectivity is placed on the mobile phase, and a simple isocratic analysis is possible. Among the stationary phases we tested, the Allure™ Biphenyl and Allure™ PFP Propyl stationary phases showed the best performance (Table 1 and Figure 1).

Developing a simple mobile phase for this application was a major concern. Ideally, to achieve ionization equilibrium, choose a mobile phase pH 2 units from the analytes' pKa. But two units below the pKa values for the tetracyclines (approximately 3.3) would be below the recommended pH limit for traditional silica-based columns, pH 2. Consequently, we chose a pH of 2.5, and we added a buffer to maintain pH. Because tetracyclines form chelates with metal ions, we chose a non-metal organic salt - ammonium phosphate - and, to minimize surface metal content, we used only columns made from high-purity Type B silica. Lastly, we chose acetonitrile as the organic solvent, because of its eluting strength and limited effect on pKa: increasing the organic composition increases pKa for acidic analytes and decreases pKa for basic analytes, but a small amount of acetonitrile lessens the effect, relative to a larger amount of methanol.

We evaluated several silica-based stationary phases, using the mobile phase described above, UV detection, and isocratic conditions. The first selection criteria we used was selectivity, which we measured by analyzing oxytetracycline and tetracycline (an impurity in oxytetracycline formulations) and determining the USP resolution and selectivity (α) between the two compounds. The Allure™ Biphenyl and Allure™ PFP Propyl stationary phases showed the best performance among the columns we tested (Table 1 and Figure 1). These results suggest that the Allure™ Biphenyl

Figure 1 Tetracycline and oxytetracycline have low pKa values that make the analysis a challenge.



Peak List	Ret. Time (min.)	Peak List	Ret. Time (min.)
1. oxytetracycline	4.92	1. oxytetracycline	5.94
* degradation peak	5.66	* degradation peak	7.08
2. tetracycline	7.10	2. tetracycline	8.57

Column:	Allure™ Biphenyl	Column:	Allure™ PFP Propyl
Cat. #:	9166565	Cat. #:	9169565
Dimensions:	150 x 4.6 mm	Dimensions:	150 x 4.6 mm
Particle size:	5μm	Particle size:	5μm
Pore size:	60Å	Pore size:	60Å

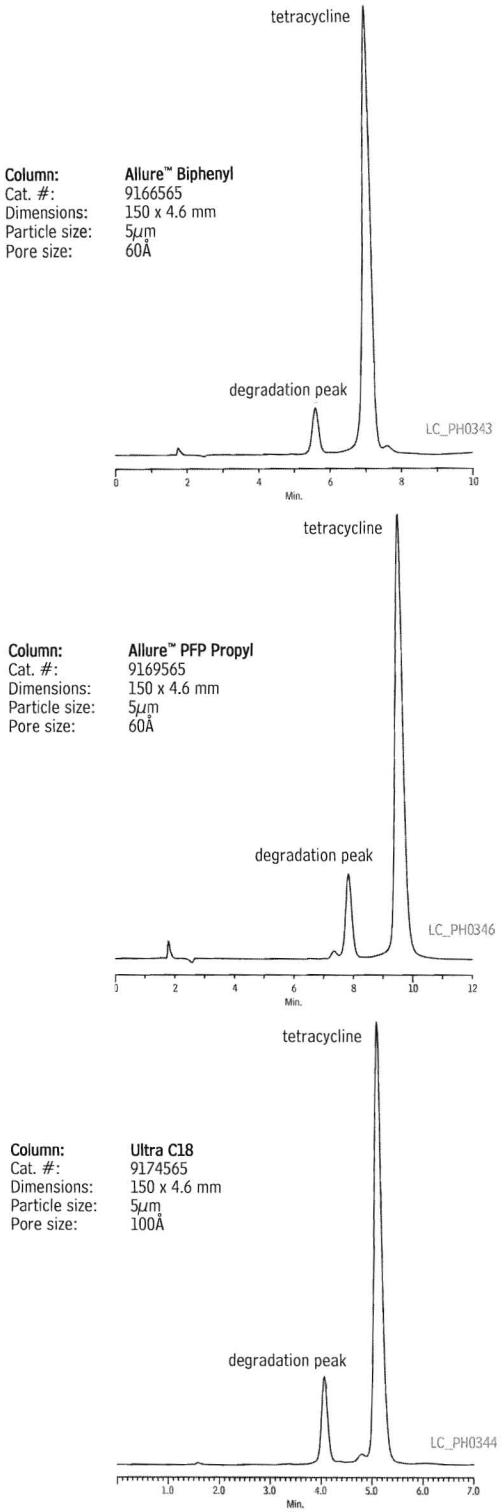
Sample:
Inj.: 20μL (Allure™ Biphenyl column) or 30μL (Allure™ PFP Propyl column)
Conc.: 100μg/mL each component
Sample diluent: methanol

Conditions:
Mobile phase: 20mM ammonium phosphate (pH 2.5):acetonitrile, 80:20
Flow: 1mL/min.
Temp.: ambient
Det.: UV @ 254 nm

Table 1 Among tested columns, Allure™ Biphenyl and Allure™ PFP Propyl columns show the best combination of resolution and selectivity for tetracycline and oxytetracycline.

Stationary Phase	USP Resolution	Selectivity (α)
Allure™ Biphenyl	5.28	1.61
Allure™ PFP Propyl	4.49	1.59
Ultra C18	3.31	1.50
Allure™ Basix	NA	1.34
Ultra C8	NA	0.47
Ultra PFP	NA	NA

Figure 2 Overall, the Allure™ Biphenyl column is the best choice for assaying the tetracycline antibiotics.



Sample:
Inj.: 5µL
Conc.: 100µg/mL each component
Sample diluent: methanol

Conditions:
Mobile phase: 20mM ammonium phosphate (pH 2.5):acetonitrile, 80:20
Flow: 1mL/min.
Temp.: ambient
Det.: UV @ 254 nm

Table 2 Allure™ Biphenyl, Allure™ PFP Propyl, and Ultra C18 columns provide excellent repeatability.

	Peak Area	Retention Time (min.)	Capacity Factor (k')	USP Tailing
Allure™ Biphenyl	best choice for tetracycline antibiotics			
Mean	2509475	7.08	5.68	1.06
Std. Dev.	36397.39	0.02	0.03	0.01
%RSD	1.45	0.36	0.45	0.49
Allure™ PFP Propyl				
Mean	2483972	9.52	8.12	1.28
Std. Dev.	22202.94	0.04	0.04	0.01
%RSD	0.89	0.45	0.53	0.43
Ultra C18				
Mean	2399803	5.13	3.73	1.21
Std. Dev.	21171.76	0.02	0.02	>0.00
%RSD	0.88	0.33	0.45	0.34

stationary phase exhibits π-π bonding with the ring structures of the tetracyclines, and the embedded polarity of the fluorinated Allure™ PFP Propyl phase interacts with tetracycline moieties. Either of these separation mechanisms increases retention, compared to a mechanism based on hydrophobicity, as exhibited by the alkyl chain of a C18 phase.

Tetracycline drug products are produced under cGMP protocols and, therefore, manufacturers are required to use validated or compendial methods, either of which require the completion of system suitability criteria (e.g., tailing factors, capacity factors, and repeatability). Consequently, we further evaluated the three stationary phases that produced the best initial results, using system suitability criteria, by assaying tetracycline.

Overall, all three columns provided excellent repeatability (Table 2). The Allure™ PFP Propyl column exhibited the greatest retention and capacity for the analytes, but exhibited the highest degree of peak tailing under these conditions (Figure 2). The Ultra C18 column also exhibited a high degree of peak tailing, and the weakest analyte retention (Figure 2). Altering the mobile phase likely would improve peak shape, but capacity factors would suffer accordingly. The Allure™ Biphenyl column proved to be the best overall choice for the tetracyclines - it exhibited good capacity, high selectivity, and the least peak tailing (Figure 2).

By selecting the stationary phase that provides the best selectivity and efficiency for tetracycline analytes - the Allure™ Biphenyl phase - analysts can exercise more control over separation and other method conditions, ultimately creating a simple, rugged, and selective method.

Allure™ Biphenyl Column

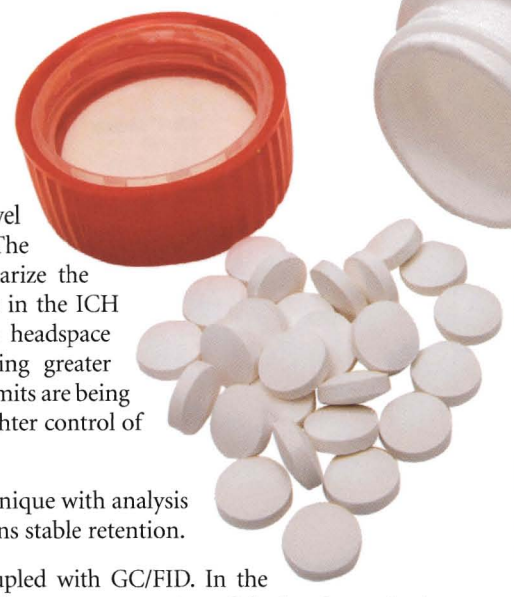
5µm Column, 4.6mm	cat. #
150mm	9166565

Analyzing Residual Solvents in Water-Soluble Articles

Dynamic Headspace Sampling Enhances Sensitivity by GC

By Rick Lake, Pharmaceutical Innovations Chemist

- Sensitivity increased 13X-30X for residual solvents (OVIs) in water.
- Excellent resolution and stable retention times, using an Rtx®-G43 column.
- Greater sensitivity makes smaller samples possible.



Residual solvents, or organic volatile impurities (OVIs), in pharmaceuticals are trace-level leftover solvents that were used in the manufacture of drug products or excipients. The International Conference on Harmonization (ICH) provides guidelines that summarize the allowable concentrations of common solvents. However, some of the detection limits in the ICH guidelines are not easily achieved through the normal sampling technique, static headspace analysis, and pharmaceutical manufacturers are becoming concerned with attaining greater sensitivity. As more toxicity data become available, maximum allowable concentration limits are being lowered. And, as active ingredient and excipient markets are becoming more global, tighter control of impurities is needed.

In our investigations, we have found that coupling a dynamic headspace sampling technique with analysis on an Rtx®-G43 column greatly increases sensitivity for residual solvents, and maintains stable retention.

Analyses for residual solvents typically are performed using headspace sampling coupled with GC/FID. In the commonly used static headspace technique, a pressurized or ballast loop system is used to extract a portion of the headspace in the sample vial for introduction into the GC. Another, more novel, technique for headspace sampling is the dynamic headspace technique. In this technique, the entire content of the vial headspace is swept onto an activated trap, which collects and concentrates the target analytes, then desorbs the analytes into the GC carrier flow. Dynamic headspace increases the sensitivity of the analysis, but high concentrations of organic solvents will cause contamination and lifetime problems with the trap and, therefore, this technique is not compatible with the use of organic solvents as diluents for water-insoluble articles. On the other hand, the technique is well suited to, and easily performed in, analyses of residual solvents in water-soluble articles.

We evaluated the sensitivity of the static and dynamic headspace techniques, using solvents in an aqueous matrix, to compare responses as they might relate to pharmaceutical analysis of residual solvents in water-soluble articles. We prepared reference standards containing the USP467 solvents at their regulatory limits in water, by adding 100µL of our USP 467 Calibration Mix #5 (cat.# 36007) to 5mL of deionized water in a 22mL headspace sampling vial. We also added approximately 1 gram of an inorganic salt, sodium sulfate, to each sample to decrease the solubility of polar compounds. This is critical for highly water-soluble volatiles, like 1,4-dioxane, as it promotes analyte transfer into the gaseous phase in the sample vial.

First, we used a traditional static headspace (loop) technique to assay a system suitability set comprised of 6 replicates (Figure 1A). The sample vial was heated, mixed, and pressurized. A six-port valve was used to fill a specified loop volume with an aliquot of the headspace, then the valve was switched to redirect the gas flow, flushing the sample into the transfer line and ultimately mixing with the GC carrier gas flow. Next, we used a dynamic headspace (trap) technique to analyze an equivalent 6-replicate system suitability set (Figure 1B). The sample vial was heated and mixed under the same conditions as used in the loop method, then a gas flow was introduced into the headspace

Table 1 Dynamic headspace sampling greatly increases sensitivity for OVIs.

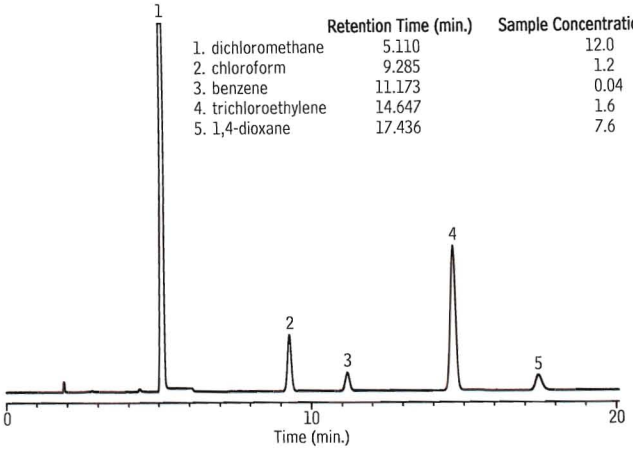
Analyte	Sample Conc. (ppm)	Concentration at Regulatory Limit (ppm)	Mean Peak Area Response		Increase in Sensitivity with Dynamic Headspace
			Static Headspace	Dynamic Headspace	
dichloromethane	12.0	600	619	18679	30X
chloroform	1.2	60	39	783	20X
benzene	0.04	2	15	313	21X
trichloroethene	1.6	80	141	3479	25X
1,4-doxane	7.6	380	20	272	13X

Table 2 Solvent retention times and resolution are equivalent for static or dynamic headspace sampling and analysis on an Rtx®-G43 column.

Solvent		Static Headspace		Dynamic Headspace	
		Retention Time (min.)	Resolution	Retention Time (min.)	Resolution
dichloromethane	Mean	5.092	—	5.139	—
	Std. Dev.	0.01	—	>0.00	—
	%RSD	0.25	—	0.04	—
chloroform	Mean	9.250	23.02	9.263	22.18
	Std. Dev.	0.02	0.26	>0.00	0.07
	%RSD	0.23	1.11	0.04	0.31
benzene	Mean	11.134	7.67	11.145	7.72
	Std. Dev.	0.03	0.08	>0.00	0.01
	%RSD	0.23	1.04	0.03	0.11
trichloroethene	Mean	14.592	11.87	14.599	11.86
	Std. Dev.	0.03	0.06	>0.00	0.01
	%RSD	0.23	0.46	0.04	0.10
1,4-dioxane	Mean	17.388	7.91	17.411	—
	Std. Dev.	0.04	0.10	0.09	—
	%RSD	0.20	1.23	0.50	—

Figure 1 USP 467 solvents by headspace analysis/GC on an Rtx®-G43 column.

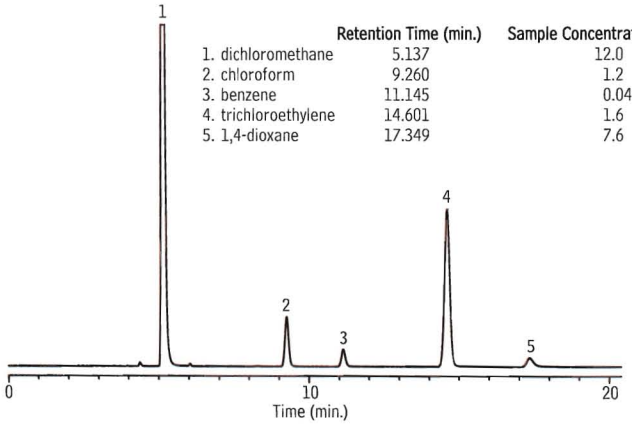
A) Static headspace (loop) technique



	Retention Time (min.)	Sample Concentration (µg/ml)
1. dichloromethane	5.110	12.0
2. chloroform	9.285	1.2
3. benzene	11.173	0.04
4. trichloroethylene	14.647	1.6
5. 1,4-dioxane	17.436	7.6

Instrument:	Teledyne Tekmar HT3	Platen/Sample Temp.:	80°C
Sample Equilibr. Time:	15.00 min.	Platen Temp. Equilibr. Time:	2.00 min.
Mixing:	level 5, 2.00 min.	Pressurize:	15 psi, 2.00 min.
Mixer Stabilize Time:	0.50 min.	Pressurize Equilibr. Time:	0.50 min.
Valve Oven Temp.:	150°C	Loop Fill Pressure:	5 psi
Transfer Line Temp.:	150°C	Loop Fill Time:	2.00 min.
Standby Flow Rate:	10mL/min.	Loop Fill Equilibr. Time:	0.50 min.
		Inject Time:	1.00 min.

B) Dynamic headspace (trap) technique



	Retention Time (min.)	Sample Concentration (µg/ml)
1. dichloromethane	5.137	12.0
2. chloroform	9.260	1.2
3. benzene	11.145	0.04
4. trichloroethylene	14.601	1.6
5. 1,4-dioxane	17.349	7.6

Instrument:	Teledyne Tekmar HT3	Trap Standby Temp.:	40°C
Sample Equilibr. Time:	15.00 min.	Platen/Sample Temp.:	80°C
Mixing:	level 5, 2.00 min.	Sweep Flow:	75mL/min., 3.00 min.
Mixer Stabilize Time:	0.50 min.	Dry Purge:	50mL/min., 5.00 min., 25°C
Valve Oven Temp.:	150°C		
Transfer Line Temp.:	150°C	Desorb Preheat:	245°C
Standby Flow Rate:	10mL/min.	Desorb:	1.00 min., 250°C
		Trap Bake:	450mL/min., 6.00 min., 300°C

Chromatography Conditions

Column:	Rtx®-G43 30m, 0.53mm ID, 3.0µm (cat.# 16085-126)
Sample:	100µL USP 467 Calibration Mixture #5 (cat.# 36007) in dimethylsulfoxide, 5mL water, ~1.0g sodium sulfate in 22mL headspace vial. Concentrations listed on figure.
Inj.:	static headspace or dynamic headspace
Inj. temp.:	180°C
Carrier gas:	helium, split 2:1
Linear velocity:	5mL/min., constant flow
Oven temp.:	40°C (20 min.), to 240°C @ 25°C/min. (hold 10 min.)
Det.:	FID @ 250°C hydrogen flow: 40mL/min.; air flow: 450mL/min.; make-up flow: 45mL/min.

of the vial, to sweep the analytes onto an activated trap. The trap, with the concentrated analytes, was dry purged to remove the water vapor, then was heated without flow to desorb the analytes. After the analytes were desorbed, the trap was back-flushed to direct the concentrated analytes onto the analytical column. Between analyses, the trap was baked at high temperature to remove all residue compounds.

When we compared the results of the system suitability analyses for the two headspace techniques, we determined that, based on area responses, the dynamic headspace method greatly enhanced sensitivity for the target OVIs: area counts were, on average, 22 times larger than for the static headspace method (Table 1). We also noted that the Rtx®-G43 capillary column provided excellent resolution among analytes, with very little drift in retention time or resolution (Table 2).

As with purge and trap systems, or other dynamic sampling systems, certain system controls must be taken into account when using a dynamic headspace technique. Factors to consider include sweeping and desorbing times and flows, adsorbent materials used to trap the analytes, and water management. In this specific application, we observed that either prolonged sample heating at 80°C or extended vial sweep times increased the water content in the sample headspace, ultimately resulting in poor peak shape for 1,4-dioxane and, if excessive, extinguishing the FID. 1,4-Dioxane has a notoriously poor partitioning efficiency and proved to be the limiting factor when setting system operating conditions. For samples heated at 80-85°C in a water matrix, a sweep time of 5 minutes or less enhanced sensitivity for all compounds while assuring proper water management.

From this work, we conclude that coupling a dynamic headspace sampling technique with analysis on an Rtx®-G43 column greatly increases sensitivity for residual solvents, and makes stable retention possible. These enhancements can lead to more achievable system suitability criteria and lower detection limits, or to effective results with smaller samples.

Rtx®-G43 Column
(fused silica with 5-meter Integra-Guard™)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.53mm	3.00	-20 to 240°C	30-Meter	16085-126

trans Fat: Resolving *cis* and *trans* FAME Isomers by GC

By Julie Kowalski, Innovations Chemist

- Highly polar Rt-2560 column resolves individual *cis* and *trans* FAME isomers.
- Analytical reference mixes for quantifying FAMEs in foods and dietary supplements.
- Use column and reference mixes to meet new *trans* fat labeling regulations.

Concern over the detrimental effects of diets high in *trans* fats has prompted the US Food and Drug Administration (FDA) to require *trans* fat content to be reported separately on food labels after January 2006. The FDA estimates that by 2009 this rule will save $900 million to $1.8 billion per year in medical costs and lost productivity. The monetary savings will far more than offset the FDA-estimated $140-250 million in one-time costs of determining amounts of *trans* fats, revising Nutrition Facts panels, and voluntarily reducing amounts of *trans* fats[1] that the food industry will incur to comply with the rule.

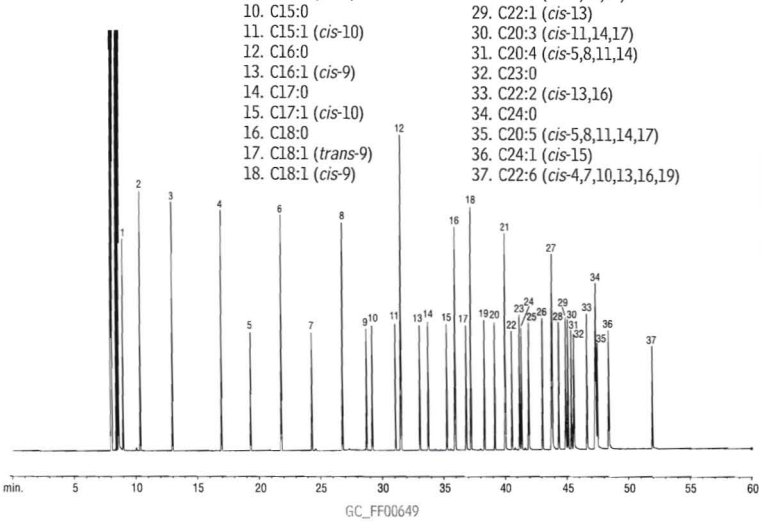
The highly polar Rt-2560 biscyanopropyl stationary phase has the selectivity needed for resolving *cis* and *trans* FAME isomers to comply with the FDA guidelines. Individual *cis* and *trans* isomers are resolved on a 100-meter Rt-2560 GC column (cat.# 13199), making this the column of choice for analyzing partially hydrogenated fats. The *trans* isomers elute before the *cis* isomers (Figure 1), a reverse of the elution order on Carbowax®-based phases such as FAMEWAX™ or Rtx®-Wax. AOAC method 996.06[2] specifies the determination of total fat content based on the fatty acid content after conversion of the fatty acids to the methyl esters, and is the accepted analytical method for determining total fat content for nutritional labeling. A 100-meter Rt-2560 column meets the requirements of this procedure, and also allows quantification of the total *trans* fat content.

To calibrate the GC system for these assays, we recommend a carefully formulated FAME mixture, such as our 37-component Food Industry FAME Mix (cat.# 35077, Figure 1) or our 28-component NLEA FAME Mix (cat.# 35078). Each of these mixes includes a gravimetric certificate of analysis to help ensure accurate quantification. To ensure correct identifications of individual C18:1 *cis* or *trans* isomers, use our *cis*/*trans* FAME Mix (cat.# 35079), as shown in Figure 1.

An Rt-2560 column is the column of choice when determining *trans* fat content and total fat content in food products. Whatever your fatty acid analysis requirements, Restek can provide the consistent-performance analytical columns and reference materials that will help you to accurately characterize your materials.

Figure 1 Food Industry FAME Mix (cat.# 35077) resolved on an Rt-2560 column.

Compound		
1. C4:0	14. C17:0	27. C22:0
2. C6:0	15. C17:1 (*cis*-10)	28. C20:3 (*cis*-8,11,14)
3. C8:0	16. C18:0	29. C22:1 (*cis*-13)
4. C10:0	17. C18:1 (*trans*-9)	30. C20:3 (*cis*-11,14,17)
5. C11:0	18. C18:1 (*cis*-9)	31. C20:4 (*cis*-5,8,11,14)
6. C12:0	19. C18:2 (*trans*-9,12)	32. C23:0
7. C13:0	20. C18:2 (*cis*-9,12)	33. C22:2 (*cis*-13,16)
8. C14:0	21. C20:0	34. C24:0
9. C14:1 (*cis*-9)	22. C18:3 (*cis*-6,9,12)	35. C20:5 (*cis*-5,8,11,14,17)
10. C15:0	23. C20:1 (*cis*-11)	36. C24:1 (*cis*-15)
11. C15:1 (*cis*-10)	24. C18:3 (*cis*-9,12,15)	37. C22:6 (*cis*-4,7,10,13,16,19)
12. C16:0	25. C21:0	
13. C16:1 (*cis*-9)	26. C20:2 (*cis*-11,14)	



Rt-2560, 100m, 0.25mm ID, 0.2µm (cat.# 13199)
Sample: Food Industry FAME Mix (cat.# 35077), 30mg/mL total FAMEs in methylene chloride
Inj.: 2.0µL split (split ratio 200:1), 4mm inlet liner (cat.# 20814)
Inj. temp.: 225°C
Carrier gas: hydrogen, constant flow
Flow rate: 1.2mL/min.
Oven temp.: 100°C (4 min. hold) to 240°C @ 3°C/min. (10 min. hold)
Det.: FID @ 250°C

References
[1] *http://www.cfsan.fda.gov/~dms/qatrans2.html#s5q1*
[2] *Official Methods of Analysis, 17th edition, AOAC International, 2000.*

free literature

High Resolution Analyses of Fatty Acid Methyl Esters (FAMEs) by Gas Chromatography

lit. cat.# 59584A

Free on request from Restek, or from your Restek distributor.

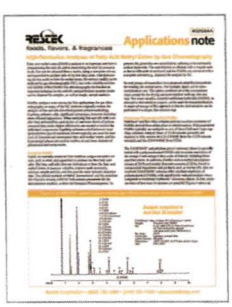
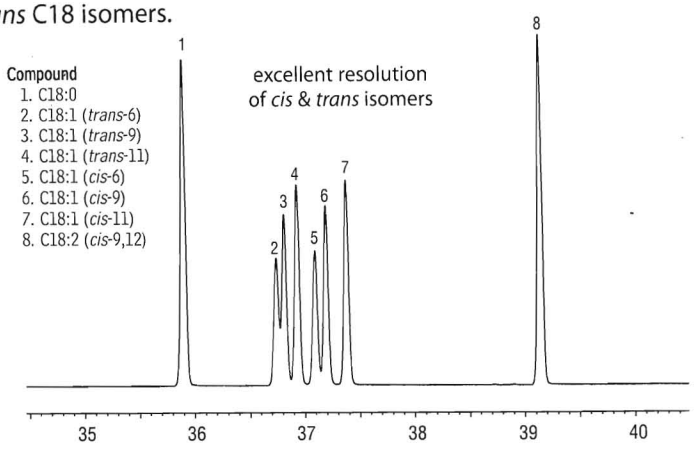


Figure 2 An Rt-2560 column resolves *cis* C18 FAME isomers from *trans* C18 isomers.



Rt-2560, 100m, 0.25mm ID, 0.2μm (cat.# 13199)
Sample: *cis/trans* FAME Mix (cat.# 35079), 10mg/mL total FAMEs in methylene chloride
Inj.: 1.0μL split (split ratio 20:1), 4mm inlet liner (cat.# 20814)
Inj. temp.: 225°C
Carrier gas: hydrogen, constant flow
Flow rate: 1.2mL/min.
Oven temp.: 100°C (4 min. hold) to 240°C @ 3°C/min. (10 min. hold)
Det.: FID @ 250°C

Rt-2560 column (fused silica)

(biscyanopropyl polysiloxane)

ID	df (μm)	temp. limits	length	cat. #
0.25mm	0.20	20 to 250°C	100-Meter	13199

Food Industry FAME Mix (37 components)
30mg/mL total in methylene chloride, 1mL/ampul
cat. # 35077 (ea.)

NLEA FAME Mix (28 components)
30mg/mL total in methylene chloride, 1mL/ampul
cat. # 35078 (ea.)

cis/trans **FAME Mix** (8 components)
10mg/mL total in methylene chloride, 1mL/ampul
cat. # 35079 (ea.)

For analysis of NLEA FAME Mix on an Rt-2560 column, please refer to our 2006 catalog or visit our website.

Genuine Restek Replacement Parts for Shimadzu HPLC Systems

By Becky Wittrig, Ph.D., HPLC Product Marketing Manager

- Keep your Shimadzu HPLC systems in top condition!
- All parts designed to meet or exceed original equipment performance.
- New items constantly being added - check our website for our complete HPLC product offering.

Restek Replacement Parts for Shimadzu HPLC Systems

Description		Model #	Similar to Shimadzu part #	qty.	cat.#
Inlet Check Valve		LC-6A, LC-10AS	228-12353-91	ea.	25287
Inlet Check Valve		LC-600, LC-9A, LC-10AD	228-18522-91	ea.	25295
Inlet Check Valve	new!	LC-10ADvp	228-39093-92	ea.	24984
Outlet Check Valve		LC-6A, LC-10AS	228-09054-93	ea.	25288
Outlet Check Valve Rebuild Kit		LC-6A, LC-10AS	228-11200-91	2-pk.	25289
Outlet Check Valve		LC-600, LC-9A, LC-10AD	228-18522-92	ea.	25282
Outlet Check Valve	new!	LC-10ADvp, LC-10ATvp	228-34976-91	ea.	24983
Plunger Seal		LC-6A	228-11999-00	ea.	25285
Plunger Seal, Polyethylene		LC-10AS	228-21975-00	ea.	25290
Plunger Seal		LC-600, LC-9A, LC-10AD	228-18745-00	ea.	25293
Plunger Seal	new!	LC-10ADvp	228-35146-00	ea.	24980
Plunger Seal, Gold	new!	LC-10ADvp	228-32628-00	ea.	24981
Plunger Seal	new!	SIL-10ADvp, LC-10ATvp	228-35145-00	ea.	24985
Plunger Rinse Seal		LC-10AS	228-28499-00	ea.	25292
Sapphire Plunger		LC-6A	228-12904-93	ea.	25286
Sapphire Plunger		LC-10AS	228-17019-93	ea.	25291
Sapphire Plunger		LC-600, LC-9A, LC-10AD	228-18523-91	ea.	25294
Needle Seal		SIL-10A, 10XL, 10ADvp	228-33355-04	ea.	25468
Rotor Seal	new!	SIL-10ADvp	228-21217-97	ea.	24986
Rotor Seal Assembly		SIL-10A, 10AXL	228-21217-91	ea.	25469
Stator Assembly		SIL-10A, 10AXL	228-21220-91	ea.	25470
Syringe, 500μL		SIL-10A, 10AXL	228-25237-04	ea.	25471
Plunger Assembly, Ceramic		LC-10ADvp	228-35601-91	ea.	25472
Plunger Assembly, Ceramic		LC-10ATvp	228-35009-92	ea.	25473
Plunger Assembly, Sapphire	new!	LC-10ADvp	228-35601-92	ea.	24982
Deuterium Lamp		SPD-6A	062-65056-02	ea.	25283
Deuterium Lamp		SPD-10A, 10AV	228-34016-02	ea.	25284

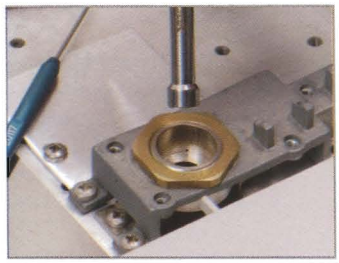
COOL TOOLS!

Restek Innovations Save You Time and Money

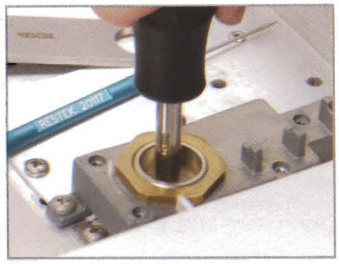
FID Jet Removal Tool for Agilent 5890/6890/6850 FIDs

- Securely grips jet in socket for easy removal or installation.
- Unique, ergonomic handle—easy to hold.

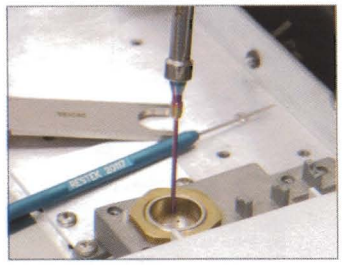
restek innovation!



Slip tool over FID jet...



loosen jet...



and remove.

Description	qty.	cat.#
FID Jet Removal Tool for Agilent 5890/6890/6850 FIDs	ea.	22328

Septum Nut Removal Tool for Agilent 5890/6890/6850 GCs

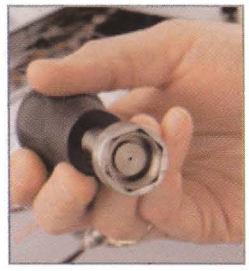
- Easily remove the septum nut without touching the heated nut—no more burned fingers!
- Unique, ergonomic handle—easy to grip.

restek innovation!

Slip tool over septum nut...

loosen nut...

and remove, avoiding hot metal surfaces.



Septum nut remains in tool until reinstalled.

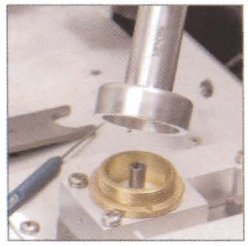
Description	qty.	cat.#
Septum Nut Removal Tool for Agilent 5890/6890/6850 GCs	ea.	24918

Spanner Wrench for Agilent 5890/6890/6850 FID Collector Assembly

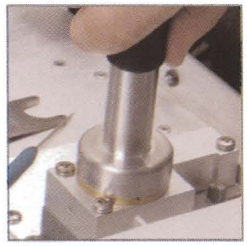
- Easily remove the nut from the FID collector without damaging the nut.
- Unique, ergonomic handle—easy to grip.

restek innovation!

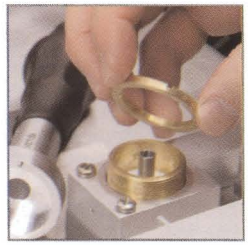
Remove FID ignitor castle.



Easily loosen nut by aligning two pins on bottom of wrench with two open slots on nut...



turn counterclockwise...



and remove.

Description	Replaces Agilent part #	qty.	cat.#
Spanner Wrench for Agilent 5890/6890/6850 FID Collector Assembly	19231-00130	ea.	22329

Headspace Vials;
Hand-held, Rechargeable, Crimpers and Decappers

By Donna Lidgett, GC Accessories Product Marketing Manager

Headspace Autosampler Vials

Description	100-pk.	1000-pk.
6mL Clear Vial	21166	21167
10mL Clear Vial, Flat Bottom	24683	24684
10mL Clear Vial, Rounded Bottom	21164	21165
20mL Clear Vial, Flat Bottom	24685	24686
20mL Clear Vial, Rounded Bottom	21162	21163
27mL Clear Vial	21160	21161

20mm Aluminum Seals w/Septa, Assembled

Description	100-pk.	1000-pk.
Silver Seal w/ PTFE/Gray Butyl Rubber	21761	21762
Silver Seal w/ PTFE/Silicone	21763	21764
Pressure Release Silver Seal w/ PTFE/Gray Butyl Rubber Septum	21765	21766
Pressure Release Silver Seal w/ PTFE/Silicone Septum	21767	21768

Hand-held Rechargeable Crimpers and Decappers

- Easy to use; comfortable grip.
- Hundreds of operations from one charge.
- Adjustable crimping force.

Powered by a standard Black & Decker Versapak® battery, these electronic tools for 11mm or 20mm caps will cycle hundreds of times on a single charge. The cycle is controlled precisely, through an internal counter, and is adjusted with two buttons on the side of the case. The tools fit comfortably in the hand and weigh approximately 600 grams. The jaws can be positioned easily around closely-spaced vials in standard autosampler trays. Each kit includes the tool, a Versapak® Gold rechargeable battery, which uses environmentally friendly NiMH technology, and a charger. Recharging generally takes 6-9 hours.

new!

Decapping has never been easier!

one...

two...

three!

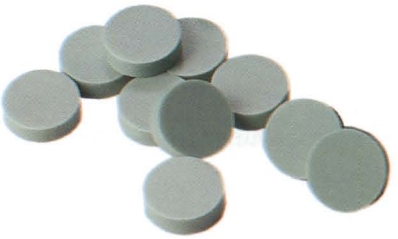
The Electronic Crimper fits around vials in standard autosampler trays. The adjustable setting provides a precision crimp, vial after vial.

Description	qty.	cat.#
11mm Electronic Crimper (110 volt battery charger)	kit	22853
11mm Electronic Crimper (220 volt European battery charger)	kit	22853-EUR
11mm Electronic Crimper (220 volt UK battery charger)	kit	22853-UK
11mm Electronic Decapper (110 volt battery charger)	kit	22854
11mm Electronic Decapper (220 volt European battery charger)	kit	22854-EUR
11mm Electronic Decapper (220 volt UK battery charger)	kit	22854-UK
20mm Electronic Crimper (110 volt battery charger)	kit	22851
20mm Electronic Crimper (220 volt European battery charger)	kit	22851-EUR
20mm Electronic Crimper (220 volt UK battery charger)	kit	22851-UK
20mm Electronic Decapper (110 volt battery charger)	kit	22852
20mm Electronic Decapper (220 volt European battery charger)	kit	22852-EUR
20mm Electronic Decapper (220 volt UK battery charger)	kit	22852-UK

Peak Performers

Avoid Septum Problems

By Donna Lidgett, GC Accessories Product Marketing Manager



- Handle septa carefully, to prevent contamination.
- Use low-bleed septa.

handy septum size chart

Instrument	Septum Diameter (mm)
Agilent (HP)	
5880A, 5890, 6890, 6850, PTV	11
5700, 5880	9.5/10
On-Column Injection	5
CE Instruments (TMQ)	
TRACE™ GC	17
Finnigan (TMQ)	
GC 9001	9.5
GCQ	9.5
GCQ w/TRACE™, PTV	17
QCQ™	9.5
TRACE™ 2000	9.5
Fisons/Carlo Erba (TMQ)	
8000 series	17
Gow-Mac	
6890 series	11
All other models	9.5
PerkinElmer	
Sigma series	11
900,990	11
8000 series	11
Auto SYS™	11
Auto SYS™ XL	11
Pye/Unicam	
All models	7
Shimadzu	
All models	Plug
SRI	
All models	Plug
Tracor	
540	11.5
550,560	9.5
220,222	12.5
Varian	
Injector type:	
Packed column	9.5/10
Split/splitless	
1078/1079	10/11
1177	9
1075/1077	11

did you know?

Restek's new Thermolite® and IceBlue™ septa are now precision molded to ensure consistent, accurate fit.

Septum Handling

All septa, regardless of their composition, puncturability, or resistance to thermal degradation, will be a source of problems if they are mishandled. Always use clean forceps or wear clean cotton gloves when handling septa; do not handle them with bare fingers, nor with powdered latex gloves—contaminants such as finger oils, perfumes, make-up, fingernail polish, skin creams, hand soaps, and talcum can be absorbed into the septum and will bleed from the septum during your analyses.

Also, follow septum and instrument manufacturers' recommendations when installing a septum. Overtightening a septum nut invariably will reduce septum lifetime by increasing septum coring and splitting problems.

Septum Bleed

All septa contain various amounts of volatile materials (e.g., silicone oils, phthalates) that can be released when the septum is heated to analysis temperatures. Septum bleed occurs when these volatiles from the septum collect on the column, then elute from the column and create baseline disturbances or extraneous (ghost) peaks in the chromatogram. This problem is prevalent in temperature-programmed analyses, because the septum volatiles collect on the column during the oven cool-down and initial hold periods. Capillary columns require much lower gas flow rates than packed columns, therefore septum volatiles are more concentrated, and bleed problems are more pronounced in capillary GC systems.

Why are Low-Bleed Septa Important?

Either baseline rise or extraneous peaks caused by septum bleed can interfere with identification and quantification of target analytes. And, because septum bleed is inconsistent, method reproducibility can be a problem. Using low-bleed septa can minimize these effects and help produce more reliable results.

Why Does Septum Puncturability Matter?

A septum that can be penetrated cleanly and easily by a syringe needle has a longer life, and consistent injections made through such a septum help ensure accurate results. The soft silicone rubber from which all Restek septa are manufactured is specially formulated for chromatographic performance, which ensures our septa are easy to puncture.

What Septum Configurations are Available, and for Which GCs?

Restek has fashioned septa for all major brands of gas chromatographs and injectors. Use the septum size chart to determine the septum diameter for your instrument or, contact us.

Which Septa Should I Use?

Thermolite® septa are a proven low-bleed champion. With a maximum temperature of 340°C, there are very few applications for which Thermolite® septa are not suitable.

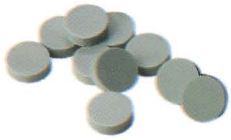
IceBlue™ septa are ideal for analysts using inlet temperatures of 250°C or below, or using solid phase microextraction (SPME) sampling techniques. IceBlue™ septa will accommodate puncturing from the large needles used in SPME, and still assure consistent injections and long lifetime.

BTO® septa are bleed and temperature optimized with a maximum temperature of 400°C, for the most demanding GC and GC/MS applications. They retain remarkable softness and pierceability at high temperatures. The CenterGuide™ can help reduce coring when used with tapered (rounded-tip) needles.

Restek Septa

- Precision molding assures consistent, accurate fit.
- Ready to use.
- Do not adhere to hot metal surfaces.
- Packaged in non-contaminating glass jars.

Septum Diameter	25-pk.	50-pk.	100-pk.
Thermolite® Septa			
5mm (3/16")	27120	27121	27122
6mm (1/4")	27123	27124	27125
7mm	27126	27127	27128
8mm	27129	27130	27131
9mm	27132	27133	27134
9.5mm (3/8")	27135	27136	27137
10mm	27138	27139	27140
11mm (7/16")	27141	27142	27143
11.5mm	27144	27145	27146
12.5mm (1/2")	27147	27148	27149
17mm	27150	27151	27152
Shimadzu Plug	27153	27154	27155
IceBlue™ Septa			
9mm		27156	27157
9.5mm (3/8")		27158	27159
10mm		27160	27161
11mm (7/16")		27162	27163
11.5mm		27164	27165
12.5mm (1/2")		27166	27167
17mm		27168	27169
Shimadzu Plug		27170	27171
BTO® Septa new!			
5mm CenterGuide™		27100	27101
6mm (1/4")		27102	27103
9mm CenterGuide™		27104	27105
9.5mm (3/8")		27106	27107
10mm		27108	27109
11mm (7/16") CenterGuide™		27110	27111
11.5mm CenterGuide™		27112	27113
12.5mm (1/2") CenterGuide™		27114	27115
17mm CenterGuide™		27116	27117
Shimadzu Plug		27118	27119



Thermolite® Septa
- Usable to 340°C inlet temperature.
- Excellent puncturability.

IceBlue™ Septa
- Usable to 250°C inlet temperature.
- General-purpose septa.
- Excellent puncturability.
- Ideal for SPME.

BTO® Septa
- CenterGuide™ design—requires less force for initial penetration.
- Usable to 400°C inlet temperature.
- Each batch GC-FID tested.
- Bleed and temperature optimized; ideal for demanding GC and GC/MS applications.

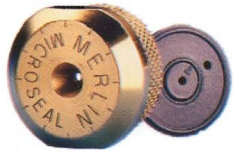
Septum Puller

- Keep several on hand in your laboratory—can be used in many different ways.
- Use hooked end for removing septa and O-rings; pointed end for removing stuck ferrules or fragments.

Description	qty.	cat.#
Septum Puller	ea.	20117

Merlin Microseal™ Septa

- Allow operation from 2 to 100psi (400 Series) or 2 to 30psi (300 Series).
- Top wiper rib improves resistance to particulate contamination; can be taken apart for cleaning.
- High resistance to wear—greatly reduces shedding of septum particles into the injection port liner, eliminating a major source of septum bleed and ghost peaks.
- Longer life—reduces the risk of septum leaks during extended automated runs.
- Maximum temperature—Agilent 6890, 5890 Series II: 325°C; Agilent 5890A: 300°C.



Microseal™ High-Pressure Septa, 400 Series (100psi)	Merlin#	Similar to Agilent#	cat.#
Standard kit (nut, 2 septa)	404	Not offered	22810
Starter kit (nut, 1 septum)	405	5182-3442	22811
Nut kit (1 nut, fits 300 & 400 series septa)	403	5182-3445	22809
High-pressure replacement septum (1 septum)	410	5182-3444	22812
Microseal™ Septa, 300 Series (30psi)			
Standard kit (nut, 2 septa)	304	5181-8833	22813
Starter kit (nut, 1 septum)	305	5181-8816	22814
Microseal replacement septum (1 septum)	310	5181-8815	22815
Replacement PTFE washers (2-pk.)	311	5182-0853	22808

Click-On Inline Super-Clean™ Traps

by Donna Lidgett, GC Accessories Product Marketing Manager

Click-On Inline Super-Clean™ Traps

- High-purity output ensures 99.9999% pure gas.
- Click-On fittings for easy, leak-tight cartridge changes; available in brass or stainless steel, 1/4" or 1/8".
- Helium-Specific Triple Trap is ideal for GC/MS.

Using the same features and benefits as Super-Clean™ base-plates and filters (see our 2006 catalog), Click-On Inline Super-Clean™ adaptor connectors allow cartridges to be exchanged without introducing air. Spring-loaded check valves seal when a filter is removed and open only when a new filter has been locked in place. There is no longer a need for loosening and tightening fittings every time a trap is changed, and your system will not become contaminated during the process.

The Triple Trap is ideal for purifying carrier gas—it contains oxygen, moisture, and hydrocarbon scrubbers in one cartridge.

please note

Super-Clean™ traps are recommended for purifying non corrosive gases with low concentrations of contaminants. For oxygen traps, the maximum concentration of oxygen in the incoming gas stream is 0.5%.

The Fuel Gas Trap is ideal for purifying flame ionization detector (FID) fuel gases, removing both moisture and hydrocarbons.

The Helium-Specific Triple Trap is ideal for purifying helium in GC/MS systems. This trap is packed and purged under helium and contains oxygen, moisture, and hydrocarbon scrubbers in one cartridge.

Trap replacement depends on the quality of the incoming gas. Use the double connector and install an indicating cartridge after the trap to indicate when a trap should be replaced.

Filter Type	Gas Quality at Outlet	Maximum Pressure	Maximum Flow (L/min.)	Use For	Capacity H_2O (g)	Capacity O_2 (mL)	Capacity Hydrocarbons (g)	Estimated Lifetime (years)
Moisture cat.#22467	>99.9999	11 bar 160psi	25	Inert carrier gas, helium, air, H_2	21	NA	NA	>3
Oxygen cat.#22468	>99.9999	11 bar 160psi	25	Inert carrier gas	NA	3000	NA	>3
Hydrocarbon cat.#22466	>99.9999	11 bar 160psi	25	Inert carrier gas, helium, air, H_2	NA	NA	36[3]	>3
Fuel Gas[1] cat.#22465	>99.9999	11 bar 160psi	25	Inert carrier gas, helium, air, H_2	10	NA	18[3]	>2
Triple[2] cat.#22464	>99.9999	11 bar 160psi	25	Inert carrier gas	6	1000	12[3]	>2

[1]Removes hydrocarbons, moisture.
[2]Removes hydrocarbons, moisture, oxygen.
[3]As *n*-butane.

Click-On Inline Super-Clean™ Trap and Connector Kits

Description	qty.	cat.#
Carrier Gas Purification Kit, 1/8" Stainless Steel Includes (2) 1/8" SS connectors and (1) oxygen/moisture/hydrocarbon triple trap	kit	22456
Carrier Gas Purification Kit, 1/8" Brass Includes (2) 1/8" brass connectors and (1) oxygen/moisture/hydrocarbon triple trap	kit	22457
Carrier Gas Purification Kit, 1/4" Stainless Steel Includes (2) 1/4" SS connectors and (1) oxygen/moisture/hydrocarbon triple trap	kit	22458
Carrier Gas Purification Kit, 1/4" Brass Includes (2) 1/4" brass connectors and (1) oxygen/moisture/hydrocarbon triple trap	kit	22459
Fuel Gas Purification Kit, 1/8" Stainless Steel Includes (4) 1/8" SS connectors and (2) hydrocarbon/moisture traps	kit	22460
Fuel Gas Purification Kit, 1/8" Brass Includes (4) 1/8" brass connectors and (2) hydrocarbon/moisture traps	kit	22461
Fuel Gas Purification Kit, 1/4" Stainless Steel Includes (4) 1/4" SS connectors and (2) hydrocarbon/moisture traps	kit	22462
Fuel Gas Purification Kit, 1/4" Brass Includes (4) 1/4" brass connectors and (2) hydrocarbon/moisture traps	kit	22463

Click-On Inline Super-Clean™ Replacement Traps

Description	qty.	cat.#
Click-On Super-Clean™ Triple Trap (removes oxygen, moisture and hydrocarbons)	ea.	22464
Click-On Super-Clean™ Fuel Gas Trap (removes moisture and hydrocarbons)	ea.	22465

Click-On Inline Super-Clean™ Ultra-High Capacity Traps

Description	qty.	cat.#
Ultra-High Capacity Hydrocarbon Trap	ea.	22466
Ultra-High Capacity Moisture Trap	ea.	22467
Ultra-High Capacity Oxygen Trap	ea.	22468

Helium-Specific Click-On Inline Super-Clean™ Trap Kits

Description	qty.	cat.#
Kits		
Helium-Specific Carrier Gas Cleaning Kit, 1/8" Stainless Steel Includes (2) 1/8" SS connectors and (1) oxygen/moisture/hydrocarbon Helium-Specific Triple Trap	kit	22469
Helium-Specific Carrier Gas Cleaning Kit, 1/8" Brass Includes (2) 1/8" brass connectors and (1) oxygen/moisture/hydrocarbon Helium-Specific Triple Trap	kit	22470
Helium-Specific Carrier Gas Cleaning Kit, 1/4" Stainless Steel Includes (2) 1/4" SS connectors and (1) oxygen/moisture/hydrocarbon Helium-Specific Triple Trap	kit	22471
Helium-Specific Carrier Gas Cleaning Kit, 1/4" Brass Includes (2) 1/4" brass connectors and (1) oxygen/moisture/hydrocarbon Helium-Specific Triple Trap	kit	22472
Replacement Trap		
Helium-Specific Triple Trap (removes oxygen, moisture and hydrocarbons)	ea.	22473

did you know?

The Helium-Specific Click-On Inline Super-Clean™ Trap is designed specifically for purification of helium in GC/MS systems!

Click-On Inline Super-Clean™ Indicator

- Color changes: oxygen-green to grey; moisture-beige to clear

Description	qty.	cat.#
Click-On Inline Super-Clean™ Indicator (oxygen, moisture plus adsorbents and hydrocarbons)	ea.	22474

tech tip

Install an indicator after the Click-On inline trap so there is no confusion about when to replace the trap.

Click-On Inline Super-Clean™ Connectors

Description	qty.	cat.#
1/8" Brass Click-On Inline Super-Clean™ Connectors	2-pk.	22475
1/8" Stainless Steel Click-On Inline Super-Clean™ Connectors	2-pk.	22476
1/4" Brass Click-On Inline Super-Clean™ Connectors	2-pk.	22477
1/4" Stainless Steel Click-On Inline Super-Clean™ Connectors	2-pk.	22478

Click-On Inline Super-Clean™ Double Connector

Description	qty.	cat.#
Click-On Inline Super-Clean™ Double Connector, stainless steel (connects trap and indicator)	ea.	22479

Wall-Mounting Clamps for Click-On Inline Super-Clean™ Traps

Description	qty.	cat.#
Wall-Mounting Clamps for Click-On Inline Super-Clean™ Traps	4-pk.	22480

Replacement O-Rings for Click-On Inline Connectors

Description	qty.	cat.#
Replacement O-Rings for Click-On Connectors	10-pk.	22481

New Rxi™ Fused Silica Columns *Continued from page 3.*

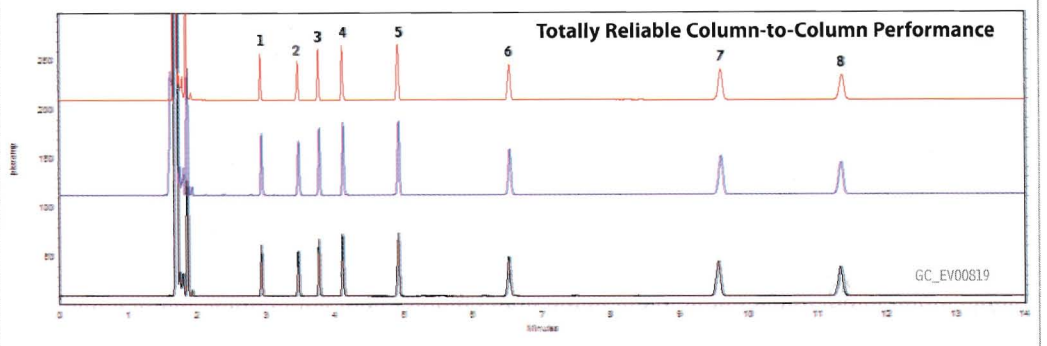
for more info

Analyses of acidic/neutral and basic drugs on Rxi™-5ms columns are described on pages 8-9 of this *Advantage*.

Totally Reliable Column-to-Column Performance

Chromatographers need to know every column they receive is going to perform in the same way as the column it replaces. Rxi™ column technology has enabled us to tighten our quality control standards, and guarantee reproducibility. Columns from each of three manufacturing batches show the excellent reproducibility assured by the new manufacturing process.

Figure 3 Three manufacturing batches of Rxi™ columns show excellent reproducibility.



Guaranteed Quality and Reliability

Rxi™ columns are already proving to be the best columns on the market, for inertness, ultra-low bleed, and column-to-column uniformity. It is our promise and commitment to you that every Rxi™ column you receive will be *exactly* as good as the one it replaces.

Typical Applications

Alcohols, amines, aromatic hydrocarbons, bile acids, drugs, EPA Methods, esters, fatty acid methyl esters (FAMEs), flavors and aromas, glycerides, halogenated hydrocarbons, herbicides, hydrocarbons, organic acids, oxygenates, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, phenols, polymers, solvents, steroids, sugars, sulfur compounds

Rxi™-5ms Columns (fused silica) new!

(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

- Nonpolar 5% dimethyl / 95% dimethylpolysiloxane phase, equivalent to USP Phase G27.
- Operating temperature range: -60 to 330/350 °C.
- Most widely used general purpose column.

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.25	-60 to 330/350°C	13420	13423
	0.50	-60 to 330/350°C	13435	13438
	1.00	-60 to 330/350°C	13450	13453
0.32mm	0.25	-60 to 330/350°C	13421	13424
	0.50	-60 to 330/350°C	13436	13439
	1.00	-60 to 330/350°C	13451	13454

For other dimensions, and additional information about Rxi™ columns, please visit our website: **www.restek.com/rxi**

Restek U.S. • 110 Benner Circle • Bellefonte, PA 16823 • 814-353-1300 • 800-356-1688 • fax: 814-353-1300 • www.restek.com
Restek GmbH • phone: +49 (0) 6172 2797 0 • fax: +49 (0) 6172 2797 77 • e-mail: info@restekgmbh.de

the RESTEK ADVANTAGE

2006.02

Allure Biphenyl™ HPLC Columns

Exclusive to Restek!

- Unique stationary phase promotes π-π interactions with aromatic and unsaturated compounds.
- Enhanced selectivity for steroids, contraceptive hormones, tetracyclines, explosives.
- Improved retention, relative to traditional phenyl phases, for unsaturated or saturated compounds.

See **page 4**.

RESTEK 20 YEARS

Turning Visions into Reality™

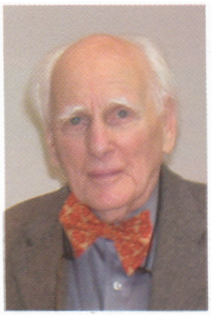
www.restek.com

theRestek Advantage
2006.02

IN THIS ISSUE

Erratum
Exempted drugs of abuse reference materials alprazolam (cat.# 34042), chlordiazepoxide HCl (cat.# 34044) and levorphanol (cat.# 34003) are at a concentration of 1mg/mL, not as listed in Advantage 2006v1.

Daily drawing winners at our PittCon® booth
Monday: Dr. S. Todd Swanson, University of Nebraska, Lincoln, NE
Tuesday: Dr. Jeffery Loo, General Motors, Milford, MI
Wednesday: Dr. Steven DuBose, Alcon Research, Fort Worth, TX
Thursday: Dr. Shawn Shanmugan, US Smokeless Tobacco, Nashville, TN
Congratulations, gentlemen, and thank you to everyone who visited our booth!



By Professor Walter Jennings ("Walt") waltj@pacbell.net
Professor Emeritus, University of California, Davis
Co-Founder, J&W Scientific, Inc.
Co-Founder, AirToxics, Ltd.

Preventive Maintenance for GC

Far too many practitioners install a new column, then ignore the enclosures that came with that column, and blissfully proceed to analysis. For reasons discussed below, most authorities would agree that the "typical" or "generic" test chromatograms that accompany batch-tested columns are essentially useless and can be ignored, but if the enclosures include a test chromatogram *that is specific for that column*, this can be valuable. The user gains a distinct advantage by purchasing individually tested columns. In batch testing, one or more columns are tested and regarded as representative of the quality of that entire batch. But the quality of a given batch of columns - - whether N, N/m, bleed, or level of response to active analytes - - normally follows a Gaussian distribution, and individual testing allows the discriminating manufacturer to identify and discard those columns on the low end of any given quality curve. With batch-tested columns, those sub-standard columns become part of the stock shipped to customers. The individually tested column offers another advantage: the test chromatogram is specific for that column under a set of specified conditions. Manufacturers using the more expensive procedures of individual testing must maintain the QC testing chromatographs in pristine condition. Chromatographs possessing even trace residues in the injectors, detectors, or gas supply lines, or faulty temperature readouts can result in the condemnation of columns that are actually good. To prevent this expensive blunder, injectors, detectors, and gas supply lines must be routinely cleaned and/or replaced; in addition, oven readouts (which do drift) require periodic recalibration, and some manufacturers do this on a weekly basis.[1]

The test chromatogram for an individually tested column illustrates the chromatogram produced by a specific test sample under specified conditions in a meticulously maintained instrument. After installing a new column, it should be conditioned in accordance with the manufacturer's recommendations. One should then make an injection of that same test mixture under the same conditions and compare the results to the test chromatogram. *Differences in theoretical plate numbers, the relative responses of test solutes, retention factors or separation factors may indicate instrumental problems that should not be ignored.*

Brandies and some wines improve with age, but most other things undergo a time-related deterioration. Neither gas chromatographs (nor unfortunately, the author) are exceptions to this generality. Eventually problems invariably emerge - - for the GC, these can take the form of unsteady baselines, erratic signal, noise spikes, ghost peaks, higher detection limits, and higher bleed. Some users become addicted to a short-term solution: they simply install a new column and the problem disappears - - for a time. This solution is especially common for those analysts under pressure to produce results rapidly because more samples are coming in the door. However, column replacement is but a temporary solution, in that the same semi-volatile contaminants that destroyed the last column are now being trapped on the new column. Until they work their way through the column and to the detector, the analyst is lulled. However, eventually they do reach the detector, and the problem recurs. In the absence of corrective actions, the interval between the need for replacement columns continues to become shorter and shorter. This dilemma is exacerbated by "real world" or dirty samples, but it also occurs, albeit less frequently, for those analyzing pristine samples.

Where do these problems originate? Few samples are truly clean. It can be educational to place a few mL of the sample on a clean watch glass, allow it to evaporate, and note the residue. In addition to these semi-volatile and non-volatile sample residues, we should be concerned with gas-borne contaminants - - with FID, these would include carrier, combustion hydrogen, make-up, and air. Most chromatographers recognize that there are different purity grades for gases, and the wary analyst specifies "five-nines-purity" (99.999%). This does not negate the need for gas filters (or traps), but usually ensures that the filters will last longer. Removal of oxygen from the gas streams is important. Even traces of oxygen attack the siloxane chain (on which most GC polymers are based), cleave Si-C bonds, and leave terminal Si-OH groups at the points of cleavage. This quickly converts the column into a "bleeder", because the terminal silanols encourage "back-biting" reactions. These split out cyclic siloxanes, primarily trimers and tetramers of (-Si-O-), generating new terminal Si-OH groups at the points of cleavage. Oxygen traps normally pay for themselves because columns experience longer lifetimes. Water traps are also important, not for the stationary phase per se, but because water or water vapor will cause most oxygen scrubbers to deteriorate rapidly. Hence good judgment dictates that the carrier should be passed first through a water trap, then through a bulk oxygen trap, and finally through an indicating oxygen trap. Traps should be mounted vertically, never horizontally. Most are filled with particulate materials and in the horizontal position the particles can settle, leaving an overhead void that provides a path of lower resistance through which most of the gas will flow.

By comparing the performance of a replacement column with the test chromatogram specific for that column, paying attention to sample composition and cleanliness, conducting proper injector and detector maintenance, and using high quality gas purifiers even with high purity gases, column lifetimes can be extended, and down times become a rarity. A regular schedule of preventive maintenance does pay dividends.

[1] Temperature exercises an exponential effect on solute retention factors (k). One of the more precise methods of re-setting the oven readout is to reserve, solely for that purpose, a "recalibration column" on which solute k values at known temperatures have been predetermined. Temperature controls are manipulated to produce the proper solute retention factors, and the readout reset to the proper value.

Inlet Seals for Agilent Instruments

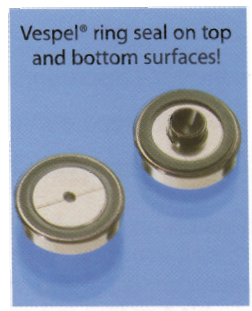
by Donna Lidgett, GC Accessories Product Marketing Manager

Dual Vespel® Ring Inlet Seals

- Vespel® ring embedded in bottom surface eliminates need for washer.
- Vespel® ring embedded in top surface reduces operator variability by requiring minimal torque to seal.
- Prevents oxygen from entering the carrier gas, increasing column lifetime.

In Agilent split/splitless injection ports, our Dual Vespel® Ring Inlet Seal greatly improves performance, relative to conventional metal-to-metal seals—it stays sealed, even after repeated temperature cycles, without retightening the reducing nut! Two soft Vespel® rings, outside the sample flow path, eliminate the need for a washer and ensure very little torque is needed to make a leak-tight seal. Tests show Dual Vespel® Ring Inlet Seals seal equally effectively at torques from 5 in. lb. to 60 in. lb.

Use a stainless steel seal for analyses of unreactive compounds. To reduce breakdown and adsorption of active compounds, use a Siltek®-treated or gold-plated seal.



best choice!

Washerless, leak-tight seals for Agilent GCs

Patent pending.

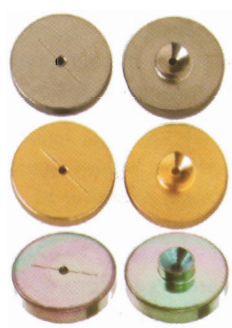
0.8mm ID Dual Vespel® Ring Inlet Seal	2-pk.	10-pk.
Siltek®	21242	21243
Gold-Plated	21240	21241
Stainless Steel	21238	21239
1.2mm ID Dual Vespel® Ring Inlet Seal	**2-pk.**	**10-pk.**
Siltek®	21248	21249
Gold-Plated	21246	21247
Stainless Steel	21244	21245

Replacement Inlet Seals for Agilent 5890/6890/6850 Split/Splitless Injection Ports

- Special grade of stainless steel that is softer and deforms more easily, creating a better seal.
- Increases column lifetime because oxygen cannot permeate into the carrier gas.
- Reduced noise benefits high-sensitivity detectors (e.g., ECDs, MSDs).
- Siltek® treatment provides inertness similar to fused silica.
- All seals include washers.

The inlet seal at the base of the Agilent 5890/6890 GC injection port contacts the sample and, because septum fragments and sample residue accumulate on the seal surface, the seal must be changed frequently to prevent adsorption of active compounds.

Use a stainless steel seal for analyses of unreactive compounds. To reduce breakdown and adsorption of active compounds, use a Siltek®-treated or gold-plated seal.



tech tip

Use a 1.2mm inlet seal with Vespel®/graphite ferrules or when installing two columns using a two-hole ferrule. Use a 0.8mm inlet seal with graphite ferrules or single capillary column installations.

Single-Column Installation, 0.8mm Opening*		0.25/0.32mm ID Dual-Column Installation, 1.2mm Opening		0.53mm ID Dual-Column Installation (1/16-inch opening)	
2-pk.	10-pk.	2-pk.	10-pk.	2-pk.	10-pk.
Stainless Steel Inlet Seal					
21315	21316	20390	20391	20392	20393
Gold-Plated Inlet Seal					
21317	21318	21305	21306	—	—
Siltek® Inlet Seal					
21319	21320	21307	21308	—	—

*0.8mm ID stainless steel inlet seal is similar to Agilent part #18740-20880, 0.8mm ID gold-plated inlet seal is similar to Agilent part #18740-20885.

Replacement Inlet Seal Washers

Description	Similar to Agilent part #	qty.	cat.#
Replacement Inlet Seal Washers	5061-5869	15-pk.	21710

Optimizing Difficult Separations of Steroids

Using an Allure™ Biphenyl HPLC Column

By Rick Lake, Pharmaceutical Innovations Chemist

- Increase resolution while using simple, isocratic conditions.
- Achieve separations not possible on a C18 column.
- Rugged and reproducible analyses.

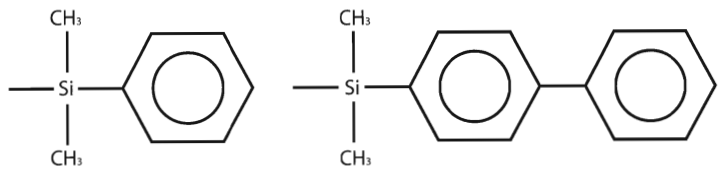
Steroids are an unusual class of compounds, in that all structural variation is centered on a common conjugated ring system, with differences in double bonding and ring constituents producing chemical diversity. Because of the consistency in their chemical structures, it can be difficult to achieve adequate separation of steroids on an alkyl (e.g., C18) HPLC stationary phase. An optimized stationary phase can be the key to these analyses.

When choosing a stationary phase, a separation mechanism that utilizes inherent differences in the chemical structures of the target analytes should be used. For analyses in which the target analytes are structurally very similar, this is especially critical. For steroids, this includes separations based on pi-pi (π-π) interactions between aromatic or unsaturated moieties: a stationary phase containing phenyl groups forms π-π bonds as the phenyl group on the stationary phase overlaps with the aromatic rings or double bonds in the analytes.

Restek chemists have made significant advancements in phenyl stationary phase chemistry, to increase retention of unsaturated compounds in reversed phase HPLC applications, while enhancing selectivity. The Allure™ Biphenyl stationary phase is a product of these advancements. A typical silica-based phenyl stationary phase consists of a single phenyl group bonded to a silica backbone (Figure 1). By developing a phase that consists of two phenyl groups bonded end-to-end, the Allure™ Biphenyl offers a more concentrated arrangement of phenyl groups, in a sterically favorable positioning (Figure 1). This phase shows markedly better selectivity for unsaturated compounds and shows a high retention capacity, similar to that of a C18 phase.[1-3]

We assayed two groups of steroids, hormones and corticosteroids, on an Allure™ Biphenyl column, to determine if separation can be enhanced by exploiting differences in π-π interactions. First, we compared performances by the Allure™ Biphenyl column and a conventional C18 column of the same dimensions, using a complex mix of steroid hormones. Under identical isocratic analytical conditions, the Allure™ Biphenyl column resolved all target compounds (Figure 2), but the C18 column showed very limited resolving power (Figure 3).

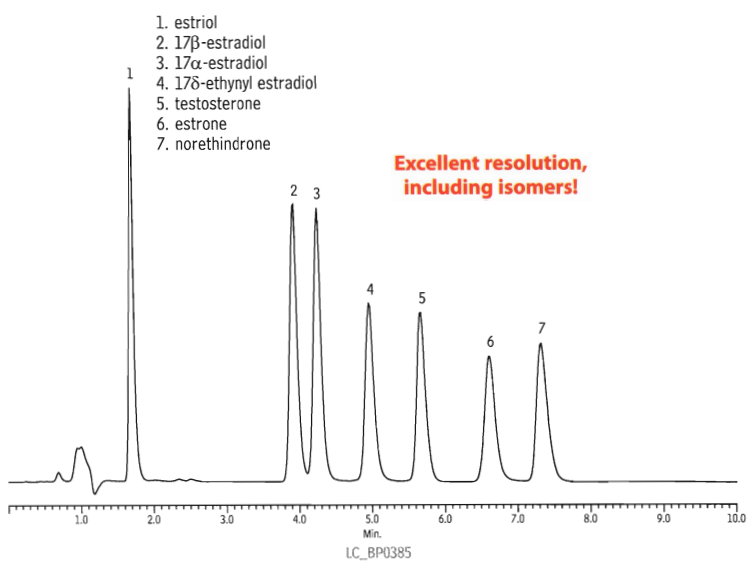
Figure 1 The unique chemistry of the Allure™ Biphenyl stationary phase creates a more concentrated phenyl arrangement in a sterically favorable positioning.



Conventional phenyl stationary phase

Allure™ Biphenyl stationary phase

Figure 2 An Allure™ Biphenyl column resolves steroid hormones in a simple, isocratic analysis.

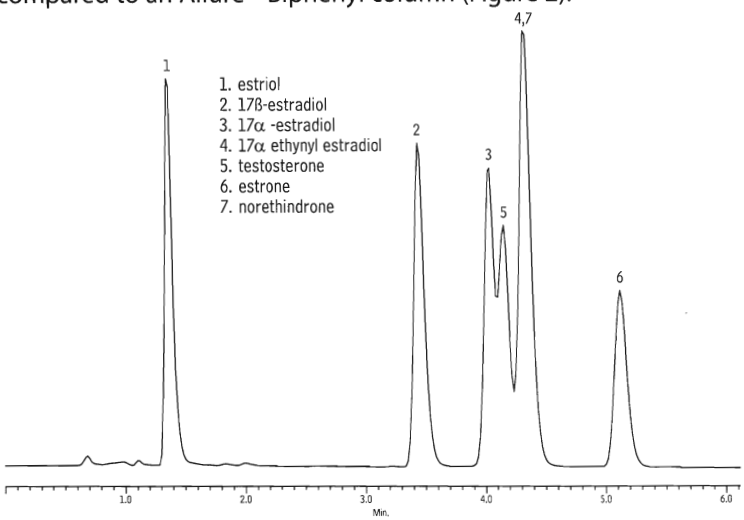


Sample:
Inj.: 10µL
Conc.: 50µg/mL each component
Sample diluent: acetonitrile:methanol, 4:1 (v/v)

Column: **Allure™ Biphenyl** (cat.# 5166365)
Dimensions: 150 x 4.6 mm
Particle size: 3µm
Pore size: 60Å

Conditions:
Mobile phase: water:acetonitrile, 50:50 (v/v)
Flow: 1.5mL/min.
Temp.: ambient
Det.: UV @ 220nm

Figure 3 A C18 column shows less resolving capability for steroids, compared to an Allure™ Biphenyl column (Figure 2).

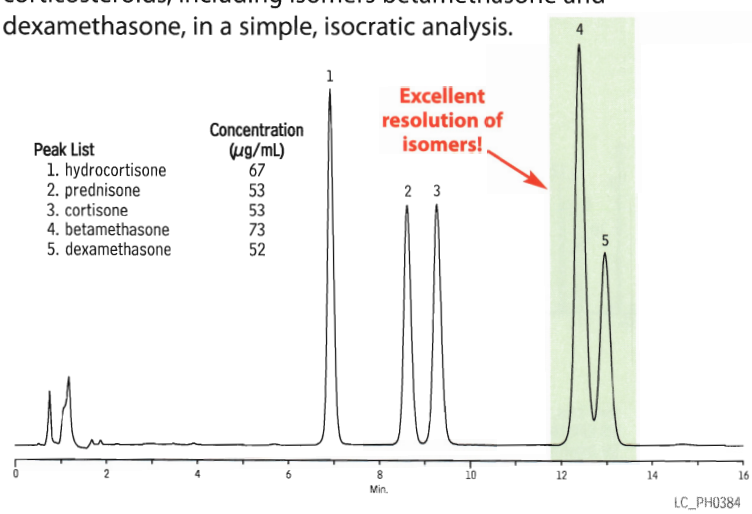


Sample:
Inj.: 10µL
Conc.: 50µg/mL each component
Sample diluent: acetonitrile:methanol, 4:1 (v/v)

Column: C18
Dimensions: 150 x 4.6 mm
Particle size: 3µm
Pore size: 100Å

Conditions:
Mobile phase: water:acetonitrile, 50:50 (v/v)
Flow: 1.5mL/min.
Temp.: ambient
Det.: UV @ 220nm

Figure 4 An Allure™ Biphenyl column resolves all target corticosteroids, including isomers betamethasone and dexamethasone, in a simple, isocratic analysis.



Sample:
Inj.: 10µL
Sample diluent: methanol

Column: Allure™ Biphenyl (cat.# 5166365)
Dimensions: 150 x 4.6 mm
Particle size: 3µm
Pore size: 60Å

Conditions:
Mobile phase: water:acetonitrile, 70:30 (v/v)
Flow: 1.5mL/min.
Temp.: ambient
Det.: UV @ 220nm

The Allure™ Biphenyl column also provided an overall increase in analyte retention – a very useful improvement relative to conventional phenyl phases.

The Allure™ Biphenyl column also showed enhanced selectivity in a second analysis, using corticosteroids. Under simple isocratic conditions, the Allure™ Biphenyl column provided baseline separation of hydrocortisone and prednisone and, more important, resolved isomers betamethasone and dexamethasone (Figure 4).

These analyses show that markedly better selectivity for steroids easily can be achieved, by using an Allure™ Biphenyl column under simple isocratic conditions. High retention capacity, similar to that of an ODS phase, also is demonstrated; a useful feature unavailable from conventional phenyl phases. By increasing π-π interactions, the Allure™ Biphenyl stationary phase offers a unique and more effective alternative to hydrophobic alkyl phases for resolving chemically similar unsaturated compounds, such as steroids.

References
1. *Superior Separations of Unsaturated Compounds by HPLC* Restek Advantage 2005v4 (lit. cat.# 580022).
2. *Improved HPLC Analysis of Steroids* Restek Application Note (lit. cat.# 580020).
3. Lake, R., and Wittrig, R., *Increasing HPLC Retention and Selectivity for Unsaturated Compounds, Using π-π Interactions* Pharmaceutical Canada, June 2006.

References 1&2 available on request.

Allure™ Biphenyl Columns

3µm Column, 2.1mm	cat. #
30mm	9166332
50mm	9166352
100mm	9166312
3µm Column, 3.2mm	
30mm	9166333
50mm	9166353
100mm	9166313
3µm Column, 4.6mm	
30mm	9166335
50mm	9166355
100mm	9166315
3µm Column, 2.1mm	
30mm (with Trident™ Inlet Fitting)	9166332-700
50mm (with Trident™ Inlet Fitting)	9166352-700
100mm (with Trident™ Inlet Fitting)	9166312-700
3µm Column, 3.2mm	
30mm (with Trident™ Inlet Fitting)	9166333-700
50mm (with Trident™ Inlet Fitting)	9166353-700
100mm (with Trident™ Inlet Fitting)	9166313-700
3µm Column, 4.6mm	
30mm (with Trident™ Inlet Fitting)	9166335-700
50mm (with Trident™ Inlet Fitting)	9166355-700
100mm (with Trident™ Inlet Fitting)	9166315-700
Allure™ Biphenyl Guard Cartridges	
10 x 2.1mm	916650212
10 x 4.0mm	916650210
20 x 2.1mm	916650222
20 x 4.0mm	916650220

New Rxi™-1ms GC Capillary Column

For Low Level GC/MS Analysis

By Robert Freeman, Environmental Innovations Chemist

- Inert, low-bleed column for reliable results from low-level GC/MS analyses.
- Save time – analyze acidic and basic compounds under the same conditions.
- Guaranteed reproducible performance, column to column.

The second column in our new Rxi™ GC column line – the Rxi™-1ms column – will provide the same outstanding performance as the Rxi™-5ms column, with equally superior inertness, ultra-low bleed, and excellent batch to batch reproducibility.

Our first test for this 100% dimethylpolysiloxane phase column was an analysis of a complex mixture of semivolatile organic compounds. The extensive target list was comprised of many classes of compounds including chloroacetanilides, chlorotriazines, triazinones, uracils, polcyclic aromatic hydrocarbons, and phthalates. Figure 1 shows peak shape and selectivity are equally good for all of these diverse compounds, and all are eluted in an acceptable analysis time.[1]

Excellent Inertness

In addition to analyzing these compounds, we analyzed an acidic compound (2,4-dinitrophenol) and a basic compound (pyridine), each at 0.5ng on column, to assess column inertness. Column activity reveals itself through poor response and peak tailing for such active compounds, and these two compounds present both varying difficulties in a GC/MS analysis and differing modes of degradation. Figure 2 shows the excellent peak shapes and responses for these compounds on the 30m x 0.25mm ID, 0.25µm film column.

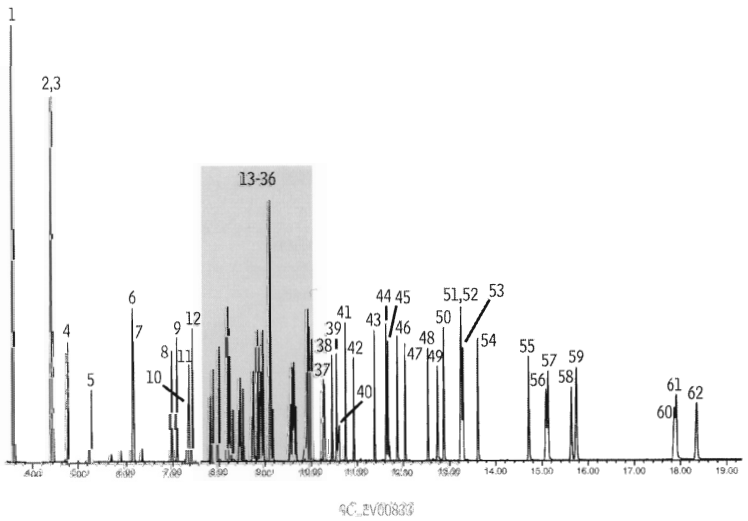
Phenols are notorious for breakdown and peak tailing, caused by interaction with the surface of an active inlet liner or an active column. Nitrophenols and pentachlorphenol, for example, very often exhibit poor peak shape and/or poor response. Figure 3 shows the 30m x 0.25mm ID, 0.25µm Rxi™-1ms column provides very good peak shapes for phenols. Peak responses are well above method requirements.

Ultra-Low Bleed

In addition to excellent inertness, Rxi™-1ms columns exhibit very low bleed. Figure 4 is focused on the end of the chromatogram for semivolatiles. At 330°C, bleed is much lower than the signals for 0.5ng of target analytes. This exceptional signal-to-noise differential for late eluting compounds assures better detection limits.

Figure 1 Excellent selectivity and peak shapes for common drinking water semivolatiles at 10ng, using an Rxi™-1ms column.

1. 2-fluorophenol (surr.)
2. bis(2-chloroethyl)ether
3. phenol-d6 (surr.)
4. 1,4-dichlorobenzene-d4 (int. std.) •
5. nitrobenzene-d5 (surr.)
6. naphthalene-d8 (int. std.) •
7. naphthalene
8. 1-methylnaphthalene
9. 2-methylnaphthalene
10. hexachlorocyclopentadiene
11. EPTC
12. 2-fluorobiphenyl (surr.)
13. 2,6-dinitrotoluene
14. dimethylphthalate
15. acenaphthylene
16. acenaphthene-d10 (int. std.) •
17. acenaphthene
18. 2,4-dinitrotoluene
19. 1-naphthalenamine
20. molinate
21. 2-naphthalenamine
22. 5-nitro-o-toluidine
23. diethylphthalate
24. fluorene
25. propachlor
26. diphenylamine
27. 2,4,6-tribromophenol (surr.)
28. simazine
29. prometon
30. atrazine
31. hexachlorobenzene
32. 4-aminobiphenyl
33. terbacil
34. phenanthrene-d10 (int. std.) •
35. phenanthrene
36. anthracene
37. metribuzin
38. acetochlor
39. alachlor
40. bromacil
41. di-*n*-butylphthalate
42. metolachlor
43. fluoranthene
44. pyrene
45. butachlor
46. *p*-terphenyl-d14 (surr.)
47. *p*-dimethylaminoazobenzene
48. benzyl butyl phthalate
49. 2-acetylaminofluorene
50. bis(2-ethylhexyl)adipate
51. benzo(a)anthracene
52. chrysene-d12 (int. std.) •
53. chrysene
54. bis(2-ethylhexyl)phthalate
55. di-*n*-octylphthalate
56. benzo(b)fluoranthene
57. benzo(k)fluoranthene
58. benzo(a)pyrene
59. perylene-d12 (int. std.) •
60. indeno(1,2,3-cd)pyrene
61. dibenzo(a,h)anthracene
62. benzo(ghi)perylene



Column:	Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)
Sample:	US EPA Method 525.2 mix: custom 525.2 calibration mix, SV Internal Standard Mix (cat.# 31206), B/N Surrogate Mix (4/89 SOW) (cat.# 31024), Acid Surrogate Mix (4/89 SOW) (cat.# 31025)
Inj.:	1.0µL, 10µg/mL each analyte (internal standards 100µg/mL), split (10:1) 4mm Drilled Uniliner® inlet liner (hole at bottom) (cat.# 20756)
Instrument:	Agilent 6890
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	50°C (hold 1 min.) to 265°C @ 20°C/min., to 330°C @ 6°Cmin. (hold 1 min.)
Det.:	Agilent 5973 MSD
Transfer line temp.:	280°C
Scan range:	35-550 amu
Solvent delay:	3.20 min.
Tune:	DFTPP
Ionization:	EI

• Internal standards at 100ng on-column.

Figure 2 An Rxi™-1ms column has excellent selectivity for basic or acidic compounds, under the same conditions. (0.5ng each; extracted ion chromatograms).

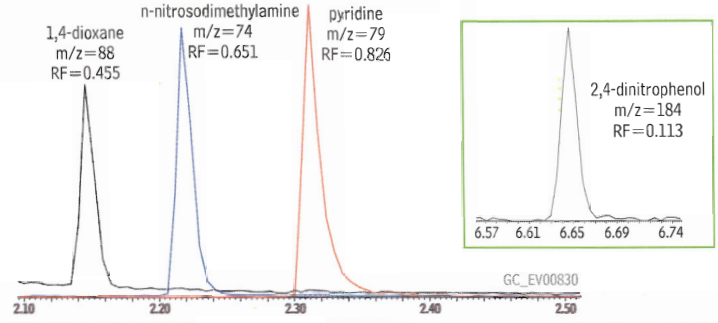
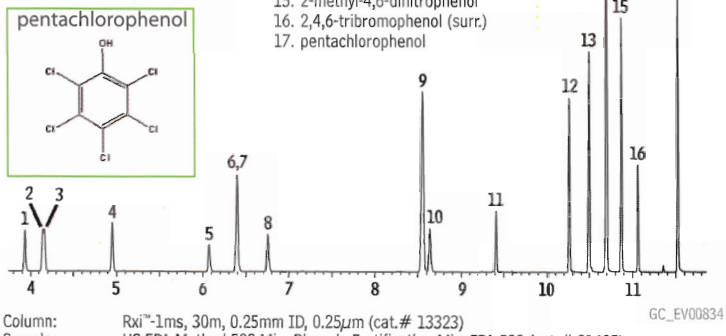


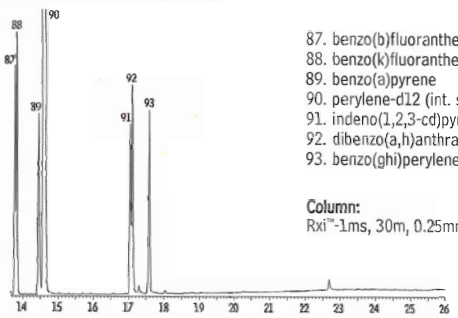
Figure 3 Acidic analytes at 5.0ng on an Rxi™-1ms column (extracted ion chromatogram).

1. phenol
2. 2-chlorophenol-d4 (surr.)
3. 2-chlorophenol
4. 2-methylphenol (*o*-cresol)
5. 2-nitrophenol
6. 2,4-dimethylphenol3,5,6-d3 (surr.)
7. 2,4-dimethylphenol
8. 2,4-dichlorophenol
9. 3-nitro-*o*-xylene (int. std.)
10. 4-chloro-3-methylphenol
11. 2,4,6-trichlorophenol
12. 2,4-dinitrophenol
13. 4-nitrophenol
14. 2,3,4,5-tetrachlorophenol (int. std.)
15. 2-methyl-4,6-dinitrophenol
16. 2,4,6-tribromophenol (surr.)
17. pentachlorophenol



Column:	Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)
Sample:	US EPA Method 528 Mix: Phenols Fortification Mix, EPA 528 (cat.# 31695), Internal Standard Mix, EPA 528 (cat.# 31696), Surrogate Standard Mix, EPA 528 (cat.# 31697)
Inj.:	1.0µL, 5µg/mL each analyte (internal standards 25µg/mL), split (10:1) 4mm Drilled Uniliner® inlet liner (hole at bottom) (cat.# 20771)
Instrument:	Agilent 6890
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	70°C (hold 0.5 min.) to 130°C @ 8°C/min., to 300°C @ 50°C/min. (hold 1 min.)
Det.:	Agilent 5973 MSD

Figure 4 Bleed profile at 330°C of Rxi™-1ms at end of 5ng on-column standard.



87. benzo(b)fluoranthene
88. benzo(k)fluoranthene
89. benzo(a)pyrene
90. perylene-d12 (int. std.)
91. indeno(1,2,3-cd)pyrene
92. dibenzo(a,h)anthracene
93. benzo(ghi)perylene

Column:
Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)

Based on these results, we highly recommend the new Rxi™-1ms column for low-level analyses that require a 100% dimethylpolysiloxane phase.

Rxi™-1ms Columns (fused silica)

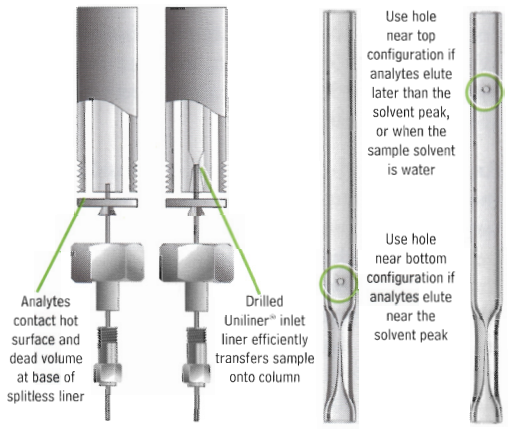
(Crossbond® 100% dimethylpolysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.18	-60 to 330/350°C	20-Meter	13302
0.20mm	0.33	-60 to 330/350°C	12-Meter	13397
0.20mm	0.33	-60 to 330/350°C	25-Meter	13398
0.20mm	0.33	-60 to 330/350°C	50-Meter	13399
0.25mm	0.25	-60 to 330/350°C	15-Meter	13320
0.25mm	0.25	-60 to 330/350°C	30-Meter	13323
0.25mm	0.25	-60 to 330/350°C	60-Meter	13326
0.25mm	0.50	-60 to 330/350°C	15-Meter	13335
0.25mm	0.50	-60 to 330/350°C	30-Meter	13338
0.25mm	0.50	-60 to 330/350°C	60-Meter	13341
0.25mm	1.00	-60 to 330/350°C	15-Meter	13350
0.25mm	1.00	-60 to 330/350°C	30-Meter	13353
0.25mm	1.00	-60 to 330/350°C	60-Meter	13356
0.32mm	0.25	-60 to 330/350°C	15-Meter	13321
0.32mm	0.25	-60 to 330/350°C	30-Meter	13324
0.32mm	0.25	-60 to 330/350°C	60-Meter	13327
0.32mm	0.50	-60 to 330/350°C	15-Meter	13336
0.32mm	0.50	-60 to 330/350°C	30-Meter	13339
0.32mm	0.50	-60 to 330/350°C	60-Meter	13342
0.32mm	1.00	-60 to 330/350°C	15-Meter	13351
0.32mm	1.00	-60 to 330/350°C	30-Meter	13354
0.32mm	1.00	-60 to 330/350°C	60-Meter	13357
0.53mm	0.50	-60 to 330/350°C	15-Meter	13337
0.53mm	0.50	-60 to 330/350°C	30-Meter	13340
0.53mm	1.00	-60 to 330/350°C	15-Meter	13352
0.53mm	1.00	-60 to 330/350°C	30-Meter	13355
0.53mm	1.50	-60 to 330/350°C	15-Meter	13367
0.53mm	1.50	-60 to 330/350°C	30-Meter	13370
0.53mm	1.50	-60 to 330/350°C	60-Meter	13373

restek innovation!

[1]The Drilled Uniliner®

To reduce the effects of surface activity in the injection port liner, and focus on the effects of the column on active analytes, we used a Drilled Uniliner® inlet liner for this work. This liner connects directly to the column, eliminating contact between the active compounds and active metal surfaces in the injector, and ensuring an inactive sample pathway for analyte transfer from the injection port to the column.



Analysis of Semivolatile Organics

Using the new Rxi™-5ms Capillary GC Column

by Robert Freeman, Environmental Innovations Chemist, and Christopher M. English, Innovations Group Leader

- Low column bleed, outstanding inertness, excellent column-to-column reproducibility.
- Symmetric peaks and good response factors for acidic or basic analytes.
- Resolve 93 analytes in less than 18 minutes.

Sub-nanogram Analysis of Semivolatile Organics

Analyzing basic or acidic semivolatile environmental pollutants at low nanogram-on-column concentrations puts demands on the entire analytical system. Using our new Rxi™-5ms column, we have developed an analytical procedure that assures good performance for both acids and bases.

Rxi™-5ms Column Offers Sensitivity for Acids and Bases

One of the most active basic compounds listed in semivolatiles methods is pyridine. This early-eluting compound can elicit poor performance in the injection port and on the column, and many currently available columns give a poor peak shape for pyridine. Columns with a slightly basic surface can perform well with pyridine, but will perform poorly with the acidic compounds, such as 2,4-dinitrophenol.

Figure 1 combines extracted ion chromatograms for the initial three US EPA Method 8270D target compounds, at 0.5ng per compound on-column. The extracted ion for 1,4-dioxane shows that injection port and oven conditions were optimized. The pyridine and N-nitrosodimethylamine peaks are symmetric, even at this low level of detection. An excessively tailing pyridine peak, or a pyridine peak smaller than that for 1,4-dioxane at the same concentration, would indicate on-column activity. With an Rxi™-5ms column, and the conditions listed for Figure 3, pyridine can be detected reliably at low concentrations.

Analytically, 2,4-dintrophenol is considered the most problematic compound in the Method 8270 target list. 2,4-Dinitrophenol and the other system performance check compounds (SPCC) - N-nitroso-di-*n*-propylamine, hexachlorocyclopentadiene, and 4-nitrophenol - must exhibit a minimum average response factor (RF) of 0.050. An optimized system generally will provide response factors greater than 0.1 for these compounds, but the lower the calibration curve for these compounds, the more difficult it is to achieve passing response factors. If any of these compounds fails to meet the Method 8270 response factor criterion, system maintenance must be performed to bring response factors to passing before samples can be

Figure 1 An Rxi™-5ms column provides sharp, easily quantified peaks for active analytes (extracted ion chromatograms).

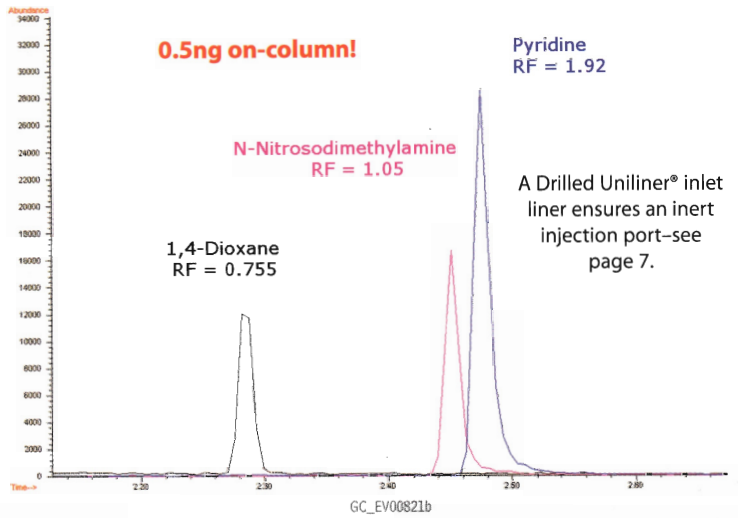


Figure 2 Excellent response for 0.5ng 2,4-dintrophenol on an Rxi™-5ms column.

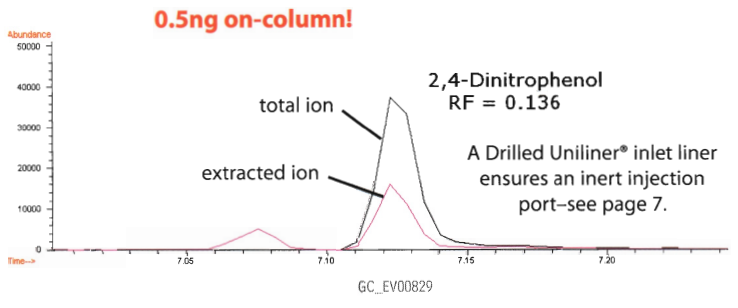
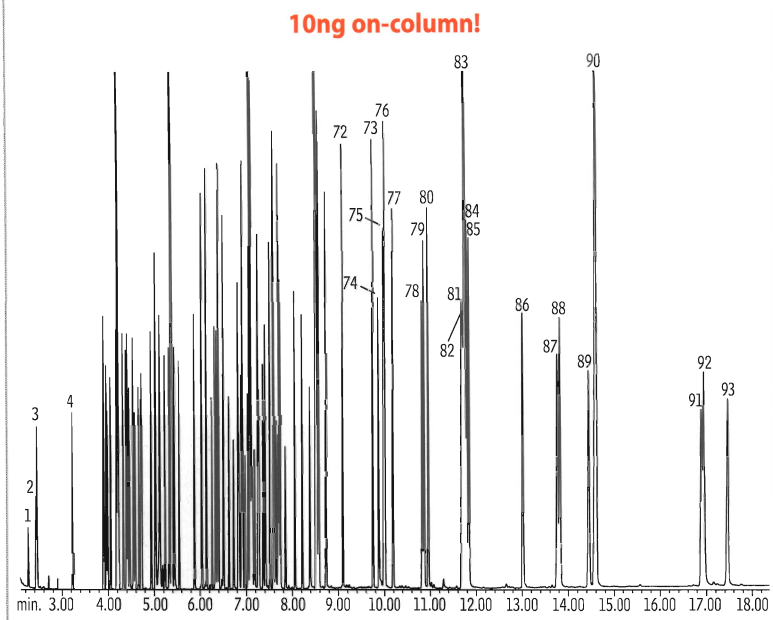


Figure 3 Separate 93 semivolatile compounds in less than 18 minutes, using an Rxi™-5ms column.



GC_EV00823

For complete identifications, please visit www.restek.com/rxi

1. 1,4-dioxane
2. N-nitrosodimethylamine
3. pyridine
4. 2-fluorophenol
5. phenol-d6
6. phenol
7. aniline
8. bis(2-chloroethyl) ether
9. 2-chlorophenol
10. 1,3-dichlorobenzene
11. 1,4-dichlorobenzene-d4
12. 1,4-dichlorobenzene
13. benzyl alcohol
14. 1,2-dichlorobenzene
15. 2-methylphenol
16. bis(2-chloroisopropyl) ether
17a. 4-methylphenol
17b. 3-methylphenol
18. N-nitroso-di-*n*-propylamine
19. hexachloroethane
20. nitrobenzene-d5
21. nitrobenzene
22. isophorone
23. 2-nitrophenol
24. 2,4-dimethylphenol
25. benzoic acid
26. bis(2-chloroethoxy)methane
27. 2,4-dichlorophenol
28. 1,2,4-trichlorobenzene
29. naphthalene-d8
30. naphthalene
31. 4-chloroaniline
32. hexachlorobutadiene
33. 4-chloro-3-methylphenol
34. 2-methylnaphthalene
35. 1-methylnaphthalene
36. hexachlorocyclopentadiene
37. 2,4,6-trichlorophenol
38. 2,4,5-trichlorophenol
39. 2-fluorobiphenyl
40. 2-chloronaphthalene
41. 2-nitroaniline
42. 1,4-dinitrobenzene
43. dimethyl phthalate
44. 1,3-dinitrobenzene
45. 2,6-dinitrotoluene
46. acenaphthylene
47. 1,2-dinitrobenzene
48. 3-nitroaniline
49. acenaphthene-d10
50. acenaphthene
51. 2,4-dinitrophenol
52. 4-nitrophenol
53. dibenzofuran
54. 2,4-dinitrotoluene
55. 2,3,4,6-tetrachlorophenol
56. 2,3,5,6-tetrachlorophenol
57. diethyl phthalate
58. 4-chlorophenyl phenyl ether
59. fluorene
60. 4-nitroanaline
61. 4,6-dinitro-2-methylphenol
62. N-nitrosodiphenylamine (as diphenylamine)
63. 1,2-diphenylhydrazine (as azobenzene)
64. 2,4,6-tribromophenol
65. 4-bromophenyl phenyl ether
66. hexachlorobenzene
67. pentachlorophenol
68. phenanthrene-d10
69. phenanthrene
70. anthracene
71. carbazole
72. di-*n*-butyl phthalate
73. fluoranthene
74. benzidine
75. pyrene-d10
76. pyrene
77. *p*-terphenyl-d14
78. 3,3'-dimethylbenzidine
79. butyl benzyl phthalate
80. bis(2-ethylhexyl) adipate
81. 3,3'-dichlorobenzidine
82. benzo(a)anthracene
83. chrysene-d12
84. chrysene
85. bis(2-ethylhexyl) phthalate
86. di-*n*-octyl phthalate
87. benzo(b)fluoranthene
88. benzo(k)fluoranthene
89. benzo(a)pyrene
90. perylene-d12
91. indeno(1,2,3-cd)pyrene
92. dibenzo(a,h)anthracene
93. benzo(ghi)perylene

Column:	Rxi™-5ms, 30m, 0.25mm ID, 0.25µm (cat.# 13423)
Sample:	US EPA Method 8270D mix: 8270 MegaMix™ (cat.# 31850), Benzoic Acid Standard (cat.# 31879), Benzidine Standard (cat.# 31852), Acid Surrogate Mix (cat.# 31025), B/N Surrogate Standard Mix (cat.# 31887), 1,4-Dioxane (cat.# 31853)
Inj.:	1.0µL, 10ppm each analyte (10ng on column), splitless (hold 0.1 min.) **4mm Drilled Uniliner® inlet liner (hole at bottom) (cat.# 20756)**
Instrument:	Agilent 6890
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	50°C (hold 0.5 min.) to 265°C @ 25°C/min., to 330°C @ 6°C/min. (hold 2 min.)
Det.:	Agilent 5973 GC/MS
Transfer line temp.:	280°C
Scan range:	35-550 amu
Solvent delay:	2 min.
Tune:	DFTPP
Ionization:	EI

analyzed. Figure 2 shows the inertness of the Rxi™-5ms column, which exhibits a response factor of 0.136 for 0.5ng on-column of 2,4-dinitrophenol.

The total ion chromatogram for our optimized analysis is shown in Figure 3. There are at least five scans across each target analyte, which assures good spectral integrity and good peak shape, and the last compound is eluted in less than 18 minutes.

The Result

The Rxi™-5ms column introduces a new generation of Restek columns that exhibit low bleed, outstanding inertness, and excellent column-to-column reproducibility.

An Rxi™-5ms column, used in an optimized system, provides excellent chromatography for Method 8270 semivolatile compounds, including difficult-to-analyze acidic or basic compounds, at low on-column concentrations. These new columns give the performance needed, at the sensitivity required, column after column.

Rxi™-5ms Columns (fused silica)

(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.18	-60 to 330/350°C	20-Meter	13402
0.18mm	0.36	-60 to 330/350°C	20-Meter	13411
0.20mm	0.33	-60 to 330/350°C	12-Meter	13497
0.20mm	0.33	-60 to 330/350°C	25-Meter	13498
0.20mm	0.33	-60 to 330/350°C	50-Meter	13499
0.25mm	0.25	-60 to 330/350°C	15-Meter	13420
0.25mm	0.25	-60 to 330/350°C	30-Meter	13423
0.25mm	0.25	-60 to 330/350°C	60-Meter	13426
0.25mm	0.50	-60 to 330/350°C	15-Meter	13435
0.25mm	0.50	-60 to 330/350°C	30-Meter	13438
0.25mm	0.50	-60 to 330/350°C	60-Meter	13441
0.25mm	1.00	-60 to 330/350°C	15-Meter	13450
0.25mm	1.00	-60 to 330/350°C	30-Meter	13453
0.25mm	1.00	-60 to 330/350°C	60-Meter	13456
0.32mm	0.25	-60 to 330/350°C	15-Meter	13421
0.32mm	0.25	-60 to 330/350°C	30-Meter	13424
0.32mm	0.25	-60 to 330/350°C	60-Meter	13427
0.32mm	0.50	-60 to 330/350°C	15-Meter	13436
0.32mm	0.50	-60 to 330/350°C	30-Meter	13439
0.32mm	1.00	-60 to 330/350°C	15-Meter	13451
0.32mm	1.00	-60 to 330/350°C	30-Meter	13454
0.32mm	1.00	-60 to 330/350°C	60-Meter	13457
0.53mm	0.25	-60 to 330/350°C	15-Meter	13422
0.53mm	0.25	-60 to 330/350°C	30-Meter	13425
0.53mm	0.50	-60 to 330/350°C	15-Meter	13437
0.53mm	0.50	-60 to 330/350°C	30-Meter	13440
0.53mm	1.00	-60 to 330/350°C	15-Meter	13452
0.53mm	1.00	-60 to 330/350°C	30-Meter	13455
0.53mm	1.50	-60 to 330/350°C	15-Meter	13467
0.53mm	1.50	-60 to 330/350°C	30-Meter	13470

tech tip

A Drilled Uniliner® inlet liner helps ensure reliable results for active compounds—see information on page 7.

8-Minute GC Analysis of Residual Solvents

Using an Rtx®-624 (G43) / Rtx®-WAX (G16) Column Pair

By Rick Lake, Pharmaceutical Innovations Chemist

- Dual-column detection/confirmation in 8 minutes.
- Columns produce desired selectivity and stable retention.
- Excellent peak shape and sensitivity, for reliable information.

The International Conference on Harmonization (ICH) publishes a guideline (Q3C) listing amounts of solvent residues that are acceptable in drug products and drug substances. The complete ICH list of regulated solvents, 61 compounds of differing chemical properties, is a challenge for separation on any single GC phase, as critical coelutions exist. Typically, residual solvents are identified by assaying samples and matching retention times with reference standards. If a response greater than the regulatory limit is obtained in a retention time window, a second sample is analyzed to confirm the compound's identity, using a column that has alternate selectivity. In some cases, GC/MS is employed for analyte verification. Assays for verification can be laborious and time intensive, and add unnecessary cost.

In the ICH guideline, residual solvents are grouped according to their toxicity. Class 1 compounds are carcinogens that pose a risk to both consumers and the environment. The use of these solvents is to be avoided but, if they are used, their use must be tightly controlled to ensure only trace level impurities in the final product. Class 2 compounds are non-genotoxic animal carcinogens, and concentrations of these compounds should be limited in pharmaceutical actives and products. Class 3 compounds have low toxic potential, and concentrations up to 0.5% are acceptable. Therefore, Class 3 compounds can be assayed by non-specific techniques, such as weight loss on drying. Because Class 2 compounds are the most likely prospects for GC analysis, we selected Residual Standards Class 2 Mix A and Residual Standards Class 2 Mix B (cat.#s 36271 and 36272, respectively) as the analytes for this work.

Because of advances in headspace technology – mainly dynamic sampling techniques – greater sensitivity now is achievable with this approach[1], and this makes a comprehensive dual-column assay feasible. By simultaneously using two columns with differing selectivities, e.g., a G43 column (Rtx®-1301 or Rtx®-624) and a G16 column (Rtx®-Wax or Stabilwax®), a single injection can be used both to detect residual solvents and to confirm their identities. Even with two columns, however, the complexity of the sample list makes it impossible for a single temperature program to provide the flexibility needed to resolve all compounds on each column. To overcome this barrier, we used a Tekmar HT3 dynamic headspace sampler and an Agilent 6890 GC equipped with a Gerstel Modular Accelerated Column Heater (MACH) System. One of the latest advances in fast GC technology, the MACH System incorporates columns encased individually in thermally controlled bundles and heated externally from the main GC oven (Figure 1).[2] This independent, low thermal mass configuration allows independent, very rapid temperature ramps, upward or downward.

Collected analytes were directed to the injection port, then were split onto the two columns via a "Y" Press-Tight® connector. Independent temperature programs for each column separated the analytes for detection on dual FIDs. Using our two columns in this novel and simple-to-use setup, we resolved all compounds in the combined reference mixes in less than 8 minutes (Figure 2) – a result not possible with a conventional GC system. There was one critical co-elution on each column, but these did not involve the same compounds, and thus posed no practical problem. Also, with the low thermal mass of the

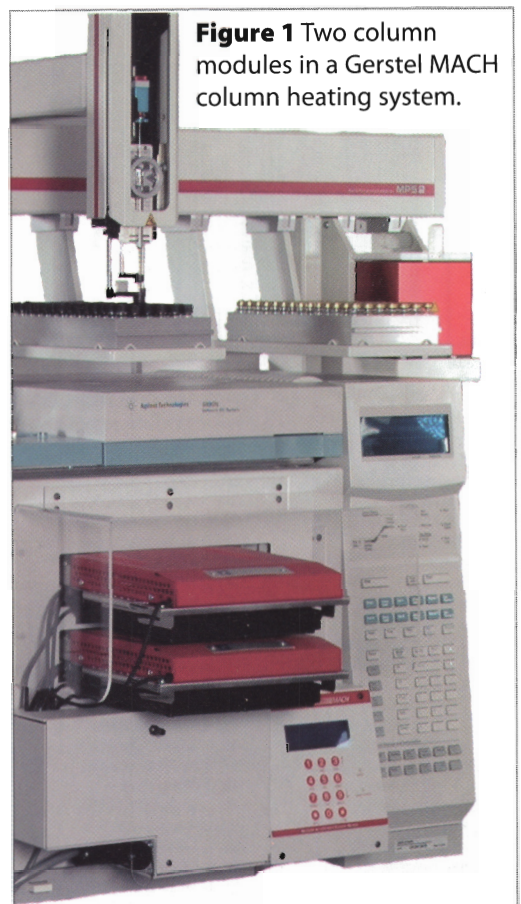
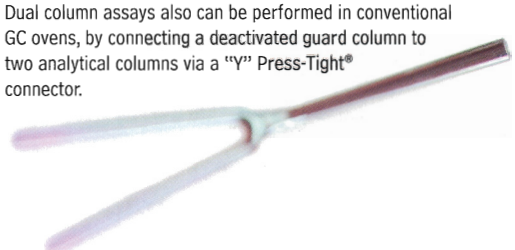


Figure 1 Two column modules in a Gerstel MACH column heating system.

The MACH system allows independent temperature programming of up to four columns, simultaneously.

tech tip

Dual column assays also can be performed in conventional GC ovens, by connecting a deactivated guard column to two analytical columns via a "Y" Press-Tight® connector.



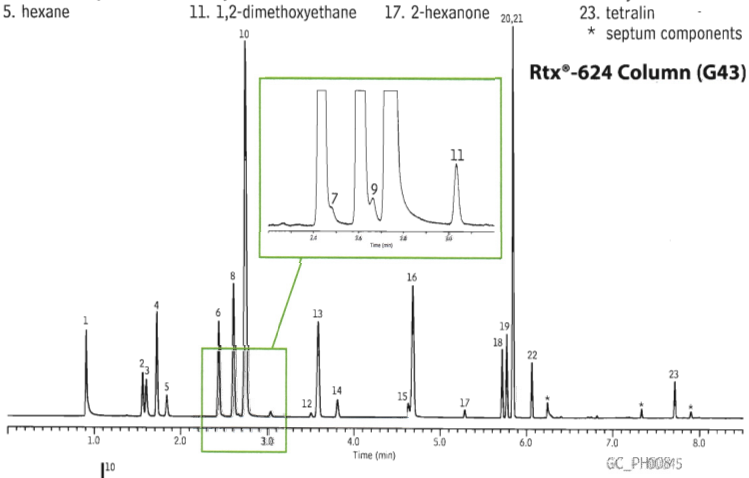
Universal "Y" Press-Tight® Connectors

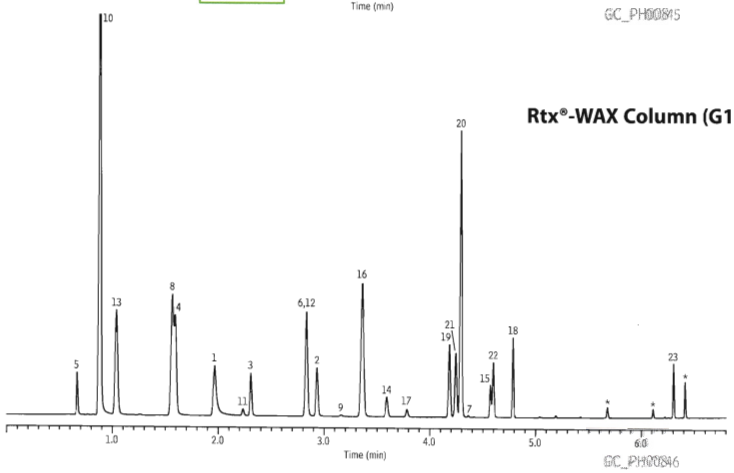
Description	cat. #
Universal "Y" Press-Tight® Connector	20405

Figure 2 Detect and confirm residual solvents with one injection, using Restek columns in a Gerstel MACH System.

1. methanol
2. acetonitrile
3. dichloromethane
4. *trans*-1,2-dichloroethylene
5. hexane
6. *cis*-1,2-dichloroethylene
7. nitromethane
8. tetrahydrofuran
9. chloroform
10. cyclohexane
11. 1,2-dimethoxyethane
12. trichloroethylene
13. methylcyclohexane
14. 1,4-dioxane
15. pyridine
16. toluene
17. 2-hexanone
18. chlorobenzene
19. ethyl benzene
20. *m*-xylene
21. *p*-xylene
22. *o*-xylene
23. tetralin

* septum components





Headspace Conditions - see our web page: www.restek.com/buzz

Instrument Conditions

Instrument:	Agilent 6890 with Gerstel Modular Accelerated Column Heater (MACH)
Column:	Column 1: Rtx®-624 (G43) 20m, 0.18mm ID, 1.00µm (cat.# 40924) Column 2: Rtx®-WAX (G16) 20m, 0.18mm ID, 0.4µm (custom)
Sample:	cat.# 36271 and cat.# 36272 diluted in 0.5mL DI water/~0.2g sodium sulfate in 20mL headspace vial; components at 0.10 - 6.00µg - see our web page.
Inj.:	headspace trap injection, split ratio 20:1, flow split after injector to two columns, using a 0.32mm ID intermediate polarity deactivated guard column (cat.# 10044) and a "Y" Press-Tight® connector (cat.# 20405)
Inj. temp.:	220°C
Carrier:	helium, constant flow
Flow rate:	Column 1: 0.85mL/min.; Column 2: 0.99mL/min.
Oven temp.:	250°C
Gerstel temps.:	Column 1: 50°C (2 min.) to 80°C at 20°C/min. (1 min.), to 200°C at 40°C/min. (2 min.) Column 2: 35°C (2 min.) to 60°C at 100°C/min. (1 min.), to 200°C at 40°C/min. (2 min.)
Det.:	FID at 250°C, hydrogen: 40mL/min., air: 450mL/min., makeup gas: 45mL/min.

1. Restek Advantage 2006 vol. 1, pp.14-15 (2006); request: lit. cat.# 580035.
2. Direct inquiries about the Gerstel MACH System to Gerstel Inc. Phone: 410-247 5885; e-mail: sales@gerstelus.com

MACH System modules, the cooldown and equilibration time between samples is considerably shorter than with a conventional GC oven.

Dynamic headspace sampling coupled with a Gerstel MACH column heating system makes possible rapid, comprehensive assays of residual solvents. By using other column combinations and other independent temperature programs, this system can be adapted to quickly resolve other complex mixes.

Residual Solvents Class 2 - Mix A (15 components)
In dimethyl sulfoxide, 1mL/ampul
cat. # 36271 (ea.)

Residual Solvents Class 2 - Mix B (8 components)
In dimethyl sulfoxide, 1mL/ampul
cat. # 36272 (ea.)

Residual Solvents Class 2 - Mix C (8 components)
In dimethyl sulfoxide, 1mL/ampul
cat. # 36273 (ea.)

European Pharmacopoeia/ICH Q3C(M) Class 2 Mix C (14 components)
In dimethyl sulfoxide, 1mL/ampul
cat. # 36274 (ea.)

European Pharmacopoeia/ICH Q3C(M) Class 2 Mix A (6 components)
In dimethyl sulfoxide, 1mL/ampul
cat. # 36275 (ea.)

Fused Silica Guard Columns/Transfer Lines

Nominal ID	Nominal OD	length	cat. #
0.32mm	0.45 ± 0.04mm	5-Meter	10044
0.32mm	0.45 ± 0.04mm	5-Meter	10044-600

gc column ordering info

To order Gerstel MACH GC Columns, call:
800-413-8160
410-247-5885
e-mail: sales@gerstelus.com

Rtx®-624 (G43) (fused silica)
(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	Module	Gerstel cat. #
0.18mm	1.00	-20 to 240°C	20-Meter	5"	015200-019-GI
0.18mm	1.00	-20 to 240°C	20-Meter	3"	015200-020-GI

Rtx®-WAX (G16) (fused silica)

ID	df (µm)	temp. limits	length	Module	Gerstel cat. #
0.18mm	0.4	-20 to 250°C	20-Meter	5"	015200-021-GI
0.18mm	0.4	-20 to 250°C	20-Meter	3"	015200-022-GI

Simple, Optimized HPLC Analysis of Catecholamines

Increase Retention by Using an Allure™ PFP Propyl Column

By Rick Lake, Pharmaceutical Innovations Chemist, and Bruce Albright, HPLC Innovations Chemist

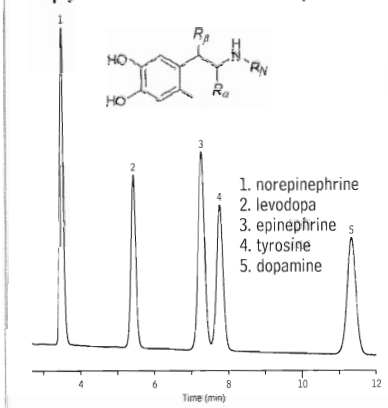
- No derivatization or ion-pairing—save time, ensure reproducible results.
- Excellent retention and resolution of low molecular weight amine compounds.
- Excellent peak shapes for reliable quantification of basic compounds.

Biogenic amines are low molecular weight intercellular messengers that relay much of the body's chemical signaling. Many synthesized drug compounds are chemically similar to these very biologically active compounds, including stimulants, hallucinogens, antidepressants, and bronchodilators.

One group of biogenic amines, the catecholamines (Figure 1), traditionally have been assayed by GC or HPLC, but either approach requires modifications. Derivatization is necessary for GC analysis, and stability issues can pose a problem. Limited retention on hydrophobic alkyl (ODS) or polar embedded (cyano) HPLC phases makes derivatization or ion-pairing techniques necessary. These modified HPLC techniques are laborious and disrupt reproducibility, and many derivatizing reagents are not LC/MS compatible.

Pentafluorophenyl HPLC phases show greater retention for compounds that have electrophilic properties, like protonated amine groups in basic compounds, and a propyl spacer between the functional group and the silica surface - a pentafluorophenyl propyl phase - further increases retention. Consequently, when an acidic mobile phase is used to induce protonation of the analytes' amine groups, the Allure™ PFP Propyl phase makes possible a simple reversed phase HPLC analysis (Figure 1). A nearly 100% aqueous mobile phase is needed, but retention of norepinephrine, the first eluting analyte, is sufficient. By changing the organic modifier, differing selectivities can be achieved (Figure 2), giving the analyst more flexibility in optimizing specific separations. By using an Allure™ PFP Propyl column, an analyst can achieve simple, reproducible analyses of catecholamines or similar low molecular weight polar compounds.

Figure 1 Superior retention of catecholamines on an Allure™ PFP Propyl column—better separations, without ion pairing.

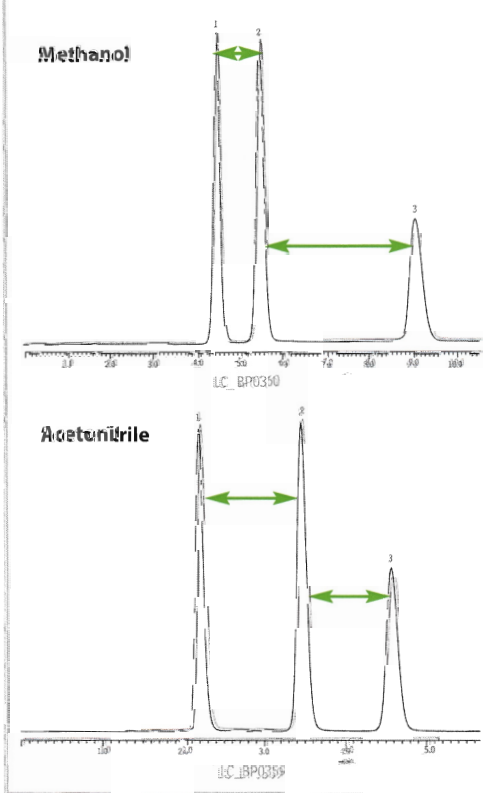


Sample:
Inj.: 10µL
Conc.: 40µg/mL each component
Sample diluent: mobile phase
Sample temp.: ambient

Column: Allure™ PFP Propyl (cat.# 9169565)
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 60Å

Conditions:
Mobile phase: 50mM ammonium phosphate (pH 3.0, adjusted with formic acid)
Flow: 1mL/min.
Temp.: 35°C
Det.: UV @ 266nm

Figure 2 Changing organic modifiers alters selectivity for catecholamines, for more flexibility in optimizing separations.



Peak List
1. levodopa
2. epinephrine
3. dopamine

Sample:
Inj.: 10µL
Conc.: 100µg/mL each component
Sample diluent: mobile phase

Column: Allure™ PFP Propyl (cat.# 9169565)
Dimensions: 150 x 4.6 mm
Particle size: 5µm
Pore size: 60Å

Conditions:
Mobile phase: 20mM ammonium formate (pH 3): methanol, 90:10
or
20mM ammonium formate (pH 3): acetonitrile, 90:10
Flow: 1mL/min.
Temp.: ambient
Det.: UV @ 280 nm

Allure™ PFP Propyl, 5µm Columns

5µm Column, 4.6mm	cat.#
150mm	9169565
150mm (with Tridet™ Inlet Fitting)	9169565-750

Allure™ PFP Propyl Guard Cartridges

Allure™ PFP Propyl	qty.	cat.#
10 x 2.1mm	3-pk.	9169502I2
10 x 4.0mm	3-pk.	9169502I0
20 x 2.1mm	2-pk.	9169502220
20 x 4.0mm	2-pk.	9169502200

80% Faster GC/MS Analysis of Essential Oils

Using a 10m x 0.10mm ID Rtx®-5 Column

by Novalina Lingga, Ph.D., Application Chemist, Shimadzu Asia Pacific, Singapore and Eberhardt Kuhn, Ph.D., International Marketing Specialist, Restek Corp.

- 5x greater sample throughput.
- Sharply reduced cost per analysis.
- Resolution and elution orders are not changed.

Essential oils are key components of perfumes, soaps, and other cosmetic products, and they find extensive use in aromatherapy. Because they have high market value, essential oils are subject to adulteration with less expensive impurities. It is, therefore, important to have reliable analytical methods to determine the purity of essential oils.

Typical analysis times for these complex samples, using a 30m x 0.25mm x 0.25µm df Rtx®-5SilMS column and "conventional" GC/MS conditions, are 18 minutes for bergamot oil and 30 minutes for patchouli oil (the analyses are posted on our website). Figure 1 shows these analyses optimized for speed, using a 10m x 0.10mm x 0.10µm df Rtx®-5 column. Analysis times were reduced to approximately 3.5 minutes for bergamot oil and 5.5 minutes for patchouli oil. This 80% reduction in analysis time increases sample throughput by a factor of 5, without sacrificing resolution or accuracy!

Relative to conventional analyses, resolution in the fast analyses is essentially unchanged for bergamot oil, and actually is slightly better for patchouli oil. Because the phase ratio (β) was kept constant at 250, the elution order of the oil components is identical for both fast and conventional analyses, allowing easy peak identification and comparison.

These results demonstrate the potential for greatly increased throughput for essential oils by using a shorter, smaller diameter column. The cost savings make this a desirable improved method for any laboratory.

Rtx®-5 Column (fused silica)

(5% diphenyl/95% dimethyl polysiloxane)

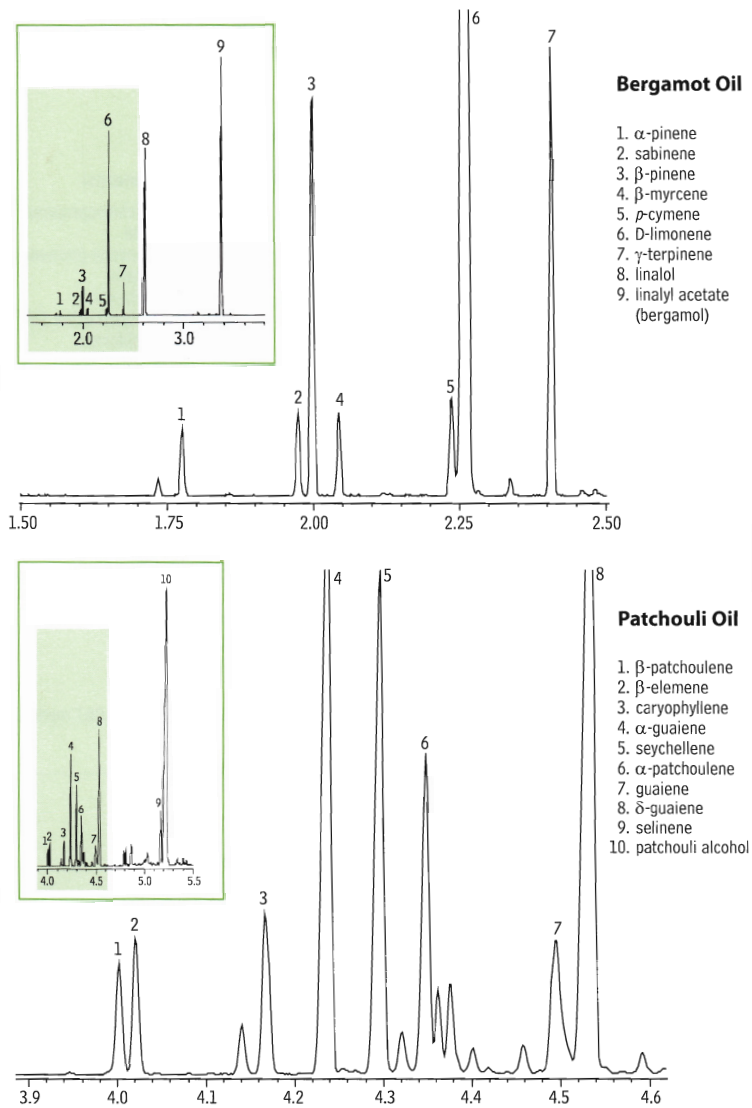
ID	df (µm)	temp. limits	length	cat. #
0.10mm	0.10	-60 to 330/350°C	10-Meter	41201

Rtx®-5Sil MS Column (fused silica)

(Selectivity similar to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	12723

Figure 1 Fast GC/MS analysis of essential oils: equivalent to conventional chromatography, 80% faster.



Column:	Rtx®-5, 10m x 0.10mm ID, 0.10µm (cat. # 41201)
Sample:	bergamot oil, 5% in acetone, 0.2µL injection
Instrument:	Shimadzu GCMS-QP2010
Injector:	split/splitless, 280°C
Injection mode:	split, split ratio = 800:1
Carrier gas:	helium
Flow control mode:	constant linear velocity (60.0 cm/sec)
Column inlet pressure:	421.5kPa @ 40°C
Column oven:	40°C – 30°C/min. – 300°C
Interface:	direct, 280°C
Ionization:	EI
Mass range:	40-500 amu

Chromatograms courtesy of Dr. Novalina Lingga, Shimadzu Asia Pacific, Singapore

Analytical Reference Materials for SOM01.1

by Ken Herwehe, Analytical Reference Materials Product Manager

- SIM compounds included in reformulated OLC 03.2 SVOA Deuterated Monitoring Compounds (DMC) mix for semivolatiles (cat.# 31810).
- SOM01.1 SVOA MegaMix™ (cat.# 33005) combines 67 semivolatiles in a single mix.
- Both ketones and non-ketones included in reformulated SOM01.1 VOA DMC Kit (cat.# 30630).

SOM01.1 defines methods for isolating, detecting, and quantitatively measuring 52 trace and low/medium level volatile, 67 semivolatile, 21 pesticide, and 9 Aroclor® target compounds in water or soil/sediment environmental samples. This document incorporates major changes to the organic methods, including separating the pesticide and Aroclor® methods, reformulating the deuterated monitoring compounds for volatiles and semivolatiles, and including selected ion monitoring (SIM) analysis. SOM01.1 calls for gas chromatography/mass spectrometry (GC/MS) and gas chromatography/electron capture detector (GC/ECD) methods for analyzing the target compounds.

please note

1. Ketone and non-ketone mixes can be purchased individually.
2. Ketone and non-ketone mixes are prepared at 500µg/mL.
3. Our methanol-d solvent (CH_3-OD; MW33)does not interfere with MS scans. Per-deuterated methanol (CD_3-OD; MW 36) interferes with MS scans.

did you know?

Our new Rxi™ capillary columns are ideal for analyses of environmental samples. For example applications, refer to the articles on **pages 6-7 and 8-9**.

For additional information, visit our website at **www.restek.com/rxi**

Volatiles: Calibration Mixes

OLC 03.2 VOA MegaMix™ (42 components)

benzene
bromochloromethane
bromodichloromethane
bromoform
carbon disulfide
carbon tetrachloride
chlorobenzene
chloroform
cyclohexane
dibromochloromethane (chlorodibromomethane)
1,2-dibromo-3-chloropropane
1,2-dibromoethane (EDB)
1,2-dichlorobenzene
1,3-dichlorobenzene
1,4-dichlorobenzene
1,1-dichloroethane
1,2-dichloroethane
1,1-dichloroethylene
cis-1,2-dichloroethylene
trans-1,2-dichloroethylene
1,2-dichloropropane
cis-1,3-dichloropropene
trans-1,3-dichloropropene
ethylbenzene
isopropylbenzene (cumene)
methyl acetate
methylcyclohexane
methyl *tert*-butyl ether (MTBE)
methylene chloride (dichloromethane)
styrene
1,1,2,2-tetrachloroethane
tetrachloroethylene
toluene
1,2,3-trichlorobenzene
1,2,4-trichlorobenzene
1,1,1-trichloroethane
1,1,2-trichloroethane
trichloroethylene
1,1,2-trichlorotrifluoroethane (Freon® 113)
m-xylene*
o-xylene
p-xylene*

2,000µg/mL each (**m*- & *p*-xylene at 1,000µg/mL) in P&T methanol, 1mL/ampul
cat. # 30492 (ea.)

1,4-Dioxane

2,000µg/mL in P&T methanol, 1mL/ampul
cat. # 30287 (ea.)

502.2 Calibration Mix #1 (gases)

bromomethane
chloroethane
chloromethane
dichlorodifluoromethane
trichlorofluoromethane
vinyl chloride

200µg/mL each in P&T methanol, 1mL/ampul
cat. # 30439 (ea.)

2,000µg/mL each in P&T methanol, 1mL/ampul
cat. # 30042 (ea.)

VOA Calibration Mix #1 (ketones)

acetone
2-butanone
2-hexanone
4-methyl-2-pentanone

5,000µg/mL each in P&T methanol:water (90:10), 1mL/ampul
cat. # 30006 (ea.)

Volatiles: DMC

SOM01.1 VOA Non-Ketone Deuterated Monitoring Compounds (11 components)

benzene-d6
chloroethane-d5
chloroform-d
1,2-dichlorobenzene-d4
1,2-dichloroethane-d4
1,1-dichloroethene-d2
1,2-dichloropropane-d6
1,3-dichloropropene-d4*
1,1,2,2-tetrachloroethane-d2
toluene-d8
vinyl chloride-d3

500µg/mL each in deuterated methanol (MeOD), 1mL/ampul
cat. # 30624 (ea.)

*Mix of *cis* and *trans* isomers. Exact proportions will be reported on the data sheet.

SOM01.1 VOA Ketone Deuterated Monitoring Compounds

2-butanone-d5
2-hexanone-d5

500µg/mL each in deuterium oxide (D_2O), 1mL/ampul
cat. # 30625 (ea.)

SOM01.1 VOA DMC Kit

kit

30624: Non-Ketones 500µg/mL
30625: Ketones 500µg/mL

1mL each of these mixtures.
cat. # 30630 (kit)

1,4-Dioxane-d8

2,000µg/mL in P&T methanol, 1mL/ampul
cat. # 30614 (ea.)

Semivolatiles: DMC

OLC 03.2 SVOA Deuterated Monitoring Compounds (DMC) (16 components)

acenaphthylene-d8
anthracene-d10
benzo(a)pyrene-d12
4-chloroaniline-d4
bis-(2-chloroethyl)ether-d8
2-chlorophenol-d4
2,4-dichlorophenol-d3
dimethylphthalate-d6
4,6-dinitro-methylphenol-d2
fluorene-d10
4-methylphenol-d8
nitrobenzene-d5
2-nitrophenol-d4
4-nitrophenol-d4
phenol-d5
pyrene-d10

2,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 31810 (ea.)

SOM01.1 Deuterated Monitoring Compound Mix SIM Compounds

fluoranthene-d10
2-methylnaphthalene-d10

2,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 33913 (ea.)

SOM01.1 Deuterated Monitoring Compound Mix w/ SIM Compounds (18 components)

acenaphthylene-d8	fluoranthene-d10
anthracene-d10	fluorene-d10
benzo(a)pyrene-d12	2-methylnaphthalene-d10
bis(2-chloroethyl)ether-d8	4-methylphenol-d8
4-chloroaniline-d4	nitrobenzene-d5
2-chlorophenol-d4	2-nitrophenol-d4
2,4-dichlorophenol-d3	4-nitrophenol-d4
dimethylphthalate-d6	phenol-d5
4,6-dinitro-2-methylphenol-d	pyrene-d10

2,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 33918 (ea.)

Semivolatiles: Calibration Mixes

SOM01.1 SVOA MegaMix™ (67 components)

acenaphthene	2,4-dinitrophenol
acenaphthylene	2,4-dinitrotoluene
acetophenone	2,6-dinitrotoluene
anthracene	diphenylamine‘
atrazine	di-*n*-octyl phthalate
benzaldehyde	fluoranthene
benzo(a)anthracene	fluorene
benzo(a)pyrene	hexachlorobenzene
benzo(b)fluoranthene	hexachlorobutadiene
benzo(ghi)perylene	hexachlorocyclopentadiene
benzo(k)fluoranthene	hexachloroethane
biphenyl	indeno(1,2,3-cd)pyrene
bis(2-chloroethoxy)methane	isophorone
bis(2-chloroethyl)ether	2-methylnaphthalene
bis(2-chloroisopropyl) ether	2-methylphenol
bis(2-ethylhexyl)phthalate	3-methylphenol*
4-bromophenyl phenyl ether	4-methylphenol*
butyl benzyl phthalate	naphthalene
ε-caprolactam	2-nitroaniline
carbazole	3-nitroaniline
4-chloro-3-methylphenol	4-nitroaniline
2-chloronaphthalene	nitrobenzene
2-chlorophenol	2-nitrophenol
4-chlorophenyl phenyl ether	4-nitrophenol
chrysene	N-nitroso-di-*n*-propylamine
dibenz(a,h)anthracene	pentachlorophenol
dibenzofuran	phenanthrene
3,3'-dichlorobenzidine	phenol
2,4-dichlorophenol	pyrene
diethyl phthalate	1,2,4,5-tetrachlorobenzene
2,4-dimethylphenol	2,3,4,6-tetrachlorophenol
dimethyl phthalate	2,4,5-trichlorophenol
di-*n*-butyl phthalate	2,4,6-trichlorophenol
4,6-dinitro-2-(dinitro-ocresol)	

1,000µg/mL each in methylene chloride (*3-methylphenol and 4-methylphenol at 500µg/mL), 1mL/ampul
cat. # 33005 (ea.)

'N-Nitroso-diphenylamine (listed analyte) decomposes to diphenylamine (mix component).

Semivolatiles: QA Mixes

SV Internal Standard Mix

acenaphthene-d10	naphthalene-d8
chrysene-d12	perylene-d12
1,4-dichlorobenzene-d4	phenanthrene-d10

2,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 31206 (ea.)

4,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 31006 (ea.)

Revised SV Internal Standard Mix (7 components)

acenaphthane-d10	naphthalene-d8
chrysene-d12	perylene-d12
1,4-dichlorobenzene-d4	phenanthrene-d10
1,4-dioxane-d8	

2,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 31885 (ea.)

4,000µg/mL each in methylene chloride, 1mL/ampul
cat. # 31886 (ea.)

SOM01.1 SVOA B/N Matrix Spike Mix

acenaphthene	N-nitroso-di-*n*-propylamine
2,4-dinitrotoluene	pyrene

5,000µg/mL each in methanol, 1mL/ampul
cat. # 33916 (ea.)

5,000µg/mL each in methanol, 5mL/ampul
cat. # 33917 (ea.)

B/N Matrix Spike Mix

acenaphthene	N-nitroso-di-*n*-propylamine
1,4-dichlorobenzene	pyrene
2,4-dinitrotoluene	1,2,4-trichlorobenzene

1,000µg/mL each in methanol, 1mL/ampul
cat. # 31004 (ea.)

5,000µg/mL each in methanol, 1mL/ampul
cat. # 31074 (ea.)

5,000µg/mL each in methanol, 5mL/ampul
cat. # 31084 (ea.)

Acid Matrix Spike Mix

4-chloro-3-methylphenol	pentachlorophenol
2-chlorophenol	phenol
4-nitrophenol	

1,500µg/mL each in methanol, 1mL/ampul
cat. # 31005 (ea.)

7,500µg/mL each in methanol, 1mL/ampul
cat. # 31075 (ea.)

7,500µg/mL each in methanol, 5mL/ampul
cat. # 31085 (ea.)

SV Tuning Compound

decafluorotriphenylphosphine (DFTPP)

2,500µg/mL in methylene chloride, 1mL/ampul
cat. # 31001 (ea.)

Organochlorine Pesticide Resolution Check Mix

(22 components)

aldrin	10µg/mL	endosulfan I	10
α-BHC	10	endosulfan II	20
β-BHC	10	endosulfan sulfate	20
δ-BHC	10	endrin	20
γ-BHC (lindane)	10	endrin aldehyde	20
α-chlordane	10	endrin ketone	20
γ-chlordane	10	heptachlor	10
decachlorobiphenyl	20	heptachlor epoxide	
dieldrin	20	(isomer B)	10
4,4'-DDD	20	methoxychlor	100
4,4'-DDE	20	2,4,5,6-tetrachloro-	
4,4'-DDT	20	*m*-xylene	10

In hexane:toluene, 1mL/ampul
cat. # 32454 (ea.)

Pesticide Surrogate Mix

decachlorobiphenyl	200µg/mL
2,4,5,6-tetrachloro-*m*-xylene	100

In P&T methanol, 1mL/ampul
cat. # 32453 (ea.)

Aroclor® 1016/1260

400µg/mL each in acetone, 1mL/ampul
400µg/mL in acetone, 1mL/ampul
cat. # 32456 (ea.)

for more info

For reference materials for OLC 03.2 analyses of pesticides or Aroclor® PCBs, please visit our website, or call your Restek representative.

free data

Available on Our Website: Lot Certificates, Datapacks, and MSDS

For complete information detailing manufacturing and testing for Restek inventoried reference standards, just visit our website at **www.restek.com** To view lot certificates and/or an MSDS, enter the catalog number of the product in the Search feature. For a free Datapack, enter the catalog number and lot number of the product, to obtain a printable pdf file.

did you know?

We have over 2,000 pure, characterized, neat compounds in our inventory! If you do not see the EXACT mixture you need listed on any of these pages, call us.

For our on-line Custom Reference Materials Request Form visit us on the web at **www.restek.com/solutions**.

Improve Characterization of Complex Protein Digests

Using Viva Wide Pore HPLC Columns

by Julie Kowalski, Innovations Chemist

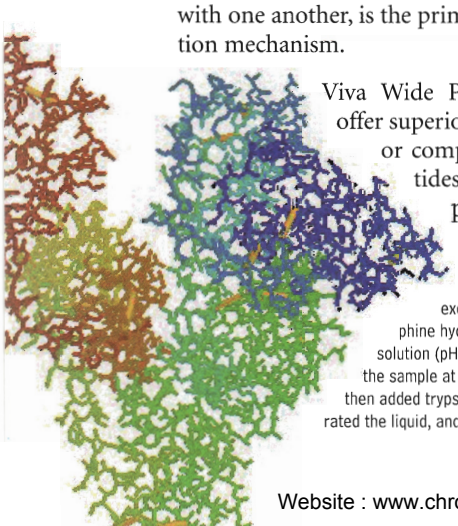
- Superior resolution—many peaks contain one or two peptides, not three or more.
- Excellent results with highly aqueous mobile phases, compatible with digest matrices.
- Restek-manufactured silica in Restek-manufactured columns.

Protein analyses often incorporate a combination of liquid chromatography and electrospray mass spectrometry. Typically, a protein sample is chemically or enzymatically digested to produce peptides, HPLC/MS is used to resolve and identify the peptides, and this information is used to search protein databases to identify the protein of interest. This type of analysis is now used in many fields, including the bioanalytical and pharmaceutical disciplines.

We tailored Viva silica specifically to provide superior chromatography for peptides and other large molecules, and we highly recommend Viva columns for analyses of protein digests. Featuring the largest available surface area in 250-350 Angstrom pores, packings prepared from Viva silica allow longer interaction between peptides and the stationary phase, affording greater resolution.

For an example analysis, we prepared a trypsin digest of bovine serum albumin (BSA).[1] We used a 150mm x 1mm ID Viva C18 column (5µm particles, cat# 9514561) to separate the peptides, which number approximately 70, and identified them through manual data analysis.

Figure 1 is a TIC chromatogram for the BSA trypsin digest. Close observation reveals the Viva C18 column has provided outstanding separation, based on the large number of discrete peaks representing only one or two peptides. In contrast, in typical results from other "wide pore" columns it is common to see three or more peptides per peak; this can reduce the number of peptides that are identified. The large number of discrete peaks in Figure 1 also indicates that peptide interaction with the Viva C18 stationary phase, rather than with one another, is the primary retention/separation mechanism.



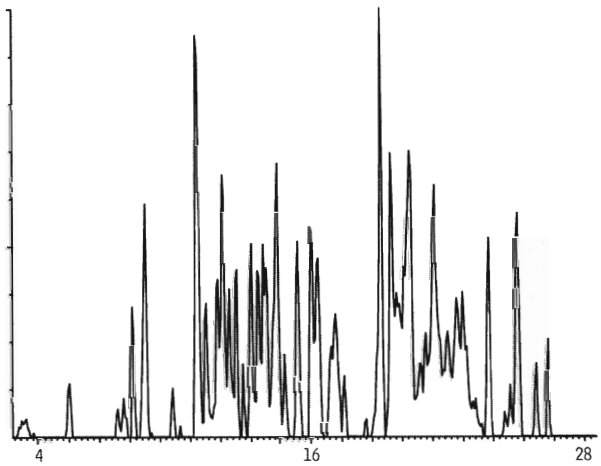
Viva Wide Pore HPLC Columns offer superior resolution of simple or complex mixtures of peptides - a critical factor in protein identifications.

[1] BSA disulfide bonds were reduced by adding a molar excess of tris(2-carboxyethyl)phosphine hydrochloride (TCEP) to a buffered solution (pH 7) containing BSA. We stored the sample at 40°C for one hour, under argon, then added trypsin to digest the protein, evaporated the liquid, and dissolved the digest in water.

Figure 1 A Viva C18 column resolves a BSA tryptic digest into many 1-2 peptide peaks, for more reliable identification.

for more info

For details on this analysis, please visit our website: **www.restek.com/bioanalytical**



Sample:
Inj.: 15µL
Conc.: bovine serum albumin tryptic digest, 16pmol/µL
Sample diluent: water/0.15% formic acid (v/v)

Column: **Viva C18** (cat. # 9514561)
Dimensions: 150mm x 1mm
Particle size: 5µm
Pore size: 300Å

Conditions:
Mobile Phase: A: water/0.15% formic acid (v/v)
B: acetonitrile/0.15% formic acid (v/v)

Time:	%B
0.0	5
4.5	5
64.0	65

Flow: 0.2mL/min.
Temp.: ambient
Det.: Micromass Quattro II

Viva C18 Columns

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 300Å
carbon load: 9%
endcap: yes
pH range: 2.5 to 10
temperature limit: 80°C

Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
5µm Columns				
30mm	9514531	9514532	9514533	9514535
50mm	9514551	9514552	9514553	9514555
100mm	9514511	9514512	9514513	9514515
150mm	9514561	9514562	9514563	9514565
200mm	9514521	9514522	9514523	9514525
250mm	9514571	9514572	9514573	9514575

Sidewinder™ Column Heaters and Heater/Cooler

By Becky Wittrig, Ph.D., HPLC Product Manager

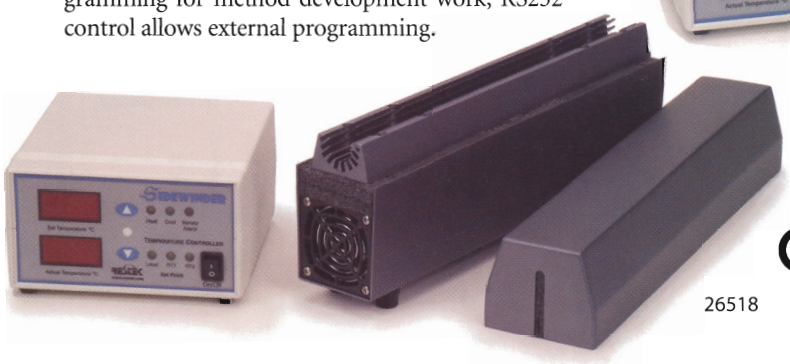
- Easy to set up!
- Lightweight and compact—require little bench space.
- Heaters operate from 5°C above ambient to 85°C, heater/cooler from 5°C to 55°C.

Temperature stability is critical for accurate and reproducible results in HPLC analyses. Sidewinder™ Column Heaters provide optimum heating performance and accuracy to within 1°C. The unique sleeve design completely encloses any analytical HPLC column up to 25cm long. State-of-the-art electronics in the 24V control unit allow fast 10Hz sampling and stability to within 0.1°C. RS232 control allows external programming.

26516

Sidewinder™ Column Heater/Coolers are designed to hold an HPLC column up to 30cm long and 7.8mm in diameter. A doubly insulated cover maintains temperature stability in the chamber to within ±0.2°C. The high performance Peltier-driven 24V control unit allows remote temperature programming for method development work; RS232 control allows external programming.

26517



26518

new and Improved!

Description	qty.	cat.#
Temperature Control Module and Long Column Holder, 25cm Holder	ea.	26516
Temperature Control Module and Short Column Holder, 10cm Holder	ea.	26517
Sidewinder™ Heater/Cooler Temperature Control Module	ea.	26518

All Sidewinder™ temperature control products carry the value recognized CE mark. Each unit meets the demanding electromagnetic emission standards of the new European Union Directives, United States standards, and Canadian standards.

MicroPulse™ Pulse Dampers

- Compact unit (2.5" x 1.5") can be placed almost anywhere.
- Small, 150µL dead volume at atmospheric pressure.
- Compatible with high pressure (stainless steel unit to 6000psi, PEEK® unit to 5000 psi).

The MicroPulse™ pulse damper improves system baseline stability while increasing system volume by only 150µL—ideal for applications in which minimizing total system volume is critical. 316 stainless steel or PEEK® option, for a wide range of applications.

Description	qty.	cat.#
MicroPulse™ Pulse Damper, Stainless Steel	ea.	25238
MicroPulse™ Pulse Damper, PEEK®	ea.	25239

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www.restek.com/hplcacc

Parker ChromGas® Hydrogen Generators

Is your lab wasting money on bottled gas?

by Barry Burger, Petroleum Chemist

- Economical, continuous source of ultra-pure hydrogen (99.9995%).
- Safe and easy to use and maintain.
- Hydrogen reduces gas costs, cuts analysis time by 50%, increases column lifetimes.

If you use 2-3 cylinders of helium and/or hydrogen per week, as carrier gas and/or fuel gas, bottled gas is an expense in the range of $15,000 to $25,000 per year•, including overhead: expenses and time involved with ordering, transporting, installing, and periodically inspecting cylinders. You also contend with unquantifiable costs, such as floor space lost to an inventory of cylinders. Helium, widely used as carrier gas, is a non-renewable resource extracted from natural gas and, because it is a petrochemical product, its cost will continue to rise, domestically and internationally. Chromatographers must look for cost effective, ultra-pure gas alternatives to supply their instruments and state-of-the-art analytical columns. Fortunately, we do have options.

Past practice in gas chromatography was to select either nitrogen or helium as the carrier gas. Hydrogen wasn't given much consideration, primarily because of flammability and storage issues, even though it offers several distinct advantages over nitrogen or helium. Now, Parker ChromGas® hydrogen generators are a safe, reliable source of ultra-pure (99.9995%) hydrogen, and effective replacements for bottled gas. A Parker ChromGas® hydrogen generator stores less than 50mL of hydrogen (less than 0.002 cubic feet) at 1 atm., or 305mL of hydrogen (0.01 cubic feet) at 6.1 atmospheres (90psig.) From a safety standpoint there is no compromise, compared to a 300 cubic foot cylinder of hydrogen at 2500 psig.

Parker ChromGas® hydrogen generators continuously produce dry, ultra-pure hydrogen by electrolytic dissociation of deionized water and hydrogen proton conduction across a membrane. The hydrogen product is dried by passing it through a coalescing filter, a drying tube, and a desiccant cartridge. Maximum output pressure, 90psig, is controlled to the point of use via a pressure adjust regulator. Other safety features include a pressure relief valve to prevent overpressurization and a

Figure 1 A simulated distillation reference mix is well resolved, in 8 minutes, showing a Parker ChromGas® hydrogen generator can meet gas volume demands.

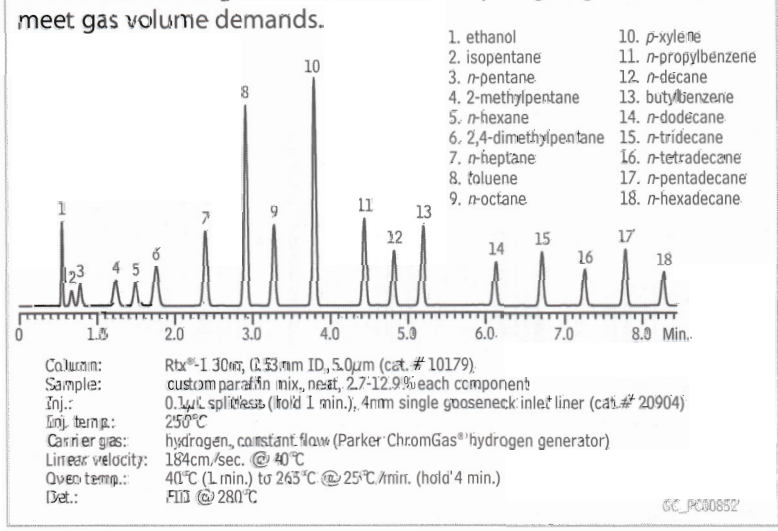
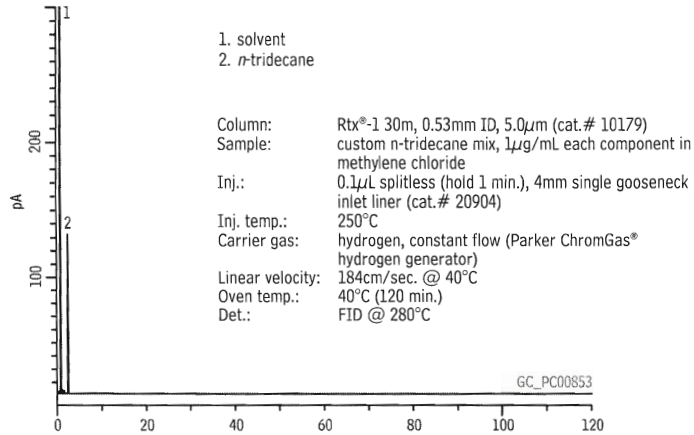


Table 1 Repeatable retention times for simulated distillation mix components confirm the hydrogen generator's steady and precise flow of carrier gas.

				Run Number / Retention Time (min.)									
Component	Mean	SD	%RSD	1	2	3	4	5	6	7	8	9	10
1. ethanol	0.547	1E-03	0.1765	0.546	0.548	0.548	0.548	0.549	0.546	0.547	0.547	0.547	0.548
2. isopentane	0.67	1E-03	0.1484	0.669	0.67	0.671	0.67	0.672	0.669	0.669	0.67	0.669	0.67
3. *n* pentane	0.779	0.001	0.169	0.777	0.779	0.78	0.779	0.781	0.777	0.778	0.779	0.778	0.78
4. 2-methylpentane	1.232	0.001	0.1198	1.229	1.231	1.233	1.232	1.234	1.23	1.232	1.232	1.232	1.233
5. *n*-hexane	1.488	0.001	0.0992	1.485	1.487	1.489	1.488	1.49	1.486	1.488	1.488	1.488	1.489
6. 2,4-dimethylpentane	1.753	0.001	0.0721	1.751	1.752	1.754	1.754	1.755	1.752	1.754	1.754	1.754	1.754
7. *n*-heptane	2.387	0.001	0.0442	2.385	2.386	2.388	2.387	2.388	2.386	2.387	2.387	2.388	2.388
8. toluene	2.904	0.001	0.0356	2.902	2.904	2.905	2.904	2.905	2.903	2.904	2.905	2.905	2.905
9. *n*-octane	3.266	7E-04	0.0214	3.264	3.265	3.266	3.266	3.266	3.265	3.266	3.266	3.266	3.266
10. *p*-xylene	3.784	7E-04	0.0195	3.783	3.784	3.785	3.784	3.784	3.783	3.784	3.784	3.785	3.785
11. *n*-propylbenzene	4.438	5E-04	0.0109	4.437	4.438	4.438	4.438	4.438	4.437	4.437	4.438	4.438	4.438
12. *n*-decane	4.809	4E-04	0.0088	4.809	4.809	4.809	4.809	4.809	4.808	4.808	4.809	4.809	4.809
13. butylbenzene	5.174	5E-04	0.0102	5.173	5.174	5.174	5.174	5.173	5.173	5.173	5.173	5.174	5.174
14. *n*-dodecane	6.116	5E-04	0.0079	6.116	6.116	6.116	6.116	6.116	6.115	6.115	6.116	6.116	6.115
15. *n*-tridecane	6.703	5E-04	0.0077	6.704	6.704	6.704	6.704	6.703	6.703	6.703	6.703	6.703	6.703
16. *n*-tetradecane	7.255	7E-04	0.0097	7.256	7.255	7.253	7.255	7.254	7.254	7.254	7.254	7.254	7.254
17. *n*-pentadecane	7.774	6E-04	0.0081	7.775	7.775	7.775	7.774	7.774	7.773	7.774	7.774	7.774	7.774
18. *n*-hexadecane	8.264	6E-04	0.0069	8.265	8.265	8.264	8.264	8.264	8.263	8.264	8.264	8.264	8.264

Figure 2 Carrier gas from a Parker ChromGas® hydrogen generator assures a stable baseline, for sensitive analyses. Performance equivalent to cylinders, at lower cost.



mass leak sensor to indicate hydrogen demand is exceeding instrument capability, in which case the generator will shut down. A low water level and/or poor quality water also will shut down the generator, to prevent damage to the electrolytic cell.

Maintaining the generator is simple. The 4-liter water reservoir may be filled at any time without shutting down the generator, eliminating the downtime associated with changing gas cylinders. At maximum hydrogen demand, the smallest generator will consume one 4-liter tank of deionized water in 8-10 days. The deionizer bags in the water tank should be replaced twice yearly. An LED indicator will illuminate when the desiccant cartridge requires regeneration.

To evaluate performance, we set up a small Parker ChromGas® hydrogen generator (90mL/min. maximum hydrogen output) to supply both carrier gas and fuel gas to an Agilent 6890 GC. We installed a 30 meter x 0.53mm ID x 5µm df Rtx®-1 column (100% polydimethylsiloxane (PDMS) phase, cat.# 10179) in the oven and set analytical parameters as specified in ASTM D-7096-05, a simulated distillation method, but substituted hydrogen for helium as the carrier gas. We used a column flow rate of 40mL/min., in the constant flow mode, which represented the optimum linear velocity for hydrogen. The 40mL/min. carrier gas flow rate, plus a 40mL/min. flow of fuel gas, was 90% of the generator's maximum output capacity, and tested the generator's capability to meet volume demands.

Figure 1 is a chromatogram of the calibration standard used for retention time-boiling point determination and response factor validation in the ASTM method. The components were well resolved and the analysis completed rapidly, in little more than 8 minutes. Reproducible retention times are vital to obtaining accurate initial boiling point (IBP) data. Table 1 shows retention times for the ASTM reference mix components were well within the method specification of ±0.05 minutes per compound, demonstrating the hydrogen generator's ability to maintain a steady and precise flow of carrier gas. Figure 2 monitors FID baseline stability over 2 hours. These figures and data clearly show that a Parker ChromGas® hydrogen generator is a dependable source of ultra-high purity carrier and fuel gas for demanding GC applications.

On average, yearly electricity and maintenance costs for operating a Parker ChromGas® hydrogen generator are approximately $225•. Offsetting the costs of purchasing and operating a generator with the savings made by not using gas cylinders indicates the generator will pay for itself in 1 to 2 years. With numbers like these, can you afford not to consider purchasing a Parker ChromGas® hydrogen generator for your laboratory?

• Cost estimate for USA, in US $.

Parker ChromGas® Hydrogen Generators

- Selectable delivery pressure: 0–100psig.
- High hydrogen purity—99.9995%.
- Greater convenience and safety.

Parker ChromGas® hydrogen generators are certified for laboratory use by Canadian Standards Association (CSA), Underwriters Laboratories (UL), and International Electrotechnical Commission (IEC) 1010.

Hydrogen Purity:	99.9995%
Outlet Port:	1/8" compression
Electrical:	117 Vac/234Vac
Pressure Control:	5 to 20 psig ±0.5%
	20 to 90 psig ±0.2%
Delivery Pressure:	2 to 30 psig ±3%
	30 to 100 psig ±2%
Shipping Weight:	40 lb (18 kg)
Dimensions:	13"H x 15"W x 14"D (33cm x 38cm x 36cm)

Description	Capacity	cat.#
Hydrogen Generator A9090	90cc/min.	22033
Hydrogen Generator A9150	160cc/min.	22034
Hydrogen Generator B9200	250cc/min.	22035
Hydrogen Generator B9400	500cc/min.	22036
Replacement Deionizer Bag (for all models, 2-pk.)		21670
Replacement Desiccant Cartridge (for all models)		21671

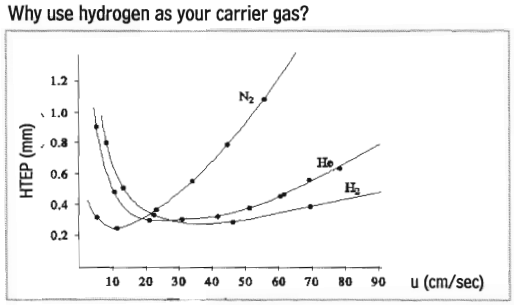
International Power Cord Sets

Location	cat.# suffix
United Kingdom (230VAC, 50/50Hz)	-550
European (230VAC, 50/60Hz)	-551
IEC Connector Only (230VAC, 50/60Hz)	-552
Japanese (200VAC, 50/60Hz)	-556
Japanese for Hydrogen (100VAC, 50/60Hz)	-554

Just add the proper suffix to the catalog number for the gas generator you are ordering.

tech tip

Why use hydrogen as your carrier gas?



Hydrogen, helium, or nitrogen - which do you choose as your carrier gas? We need only look at the van Deemter curves to see the advantages of hydrogen as a carrier gas.Nitrogen generates the highest column efficiency (HETP = 0.22mm), but at an optimum velocity of only 8-10 cm/sec. This great sacrifice in the speed of analysis generally makes nitrogen a poor choice. Column efficiency is slightly reduced with helium (HETP = 0.29mm), but optimum linear velocity is 19-22 cm/sec. With an optimum linear velocity of 35-42cm/sec., hydrogen combines high column efficiency (HETP = 0.28mm) with analysis times 4x faster than nitrogen and 2x faster than helium, thus reducing costs per analysis. Linear velocities of up to 75-80cm/sec. can be used with only a small decrease in column efficiency. Another benefit: lower temperatures are needed to elute analytes, increasing column longevity.

GC Inlet Liner Deactivations for Basic Drug Analysis

By Kristi Sellers, Clinical/Forensic Innovations Chemist, and Lydia Nolan, Innovations Chemist

- Base-deactivated inlet liners are inert to basic drugs, for greater responses.
- Inertness of Rtx®-5Amine column is enhanced for basic compounds.
- Use this liner / column combination for the lowest %RSDs for basic drugs.

Clinical and forensic toxicologists are required to detect low levels of abused drugs in body fluids and confirm their presence by GC/MS. Typical limits of detection are 1-15ng/mL, depending on the sample matrix. For basic drugs (e.g., Figure 1), selecting the proper surface treatment for the GC inlet liner is important, because this parameter can affect responses. The surface of a glass inlet liner contains active silanol groups (Si-OH) that can act as electron pair acceptors, and react with nitrogen or oxygen electron pair donors in basic drug molecules (Figure 2).[1] These reactions usually are rapid and reversible, but they are expressed chromatographically as broad, tailing peaks and/or reduced responses. To eliminate these acid-base reactions, make chromatographic peaks sharp, Gaussian, and easy to integrate, and thereby help ensure reproducible and accurate responses, the -OH groups on the glass surface must be deactivated.

Using GC/FID responses, we evaluated several alternatives for deactivating inlet liners, to determine maximum sensitivity for basic drugs. We prepared reference standards of the free base forms of alprazolam, benzphetamine, cocaine, codeine, ketamine, methadone, and phencyclidine (Figure 1) at 100, 50, 25, 10, and 5 ng/mL concentrations, then analyzed the drugs on a base-deactivated 15m, 0.25mm ID, 0.25µm Rtx®-5Amine column (5% diphenyl/95% dimethylpolysiloxane stationary phase), using a 4mm single gooseneck inlet liner that was untreated, deactivated through an intermediate polarity deactivation process (standard liner deactivation procedure), deactivated through a base deactivation process, or deactivated through the Siltek® deactivation process. We obtained three replicate analyses for each reference standard-liner treatment combination, and evaluated the response data statistically to determine which deactivation treatment maximized sensitivity and reproducibility. We used these results to generate box plots that display the range of data distribution, or variation – an indication of the reproducibility of the performance. We chose phencyclidine (PCP) and cocaine plots to represent the nitrogen-containing and nitrogen/oxygen-containing drugs, respectively (Figure 2). The line in each box indicates the mean response.

Figure 1 Nitrogen- and oxygen-containing compounds can react with silanol groups on glass surfaces, causing poor chromatography.

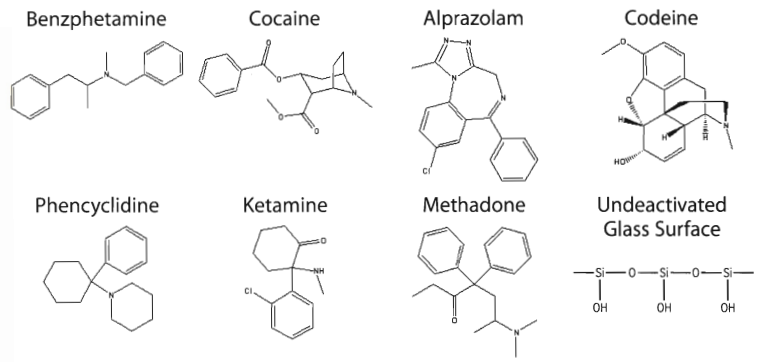


Figure 2 A base-deactivated inlet liner provides highest mean responses for PCP.

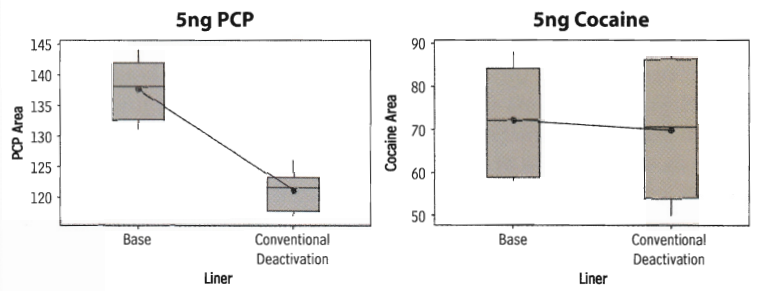
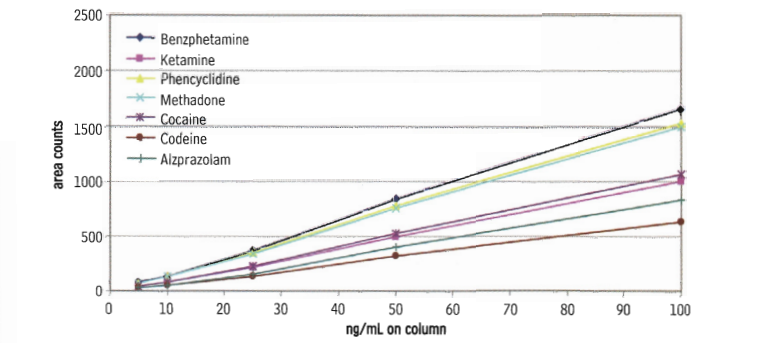
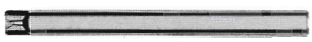


Figure 3 Linearity plots for all drugs, analyzed using a base-deactivated inlet liner and an Rtx®-5Amine column.



Base Deactivated Inlet Liners for Basic Drug Analysis

For Agilent GCs	ea.	cat.# 5-pk.	25-pk.
Gooseneck Splitless (4.0mm ID** x 6.5mm OD x 78.5mm) 	20798-210.1	20799-210.5	20800-210.25
Gooseneck Splitless w/ Base Deactivated Wool (4.0mm ID** x 6.5mm OD x 78.5mm) 	20798-211.1	20799-211.5	20800-211.25
Split Straight w/ Base Deactivated Wool (4.0mm ID** x 6.3mm OD x 78.5mm) 	20781-211.1	20782-211.5	20783-211.25
Cyclosplitter® (4.0mm ID** x 6.3mm OD x 78.5mm) 	20706-210.1	20707-210.5	20708-210.25

**Nominal ID at syringe needle expulsion point.
For liners for other instruments, refer to our catalog or website.

Base-Deactivated Inlet Liners

qty.	Base-Deactivated		Base-Deactivated w/ Base-Deactivated Wool	
each	-210.1	addl. cost	-211.1	addl. cost
5-pk.	-210.5	addl. cost	-211.5	addl. cost
25-pk.	-210.25	addl. cost	-211.25	addl. cost

For base-deactivated inlet liners, add the corresponding suffix number to the liner catalog number.

Base-Deactivated Wool

Ideal for amines and other basic compounds.

Description	qty.	cat.#
Base-Deactivated Wool	10 grams	20999

Mini Wool Puller/Inserter

Insert and remove wool plugs easily.

Description	qty.	cat.#
Mini Wool Puller/Inserter	2-pk.	20114

Not recommended for use with double gooseneck liners.

Inlet Liner Removal Tool

- Easily remove liner from injector—no more burned fingers.
- Made from high-temperature silicone.
- Won't chip or crack the liner.

Description	qty.	cat.#
Inlet Liner Removal Tool	3-pk.	20181

Rtx®-5 Amine Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (μm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 300/315°C	30-Meter	12323
0.25mm	0.25	-60 to 300/315°C	15-Meter	12320

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The data show that undeactivated liners and liners that received intermediate polarity treatment provided poorer responses or reproducibility, compared to base-deactivated or Siltek® treated liners, due to the acidic nature of the undeactivated glass surface or to a small but influential number of residual acidic sites remaining on the intermediate polarity deactivated surface.

Because the undeactivated liners and intermediate polarity treated liners exhibited either low mean response or high variation, we reanalyzed the data, excluding these treatments and comparing the remaining data (for base-deactivated liners and Siltek® treated liners) for responses and reproducibility. As shown by the examples in Figure 2, base-deactivated liners and Siltek® treated liners performed equally well for cocaine, but the base-deactivated liners yielded the best responses and reproducibility for PCP. Ultimately, a base-deactivated liner would give the best overall performance. Figure 3 shows the linearity plots for all analyzed drugs, obtained using a base-deactivated liner and an Rtx®-5Amine column. Low %RSD values for ketamine (3%), phencyclidine (2%), methadone (2%), cocaine (3%), codeine (5%), and alprazolam (12%) confirm the reproducibility of data obtained from this combination.

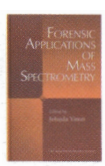
Because nitrogen- and oxygen-containing drugs react with silanol groups on glass surfaces, it is important to use properly deactivated glass inlet liners when analyzing these compounds by GC. This work demonstrates that a base-deactivated inlet liner, used in combination with a base-deactivated column, produces high and reproducible responses for basic drugs.

Reference

1. Seyhan N. and D.C. Ege, *Organic Chemistry* Health and Company, 1984, pp.124-136.

recommended reading

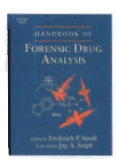
Forensic Applications of Mass Spectrometry



Applies current developments in mass spectrometry to forensic analyses. Techniques discussed include capillary GC/MS, thermospray LC/MS, tandem mass spectrometry, (MS/MS), pyrolysis GC/MS and isotope ratio mass spectrometry.

J. Yinon, CRC Press LLC, 1994, 320pp., ISBN 0-8493-8252-1 **cat.# 23056** (ea.)

Handbook of Forensic Drug Analysis



Provides in-depth, up-to-date methods and results. Chapters by leading researchers discuss the various forms of drugs, as well as the origin and nature of samples.

F. Smith and J. Siegel, Elsevier Academic Press, 2004, 584pp., ISBN 0-12-650641-8 **cat.# 23055** (ea.)

Instrument Innovations!

New Injection Port Can Simplify Life in Your Laboratory

by Donna Lidgett, GC Accessories Product Marketing Manager

- No kinked or broken gas lines.
- Change inlet liners faster, easier, and eliminate touching hot surfaces.
- Excellent for Agilent 5890, 6850, or 6890 GCs; especially advantageous with Agilent GCs equipped with autosamplers.

EZ Twist Top™ Split/Splitless Injection Port for Agilent GCs

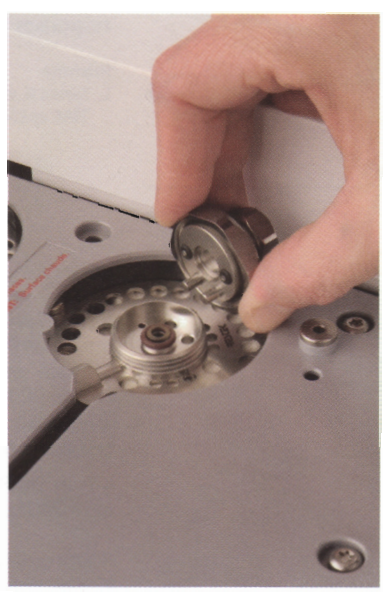
Injection port maintenance should be performed prior to installing any capillary column, and on a routine basis, based on the number of injections made and the cleanliness of the samples. For optimum system performance, the injection port liner must be free of sample residue, septum particles, and ferrule fragments, so proper maintenance includes replacing the injection port liner, critical seals, and septum. Peak shape degradation, poor reproducibility, sample decomposition, and ghost peaks all are associated with using a dirty liner. Frequent septum replacement prevents fragmentation and leaks. Multiple injections and continuous exposure to hot injection port surfaces will decompose the septum and can create particles that can fall into the injection port liner, where they become a potential source of ghost peaks, loss of inertness, and occluded carrier gas flow. Therefore, changing septum and inlet liners frequently is essential to maintaining optimum system performance.

Using Restek's new, unique EZ Twist Top™ Injection Port, and Restek Cool Tools (Septum Nut Removal Tool, cat. # 24918, and Inlet Liner Removal Tool, cat. # 20181—order separately), you can reduce maintenance time and frustration, and eliminate tangled gas lines and damage that leads to leaks, while avoiding direct contact with hot metal and glass surfaces.

The gas lines are attached to the EZ Twist Top™ Shell Weldment (bottom) instead of the weldment (top). Once the injection port is installed the gas lines are under the GC cover and do not interfere with routine injection port maintenance, as shown in Figure 1. To remove the weldment and access the liner, simply slip the Weldment Removal Tool (included in the complete injection port kit) over the weldment (Figure 2), twist, and remove the weldment. For speed and efficiency, the weldment stays secured in the tool until you are ready to reattach it. Changing inlet liners in original equipment injection ports was complicated by the gas lines and sampling tray. Our new injection port makes changing the liner a quick and simple task.

Figure 1

The old way: gas lines can be damaged during routine injection port maintenance.



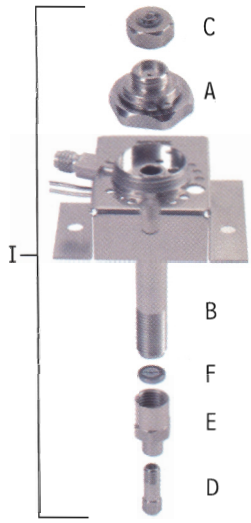
The new way: with the EZ Twist Top™ Injection Port, the gas lines are under the GC cover and are not disturbed during maintenance.

Figure 2

Simply slip the Weldment Removal Tool over the weldment, then twist and remove the weldment. For speed and efficiency, the weldment stays secured in the Weldment Removal Tool until you reattach it.

EZ Twist Top™ Split/Splitless Injection Port for Agilent 5890 GCs

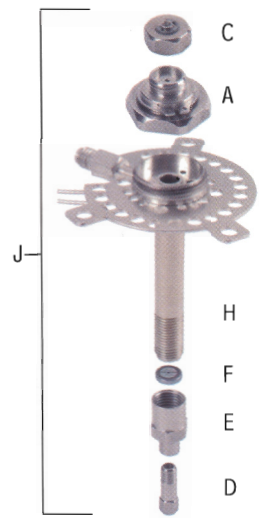
Description	qty.	cat.#
I) **Complete injection port assembly** includes: split/splitless weldment, shell weldment, 2 weldment O-rings, Siltek® Dual Vespel® Ring inlet seal, septum nut, reducing nut, stainless steel capillary nut and weldment tool	kit	22725
Siltek® complete injection port assembly includes: Siltek® split/splitless weldment, Siltek® shell weldment, 2 weldment O-rings, Siltek® Dual Vespel® Ring inlet seal, septum nut, reducing nut, stainless steel capillary nut and weldment tool	kit	22726
A) Split/Splitless Weldment for Agilent 5890/6890/6850 GCs (2 weldment O-rings are installed on the weldment)	ea.	22724
Siltek® Split/Splitless Weldment for Agilent 5890/6890/6850 GCs (2 weldment O-rings are installed on the weldment)	ea.	22732
B) Shell Weldment for Agilent 5890 GCs	ea.	22727
Siltek® Shell Weldment for Agilent 5890 GCs	ea.	22731
Weldment O-rings	10-pk.	22729
C) Autosampler & PTV Septum Nut (for 23-gauge needles)	ea.	20631
D) Stainless Steel Capillary Column Nut (for use with standard 1/16" ferrules)	2-pk.	20883
E) Reducing Nut	ea.	22078
F) Siltek® 0.8mm ID Dual Vespel® Ring Inlet Seal	2-pk. 10-pk.	21242 21243
G) Weldment Removal Tool for Agilent 5890/6890/6850 GCs	ea.	22728



Septum Nut Removal Tool (sold separately)

EZ Twist Top™ Split/Splitless Injection Port for Agilent 6890/6850 GCs

Description	qty.	cat.#
J) **Complete injection port assembly** includes: split/splitless weldment, shell weldment, 2 weldment O-rings, Siltek® Dual Vespel® Ring inlet seal, septum nut, reducing nut, stainless steel capillary nut and weldment tool	kit	22721
Siltek® complete injection port assembly includes: Siltek® split/splitless weldment, Siltek® shell weldment, 2 weldment O-rings, Siltek® Dual Vespel® Ring inlet seal, septum nut, reducing nut, stainless steel capillary nut and weldment tool	kit	22722
A) Split/Splitless Weldment for Agilent 5890/6890/6850 GCs (2 weldment O-rings are installed on the weldment)	ea.	22724
Siltek® Split/Splitless Weldment for Agilent 5890/6890/6850 GCs (2 weldment O-rings are installed on the weldment)	ea.	22732
H) Shell Weldment for Agilent 6890/6850 GCs	ea.	22723
Siltek® Shell Weldment for Agilent 6890/6850 GCs	ea.	22730
Weldment O-rings	10-pk.	22729
C) Autosampler & PTV Septum Nut (for 23-gauge needles)	ea.	20631
D) Stainless Steel Capillary Column Nut (for use with standard 1/16" ferrules)	2-pk.	20883
E) Reducing Nut	ea.	22078
F) Siltek® 0.8mm ID Dual Vespel® Ring Inlet Seal	2-pk. 10-pk.	21242 21243
G) Weldment Removal Tool for Agilent 5890/6890/6850 GCs	ea.	22728



EZ Twist Top™ Split/Splitless Injection Port with Optional Split Vent for Agilent 6890/6850 GCs

Description	qty.	cat.#
Complete injection port assembly includes: split/splitless weldment, shell weldment, 2 weldment O-rings, Siltek® Dual Vespel® Ring inlet seal, septum nut, reducing nut, stainless steel capillary nut and weldment tool	kit	22735
Siltek® complete injection port assembly includes: Siltek® split/splitless weldment, Siltek® shell weldment, 2 weldment O-rings, Siltek® Dual Vespel® Ring inlet seal, septum nut, reducing nut, stainless steel capillary nut and weldment tool	kit	22736
Optional Split/Splitless Shell Weldment (for use with large canister type filter)	ea.	22733
Siltek® Optional Split/Splitless Shell Weldment (for use with large canister type filter)	ea.	22734

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- chiral phases
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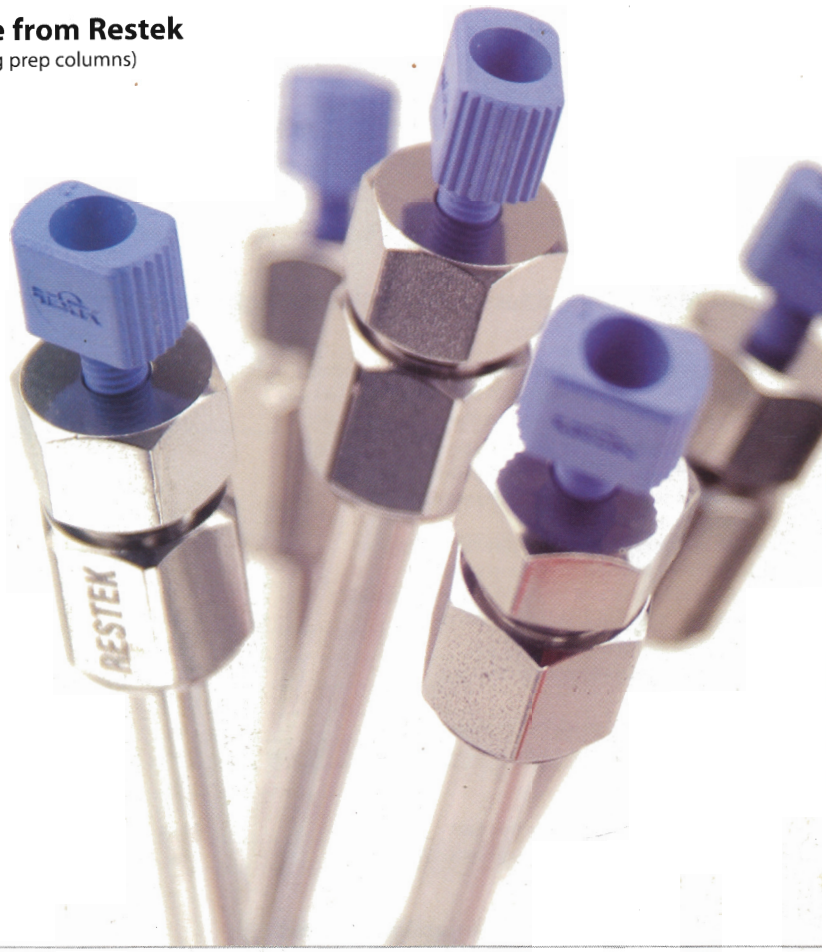

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IN THIS ISSUE

Comprehensive 2D Gas Chromatography – Making GC Separations Work Harder

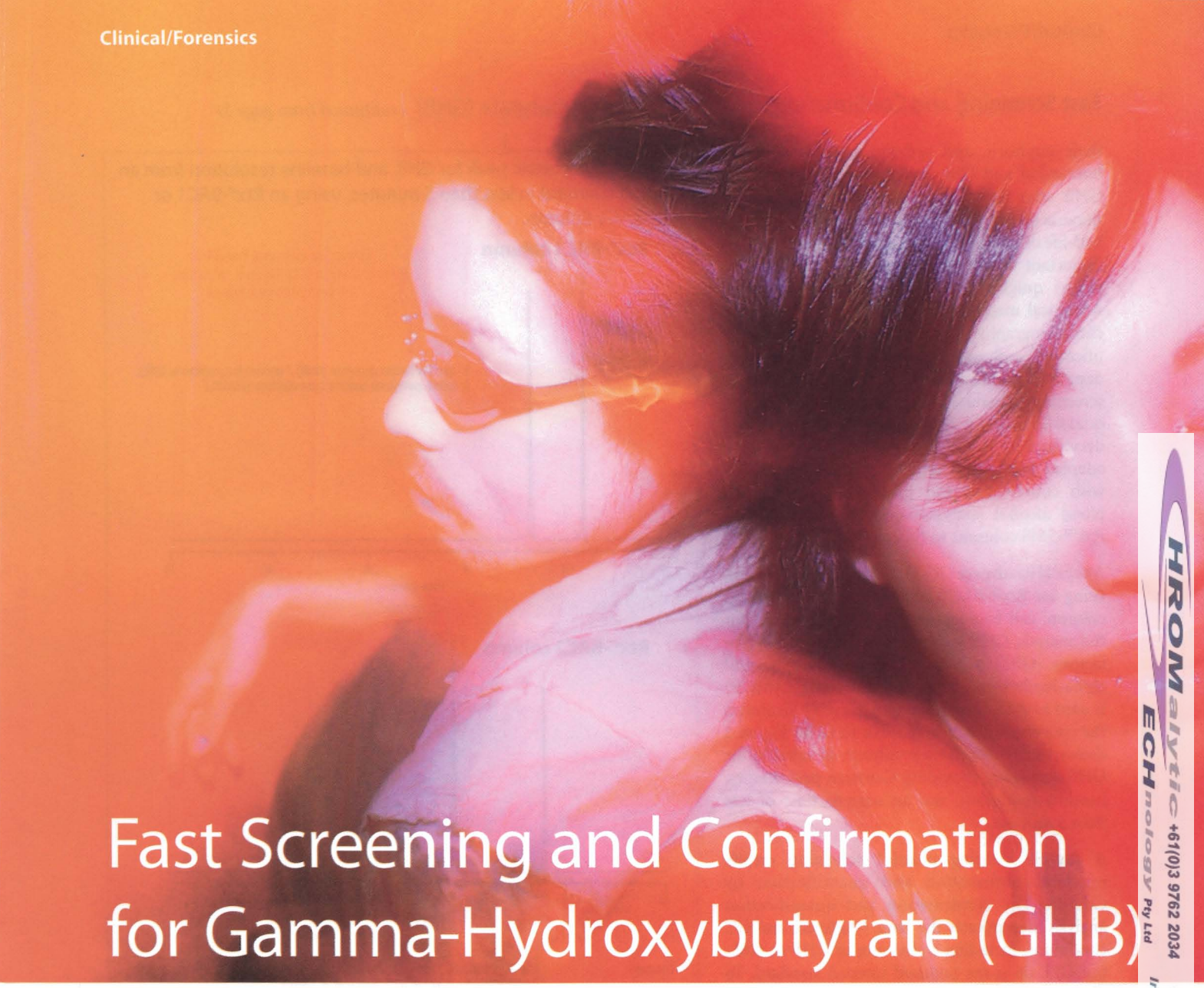
By Dr. Philip Marriott, Professor of Chemistry, RMIT University, Melbourne, Australia, philip.marriott@rmit.edu.au

We are entering a period in its development where the expectations of comprehensive two-dimensional gas chromatography (GC×GC) should – justifiably – match the rhetoric. Since its inception about 15 years ago, researchers who have made it their (life) goal to develop and promote GC×GC have waxed lyrical about the advantages of GC×GC to the GC community. If we were to list the three primary contributions that are often ascribed to GC×GC, these would be: (i) greater separation capacity; (ii) greater sensitivity; and (iii) retention structure in the 2D data presentation that permits the analyst to identify, or predict the identity of, related compounds based on the molecular properties that control retention. At this point, I should admit that I count myself guilty of being amongst those who have promulgated these advantages! Further, I also strongly support the position of GC×GC, and the benefits it holds for volatile and semi-volatile chemical analysis. And if these benefits are indeed general outcomes of GC×GC, then it is only logical that, sooner or later, this coupled column technique will supplant the single-column method that has served us so well for many years. But we might query whether single column GC has really served us so well. Admittedly, it has been just about all we had, so we have had to learn to live with its inherent limitations. Just as we might have recognised, and been frustrated by, the limited separation capacity of single column GC (i.e., as we searched for more complete understanding of the molecular composition of complex samples), analysts turned their attention to GC/MS which became routinely available. Considerable effort was devoted to implement solutions based on mass-detection to provide the necessary unique identification of individual compounds in (grossly) overlapping chromatograms. The mantra that MS can solve (all) our overlap problems probably became a crutch that somewhat numbed our realisation, according to my Research Group's philosophy, that often "the only Solution is better Resolution".

So, now that we have this new tool, what does it mean to the analyst? Well, in a simple answer – everything! With extra separation, the rationale for having to rely on MS for compound measurement (as opposed to identification) might now be negotiable. This is a considerable conceptual departure from the classical reliance on GC/MS. Extra sensitivity is a useful property to analysts, but this may be a lesser advantage of GC×GC. The ability to remove column bleed from solute elution does have benefits (when doing GC×GC/MS). The most significant advantage is separation power. To be able to resolve many more compounds immediately enables a much more complete 'picture' of the composition of a sample. Picture is used deliberately here, since the 2D GC presentation is very much akin to a picture. The comparison of 1D GC results is via a conventional GC trace – a one-dimensional time-response plot. The comparison is limited by the extent to which peaks coincide, or give multiple compound responses at one point. In GC×GC, the greater separation and picture-style GC plot means that we can simply compare two 2D pictures. Each compound now resides in its own 2D location which is determined by, or depends upon, the specific chemical-physical properties of a molecule which generate the peak position though specific interactions with the column stationary phases. The 2D plot has been called a chemical property retention map, which has axes controlled by retention mechanisms on each of the two columns. Choice of column phases is crucial to the effectiveness with which compounds are located within the available 2D space. Here, we will not consider how we generate the GC×GC experiment (i.e., the modulation methods used), however a few comments on the column selection are warranted in this text.

In GC×GC we usually couple a long 1D column directly to a short 2D column (or a regular elution column to a fast elution column). The second column has to work hard! We ask it to resolve peaks that are overlapping on the 1D column. Being about 1 m in length, with a need to complete continual, on-the-fly analyses of effluent from the 1D column within about 4-5 s, performance is everything. We use high carrier flow and narrow bore columns, but actual conditions are flexible. We now commonly find some regions of 1D GC analyses where up to 5 – 10 or more compounds co-elute. This is clearly beyond the scope of MS

Continued on page 31.



Fast Screening and Confirmation for Gamma-Hydroxybutyrate (GHB)

Using Restek Columns in Headspace GC or GC/MS Systems

By Kristi Sellers, Clinical/Forensic Innovations Chemist

- Use an Rtx®-BAC1 column or Rtx®-BAC2 column for GHB screening.
- Confirm and quantify positive GHB screens by using an Rxi™-5ms column.
- Fast, reliable screening; accurate confirmation and quantification.

In the last ten years, gamma-hydroxybutyrate (GHB) and its related products 1,4-butanediol and gamma-butyrolactone (GBL) have been identified as abused substances in cases of driving under the influence and drug facilitated sexual assault. Currently, GHB is regulated as a federally controlled Schedule I drug. The rise in use of GHB and GHB-type products as recreational drugs is primarily due to

Continued on page 4.

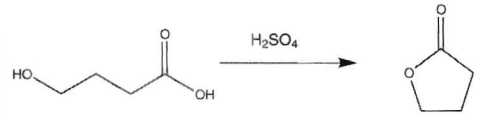
Fast Screening and Confirmation for Gamma-Hydroxybutyrate (GHB) *(continued from page 3)*

their euphoric and sedative properties. 1,4-butanediol and GBL are quickly metabolized to GHB after ingestion and are analyzed as such. Because GHB is endogenous in humans, and has a half-life of one hour or less after injection, it is very important to collect biofluids (typically blood or urine) quickly for toxicological investigation. Analytical methods for GHB usually employ gas chromatography and mass spectrometry for quantification and confirmation. The methodology described here establishes a headspace GC-FID screening procedure followed by confirmation and quantification by headspace GC/MS, and was developed by the FBI Chemistry Unit.[1] We have adapted Rtx®-BAC1 and Rtx®-BAC2 columns—with court-tested and proven performance in blood alcohol analyses—and new, highly inert Rxi™-5MS columns to the methods.

A typical headspace GC-FID blood alcohol system, using an Rtx®-BAC1 column or an Rtx®-BAC2 column, can be adapted for GHB screening. For the analysis, GHB is converted to gamma-butyrolactone (GBL) to improve chromatography, and alpha-methylene-gamma-butyrolactone (AMGB) is used as the internal standard. Figure 1 illustrates the conversion reaction of GHB to GBL. Figure 2 shows that either Restek column is suitable for GHB screening, providing Gaussian peak shapes, baseline resolution, and an analysis time of less than 5 minutes.

A sample yielding positive screening results requires confirmation and quantification by GC/MS. The confirmation and quantification analysis incorporates the same headspace and GC conditions, including conversion of GHB to GBL, but GBL-d6 is the required internal standard. To illustrate GBL and GBL-d6 separation and peak shape on an Rxi™-5ms column we analyzed 1µL of a standard, using GC/MS. (Figure 3). This typical liquid injection shows the two compounds are partially resolved on the Rxi™-5ms column, and positively identified using full scan. Then, extracted ion data (EI) were obtained(Figure 4). After positive identification, GHB is quantified by comparing the areas of the deuterated and undeuterated GBL extracted ions.

Figure 1 Conversion of GHB to GBL.

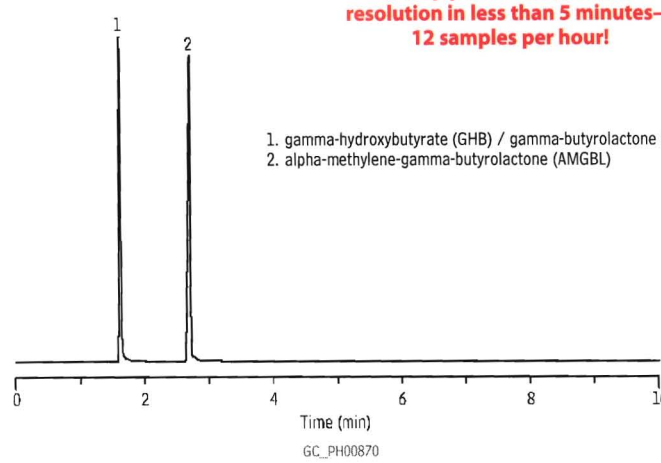


gamma-hydroxybutyrate (GHB) — gamma-butyrolactone (GBL)

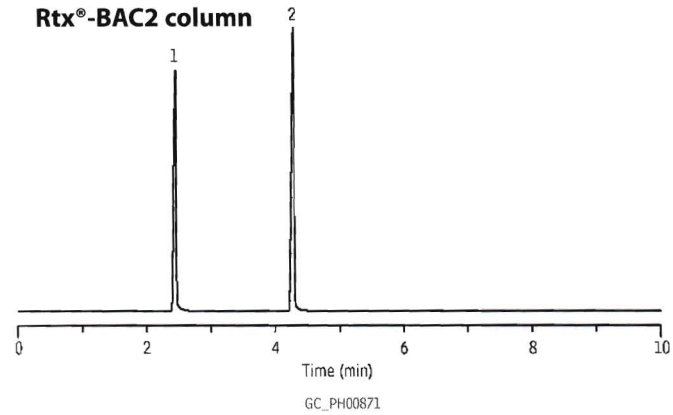
Figure 2 Symmetric peak for GHB, and baseline resolution from an internal standard in less than 5 minutes, using an Rtx®-BAC1 or Rtx®-BAC1 Column.

Rtx®-BAC1 column

Sharp peaks and baseline resolution in less than 5 minutes—12 samples per hour!



Rtx®-BAC2 column



Column:	Rtx®-BAC1 30m, 0.32mm ID, 1.8µm (cat.# 18003) and Rtx®-BAC2 30m, 0.32mm ID, 1.2µm (cat.# 18002), connected via universal "Y" Press-Tight® connector (cat.# 20405)
Sample:	GHB, GBL, α-methylene-γ-butyrolactone (AMGBL), 10µg/mL each in water
Inj.:	1.0mL headspace, split (split ratio 1:10), 1mm split inlet liner (cat.# 20972)
Inj. temp.:	200°C
Carrier gas:	helium, constant pressure
Linear velocity:	44cm/sec. @ 50°C
Oven temp.:	50°C (3 min.) to 150°C @ 20°C/min. (hold 7 min.)
Det:	FID @ 240°C
Headspace autosampler:	Teledyne/Tekmar HT3
Sample/platen temp.:	100°C
Sample equilibration:	15 min.
Mixing time:	5 min.
Vial pressure:	10psig
Vial pressurization time:	2 min.
Loop fill time:	2 min.
Transfer line temp.:	120°C

Drug-Facilitated Sexual Assault: A Forensic Handbook

This unique handbook educates readers about how drugs are used in sexual assaults. It is important reading for any involved in investigating these crimes, including forensic scientists, law enforcement officers, lawyers, toxicologists, and medical professionals.

M. LeBeau and A. Mozayani, Eds., Academic Press, 2001, 326pp., ISBN 0-12-440261-5 **cat.# 23054** (ea.) $84.95

Figure 3 An Rxi™-5ms column provides the symmetric peaks and resolution needed for reliable confirmation of GHB.

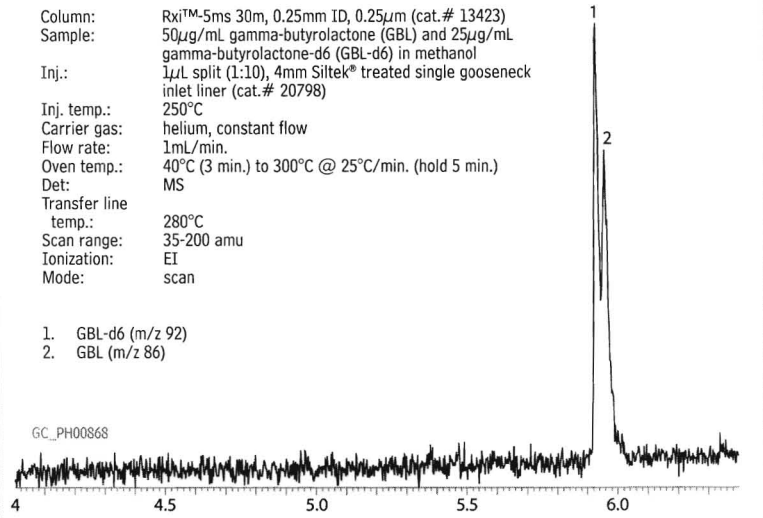
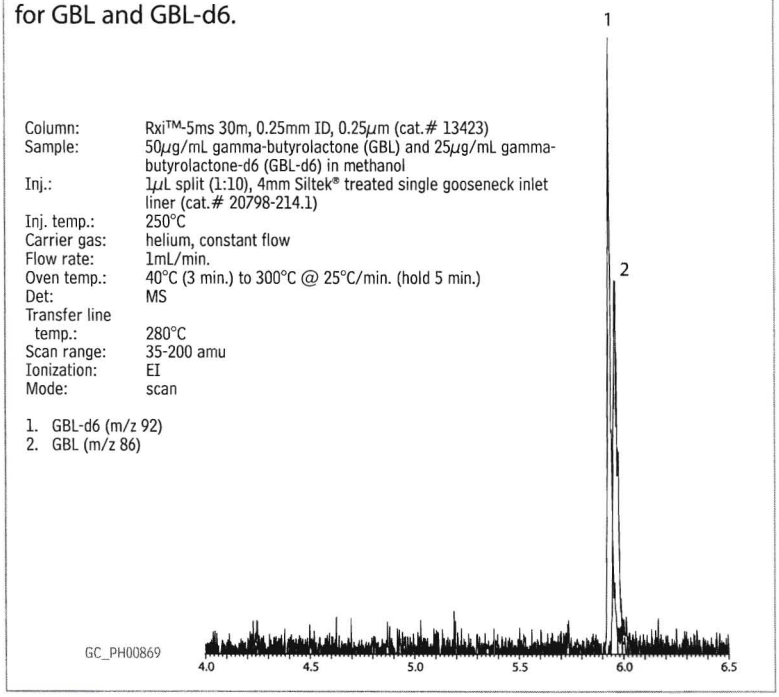


Figure 4 Overlay of extracted ion chromatograms for GBL and GBL-d6.



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Clinical/Forensics Products & Applications for GC & HPLC (2006/07 Edition)
This 64-page catalog presents a wealth of information about clinical and forensic analyses by GC and HPLC. Clinical applications include analgesics, antihistamines, cardiac medications, CNS depressants, cold & sinus medications, steroids, and more; forensic applications include arson accelerants, anesthetics, barbiturates, blood alcohol, butyrolactone, cannabinoids, cocaine, opiates, and more. Includes references to many additional free Restek publications that discuss specific subjects in detail. **lit. cat.# 59989A**

Because this methodology for analyzing GHB in biofluids employs sample introduction through a headspace technique, the need for injector and column maintenance is dramatically reduced. The use of an existing headspace GC system for blood alcohol analysis eliminates the need for additional equipment and allows rapid and reliable screening, using the same Rtx®-BAC1 or Rtx®-BAC2 column. For positive results, an Rxi™-5ms column in a GC/MS system provides accurate confirmation and quantification of GHB.

Reference
1 LeBeau, M.A., M.A. Montgomery, M.L Miller, and S.G. Burmeister, J. Anal. Toxicol. 24 (6): 421-428 (Sept. 2000).

Rtx®-BAC1 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	1.80	-20 to 240/260°C	30-Meter	18003	$485
0.53mm	3.00	-20 to 240/260°C	30-Meter	18001	$510

Rtx®-BAC2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	1.20	-20 to 240/260°C	30-Meter	18002	$485
0.53mm	2.00	-20 to 240/260°C	30-Meter	18000	$510

Rxi™-5ms Columns (fused silica)

(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.18	-60 to 330/350°C	20-Meter	13402	$370
0.18mm	0.36	-60 to 330/350°C	20-Meter	13411	$370
0.20mm	0.33	-60 to 330/350°C	12-Meter	13497	$230
0.20mm	0.33	-60 to 330/350°C	25-Meter	13498	$365
0.20mm	0.33	-60 to 330/350°C	50-Meter	13499	$630
0.25mm	0.25	-60 to 330/350°C	15-Meter	13420	$260
0.25mm	0.25	-60 to 330/350°C	30-Meter	13423	$435
0.25mm	0.25	-60 to 330/350°C	60-Meter	13426	$780
0.25mm	0.50	-60 to 330/350°C	15-Meter	13435	$260
0.25mm	0.50	-60 to 330/350°C	30-Meter	13438	$435
0.25mm	0.50	-60 to 330/350°C	60-Meter	13441	$780
0.25mm	1.00	-60 to 330/350°C	15-Meter	13450	$260
0.25mm	1.00	-60 to 330/350°C	30-Meter	13453	$435
0.25mm	1.00	-60 to 330/350°C	60-Meter	13456	$780

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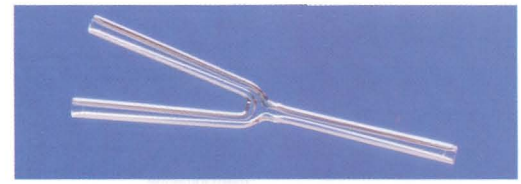

Description		ea./price
Universal "Y" Press-Tight® Connector	20405	$61
Deactivated Universal "Y" Press-Tight® Connector	20405-261	$62
Siltek® Treated Universal "Y" Press-Tight® Connector	20485	$63

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Drugs of Abuse Analytical Reference Materials

by Ken Herwehe, Analytical Reference Materials Product Manager

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1000µg/mL in 1 mL purge & trap methanol

Compound	cat.#	price
Benzodiazepines		
alprazolam	34042	$23
bromazepam	34043	$23
chlordiazepoxide	34044	$23
clobazam	34045	$23
clonazepam	34046	$23
diazepam	34047	$23
flunitrazepam	34049	$23
flurazepam	34050	$23
lorazepam	34051	$23
nitrazepam	34053	$23
oxazepam	34054	$23
prazepam	34055	$23
temazepam	34056	$23
triazolam	34057	$23
Cocaine & Metabolites		
cocaine	34015	$23
benzoylecgonine	34016	$23
ecgonine	34017	$23
ecgonine methyl ester	34018	$23
Methadone & Metabolites		
methadone	34005	$23
Amphetamines & Metabolites		
d-amphetamine	34020	$23
(+)methamphetamine	34021	$23
Opiates & Metabolites		
codeine	34000	$23
hydrocodone	34002	$23
hydromorphone	34063	$23
morphine	34006	$23
oxycodone	34007	$23
oxymorphone	34065	$23
Cannibinoid & Metabolites		
cannabidiol	34011	$23
cannabinol	34010	$23
Barbituates		
amobarbital	34028	$23
aprobarbital	34029	$23
barbital	34030	$23
butabarbital	34031	$23
butalbital	34032	$23
DL-glutethimide	34058	$23
hexobarbital	34033	$23
mephobarbital	34034	$23
methohexital	34035	$23
pentobarbital	34036	$23
phenobarbital	34037	$23
secobarbital	34038	$23
talbutal	34039	$23
thiamylal	34040	$23
thiopental	34041	$23
Other		
benzphetamine	34022	$23
cocaethylene*	34066	$23
fenfluramine	34023	$23
levorphanol	34003	$23
meperidine	34004	$23
meprobamate	34059	$23
methaqualone	34064	$23
methyprylon	34060	$23
pentazocine	34062	$23
phencyclidine	34027	$23
phendimetrazine	34025	$23
phenmetrazine	34026	$23
phentermine	34024	$23
dextro-propoxyphene	34008	$23
thebaine	34009	$23

*1000µg/mL in 1mL acetonitrile.

Blood Alcohol Standards

Compound	qty.	cat.#	price
0.015g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36232	$26
1mL/ampul	10-pk.	36332	$41
5mL/ampul	ea.	36240	$26
20mL/ampul	ea.	36248	$46
0.02g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36233	$26
1mL/ampul	10-pk.	36333	$41
5mL/ampul	ea.	36241	$26
20mL/ampul	ea.	36249	$46
0.025g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36234	$26
1mL/ampul	10-pk.	36334	$41
5mL/ampul	ea.	36242	$26
20mL/ampul	ea.	36250	$46
0.04g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36235	$26
1mL/ampul	10-pk.	36335	$41
5mL/ampul	ea.	36243	$26
20mL/ampul	ea.	36251	$46
0.05g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36257	$26
1mL/ampul	10-pk.	36259	$41
5mL/ampul	ea.	36258	$26
20mL/ampul	ea.	36260	$46
0.08g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36262	$26
1mL/ampul	10-pk.	36264	$41
5mL/ampul	ea.	36263	$26
20mL/ampul	ea.	36265	$46
0.1g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36236	$26
1mL/ampul	10-pk.	36336	$41
5mL/ampul	ea.	36244	$26
20mL/ampul	ea.	36252	$46
0.15g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36237	$26
1mL/ampul	10-pk.	36337	$41
5mL/ampul	ea.	36245	$26
20mL/ampul	ea.	36253	$46
0.2g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36238	$26
1mL/ampul	10-pk.	36338	$41
5mL/ampul	ea.	36246	$26
20mL/ampul	ea.	36254	$46
0.3g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36239	$26
1mL/ampul	10-pk.	36339	$41
5mL/ampul	ea.	36247	$26
20mL/ampul	ea.	36255	$46
0.4g/dL forensic ethanol solution			
1mL/ampul	5-pk.	36266	$26
1mL/ampul	10-pk.	36268	$41
5mL/ampul	ea.	36267	$26
20mL/ampul	ea.	36269	$46

Blood Alcohol Mix Resolution Control Standard

(8 components)

acetaldehyde
acetone
acetonitrile
ethanol (NIST certified value)
ethyl acetate
isopropanol
methanol
methyl ethyl ketone

0.100g/dL each in water, 1mL/ampul

cat. # 36256 (ea.) $31

Analytical Reference Materials for Semivolatile Pollutants

Drinking Water: US EPA Method 525.2

by Ken Herwehe, Analytical Reference Materials Product Manager

did you know?

We have over 2,000 pure, characterized, neat compounds in our inventory! If you do not see the EXACT mixture you need listed on any of these pages, call us.

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free data

Available on Our Website: Lot Certificates, Datapacks, and MSDS

For complete information detailing manufacturing and testing for Restek inventoried reference standards, just visit our website at **www.restek.com** To view lot certificates and/or an MSDS, enter the catalog number of the product in the Search feature. For a free Datapack, enter the catalog number and lot number of the product, to obtain a printable pdf file.

Organochlorine Pesticide Mix #2 (Rev), Method 525.2 (8 components)

chlorobenzilate
chloroneb
chlorothalonil
DCPA methyl ester (Dacthal®)
heptachlor epoxide (isomer A)
trans-nonachlor
cis-permethrin
trans-permethrin

500µg/mL each in acetone, 1mL/ampul
cat. # 33011 (ea.) $35

Organonitrogen Pesticide Mix #1 (Rev), Method 525.2 (37 components)

alachlor
ametryn
atraton
atrazine
bromacil
butachlor
butylate
chlorpropham
cyanazine (Bladex)
cycloate
diphenamid
EPTC
etridiazole (Terrazole®)
fenarimol
fluridone (Sonar®)
hexazinone (Velpar®)
metolachlor
metribuzin
MGK-264
molinate
napropamide (Devrinol®)
norflurazon
pebulate
prometon
prometryne
pronamide (propyzamide)
propachlor
propazine
simazine
simetryn
tebuthiuron
terbacil
terbutryn
triadimefon
tricyclazole (Beam)
trifluralin
vernolate

500µg/mL each in acetone, 1mL/ampul
cat. # 33012 (ea.) $195

Organophosphorus Pesticide Mix #1 (Rev), Method 525.2 (7 components)

chlorpyrifos (Dursban®)
dichlorvos (DDVP)
disulfoton sulfone
ethoprop (ethoprophos)
methyl paraoxon (Parathion methyl-O-analog)
mevinphos (Phosdrin)
stirofos (tetrachlorvinphos)

500µg/mL each in acetone, 1mL/ampul
cat. # 33013 (ea.) $45

Method 525.2 Semivolatile Mix (revised)

(28 components)

acenaphthylene
anthracene
benzo(a)anthracene
benzo(a)pyrene
benzo(b)fluoranthene
benzo(ghi)perylene
benzo(k)fluoranthene
benzylbutylphthalate
bis(2-ethylhexyl)adipate
bis(2-ethylhexyl)phthalate
chrysene
dibenzo(a,h)anthracene
diethylphthalate
dimethylphthalate
di-*n*-butylphthalate
2,4-dinitrotoluene
2,6-dinitrotoluene
di-*n*-octylphthalate
fluoranthene
fluorene
hexachlorobenzene
hexachlorocyclopentadiene
indeno(1,2,3-cd)pyrene
isophorone
naphthalene
pentachlorophenol*
phenanthrene
pyrene

1,000µg/mL each in acetone, (*pentachlorophenol at 4,000µg/mL), 1mL/ampul
cat. # 31899 (ea.) $80

Method 525.2 PCB Congener Mix (8 components)

2-chlorobiphenyl (BZ#1)
2,3-dichlorobiphenyl (BZ#5)
2,4,5-trichlorobiphenyl (BZ#29)
2,2',4,4'-tetrachlorobiphenyl (BZ#47)
2,2',3',4,6-pentachlorobiphenyl (BZ#98)
2,2',4,4',5,6'-hexachlorobiphenyl (BZ#154)
2,2',3,3',4,4',6-heptachlorobiphenyl (BZ#171)
2,2',3,3',4,5',6,6'-octachlorobiphenyl (BZ#200)

200µg/mL each in acetone, 1mL/ampul
cat. # 32420 (ea.) $56

Organochlorine Pesticide Mix AB # 3

(20 components)

aldrin
α-BHC
β-BHC
δ-BHC
γ-BHC (lindane)
α-chlordane
γ-chlordane
4,4'-DDD
4,4'-DDE
4,4'-DDT
dieldrin
endosulfan I
endosulfan II
endosulfan sulfate
endrin
endrin aldehyde
endrin ketone
heptachlor
heptachlor epoxide (B)
methoxychlor

2,000µg/mL each in hexane:toluene (1:1), 1mL/ampul
cat. # 32415 (ea.) $71

Method 525.2 Nitrogen/Phosphorus Pesticide Mix #2 (6 components)

carboxin
diazinon
disulfoton
fenamiphos
merphos
terbufos

1,000µg/mL each in acetone, 1mL/ampul
cat. # 32423 (ea.) $64

Metribuzin

1,000µg/mL in acetone, 1mL/ampul
cat. # 32436 (ea.) $23

Method 525.2 Fortification Recovery Standard

p-terphenyl-d14

1,000µg/mL in methylene chloride, 1mL/ampul
cat. # 31828 (ea.) $23

Method 525.2 Internal Standard Mix

acenaphthene-d10
chrysene-d12
phenanthrene-d10

1,000µg/mL each in acetone, 1mL/ampul
cat. # 31825 (ea.) $27

Method 525.2 Surrogate Standard Mix

2-nitro-*m*-xylene
perylene-d12
pyrene-d10
triphenylphosphate

1,000µg/mL each in acetone, 1mL/ampul
cat. # 31826 (ea.) $27

Method 525.2 GC/MS Performance Check Mix

4,4'-DDT
DFTPP (decafluorotriphenylphosphine)
endrin

1,000µg/mL each in acetone, 1mL/ampul
cat. # 31827 (ea.) $27

Rapid Analysis of Steroid Hormones by GC/MS

Using the New Rxi™-1ms Column

By Kristi Sellers, Clinical/Forensic Innovations Chemist

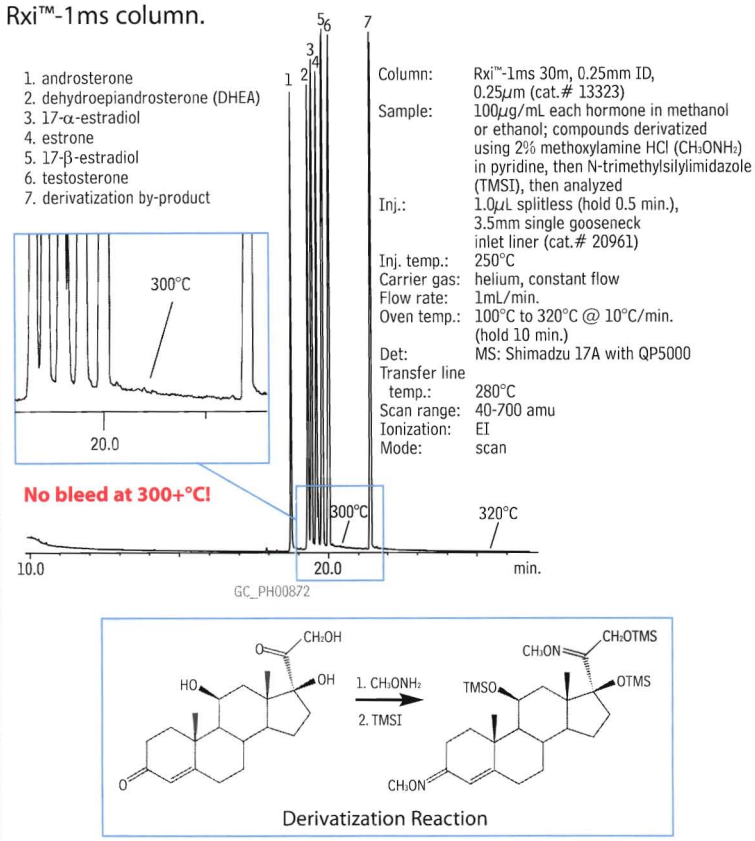
- Resolve 6 common steroid hormones in less than 25 minutes.
- Ultra-low bleed column greatly reduces background interferences.
- Stable performance at 300°C or above.

Determinations of urinary steroid hormones are widely used for diagnosing and monitoring many health conditions, including bio-identical hormone replacement, menopause, Cushing's syndrome, Addison's disease, adrenal fatigue, and others[1]. Many clinical laboratories use gas chromatography and mass spectrometry (GC/MS) as the primary analytical method for identification and quantification. A capillary GC column with a thin film (0.25µm or less) of 100% dimethylpolysiloxane is the column of choice for many analysts, because this stationary phase has the highest operating temperature available. Temperatures exceeding 300°C are required to elute the high molecular weight (250-400 Dalton) hormones in a reasonable analysis time while maintaining and Gaussian peak shape resolution. A phase film thickness of 0.25µm or less minimizes column bleed at these high temperatures. Also, in order to provide reliable quantification, the column must exhibit the inertness necessary to produce symmetric peaks and reproducible results.

Our new Rxi™-1ms column, designed for GC-MS applications, provides the ultra-low bleed and exceptional inertness needed for analyzing urinary steroid hormones. For this application we derivatized six sex hormones, using methoxylamine HCl and trimethylsilyl imidazole (Figure1) to improve chromatography. Figure 1 shows this variety of derivatized steroid sex hormones, analyzed in less than 25 minutes by using an Rxi™-1ms column. Note that these compounds elute at temperatures near or above 300°C and that bleed from the Rxi™-1ms column is negligible at these temperatures. The Rxi™-1ms column exhibits the inertness needed to produce Gaussian peaks and excellent resolution.

Because GC/MS analysis of urinary steroid hormones is a demanding application, it is important to use the lowest bleed, most inert column available. The new Rxi™-1ms column meets these requirements better than any column we have tested, and we recommend it as the column of choice for this application.

Figure 1 Negligible bleed, Gaussian peaks, and fast results characterize analyses of derivatized steroids on an Rxi™-1ms column.



Reference

1. http://www.meridianvalleylab.com/steroid_dept.html

Rxi™-1ms Column (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13323	$435

For other dimensions, please vist our website.

Enhanced Resolution of Endocrine Disrupting Hormones

Using an Allure® Biphenyl Column and LC-TOFMS

By Robert Freeman, Environmental Innovations Chemist, Rick Lake, Pharmaceutical Innovations Chemist, and Lydia Nolan, Innovations Chemist

- Enhanced selectivity for closely related hormones.
- Complete resolution of 7 common sex hormones in less than 8 minutes.
- Increased confidence in identifications, using a LECO TOFMS system.

Endocrine disrupting chemicals in the environment are a topic of growing concern. Evidence suggests that the developmental and reproductive systems of both fish and wildlife have been affected.[1] A variety of commonly used chemicals have endocrine disrupting properties, but the sex hormones (estrogens, progestogens and androgens) carry the most estrogenic potency.[2] The primary sources are believed to be human excretion and agriculture runoff. Since these compounds generally are not affected by standard wastewater treatment practices, it is believed they are routinely discharged into receiving streams. For this reason, we sought to develop a procedure to detect endocrine disrupting hormones in aqueous matrices.

Chemically, the sex hormones are steroids. Steroids are a unique class of compounds, in that all structural variation is centered on a common conjugated ring system (Figure 1), from which double bonding and various functional groups produce chemical diversity. Estrogens possess a hydroxyl group at position 3, while progestogens and androgens possess a carbonyl group (Figure 2). Typically a complex functional group at position 17 denotes a synthetically produced steroid.

Because steroids are neutral compounds, we evaluated both alkyl (i.e., C18) and phenyl stationary phases to determine the optimum phase for resolving steroid hormones. Alkyl stationary phases separate analytes on the basis of overall hydrophobicity. Phenyl phases offer a different separation mechanism: interactions among π-π electrons, between the phenyl ligand and the analytes. often, these π-π interactions can produce alternate and enhanced selectivity.[3]

A downside to phenyl phases is that they typically show only moderate retention, compared to octadecylsilyl (ODS) alkyl phases. In contrast, the Allure® Biphenyl phase – a surface chemistry consisting of two phenyl groups bonded end-to-end – provides a greater concentration of phenyl groups, in sterically favorable positioning, and thereby increases π-π interactions. An Allure® Biphenyl column exhibits an overall increase in retention capacity and analyte interaction, and provides highly effective separations of compounds exhibiting differences in π-π interactions (Figure 3).

Figure 1 Separations of steroids are especially challenging because all steroid molecules are based on a common structure.

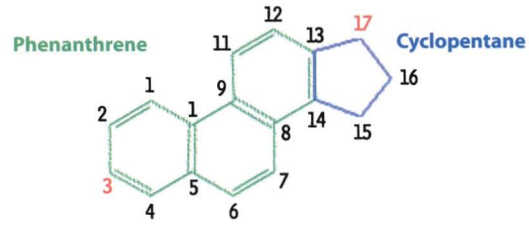
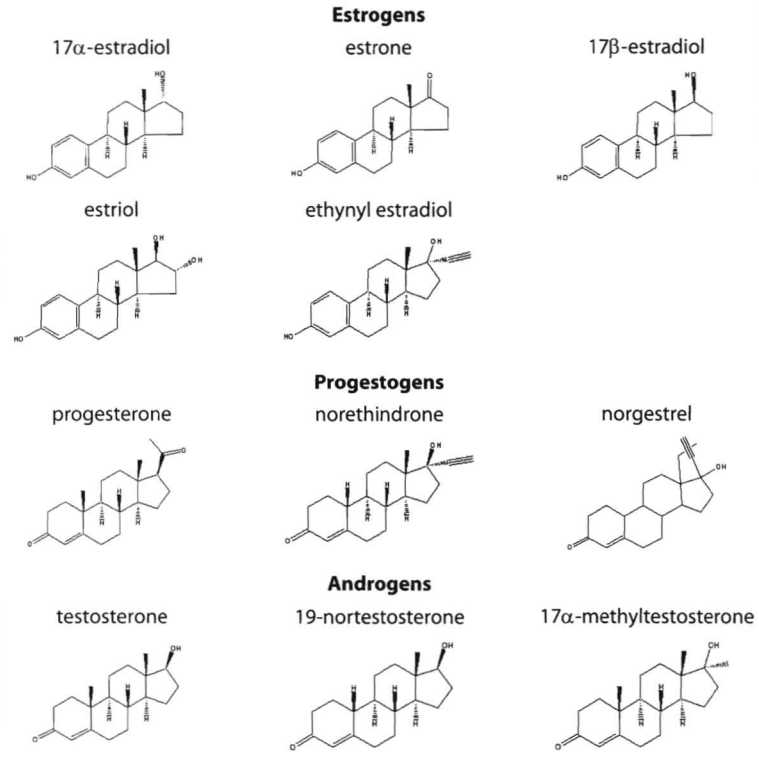


Figure 2 Estrogens include a hydroxyl group at position 3, progestogens and androgens include a carbonyl group.



Acknowledgement
We are grateful to Paul Kennedy, Ph.D, LECO Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396, for his assistance with this analysis.

Figure 3 An Allure® Biphenyl column provides superior selectivity and retention for steroids (UV detection).

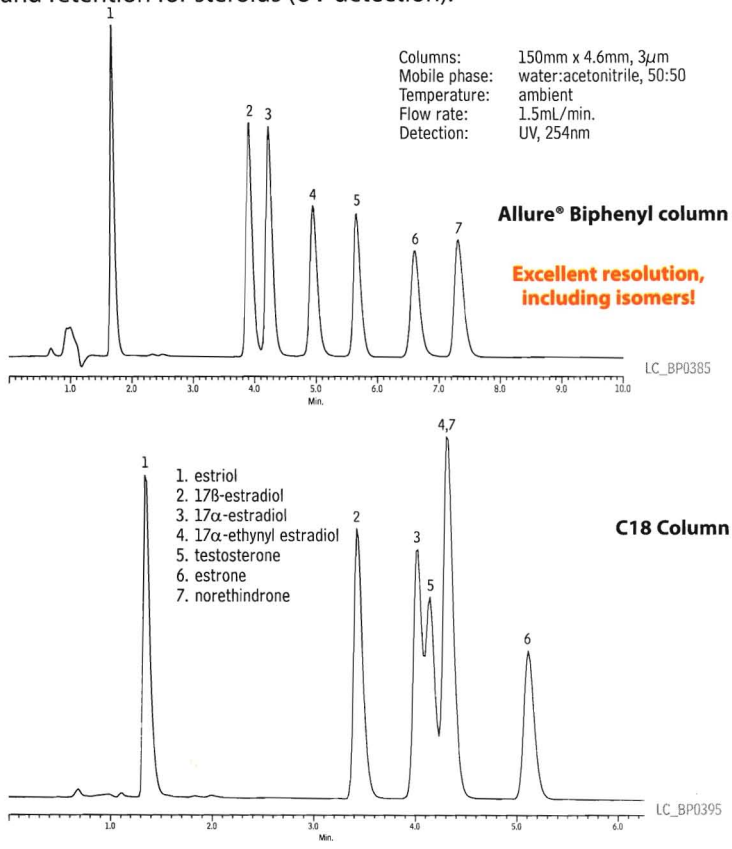
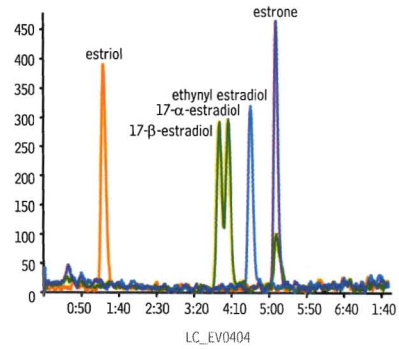


Figure 4 Sensitive analysis of steroids, using an Allure® Biphenyl column, and LECO Unique® TOFMS.



Estrogens at 5ng on column (-ESI)

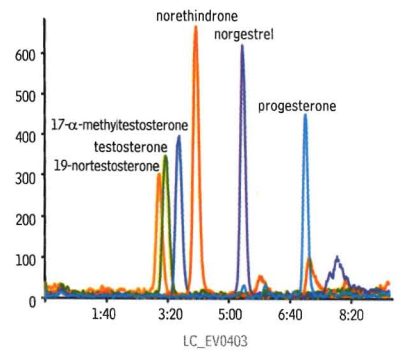
Sample:
Inj.: 5µL
Sample diluent: acetonitrile

Column: Allure® Biphenyl (cat.# 9166352)
Dimensions: 50 x 2.1 mm
Particle size: 3µm
Pore size: 60Å

Conditions:
Mobile phase: A) water + 1mM NH_4OH
B) acetonitrile

Time (min.)	%B
0	45
5	70
8	70
9	45
15	45

Flow: 0.3mL/min.
Temp.: ambient
Det.: Leco Unique® TOFMS



Androgens at 1ng on column (+ESI)

To monitor steroid sex hormones in water, we first developed an extraction procedure, using styrene-divinylbenzene solid phase extraction disks and methyl *tert*-butyl ether (MTBE) as the extraction solvent. We conditioned the extraction disks with acetonitrile and MTBE to remove any potential interferences. After rinsing the disk with distilled water and loading the disks with one liter of sample we used 10mL of MTBE to elute the sample. Prior to analysis the final 10mL extract was concentrated to 2mL and exchanged to acetonitrile.

We recognized that the complexity of environmental sample mixtures and matrices often would make difficult a complete chromatographic separation of the steroid sex hormones by HPLC, and qualitative detection with a non-selective detector (UV-Vis). Mass spectrometry, with secondary separation based on m/z, increased our confidence in the qualitative identifications. We selected LECO Corporation's Unique® LC-TOFMS system for its high data acquisition rate – 100 spectra/sec. The ChromaTOF® software Peak Find algorithm can deconvolute closely eluting peaks, and mass can be determined accurately, to within 5ppm, to calculate possible molecular formula. Because the ionization potential differs among the groups of steroid sex hormones, both negative and positive ESI was used. The estrogens were amenable to negative ESI, while the androgens and progestogens showed much greater sensitivity when we used positive ESI (Figure 4). We believe this difference is because of the differing functional groups at position 3.

These analyses demonstrate that the Allure® Biphenyl stationary phase, through π-π interactions, offers excellent selectivity for compounds with unsaturation differences in their hydrocarbon ring structures. Additionally, the secondary separation power of the Unique® TOFMS system and ChromaTOF® software allows overall analysis time to be reduced, through optimized column dimensions and run conditions, while qualitative identification is maintained.

References
1 http://www.epa.gov/scipoly/oscpendo/
2 Kuster, M., M.J. Lopez, and D. Barcelo, Estrogens and Progesterons in Wastewater, Sludge, Sediments, and Soil, pp. 3 Handbook of Environmental Chemistry
3 http://www.restek.com/fantasia/pdfCache/580020.pdf

Allure® Biphenyl Columns

3µm Column, 2.1mm	cat. #	price
30mm	9166332	$364
50mm	9166352	$364
100mm	9166312	$390
3µm Column, 4.6mm	**cat. #**	**price**
30mm	9166335	$364
50mm	9166355	$364
100mm	9166315	$390

For other column dimensions, and columns with 5µm packing, please visit our website.

New Rxi™-1ms Capillary GC Column

For Low Level GC/MS Analyses

By Robert Freeman, Environmental Innovations Chemist

- Inert, low-bleed column for reliable results.
- Save time – analyze acidic and basic compounds under the same conditions.
- Guaranteed reproducible performance, column to column.

The second column in our new Rxi™ GC column line – the Rxi™-1ms column – will provide the same outstanding performance as the Rxi™-5ms column, with equally superior inertness, ultra-low bleed, and excellent batch to batch reproducibility.

Our first test for this 100% dimethylpolysiloxane phase column was an analysis of a complex mixture of semivolatile organic compounds. The extensive target list was comprised of many classes of compounds including chloroacetanilides, chlorotriazines, triazinones, uracils, polcyclic aromatic hydrocarbons, and phthalates. Figure 1 shows peak shape and selectivity are equally good for all of these diverse compounds, and all are eluted in an acceptable analysis time.

Excellent Inertness

In addition to analyzing these compounds, we analyzed an acidic compound (2,4-dinitrophenol) and a basic compound (pyridine), each at 0.5ng on column, to assess column inertness. Column activity reveals itself through poor response and peak tailing for such active compounds, and these two compounds present both varying difficulties in a GC/MS analysis and differing modes of degradation. Figure 2 shows the excellent peak shapes and responses for these compounds on the 30m x 0.25mm ID, 0.25µm film column.

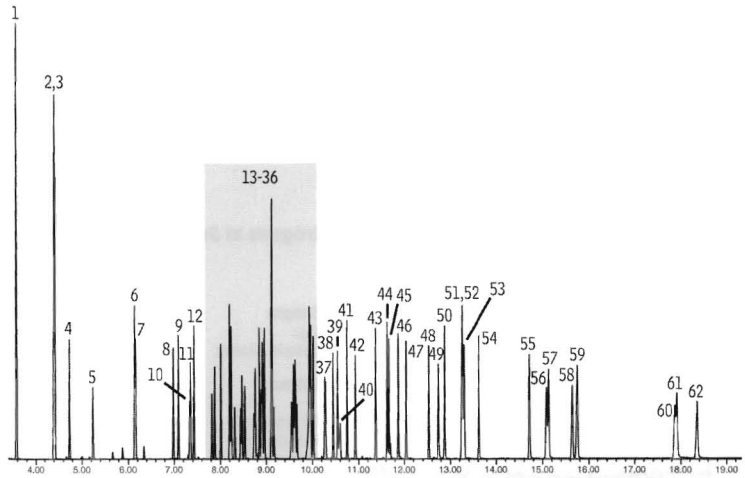
Phenols are notorious for breakdown and peak tailing, caused by interaction with the surface of an active inlet liner or an active column. Nitrophenols and pentachlorphenol, for example, very often exhibit poor peak shape and/or poor response. Figure 3 shows the 30m x 0.25mm ID, 0.25µm Rxi™-1ms column provides very good peak shapes for phenols. Peak responses are well above method requirements.

Ultra-Low Bleed

In addition to excellent inertness, Rxi™-1ms columns exhibit very low bleed. Figure 4 is focused on the end of the chromatogram for semivolatiles. At 330°C, bleed is much lower than the signals for 0.5ng of target analytes. This exceptional signal-to-noise differential for late eluting compounds assures better detection limits.

Figure 1 Excellent selectivity and peak shapes for common drinking water semivolatiles at 10ng, using an Rxi™-1ms column.

1. 2-fluorophenol (surr.)
2. bis(2-chloroethyl)ether
3. phenol-d6 (surr.)
4. 1,4-dichlorobenzene-d4 (int. std.) •
5. nitrobenzene-d5 (surr.)
6. naphthalene-d8 (int. std.) •
7. naphthalene
8. 1-methylnaphthalene
9. 2-methylnaphthalene
10. hexachlorocyclopentadiene
11. EPTC
12. 2-fluorobiphenyl (surr.)
13. 2,6-dinitrotoluene
14. dimethylphthalate
15. acenaphthylene
16. acenaphthene-d10 (int. std.) •
17. acenaphthene
18. 2,4-dinitrotoluene
19. 1-naphthalenamine
20. molinate
21. 2-naphthalenamine
22. 5-nitro-o-toluidine
23. diethylphthalate
24. fluorene
25. propachlor
26. diphenylamine
27. 2,4,6-tribromophenol (surr.)
28. simazine
29. prometon
30. atrazine
31. hexachlorobenzene
32. 4-aminobiphenyl
33. terbacil
34. phenanthrene-d10 (int. std.) •
35. phenanthrene
36. anthracene
37. metribuzin
38. acetochlor
39. alachlor
40. bromacil
41. di-*n*-butylphthalate
42. metolachlor
43. fluoranthene
44. pyrene
45. butachlor
46. *p*-terphenyl-d14 (surr.)
47. *p*-dimethylaminoazobenzene
48. benzyl butyl phthalate
49. 2-acetylaminofluorene
50. bis(2-ethylhexyl)adipate
51. benzo(a)anthracene
52. chrysene-d12 (int. std.) •
53. chrysene
54. bis(2-ethylhexyl)phthalate
55. di-*n*-octylphthalate
56. benzo(b)fluoranthene
57. benzo(k)fluoranthene
58. benzo(a)pyrene
59. perylene-d12 (int. std.) •
60. indeno(1,2,3-cd)pyrene
61. dibenzo(a,h)anthracene
62. benzo(ghi)perylene



GC_EV00833

Column:	Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)
Sample:	US EPA Method 525.2 mix: custom 525.2 calibration mix, SV Internal Standard Mix (cat.# 31206), B/N Surrogate Mix (4/89 SOW) (cat.# 31024), Acid Surrogate Mix (4/89 SOW) (cat.# 31025)
Inj.:	1.0µL, 10µg/mL each analyte (internal standards 100µg/mL), split (10:1) 4mm Drilled Uniliner® inlet liner (hole at bottom) (cat.# 20756)
Instrument:	Agilent 6890
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	50°C (hold 1 min.) to 265°C @ 20°C/min., to 330°C @ 6°Cmin. (hold 1 min.)
Det.:	Agilent 5973 MSD
Transfer line temp.:	280°C
Scan range:	35-550 amu
Solvent delay:	3.20 min.
Tune:	DFTPP
Ionization:	EI

• Internal standards at 100ng on-column.

Drilled Uniliner®—see page 11.

Figure 2 An Rxi™-1ms column has excellent selectivity for basic or acidic compounds, under the same conditions. (0.5ng each; extracted ion chromatograms).

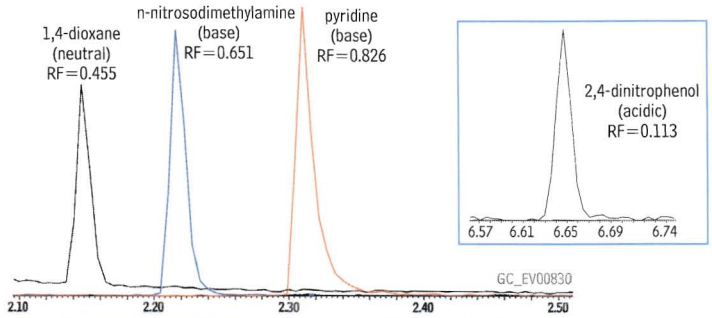
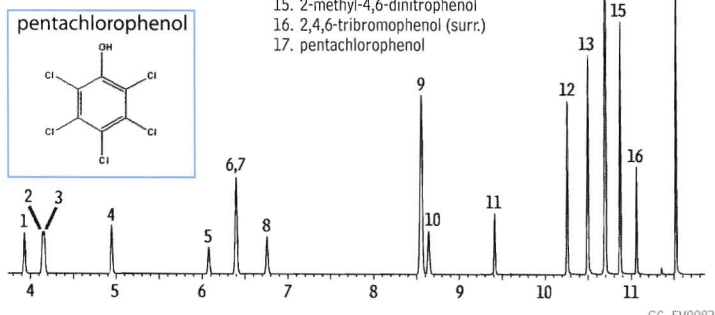


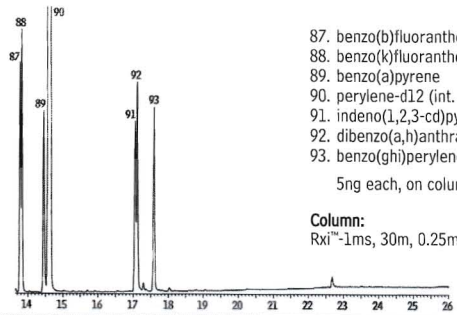
Figure 3 Acidic analytes at 5.0ng on an Rxi™-1ms column (extracted ion chromatogram).

1. phenol
2. 2-chlorophenol-d4 (surr.)
3. 2-chlorophenol
4. 2-methylphenol (*o*-cresol)
5. 2-nitrophenol
6. 2,4-dimethylphenol3,5,6-d3 (surr.)
7. 2,4-dimethylphenol
8. 2,4-dichlorophenol
9. 3-nitro-*o*-xylene (int. std.)
10. 4-chloro-3-methylphenol
11. 2,4,6-trichlorophenol
12. 2,4-dinitrophenol
13. 4-nitrophenol
14. 2,3,4,5-tetrachlorophenol (int. std.)
15. 2-methyl-4,6-dinitrophenol
16. 2,4,6-tribromophenol (surr.)
17. pentachlorophenol



Column:	Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)
Sample:	US EPA Method 528 Mix: Phenols Fortification Mix, EPA 528 (cat.# 31695), Internal Standard Mix, EPA 528 (cat.# 31696), Surrogate Standard Mix, EPA 528 (cat.# 31697)
Inj.:	1.0µL, 5µg/mL each analyte (internal standards 25µg/mL), split (10:1) 4mm Drilled Uniliner® inlet liner (hole at bottom) (cat.# 20756)
Instrument:	Agilent 6890
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	70°C (hold 0.5 min.) to 130°C @ 8°C/min., to 300°C @ 50°C/min. (hold 1 min.)
Det.:	Agilent 5973 MSD

Figure 4 Exceptionally low bleed for Rxi™-1ms columns at 330°C.



87. benzo(b)fluoranthene
88. benzo(k)fluoranthene
89. benzo(a)pyrene
90. perylene-d12 (int. std.)
91. indeno(1,2,3-cd)pyrene
92. dibenzo(a,h)anthracene
93. benzo(ghi)perylene

5ng each, on column

Column:
Rxi™-1ms, 30m, 0.25mm ID, 0.25µm (cat.# 13323)

Based on these results, we highly recommend the new Rxi™-1ms column for low-level analyses that require a 100% dimethylpolysiloxane phase.

Rxi™-1ms Columns (fused silica)

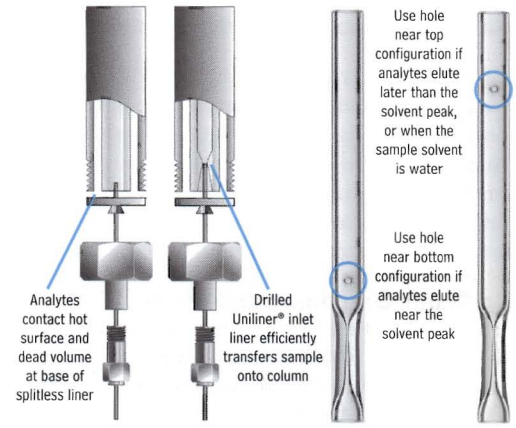
(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.18	-60 to 330/350°C	20-Meter	13302	$370
0.20mm	0.33	-60 to 330/350°C	12-Meter	13397	$230
0.20mm	0.33	-60 to 330/350°C	25-Meter	13398	$365
0.20mm	0.33	-60 to 330/350°C	50-Meter	13399	$630
0.25mm	0.25	-60 to 330/350°C	15-Meter	13320	$260
0.25mm	0.25	-60 to 330/350°C	30-Meter	13323	$435
0.25mm	0.25	-60 to 330/350°C	60-Meter	13326	$780
0.25mm	0.50	-60 to 330/350°C	15-Meter	13335	$260
0.25mm	0.50	-60 to 330/350°C	30-Meter	13338	$435
0.25mm	0.50	-60 to 330/350°C	60-Meter	13341	$780
0.25mm	1.00	-60 to 330/350°C	15-Meter	13350	$260
0.25mm	1.00	-60 to 330/350°C	30-Meter	13353	$435
0.25mm	1.00	-60 to 330/350°C	60-Meter	13356	$780
0.32mm	0.25	-60 to 330/350°C	15-Meter	13321	$280
0.32mm	0.25	-60 to 330/350°C	30-Meter	13324	$460
0.32mm	0.25	-60 to 330/350°C	60-Meter	13327	$820
0.32mm	0.50	-60 to 330/350°C	15-Meter	13336	$280
0.32mm	0.50	-60 to 330/350°C	30-Meter	13339	$460
0.32mm	0.50	-60 to 330/350°C	60-Meter	13342	$820
0.32mm	1.00	-60 to 330/350°C	15-Meter	13351	$280
0.32mm	1.00	-60 to 330/350°C	30-Meter	13354	$460
0.32mm	1.00	-60 to 330/350°C	60-Meter	13357	$820
0.53mm	0.50	-60 to 330/350°C	15-Meter	13337	$310
0.53mm	0.50	-60 to 330/350°C	30-Meter	13340	$515
0.53mm	1.00	-60 to 330/350°C	15-Meter	13352	$310
0.53mm	1.00	-60 to 330/350°C	30-Meter	13355	$515
0.53mm	1.50	-60 to 330/350°C	15-Meter	13367	$310
0.53mm	1.50	-60 to 330/350°C	30-Meter	13370	$515
0.53mm	1.50	-60 to 330/350°C	60-Meter	13373	$880

restek innovation!

The Drilled Uniliner®

To reduce the effects of surface activity in the injection port liner, and focus on the effects of the column on active analytes, we used a Drilled Uniliner® inlet liner. This liner eliminates contact between the active compounds and active metal surfaces in the injector, ensuring an inactive sample pathway for analyte transfer from the injection port to the column. For more information, request **lit. cat.# 59877**.



GC/MS Low-Level for Semivolatiles in Drinking Water

Excellent Responses at 10ng On Column, Using an Rxi™-5ms Column

By Robert Freeman, Environmental Innovations Chemist

- Inert, ultra- low bleed column improves low level analyses.
- Excellent peak shapes and responses for active analytes.
- Drilled Uniliner® inlet liner minimizes sample breakdown in the injector.

Semivolatile organic chemical contaminants in drinking water are target compounds in many analytical methods, worldwide. US EPA Method 525.2, for example, is a general purpose solid-phase extraction/GC/MS procedure for identifying and quantifying a wide range of semivolatile compounds. Analytes, and introduced internal standards and surrogates, are extracted from a 1-liter water sample by passing the sample through a solid phase extraction disk containing a bonded C18 phase (e.g., Resprep™-C18, cat. #24004). Target compounds are trapped on the disk, then eluted in a small amount of solvent. The extract is concentrated by evaporating the solvent, and the sample components are separated, identified, and quantified by GC/MS.

As is true for many other semivolatiles methods, the extensive target compound list for Method 525.2 encompasses numerous classes of analytes. These diverse compounds present varying difficulties in the analysis, including differing modes of degradation. Coupled with the continual need for lower levels of detection, these challenges make extreme demands on the chromatography column, and the analysis requires an inert, thermally stable, low-bleed stationary phase. To meet these needs, we recommend a 30 meter, 0.25mm ID, 0.25µm Rxi™-5ms column. Enhanced surface deactivation provides Rxi™-5ms columns with exceptional inertness and ultra-low bleed, ensuring resolution and symmetric peaks for these difficult analytes.

Figure 1 shows the total ion chromatogram for 88 semivolatiles commonly analyzed in drinking water, and listed in US EPA Method 525.2, at 10ng each on an Rxi™-5ms column. Resolution and peak shapes are exceptionally good.

To minimize analyte degradation in the injection port, and discrimination among analytes by molecular weight, we recommend installing a Drilled Uniliner® inlet liner in the injection port. This liner forms a Press-Tight® seal with the inlet end of the column, eliminating contact between the sample and the hot metal surfaces in the injection port and assuring near-complete sample transfer. The small hole in the wall of the liner allows the liner to be used with split/splitless injections. As an additional precaution to minimize analyte breakdown, we use a pulsed splitless injection (50psi / 0.3 min.; 1µL sample) to reduce the time the analytes spend in the injection port.

Exceptional inertness and ultra-low bleed enable an Rxi™-5ms column to perform exceptionally well in analyses of complex mixtures of semivolatile compounds. We recommend pairing an Rxi™-5ms column with our recently revised analytical reference mixes for semivolatile pollutants in water, listed on page 16 of this *Advantage*. Restek can provide all the materials needed for a semivolatiles analysis: extraction disks, analytical reference materials, and a column capable of excellent responses for all target analytes at low on-column concentrations.

Rxi™-5ms Columns (fused silica)

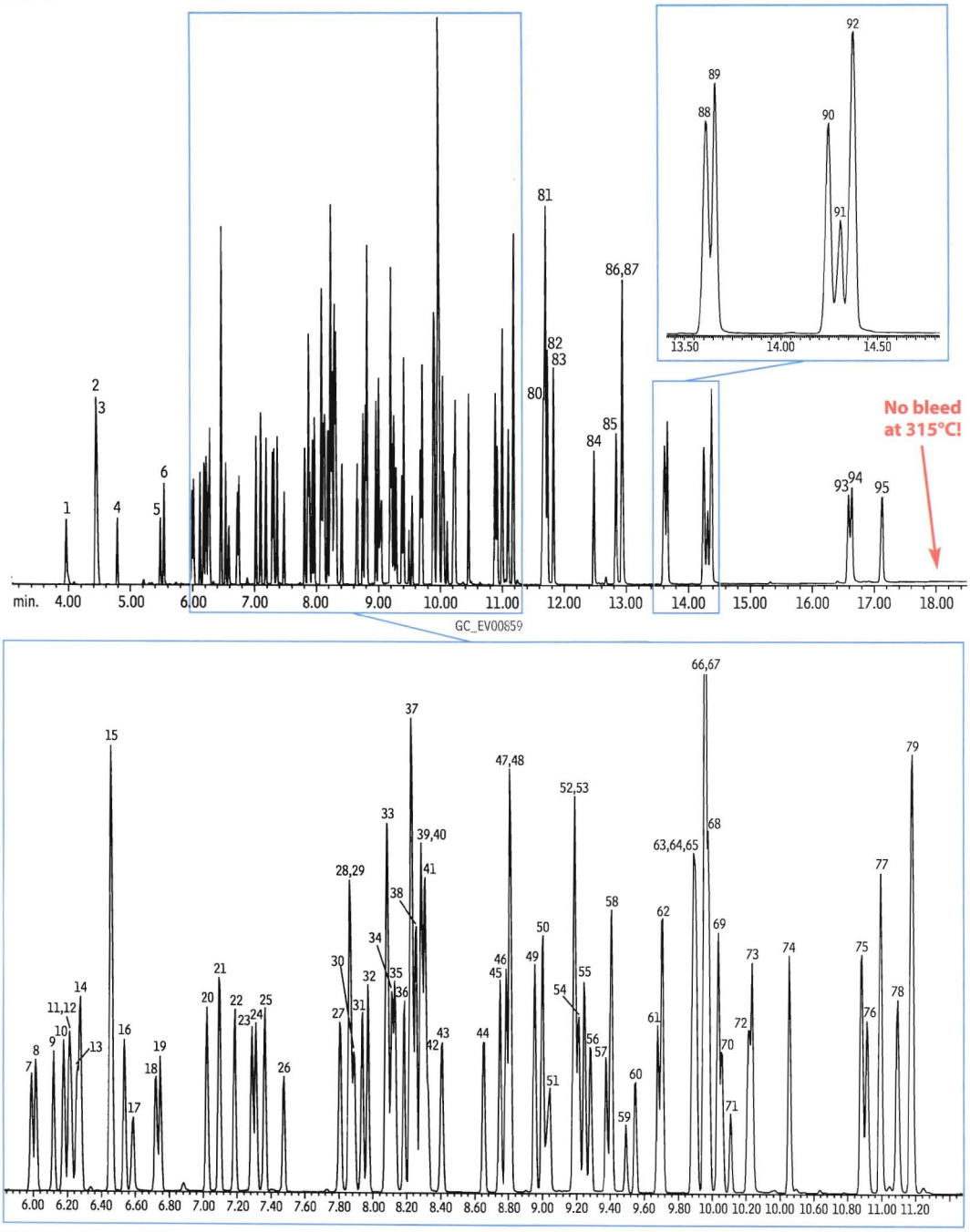
(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13423	$435

for more info

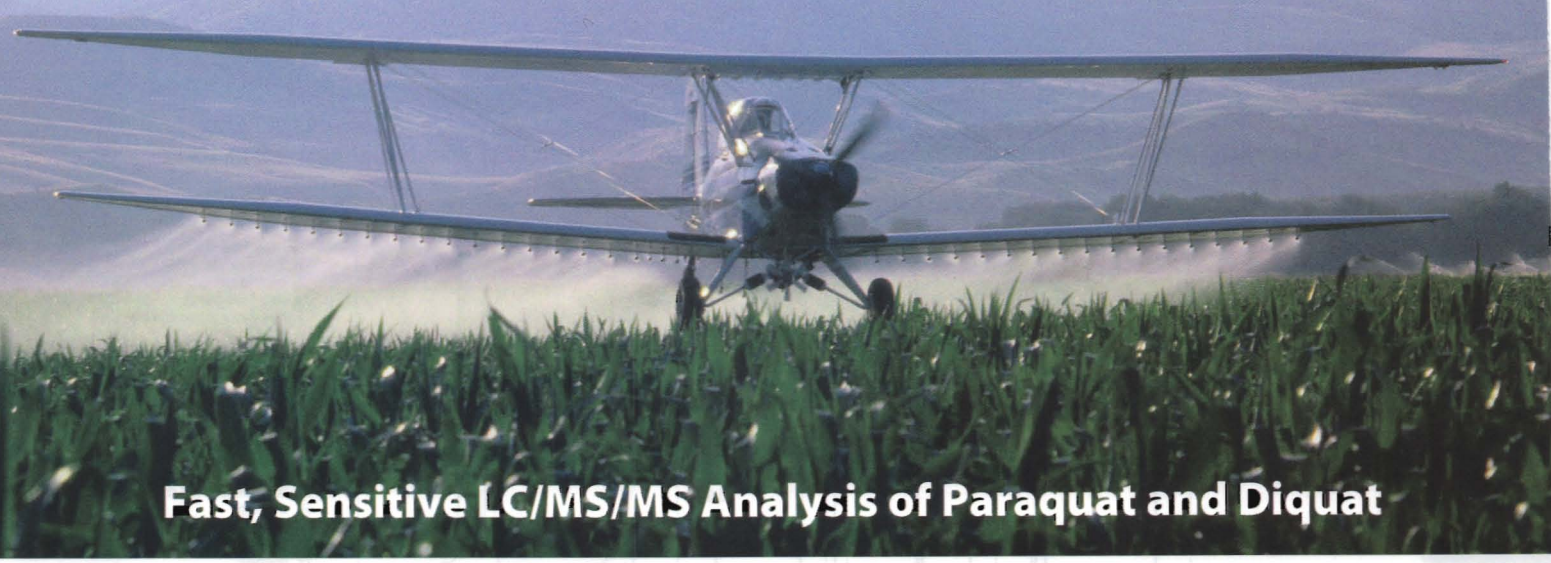
For more information about Drilled Uniliner® inlet liners see page 11 and request **lit. cat.# 59877**, or visit our website: www.restek.com

Figure 1 Excellent resolution and symmetric peaks for commonly analyzed drinking water semivolatiles, at 10ng each on an Rxi™-5ms column.



1. isophorone
2. 2-nitro-*m*-xylene (SS)
3. naphthalene
4. dichlorvos (DDVP)
5. hexachlorocyclopentadiene
6. EPTC
7. mevinphos
8. butylate
9. vernolate
10. dimethyl phthalate
11. pebulate
12. etridiazole (Terrazole®)
13. 2,6-dinitrotoluene
14. acenaphthylene
15. acenaphthene-d10 (IS)
16. chlorneb
17. tebuthiuron
18. 2,4-dinitrotoluene
19. molinate
20. diethyl phthalate
21. fluorene
22. propachlor
23. ethoprop (ethoprophos)
24. cycloate
25. chlorpropham
26. trifluralin
27. atraton
28. hexachlorobenzene
29. prometon
30. simazine
31. atrazine
32. propazine
33. pentachlorophenol
34. terbufos
35. pronamide (propyzamide)
36. diazinon
37. phenanthrene-d10 (IS)
38. phenanthrene
39. disulfoton
40. methyl paraoxon
41. anthracene
42. terbacil
43. chlorothalonil
44. metribuzin
45. simetryn
46. ametryn
47. alachlor
48. prometryn
49. terbutryn
50. di-*n*-butyl phthalate
51. bromacil
52. cyanazine (Bladex)
53. metalochlor
54. chlorpyrifos (Dursban®)
55. triademefon
56. Dacthal® (DCPA)
57. MGK-264 (isomer A)
58. diphenamid
59. MGK-264 (isomer B)
60. merphos
61. heptachlor epoxide
62. fluoranthene
63. stirofos
64. disulfoton sulfone
65. butachlor
66. pyrene-d10 (SS)
67. fenamiphos
68. pyrene
69. napropamide (Devrinol®)
70. *trans*-nonachlor
71. merphos oxide
72. tricyclazole (Beam)
73. carboxin
74. chlorobenzilate
75. butyl benzyl phthalate
76. norflurazon
77. bis(2-ethylhexyl) adipate
78. hexazinone (Velpar®)
79. triphenylphosphate (SS)
80. benzo(a)anthracene
81. chrysene-d12 (IS)
82. chrysene
83. bis(2-ethylhexyl) phthalate
84. fenarimol
85. *cis*-permethrin
86. trans-permethrin
87. di-*n*-octyl phthalate
88. benzo(b)fluoranthene
89. benzo(k)fluoranthene
90. benzo(a)pyrene
91. fluridone (Sonar®)
92. perylene-d12 (SS)
93. indeno(1,2,3-cd)pyrene
94. dibenzo(a,h)anthracene
95. benzo(ghi)perylene

Column:	Rxi™-5ms, 30m, 0.25mm ID, 0.25µm (cat.# 13423)
Sample:	US EPA Method 525.2 mix, 10µg/mL each analyte, 25µg/mL each internal standard and surrogate: Method 525.2 Semivolatile Mix (cat.# 31899), Organonitrogen Pesticide Mix #1 (cat.# 33012), Organonitrogen Pesticide Mix #2 (cat.# 33011), Organophosphate Pesticide Mix #1 (cat.# 33013), Nitrogen/Phosphorous Pesticide Mix #2 (cat.# 32423), Method 525.2 Internal Standard Mix (cat.# 31825), Method 525.2 Surrogate Standard Mix (cat.# 31826)
Instrument:	Agilent 6890
Inj.:	1.0µL, pulsed splitless injection: 50psi (0.3 min.), 80mL/min. (0.15 min.), gas saver 15mL/min. (1 min.), 4mm Drilled Uniliner® inlet liner, hole near bottom (cat#20771)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2mL/min.
Oven temp.:	90°C (1 min.) to 270°C @ 20°C/min., to 315°C @ 6°C/min.
Det.:	Agilent 5973 MSD
Interface line temp.:	280°C
Scan range:	35-550 amu
Solvent delay:	3.00 min.
Tune:	DFTPP
Ionization:	EI



Fast, Sensitive LC/MS/MS Analysis of Paraquat and Diquat

Using an API 3200™ Mass Spectrometer and an Ultra Quat HPLC Column

Houssain El Aribi, Ph.D., LC/MS Product and Application Specialist, MDS SCIEX*, Becky Wittrig, Ph.D., HPLC Product Manager, C. Vernon Bartlett, HPLC R&D Scientist, and Julie Kowalski, Innovations Team Chemist, Restek Corporation

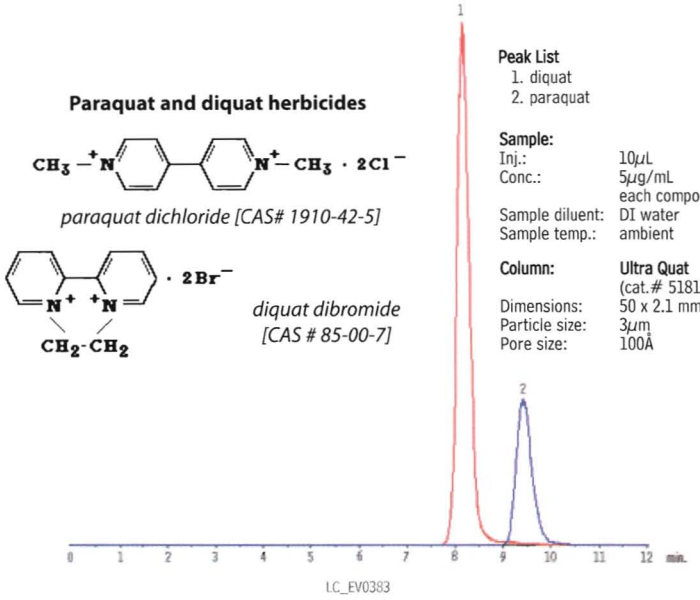
- Complete resolution of paraquat & diquat – with a simple, isocratic mobile phase!
- Superior sensitivity—5ppb paraquat or 0.1ppb diquat—without preconcentration.
- Significantly faster than conventional methodologies.

Restek chemists designed the Ultra Quat HPLC column specifically for analyses of quaternary amine compounds. This unique column makes possible a simple HPLC/UV analysis for paraquat and diquat[1] – a significant improvement over alternative methodologies. Now, in collaboration with scientists at MDS Sciex, we have developed a fast, highly sensitive LC/MS method for analyzing these challenging target compounds.

Charged quaternary amines, such as paraquat and diquat, exhibit little or no retention on C18 or other alkyl stationary phases. In our HPLC/UV procedure, our Ultra Quat mobile phase modifier (Ultra Quat Reagent Solution, cat.# 32441) increases the interactions between paraquat and diquat and the Ultra Quat stationary phase, providing the necessary retention and resolution. For compatibility with MS detection, however, we needed a volatile mobile phase additive. Low concentrations of heptafluorobutyric acid (HFBA) effectively shield the positive charges of paraquat and diquat, increasing interactions between the quaternary amines and the Ultra Quat stationary phase.

Figure 1 shows the excellent separation of paraquat and diquat, at a concentration of 5µg/mL each in water, achieved by using an API 3200™ mass spectrometer. We used multiple reaction monitoring (MRM) – a standard technique for quantitative LC/MS/MS – for this application. In MRM, pairs of target precursor ions and unique fragment ions are used for quick and accurate identification of target species. Collision induced

Figure 1 Fast, sensitive LC/MS/MS analysis of paraquat and diquat, using an API 3200™ mass spectrometer and an Ultra Quat HPLC column.



Conditions:

Mobile phase:	10mM heptafluorobutyric acid:acetonitrile, 95:5 (v/v)
Flow:	0.3mL/min.
Temp.:	ambient
Det.:	Applied Biosystems API 3200™
Interface:	electrospray
Ion Mode:	positive
Temp.:	600°C
Ion Spray™ voltage:	5500V
Collision exit potential:	3V
Curtain Gas™:	15psi
Gas supply 1:	70psi
Gas supply 2:	60psi
Quantitation:	(MRM)
Q1/Q3:	unit resolution
Dwell time:	200 ms

Precursor Ion (amu)	Fragment Ion (amu)	DP (V)	Collision Energy (eV)
diquat, 183+	157+	30	30
paraquat, 93 (2+)	171+	20	20

*Data courtesy of Houssain El Aribi, Ph.D., LC/MS Product and Application Specialist, MDS SCIEX, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8

Table 1 MRM transitions and MS conditions used to generate CID spectra for paraquat and diquat.

Precursor Ion (m/z)	Fragment Ions (m/z)	DP (V)	Collision Energy (eV)
Paraquat [M^{2+} - H^+] 185	170a 169b	40	30
Paraquat-d8 [M^{2+} - D^+] 193 (int. std.)	178a	40	30
Diquat [M^{2+} - H^+] 183	157a 168b	35	30
Diquat-d4 [M^{2+} - D^+] 186 (int. std.)	158a	35	30

Figure 2 CID spectra for paraquat$^+$ at CE = 25eV.

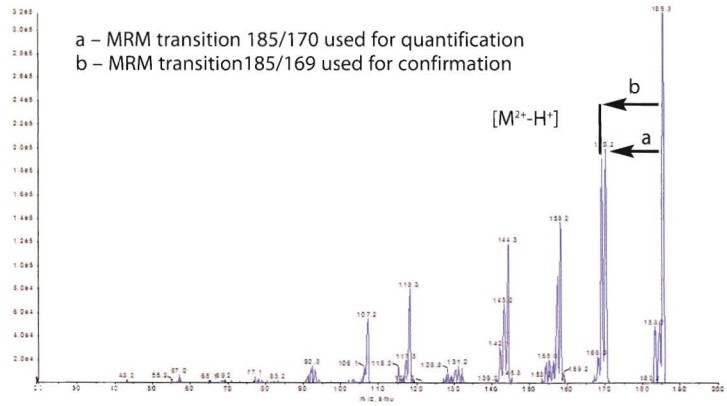
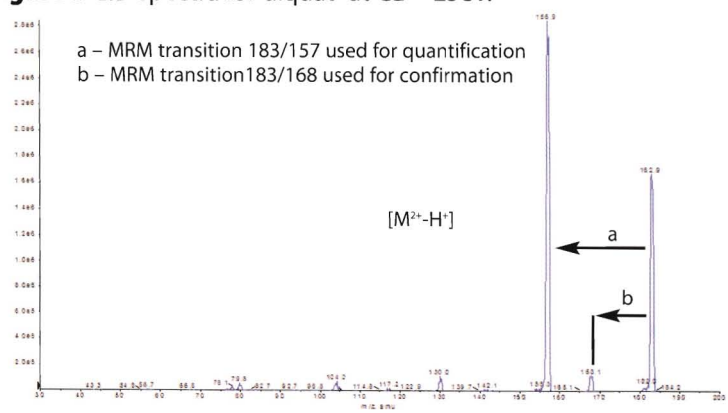


Figure 3 CID spectra for diquat$^+$ at CE = 25eV.



free literature

Simple, Sensitive HPLC/UV Analysis for Paraquat and Diquat Using High-Recovery Solid Phase Extraction and an Ultra Quat HPLC Column

These highly charged quaternary amines are poorly retained on alkyl stationary phases. Using only acetonitrile, water, and a solvation-blocking reagent, our separation system alters the interactions among analyte, mobile phase, and stationary phase, and promotes solubility of the analytes in the stationary phase. In our system, the detection limit is 6ppb for either herbicide, and the analysis is completed in less than 10 minutes. An optimized solid phase extraction cartridge concentrates the herbicides for the analysis. **lit. cat.# 580006**

Environmental HPLC: Applications-Columns-Reference Materials

Restek HPLC columns support environmental HPLC applications with rapid analysis times and effective analyte resolution. Sample turn-around can be 50% faster, or more, than with alternative columns. In addition, we prepare analytical reference materials and sample clean-up products for these methods. Applications in this publication include polyaromatic hydrocarbons, carbamates, phenoxyacid herbicides, explosives, carbonyls, and paraquat/diquat. **lit. cat.# 59741A**

dissociation (CID) is used to generate the fragment ions. CID spectra for paraquat and diquat are shown in Figures 2 and 3. This approach has been used in many pharmaceutical and environmental applications, to generate unmatched limits of detection or quantification, precision, and accuracy. For accurate quantification, we used paraquat-d8 and diquat-d4 as internal standards (Table 1), to compensate for matrix effects and to correct for random and systematic errors in separation and detection.

For triplicate injections of 8 concentrations of analytes in deionized water and in lake water, from 5µg/100mL to 100µg/100mL for paraquat and from 0.1µg/100mL to 100µg/100mL for diquat, correlation coefficients for calibration curves were >0.995, using a linear fit and 1/x weighting factor. These results indicate that quantification can be performed with good linearity and sensitivity. Minimum detection limits (MDL) for the method, for paraquat and diquat in deionized water, were 5µg/L and 0.1µg/L, respectively.

LC/MS is a powerful tool for analyses of challenging environmental contaminants. In LC/MS analyses of paraquat and diquat, the combination of an Applied Biosystems API 3200™ mass spectrometer and an Ultra Quat HPLC column ensures fast, sensitive, and accurate results.

Reference

1. *Simple, Sensitive HPLC/UV Analysis for Paraquat and Diquat, Using High-Recovery Solid Phase Extraction and an Ultra Quat HPLC Column* Applications Note 580006, Restek Corporation, Feb. 2006.

Reference available from Restek on request.

Ultra Quat Columns & Guard Cartridges

5µm Column, 4.6mm	cat. #	price
150mm	9181565	$399
150mm (with Trident™ Inlet Fitting)	9181565-700	$414
Ultra Quat Guard Cartridges		
10 x 2.1mm	918150212	$131
10 x 4.0mm	918150210	$131
20 x 2.1mm	918150222	$131
20 x 4.0mm	918150220	$131

Paraquat & Diquat Calibration Mix

diquat dibromide paraquat dichloride

1,000µg/mL each in water, 1mL/ampul

cat. # 32437 (ea.) $26

free literature

HPLC Essentials

Genuine Restek Replacement Parts will keep your Agilent, Beckman, Hitachi, PerkinElmer, Shimadzu, Thermo Separation Products, or Waters system running smoothly and chromatography sharp. Restek parts equal or exceed the performance of original components. **lit. cat.# 59012A**

Assaying Local Anesthetics by GC/FID

Optimizing System Suitability, Using an Rxi™-5ms Column

By Rick Lake, Pharmaceutical Innovations Chemist

- Rxi™-5ms column assures excellent peak shapes for basic compounds.
- Stable, reproducible retention times.
- Easy conformance to stringent system suitability criteria.

Local anesthetics are biologically active compounds that reversibly inhibit the propagation, or broadcasting, of signals along nerve cell pathways. Because of this action, they are widely used as drug compounds to produce temporary analgesia (loss of pain) and paralysis (loss of muscle movement). Anesthetic compounds are formulated into a large number and wide variety of drug products, ranging from over-the-counter topical ointments to clinical injectables, and they often are formulated in combination with other active ingredients. Therefore, many analyses of local anesthetics involve manufacturing assays, like potency and stability assays, which require high-throughput and reproducible results. These assays require the fulfillment of system suitability criteria and, for this reason, we investigated assaying local anesthetics by GC/FID, using common system suitability parameters as evaluation criteria.

By GC standards, a local anesthetic is a high molecular weight, weakly basic, active compound. We took these characteristics into account when we chose the column and inlet liner for this application. Considering that these analytes are basic and active, the deactivation of the inlet liner and capillary column is very important. For superior inertness, we chose to use an Rxi™-5ms column.

When analyzing high molecular weight compounds – the normal case in pharmaceutical assays – discrimination and irreproducible injections sometimes occur, primarily due to incomplete vaporization of the analytes. This can be especially problematic for analysts who must meet stringent system suitability criteria. Some liners, like the laminar cup and cup splitter, were designed specifically for samples containing high molecular weight compounds. These liner designs aid in sample vaporization, but at a cost of reduced internal volumes and intricate flow paths that can cause poor reproducibility when such liners are used with a solvent that has a large expansion volume, like methanol. In this application, we used our conventional, intermediate polarity deactivated, split liners packed with intermediate polarity deactivated wool. Wool in the liner provides a large surface area, for rapid vaporization, but the liner still delivers a uniform vapor cloud to the split point.

Under these conditions, chromatography from a six-replicate system suitability analysis (Figure 1) was well within normal acceptance criteria (Table 1). USP tailing, approximately 1.00 for all analytes, shows the exceptional inertness of the Rxi™-5ms column. In addition, retention times and area responses were extremely stable. The Rxi™-5ms column, coupled with an appropriate inlet liner, provides the stability and deactivation necessary to afford easier conformance to system suitability criteria. The 10-minute analysis time for these compounds ensures high sample throughput.

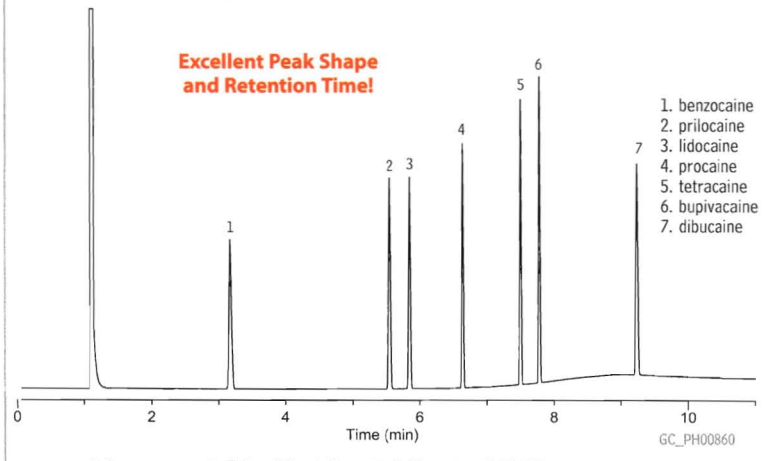
Rxi™-5ms Column (fused silica)

(Crossbond® 5% diphenyl / 95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.53mm	1.00	-60 to 330/350°C	30-Meter	13455	$515

For other dimensions, see page 5.

Figure 1 An Rxi™-5ms column provides excellent peak shape and stable retention times for basic compounds, for easier conformance to system suitability criteria.



Column:	Rxi™-5ms 30m, 0.53mm ID, 1.00µm (cat.# 13455)
Sample:	50µg/mL each component in methanol
Inj.:	1.0µL split (10:1), 4mm split inlet liner with wool (cat # 20781)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	5.0mL/min.
Oven temp.:	200°C (4 min.) to 320°C @ 30°C/min. (hold 3 min.)
Det.:	FID @ 300°C

Table 1 An Rxi™-5ms column provides exceptionally stable retention times and area responses.

Compound	Peak Area (%RSD)	Retention Time (%RSD)	USP Tailing	Efficiency
benzocaine	0.85	0.03	1.00	55858
prilocaine	1.36	0.02	1.00	(isothermal)
lidocaine	1.01	0.02	1.00	
procaine	1.83	0.03	1.00	
tetracaine	1.78	0.01	1.00	
bupivacaine	1.64	0.02	1.02	
dibucaine	1.17	0.06	1.00	
Mean	1.38	0.03	1.00	

six-replicate system suitability analysis

Optimized RP-HPLC Method for Hydroxybenzoic Acids

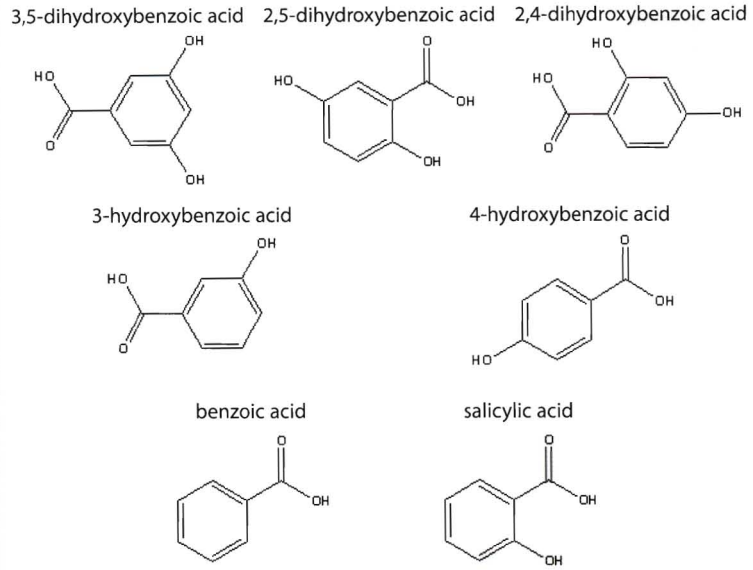
Balanced Retention for a Range of Polarities, Using an Ultra Aqueous C18 Column

By Rick Lake, Pharmaceutical Innovations Chemist

- Useful retention of more polar and less polar analytes.
- Ultra Aqueous C18 column is compatible with 100% aqueous mobile phases.
- Ideal for samples that encompass a broad range of analyte polarity.

Hydroxybenzoic acids are important pharmacological compounds. They serve as active drug substances (aspirin, for example), as well as preservatives in drug products. In some cases, they represent impurities in drug products. Their analysis sometimes can be difficult, not only because they represent a wide range of applications, but primarily because they encompass a wide range of polarity. Chemically, benzoic acid, the basic structure for these analytes, consists of a benzene ring with a carboxyl group (Figure 1). Hydroxybenzoic acids share the same basic structure, but contain additional hydroxyl groups on the benzene ring (Figure 1). The additional hydroxyl groups' varied positions and numbers create differences among the analytes' overall polarity and solubility. Because these compounds represent such varying chemistry and polarity, finding an alkyl (C18) HPLC column that can effectively assay them all could be very difficult, but such a column could be of value for resolving these compounds from active drugs or from chemically similar impurities.

Figure 1 Hydroxybenzoic acids share the same basic structure, but have varying polarity.

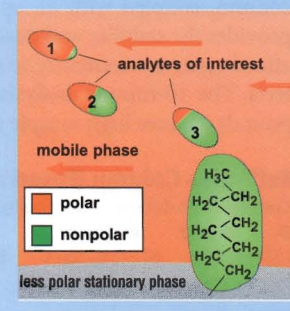


Using identical conditions, we analyzed a group of hydroxybenzoic acids on a conventional C18 stationary phase column, on a C18 column with a polar group within (intrinsic to) the alkyl bonded phase (an IBD phase*), and on an Ultra Aqueous C18 column. Our objective was to find the optimum stationary phase for resolving analytes with a varying number of polar functional groups.

Overall, the Ultra Aqueous C18 column provided the best balance of retention for more polar and less polar analytes (Figure 2A), completely resolving our test mix when used with a simple gradient mobile phase. The conventional C18 column exhibited retention very similar to that of the Ultra Aqueous C18 column for the less polar analytes, benzoic acid and salicylic acid, but it showed less retention and resolution for the more polar compounds (Figure 2B). The intrinsically base deactivated column, on the other hand, exhibited opposite characteristics – retention similar to the Ultra Aqueous C18 column for the more polar compounds, but little retention of the less polar compounds (Figure 2C).

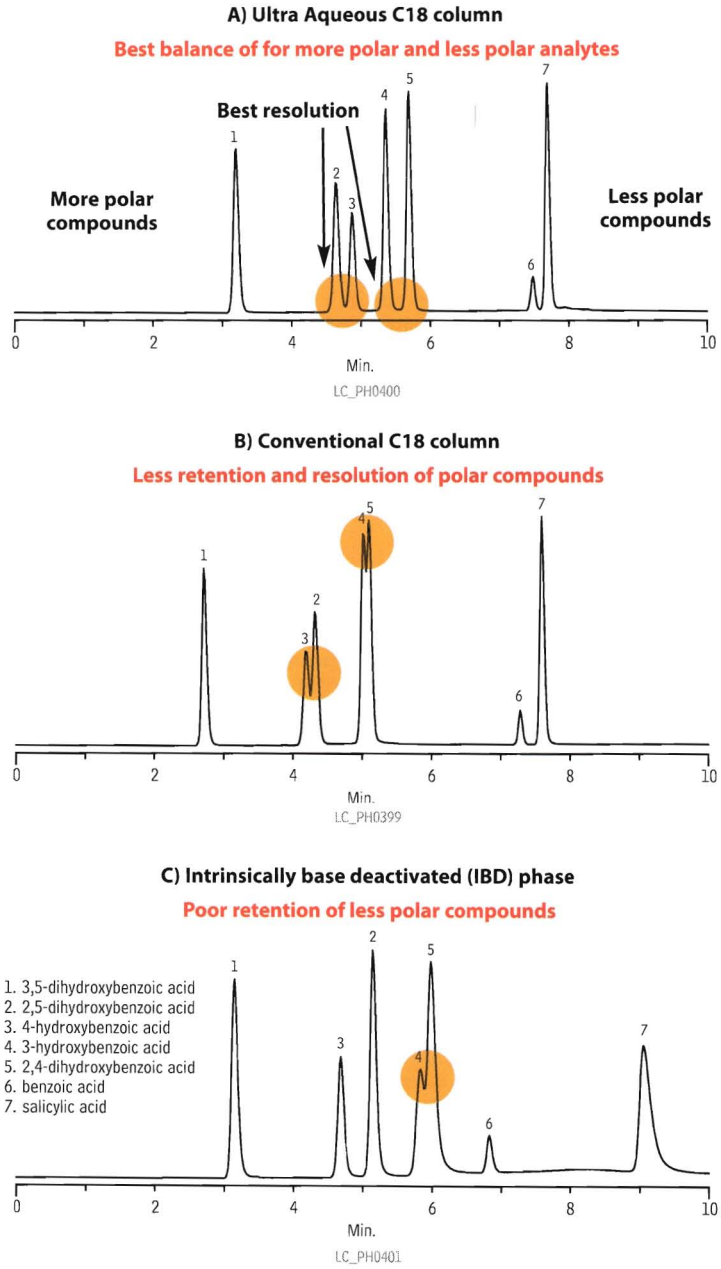
Options for Analyzing Polar Compounds

Many types of alkyl phases currently are available to the analyst, making column selection difficult. Although all alkyl phases possess the same basic structure – a specific length of alkyl chain bonded to a silica surface (typically C1-C30, with C18 being the most common) – various attached polar groups create selectivity and retention differences among columns. For example, a conventional C18 phase is comprised of a monomerically bonded straight 18 carbon alkyl chain, meaning every alkyl chain has a single, direct attachment to the silica surface. These phases are excellent for retaining nonpolar compounds, but they show very limited retention for polar compounds. One common bonding technique for increasing retention of polar compounds on an alkyl phase is to attach a polar group within, or intrinsic to, the alkyl phase. These phases, known as intrinsically base deactivated (IBD) phases, show increased retention for polar compounds because the embedded polar groups are capable of interaction with polar portions of analyte molecules. (These phases also have a deactivating effect on basic compounds, by creating an electrostatic barrier.) Polarity also can be added to an alkyl phase by adding polar end caps to active sites on the silica surface, or by adding polar side chains to the alkyl attachment. Interactions with polar compounds also can be increased through the use of a polymeric bonding chemistry.



Retention by Reversed Phase (RP) HPLC

Figure 2 An Ultra Aqueous C18 column shows suitable retention for polar or nonpolar compounds, providing enhanced selectivity for a broad range of polarity.



Sample:
Inj.: 5µL
Conc.: 50µg/mL each component
Sample diluent: mobile phase

Column: Ultra Aqueous C18 (cat.# 9178568)
Dimensions: 150 x 4.6 mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: A: 20mM potassium phosphate (pH 2.5); B: acetonitrile

Time (min.)	%B
0.00	20
5.00	50
10.00	50

Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 210nm

It is well documented that Ultra Aqueous C18 columns are compatible with 100% aqueous mobile phases, because the stationary phase has sufficient polar character to prevent dewetting or hydrophobic collapse.[1] Our current analyses reveal yet another advantage to the slight polar character of this column: by providing the best resolution of analytes exhibiting a wide range of polarity, the Ultra Aqueous C18 column demonstrates that it also can be used to retain, and separate, more polar or less polar compounds – or mixtures of both.

Reference

1 *Ultra Aqueous C18 HPLC Columns: Achieve Stable Retention in 100% Aqueous Mobile Phase* Restek Corporation, 2002 (lit. cat.# 59371). Reference available on request.

*The intrinsically base deactivated (IBD) phase shows increased retention for polar compounds, because the embedded polar groups are capable of interaction with polar portions of analyte molecules.

Ultra Aqueous C18 Columns

3µm Column, 2.1mm	cat. #	price
30mm	9178332	$344
50mm	9178352	$344
100mm	9178312	$370
3µm Column, 3.2mm	**cat. #**	**price**
30mm	9178333	$344
50mm	9178353	$344
100mm	9178313	$370
3µm Column, 4.6mm	**cat. #**	**price**
30mm	9178335	$344
50mm	9178355	$344
100mm	9178315	$370
5µm Column, 2.1mm	**cat. #**	**price**
30mm	9178532	$319
50mm	9178552	$319
100mm	9178512	$344
150mm	9178562	$370
200mm	9178522	$396
250mm	9178572	$423
5µm Column, 3.2mm	**cat. #**	**price**
30mm	9178533	$319
50mm	9178553	$319
100mm	9178513	$344
150mm	9178563	$370
200mm	9178523	$396
250mm	9178573	$423
5µm Column, 4.6mm	**cat. #**	**price**
30mm	9178535	$319
50mm	9178555	$319
100mm	9178515	$344
150mm	9178565	$370
200mm	9178525	$396
250mm	9178575	$423

All columns also available with Trident™ integrated guard column configuration. Call for more details.

GC Analysis of Total Reduced Sulfurs at ppbv Levels

Using an Rxi™-1ms Column and Sulfur Chemiluminescence Detection

by Silvia Martinez, Innovations Chemist

- Reliable results for ppbv concentrations of highly active sulfur compounds.
- Inert, low bleed column resolves all analytes.
- Column compatible with SCD and other sulfur-specific detectors.

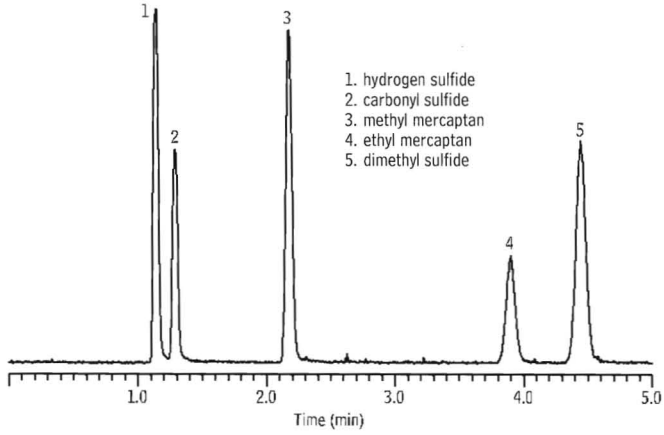
Through the Clean Air Act, the United States Environmental Protection Agency (US EPA) regulates and limits the emission of toxic air pollutants. The determination of total reduced sulfurs, as required by CFR Title 40, requires the use of methods and equipment capable of providing full resolution as well as high sensitivity. Methods TO-15, TO-16 and TO-16A describe GC procedures that apply to the determination of reduced sulfurs from stationary sources, such as recovery furnaces, lime kilns, smelt dissolving tanks, fuel gas combustion devices, tail gas control units, and others. Method TO-16 specifies detectable concentrations of ppbv levels for dimethyl disulfide, dimethyl sulfide, hydrogen sulfide, and methyl mercaptan. While these methods do not specify the analytical GC column to use, they do state that the column must resolve the sulfur compounds.

Our new 100% dimethylpolysiloxane column, the Rxi™-1ms column, provides the ultra-low bleed required for low level detection and quantification of sulfur compounds. Its exceptional inertness allows complete separation of these very reactive compounds, with excellent peak shape, at ppbv levels. When this column is coupled with a sulfur chemiluminescence detector (SCD), the analysis is fast and simple.

For our example analysis, we collected a 20mL sample of a gaseous mixture of hydrogen sulfide, carbonyl sulfide, methyl mercaptan, ethyl mercaptan, and dimethyl sulfide in helium, using a Sulfinert®-treated stainless steel sample loop. We transferred the sample to a SilcoCan™ air monitoring canister and pressurized the can to 30psig with dry nitrogen. The Sulfinert® passivation treatment on both the sample loop and canister prevented adsorption losses of the highly active sulfur compounds. We introduced a 1mL aliquot of the diluted gaseous mixture into the Rxi™-1ms column via a second Sulfinert®-treated stainless-steel sample loop, using helium as a carrier, and analyzed the sample isothermally at 30°C.

Figure 1 shows the chromatography for the reduced sulfur compounds, demonstrating full resolution in less than 5 minutes. For collecting, storing, and analyzing active sulfur compounds at levels as low as single parts per billion, the performances of Sulfinert® passivated containers and transfer systems, and inert Rxi™-1ms columns, simply can't be equaled.

Figure 1 Total resolution of reduced sulfur compounds, in less than 5 minutes, using an Rxi™-1ms column.



Column:	Rxi™-1ms, 30m, 0.32mm ID, 4.00µm (cat.# 13396)
Sample:	hydrogen sulfide, carbonyl sulfide, methyl mercaptan, ethyl mercaptan, dimethyl sulfide, 100 ppbv each in helium
Inj.:	1.mL splitless, direct
Sample loop temp.:	30°C
Carrier gas:	helium, constant pressure
Linear velocity:	48cm/sec. @ 30°C
Oven temp.:	30°C
Det.:	sulfur chemiluminescence detector
Det. temp.:	800°C

Sample storage & transfer:
SilcoCan™ air monitoring canister with Siltek® treated 1/4" valve (cat.# 24182-650); Sulfinert® treated gas sample loop, 1cc (cat.# 22848); Sulfinert® treated gas sample loop, 10cc (custom order)

Rxi™-1ms Columns (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	4.00	-60 to 330/350°C	30-Meter	13396	$460

for more info

SilcoCan™ Canisters

The best alternative for ambient air monitoring

Recovery data for low ppb levels of active sulfur-containing compounds show why SilcoCan™ canisters are the best choice for monitoring TO-14, TO-15, or reactive sulfur compounds. lit. cat.# 59011A

Sulfinert®-Treated Sample Cylinders

Store Active Sulfur Compounds at ppb Levels

by Neil Mosesman, Air Sampling Products Manager

- Stable storage of sulfur compounds at ppb levels.
- D.O.T. rated to 1800psi at room temperature.
- High quality cylinders manufactured by Swagelok®.

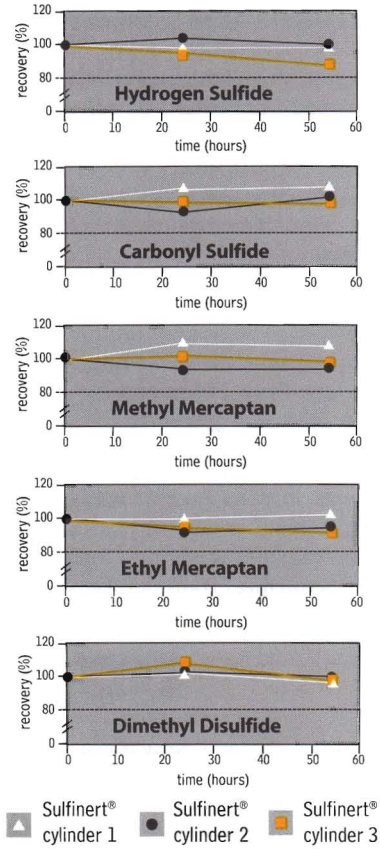
Refinery and natural gas samples often contain trace amounts of sulfur-containing compounds which can interfere with reactions or poison catalysts in petrochemical processes. Because sulfur compounds quickly react with stainless steel surfaces, accurate determination of these compounds is impossible when samples are collected and stored in untreated sample cylinders. Restek's Sulfinert® passivation technique bonds an inert silica layer into the surface of stainless steel, preventing active compounds from reacting with or adsorbing to the steel.

To characterize Sulfinert® surfaces, we tested the stability of 17ppbv standards of sulfur compounds in three Sulfinert® sample cylinders over a 54-hour period. Dimethyl sulfide, which is not adsorbed by stainless steel, was used as an internal standard.

The Sulfinert®-treated cylinders were inert to the reactive sulfur compounds over the 54-hour test period (Figure 1). Hydrogen sulfide exhibited greater than 85% recovery; methyl mercaptan, ethyl mercaptan, carbonyl sulfide, and dimethyl disulfide exhibited greater than 90% recovery.

Sulfinert®-treated gas sampling equipment is ideal for collecting and storing samples containing ppb levels of sulfur compounds, such as natural gas or beverage-grade carbon dioxide. Sulfinert® treatment ensures that sulfur compounds or other highly active compounds remain stable during transport from the field to the laboratory.

Figure 1 Stability of sulfur compounds is remarkable in Sulfinert®-treated cylinders.



Sulfinert® Treated Swagelok® Sample Cylinders

These cylinders are made from 304 grade stainless steel with 1/4" female NPT threads on both ends.

Size	qty.	cat.#	price
75cc	ea.	24130	$202
150cc	ea.	24131	$228
300cc	ea.	24132	$233
500cc	ea.	24133	$258
1000cc	ea.	24134	$430
2250cc	ea.	21394	$829

Sulfinert® Treated Alta-Robbins Sample Cylinder Valves

- All wetted parts Sulfinert® treated for inertness.
- Compatible with Sulfinert® treated Swagelok® sample cylinders.
- Large, durable, Kel-F® seat ensures leak-free operation.

Description	qty.	cat.#	price
1/4" NPT Exit	ea.	21400	$177
1/4" Compression Exit	ea.	21401	$177
1/4" NPT with Dip Tube*	ea.	21402	$253
1/4" NPT with 2850psi Rupture Disk	ea.	21403	$354
1/4" NPT Male Inlet x 1/4" Female Outlet with 2850psi Rupture Disk	ea.	21404	$354

*Specify dip tube length or % outage when ordering (maximum length = 5.25"/ 13.3cm)

How Good is Your PONA Column?

Data-Based Decisions Help Simplify the Choice

By Barry L. Burger, Innovations Chemist

- When tested, most PONA columns do not meet ASTM D-6730 method specifications.
- Restek 100-meter PONA column meets all ASTM specifications.
- Restek PONA column is compatible with hydrogen carrier gas.

So, you're ready to purchase a PONA column. But, with all the options available today, which manufacturer do you purchase the column from, and what criteria do you consider in making your selection? Do you select the most expensive column, thinking higher price means quality, and therefore higher performance? Or, do you take the advice of the guy in the laboratory down the hall when he tells you it doesn't matter whose column you buy – they are all the same? That statement cannot be further from reality. Many variables affect how well a column will perform in the demanding ASTM D-6730 method: column length and ID, polymer deposition, and column deactivation, to name a few. These all vary among manufacturers, and the effects of these variations are substantiated by data.

To assist you in making a data-based decision when selecting a PONA column for use in the ASTM D-6730 method, Restek purchased designated versions of the 100 meter x 0.25mm ID x 0.5df PONA column from four vendors. We evaluated these columns, and our own Rtx™-1PONA column, using the proposed D-6730 method that calls for hydrogen carrier gas, which reduces tridecane retention time from 140 minutes to approximately 70 minutes. (For more advantages of using hydrogen as the carrier gas, see *Advantage* 2006.02, pages 18-19.)

We performed the comparisons using an Agilent 6890 GC equipped with a flame ionization detector and ChemStation data collection software. In all analyses we used hydrogen carrier gas in the constant flow mode, adjusted the dead time to 3.50 ±0.05 minutes at 35°C, and set a split ratio of 150:1. Data presented here were generated at 35°C, as specified by the ASTM method, to determine if a column is suitable for adding a tuning column and performing the PONA analysis. We used Transition Labs' (Golden, Colorado) DHA Oxy-Setup mix (Transition Labs part number 94100) for this determination. We evaluated all five columns under the same conditions, and measured each against the specifications for ASTM D-6730, as follows:

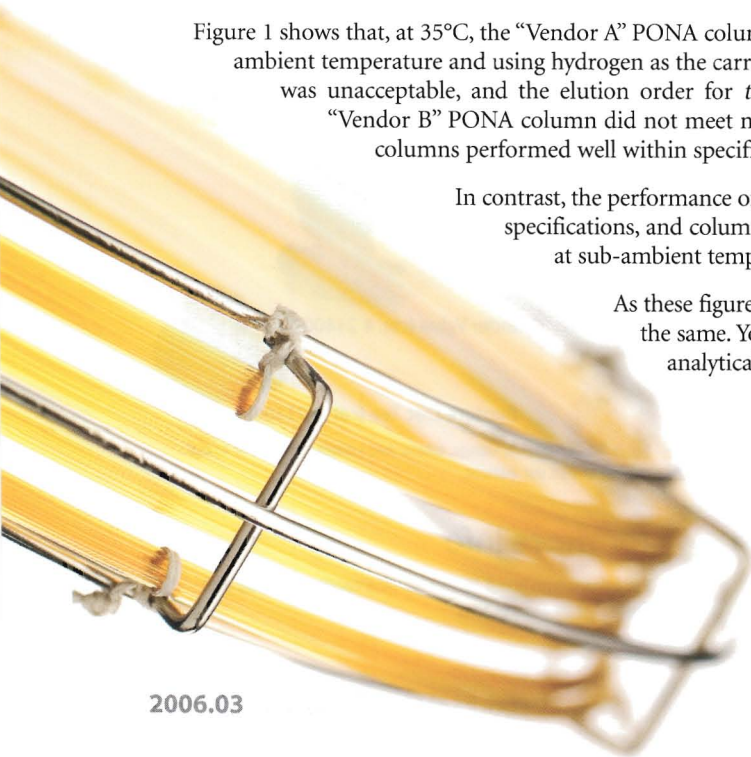
Parameter	**ASTM D-6730 Specification**
theoretical plates for C5:	450,000 - 550,000
K′ for C5:	0.45 - 0.50
peak asymmetry for *t*-butanol:	>1.00 - <5.00
resolution of *t*-butanol/2-methylbutene-2:	3.25 - 5.25

On opening the competitor PONA column containers we discovered that only one of the four manufacturers provided QA data pertinent to the ASTM 6730 method – each of the other three provided a chromatogram of a sample unrelated to the method. Further, one column did not meet the ASTM D-6730 minimum efficiency specification of 450,000 theoretical plates.

Figure 1 shows that, at 35°C, the "Vendor A" PONA column did not meet ASTM D-6730 method specifications. Further, at sub-ambient temperature and using hydrogen as the carrier gas, per ASTM D-6730 method, peak asymmetry for the oxygenates was unacceptable, and the elution order for *t*-butanol and 2-methylbutene-2 was reversed. Similarly, at 35°C, the "Vendor B" PONA column did not meet method specifications. At 35°C, the "Vendor C" and "Vendor D" PONA columns performed well within specifications, but column efficiency was less than ideal.

In contrast, the performance of the Restek PONA column at 35°C was well within ASTM 6730 method specifications, and column efficiency exceeded the specification. The column also performed well at sub-ambient temperature and using hydrogen as the carrier gas.

As these figures show, all PONA columns – or any columns, for that matter – are *NOT* the same. You the customer, have the final say about which vendor to select for your analytical column needs. If you make data based decisions, you can choose wisely.



Rtx®-1PONA Column (fused silica)

(Crossbond® 100% dimethyl polysiloxane)*

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.50	-60 to 300/340°C	100-Meter	10195	$810

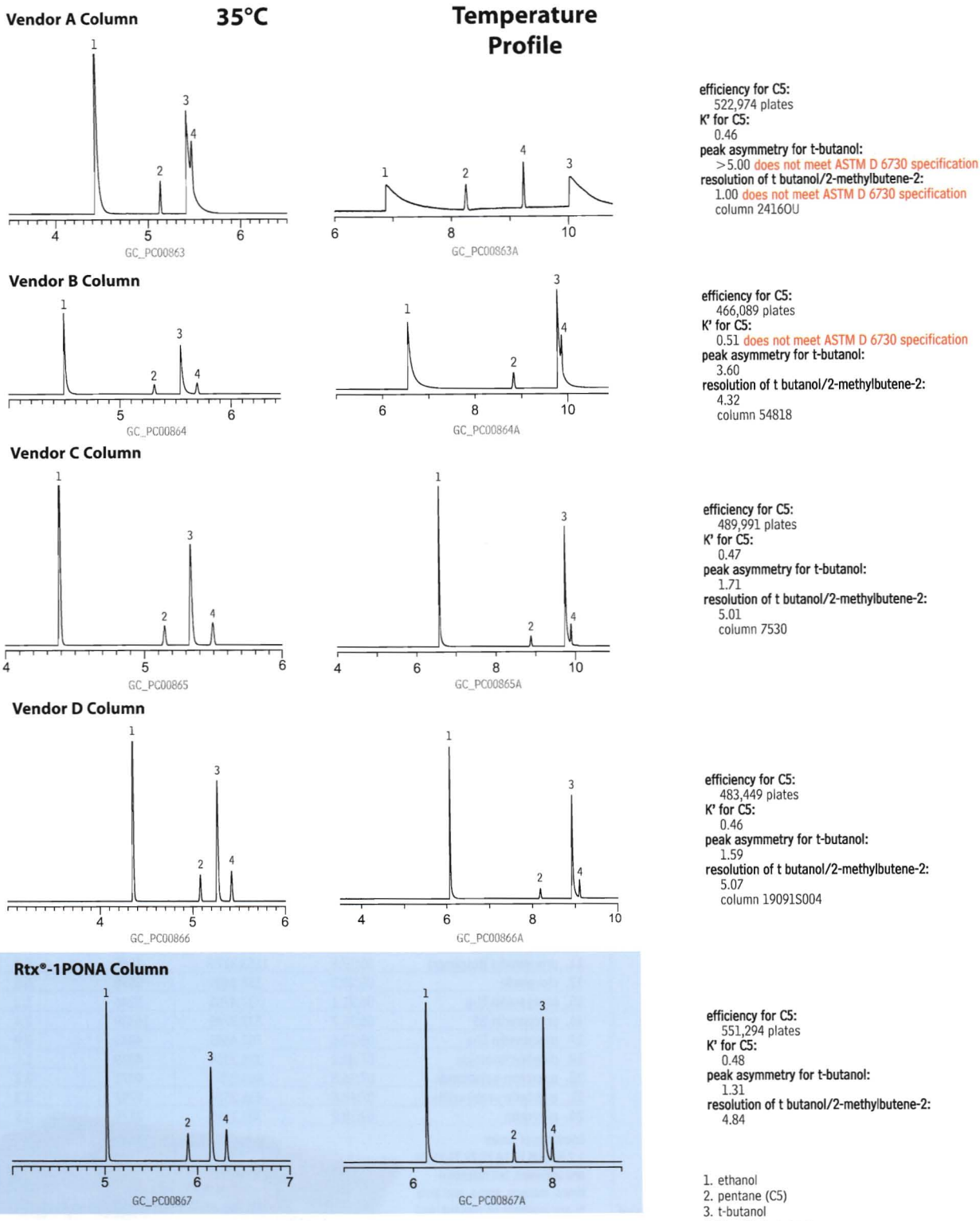
*Optimized phase for hydrocarbon analysis

Rtx®-5PONA Tuning Column (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	1.0	-60 to 325°C	5-Meter	10196	$75

Figure 1 An Rtx-1PONA column offers superior performance for ASTM D-6730 method specifications.



Column:	100m, 0.25mm ID, 0.50μm
Sample:	DHA Oxy-Setup mix (Transition Labs #94100)
Inj.:	0.01μL split (split ratio 150:1)
Inj. temp.:	275°C
Carrier gas:	hydrogen
Linear velocity:	48cm/sec.
Oven temp.:	35°C and Method D 6730 temperature profile
Det.:	FID
Det. temp.:	300°C

Temperature Profile

Column A:	5°C > 8.23 min. > 22°C/min. > 48 min.
Column B:	5°C > 8.84 min. > 22°C/min. > 48 min.
Column C:	5°C > 8.87 min. > 22°C/min. > 48 min.
Column D:	5°C > 8.19 min. > 22°C/min. > 48 min.
Rtx®-1PONA:	5°C > 8.20 min. > 22°C/min. > 48 min.

Rapid, Reproducible HPLC Analysis for Flavonoids in Cocoa

Using a LECO Unique® LC-TOFMS System and an Ultra Aqueous C18 Column

By Julie Kowalski, Restek Innovations Chemist, and Brian Shofran, LECO Corporation

- 15-minute screening for flavonoids.
- Excellent selectivity, using an Ultra Aqueous C18 column.
- Reliable identifications and reproducible results for complex samples.

Flavonoids are complex polyphenolic compounds, with diverse aromatic substitutions, that contribute to color, flavor, fragrance—and toxicity—of many foods. Interest in flavonoids has exploded because of links to antioxidant activity and, possibly, to control and prevention of disease.[1,2] Flavonoid contents of foods have been difficult to study, due to sample complexity and generally low abundances of the target compounds. Cocoa is rich in the flavan-3-ol flavonoids, including catechin, epicatechin, and procyanidin (Figure 1), and these are screened for as marker compounds. In finished chocolate and cocoa products, amounts of flavonoids depend primarily on the amounts of nonfat cocoa solids, on bean type, and on processing. Flavonoids can be destroyed by heat or other processing, like dutching, which is common in the production of cocoa and chocolate products.

We developed a rapid screening method for catechin, epicatechin, and procyanidin content, and screened commercial cocoa products for flavan-3-ol content. We prepared samples by mixing the cocoa products with liquid nitrogen, powdering the frozen mixes, and extracting samples with deionized water: methanol (1:4). Extracts were

Figure 1 Flavan-3-ol flavonoids are screened for as marker compounds.

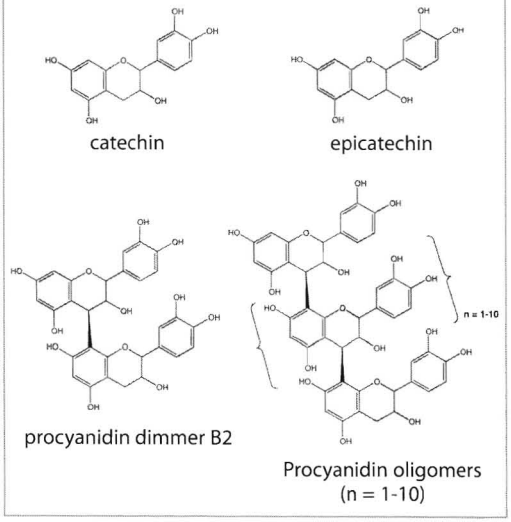
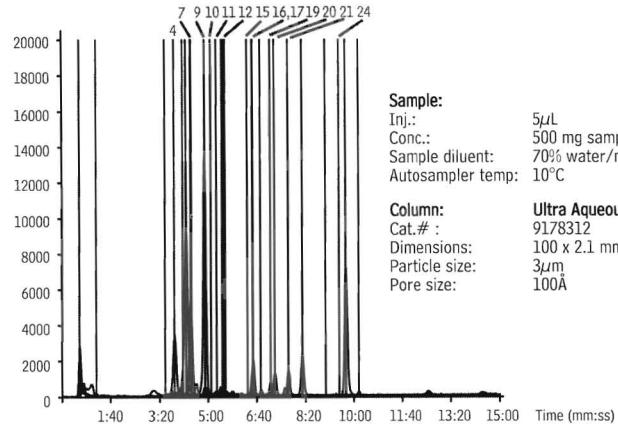


Figure 2 Extracted ion chromatogram of a cacao sample.



Sample:
Inj.: 5µL
Conc.: 500 mg sample extract
Sample diluent: 70% water/methanol
Autosampler temp: 10°C

Column: Ultra Aqueous C18
Cat.# : 9178312
Dimensions: 100 x 2.1 mm
Particle size: 3µm
Pore size: 100Å

Conditions:
Mobile phase: A: 0.1% formic acid in water; B: acetonitrile:methanol, 50:50 (v/v)

Time (min.)	%B
0	10
10	60
15	60

Numbered peaks are listed in Table 1

Flow: 400µL/min.
Temp.: 30°C
Det.: UV @ 210nm

Mass Spectrometry
Instrument: Leco Unique® LC-TOFMS High Flow ESI Source
ESI voltage: (-) 3500 V
Desolv. temp.: 300°C
Nebulizer pres.: 375kPa
Desolv. gas: nitrogen, 7L/min.
Interface temp.: 100°C
Nozzle: (-) 160V
Data acq. rate: 4 spectra/sec.

Table 1 Components in the cacao sample.

Peak	RT (min:sec)	Unique Mass	Area	Area %
4. catechin (monomer)	03:50.4	289.1818	28618	6.7
7. procyanidin B2	04:24	577.3722	34559	8.0
9. epicatechin	04:53.8	289.1841	93682	21.8
10. procyanidin C1	05:06.2	865.5671	10221	2.4
11. procyanidin (tetramer)	05:17.8	1153.8179	1585	0.4
12. clovamide	05:29.3	358.2409	3528	0.8
15. procyanidin II-g	06:21.1	737.4785	5246	1.2
16. procyanidin B5	06:31.7	577.3745	10339	2.4
17. procyanidin II-a	06:32.6	707.4643	4043	0.9
19. dideoxyclovamide	07:08.2	326.2384	4839	1.1
20. quercetin-galactoside	07:16.8	463.279	9471	2.2
21. quercetin-arabinoside	07:44.6	433.2524	9797	2.3
24. quercetin	09:30.2	301.1595	2179	0.5

Identities of peaks 1,2,3,5,6,8,13,14,18,22,23,25,26 are unknown, but retention times, masses, areas, and area % are available on request, and will be listed in our next Buzz electronic newsletter.

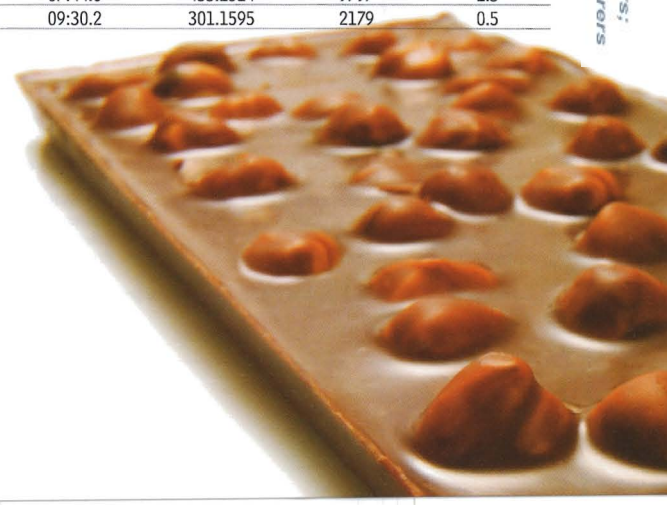
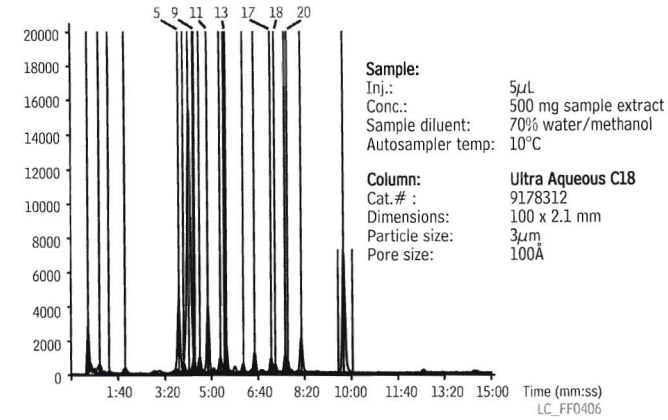


Figure 3 The flavonoid composition of cocoa powder is readily distinguished from that of cacao, using our column and detection system.



Conditions:
Mobile phase: A: 0.1% formic acid in water; B: acetonitrile:methanol, 50:50 (v/v)

Time (min.)	%B
0	10
10	60
15	60

Flow: 400µL/min.
Temp.: 30°C
Det.: UV @ 210nm

Numbered peaks are listed in Table 2

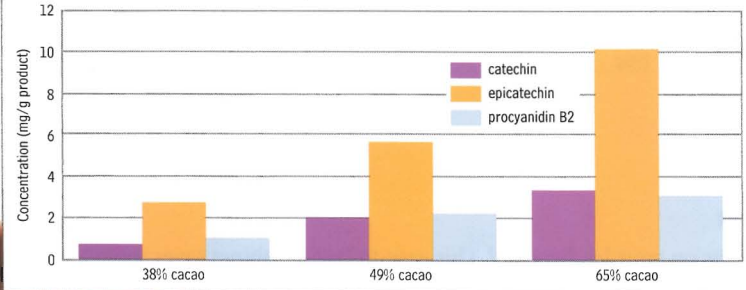
Mass Spectrometry
Instrument: Leco Unique® LC-TOFMS High Flow ESI Source
ESI voltage: (-) 3500 V
Desolv. temp.: 300°C
Nebulizer pres.: 375kPa
Desolv. gas: nitrogen, 7L/min.
Interface temp.: 100°C
Nozzle: (-) 160V
Data acq. rate: 4 spectra/sec.

Table 2 Flavonoid components in cocoa powder exhibit virtually the same retention times as in cacao.

Peak	RT (min:sec)	Unique Mass	Area	Area %
5. catechin (monomer)	03:50.4	289.1806	35151	8.7
9. procyanidin B2	04:25.0	577.3661	3928	1.0
11. epicatechin	04:52.8	289.1802	28030	6.9
13. procyanidin C1	05:28.3	358.2432	3287	0.8
17. procyanidin (tetramer)	07:08.2	326.2279	7088	1.8
18. clovamide	07:16.8	463.2485	6002	1.5
20. procyanidin II-g	07:43.7	433.2532	6047	1.5

Identities of peaks 1,2,3,4,6,7,8,10,12,14,15,16,19,21,22 are unknown, but retention times, masses, areas, and area % are available on request, and will be listed in our next Buzz electronic newsletter.

Figure 4 Concentrations of flavonoids in Venezuelan cacao, determined using an Ultra Aqueous C18 column and a LECO Unique® LC-TOFMS system.



centrifuged, concentrated, and filtered.[3] For a detailed description of sample preparation, refer to the LECO website www.leco.com.

An Ultra Aqueous C18 column is an excellent choice for this analysis, because it is designed to perform reversed phase separations well and reproducibly when the mobile phase has a high aqueous content. Using a 100mm x 2.1mm Ultra Aqueous C18 column and the automated peak find LECO ChromaTOF software in the Unique® LC-TOFMS system, we separated and identified 26 flavonoid compounds in a cacao sample (Figure 2 and Table 1).*

Next, using the automated peak find software in ChromaTOF, we identified flavonoids in cocoa powder (Figure 3 and Table 2). Processing of cacao reduces the amount of catechins and procyanidins in cocoa components. If an alkalizing step is present in the process, this also leads to a remarkable decrease in the content of catechins and procyanidins. For peaks identified in the cocao and cocoa powder samples, retention time did not differ by more than 0.01 seconds (Tables 1 and 2). The analysis was completed and conditions returned to the initial mobile phase composition in 15 minutes.

Subsequently, we analyzed three samples from Venezuela, containing differing amounts of cacao. Quantitative results were determined through ChromaTOF. Analytical results for these samples are shown in Figure 4. As expected, based on data in Table 1, epicatechin was substantially higher than catechin in each sample. Also as expected, catechin, epicatechin, and procyanidin B2 content increased with increasing amounts of cacao.

A LECO Unique® LC-TOFMS system and an Ultra Aqueous C18 column assure rapid, excellent resolution, reliable identification and quantification, and highly reproducible retention times for flavonoid compounds – even in very complex mixtures.

References
1. Prior, R.L., *et al., Procyanidin and catechin content and antioxidant capacity of cocoa and chocolate products, J. Agric. Food Chem.* 54: 4057-4061 (2006).
2. Hurst, W.J., *et al., Antioxidant activity and polyphenols and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States, J. Agric. Food Chem.* 54: 4062-4068 (2006).
3. Andreas-Lacueva, *et al., An LC method for the analysis of cocoa phenolics, LC*GC Eur.* 902-905 (2000).

LECO Corporation, 14950 Technology Court, Fort Myers, FL, 33912

*Cacao is the sum of the products derived from the cacao bean – chocolate liquor, cocoa, and cocoa butter[2].

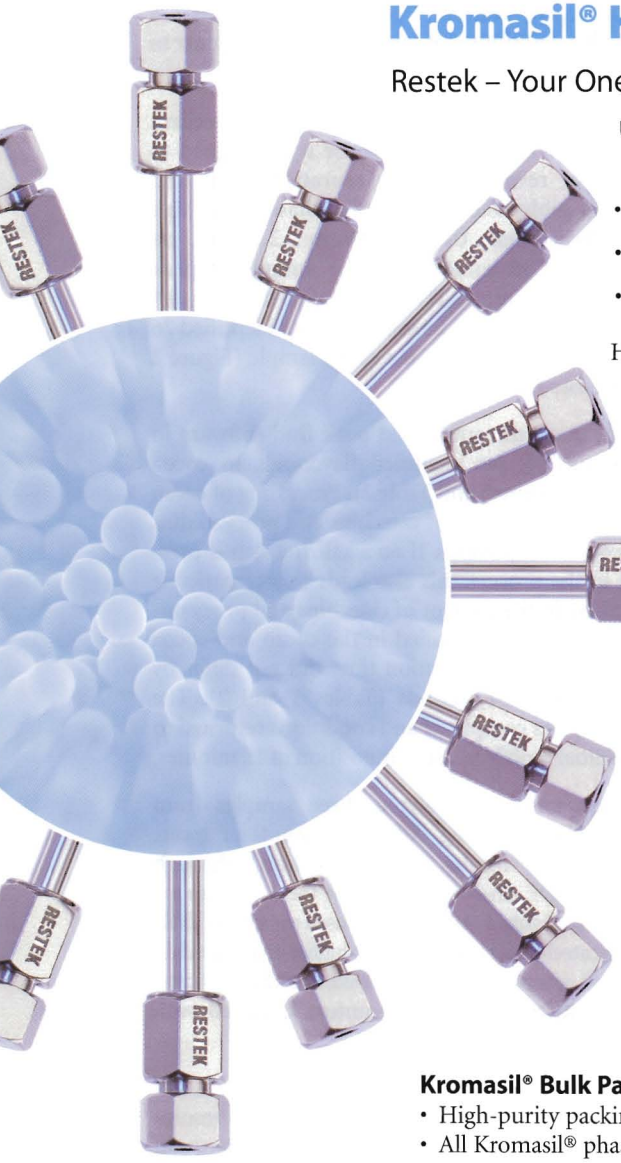
Ultra Aqueous C18, 5µm Column

3µm Column, 2.1mm	cat. #	price
100mm	9178312	$370

Kromasil® HPLC Bulk Packing Materials

Restek – Your One Source for Kromasil® Bulk Packing Materials

By Becky Wittrig, Ph.D., HPLC Product Manager



- The Kromasil® HPLC products you know and trust – available from Restek!
- Perfectly spherical, totally porous bulk silica products.
- Wide range of bonded phases and particle sizes.

HPLC grade silica materials differ greatly from one manufacturer to another. Factors that affect the selectivity of a silica substrate include the surface area and chemical purity of the substrate, the pore structure, and the pore diameter distribution. Kromasil® HPLC silica products consist of highly spherical, porous particles in sizes from 3.5µm to 16µm and larger. The surface properties of the silica have been optimized, including a narrow pore size distribution and well-defined pore structure. For chromatographic separations, this ensures higher efficiencies, smaller pressure drops, and excellent lot-to-lot reproducibility.

Kromasil® spherical silicas are produced using a sol-gel technique, which yields a mechanically strong particle with a large surface area (330m2/g for 100Å silica). Metal impurities are carefully monitored, as trace metals in the silica structure increase the surface acidity and can lead to tailing peaks for basic or chelating compounds. Typical metal content for Kromasil® silicas is shown in Table 1.

Table 1 Typical chemical purity for Kromasil® spherical silicas.

Metal	Content (ppm)
Na	<25
Al	<10
Fe	<10

Based on AAS or ICP measurements.

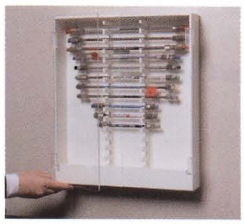
Kromasil® bulk packings are available from Restek in a wide range of particle sizes and bonded phases. For more information, please contact Restek technical service at **800-356-1688** or **814-353-1300, ext. 4.**

Kromasil® Bulk Packings

- High-purity packing materials in 10 and 16µm.
- All Kromasil® phases available.

Description	min. qty.	cat.#	200-499 grams	500-999 grams	≥1000 grams
Kromasil® 100Å Silica, 10µm	200g	92000	$7.93/gram	$6.90/gram	$6.13/gram
Kromasil® 100Å Silica, 16µm	200g	92001	$6.35/gram	$5.52/gram	$4.91/gram
Kromasil® 100Å C8, 10µm	200g	92030	$11.90/gram	$10.35/gram	$9.20/gram
Kromasil® 100Å C8, 16µm	200g	92031	$9.52/gram	$8.28/gram	$7.36/gram
Kromasil® 100Å C18, 10µm	200g	92040	$11.90/gram	$10.35/gram	$9.20/gram
Kromasil® 100Å C18, 16µm	200g	92041	$9.52/gram	$8.28/gram	$7.36/gram
Kromasil® 100Å Chiral DMB, 10µm	200g	92080	$34.88/gram	$30.33/gram	$26.96/gram
Kromasil® 100Å Chiral DMB, 16µm	200g	92081	$27.90/gram	$24.26/gram	$21.57/gram
Kromasil® 100Å Chiral TBB, 10µm	200g	91990	$34.88/gram	$30.33/gram	$26.96/gram
Kromasil® 100Å Chiral TBB, 16µm	200g	91991	$27.90/gram	$24.26/gram	$21.57/gram

Other phases and particle sizes available on request.



30-Column Storage Cabinet

Tired of stacks of HPLC columns on your lab benches? This easy-to-install cabinet saves space and protects columns; the hinged door is clear to allow quick identification of column labels or tags.

Description	dimensions	qty.	cat.#	price
30 Column Cabinet	17 3/8 x 15 x 2 7/8"	ea.	25159	$119

*Please note: Columns in photograph are not included.

Cool Tools!

For Thermo Instruments

Jet Removing Tool for Thermo GCs: Focus GC / TRACE™ GC / Ultra/TRACE™ GC x GC

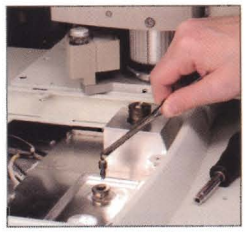
new for 2006

- Unique, ergonomic handle—easy to grip.
- Easily loosens the FID jet.

Remove FID cell assembly.

Slip tool over FID jet.

Turn counterclockwise to loosen jet.



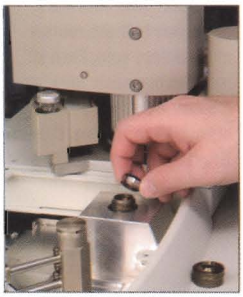
Use tweezers (cat. #20101) to remove jet.

Description	Similar to TF part #	qty.	cat.#	price
Jet Removing Tool for Thermo	205-019-00	ea.	24936	$50

Liner Cap Removing Tool for Thermo GCs: Focus GC / TRACE™ GC / Ultra/TRACE™ GC x GC

new for 2006

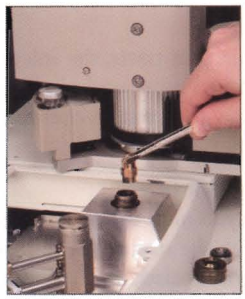
- Unique, ergonomic handle—easy to grip.
- Easily loosens the liner cap from the injector.



Remove septum cap, septum holder, septum, and septum support.

Place tool on liner cap. Align two pins on bottom of tool with two open slots on liner cap.

Turn counterclockwise to loosen liner cap.

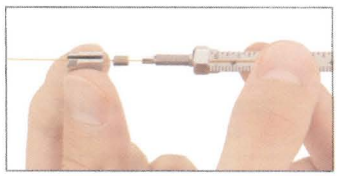


Use tweezers (cat. #20101) to remove liner cap.

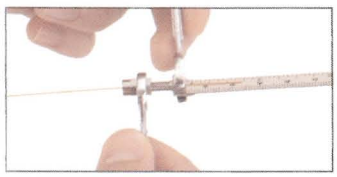
Description	Similar to TF part #	qty.	cat.#	price
Liner Cap Removing Tool for Thermo	205-070-10	ea.	24937	$50

Capillary Installation Gauge for TRACE™ and Focus SSL (M4 Ferrules)

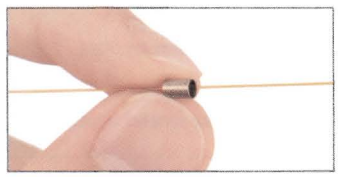
- Seats ferrule onto column for consistent installations.
- Prevents crushed column ends.
- Made from high-quality stainless steel.



Install nut and ferrule onto column. Cut column end squarely. Slide column into installation gauge to recommended insertion distance. Finger-tighten column nut.



Tighten assembly to ensure a properly seated ferrule. Loosen assembly and remove column and column nut.



The ferrule will be properly seated, and should remain in place when light force is applied. If it slides loosely on the column, repeat procedure.

Description	qty.	cat.#	price
Capillary Installation Gauge for TRACE™ & Focus SSL (M4 ferrules)	ea.	22330	$70

download this

Cool Tools for GC and HPLC
Restek innovation saves you time and money.

lit. cat.# 59879

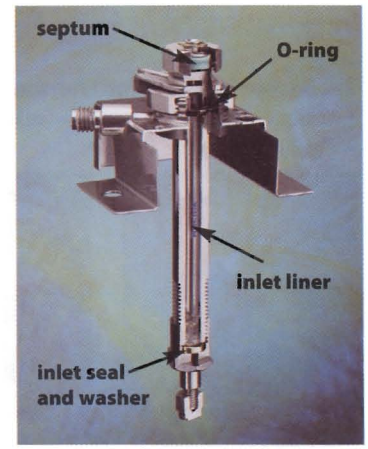
restek
innovation

Easily seat ferrules for consistent installations!

Peak Performers

Injection Port Maintenance with FastPack™ Inlet Kits

by Donna Lidgett, GC Accessories Product Manager



What are the benefits of using a FastPack™ Inlet Kit?

FastPack™ inlet kits include all the parts needed to maintain your system. Injection port maintenance should be performed prior to installing any capillary column, and on a routine basis, based on the number of injections made and the cleanliness of the samples. Maintenance includes replacing the injection port (inlet) liner, the critical inlet seals, and the septum.

Why replace an injection port liner?

For optimum column performance, the injection port (inlet) liner must be free of septum particles, sample residue, or ferrule fragments. Peak shape degradation, poor reproducibility, sample decomposition, and ghost peaks all are associated with using a dirty (contaminated) liner.

Why replace the critical seal?

The critical seal must fit tightly around the inlet liner, to prevent carrier gas from leaking around the outside of the liner. Replace the critical seal prior to installing an inlet liner.

Why replace the septum?

The septum maintains a leak-tight seal that excludes air from the inlet. Frequent replacement prevents fragmentation and leaks. Multiple injections and continuous exposure to hot injection port surfaces will decompose the septum and can create particles, which can fall into the inlet liner. Septum particles are a potential source of ghost peaks, loss of inertness, and carrier gas flow occlusion. Allow a new septum sufficient time to condition in the injector, to reduce the incidence of ghost peaks. To avoid contamination, always use forceps when handling septa.

FastPack™ Inlet Kits are a great way to make routine maintenance easy. Each kit includes one each: inlet liner (choose from four popular styles), Viton® O-ring, 0.8mm ID gold-plated inlet seal, inlet seal washer, 11mm Thermolite® septum.

Why replace the inlet seal?

In Agilent split/splitless injection ports, the inlet seal sits at the base of the injector. Dirt, non-volatile residue, septum fragments, and other undesirable materials contaminate the inlet seal and decrease analytical linearity. The only way to maintain optimum performance is by frequently changing the inlet seal and ensuring the seal is leak-tight.

FastPack™ Inlet Kits for Agilent GCs

- Convenient: all the parts you need in one package—no hunting for individual items.
- Economical: costs less than the sum of the individual parts.
- Clean: Mylar® bag is factory sealed; no contamination of the products from weeks in the lab.

1 pack includes 5 maintenance kits

Deactivated Liner	cat.#	1 pack (5 kits)	5-19 packs	20 or more packs
4mm Splitless	21101	$193/pk.	$183/pk.	$173/pk.
4mm Splitless Gooseneck	21102	$213/pk.	$203/pk.	$193/pk.
4mm Splitless Double Gooseneck	21103	$253/pk.	$241/pk.	$228/pk.
4mm Split with Wool*	21104	$203/pk.	$193/pk.	$183/pk.

*Liner dimensions are 4mm ID, 6.3mm OD, 78.5mm long. Liners in other kits are 6.5mm OD.

Liners for Agilent/Finnigan GCs	Benefits/Uses	ID*/OD & Length (mm)	Similar to Agilent part #	cat.#/price ea.	5-pk.	25-pk.
2mm Splitless	trace samples <2µL	2.0 ID 6.5 OD x 78.5	5181-8818 (ea.) 5183-4703 (5-pk.) 5183-4704 (25-pk.)	20712 $23	20713 $77	20714 $233
4mm Splitless	trace samples >2µL	4.0 ID 6.5 OD x 78.5	210-3003 (ea.) 210-3003-5 (5-pk.)	20772 $19	20773 $58	20774 $233
Siltek® 4mm Splitless	trace samples >2µL	4.0 ID 6.5 OD x 78.5	—	20772-214.1 $24	20773-214.5 $78	20774-214.25 $322
Gooseneck Splitless (4mm) w/ Wool†	trace samples >2µL	4.0 ID 6.5 OD x 78.5	5062-3587 (ea.) 5183-4693 (5-pk.) 5183-4694 (25-pk.)	22405 $29	22406 $81	22407 $329
4mm Split w/ Wool	universal, use with Agilent 7673 autosampler	4.0 ID 6.3 OD x 78.5	19251-60540 (ea.) 5183-4691 (5-pk.) 5183-4692 (25-pk.)	20781 $23	20782 $67	20783 $274
Siltek® 4mm Split w/ Wool	universal, use with Agilent 7673 autosampler	4.0 ID 6.3 OD x 78.5	—	20781-213.1 $42	20782-213.5 $116	20783-213.25 $442

Liners for Varian 1075/1077 GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to Varian part #	ea.	cat.#/price 5-pk.	25-pk.
 2mm Splitless	trace samples <2µL	2.0 ID 6.3 OD x 74	01-900109-05	20721 $35	20722 $97	20723 $437
 4mm Splitless	trace samples >2µL	4.0 ID 6.3 OD x 74	01-900109-05	20904 $25	20905 $97	20906 $437
 Splitter w/ Wool	universal, use with rapid autosamplers	4.0 ID 6.3 OD x 72	01-900109-01	20792 $35	20793 $111	20794 $501

Liners for Varian 1177 GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to Varian part #	ea.	cat.#/price 5-pk.	25-pk.
 4mm Split w/Glass Frit	universal	4.0 ID 6.3 OD x 78.5	39-26119-36	21045 $39	21046 $171	—
 4mm Split w/ Wool	universal	4.0 ID 6.3 OD x 78.5	39-26119-34	—	21079 $67	—

Liners for Varian 1078/1079 GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to Varian part #	ea.	cat.#/price 5-pk.	25-pk.
 1078/1079 Split w/ Frit	dirty samples, non-active compounds	3.4 ID 5.0 OD x 54	03-918464-00	21708 $39	21709 $171	—
 1078/1079 Splitless	trace samples <2µL	2.0 ID 5.0 OD x 54	03-918466-00	21711 $29	21712 $113	—

Liners for Shimadzu GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to Shimadzu part #	ea.	cat.#/price 5-pk.	25-pk.
 17A & 2010 Split/Splitless w/ Wool	universal, for most common analyses	3.5 ID 5.0 OD x 95	221-41444-00	20955 $25	20956 $89	20957 $317
 Siltek® 17A & 2010 Split/Splitless w/ Wool	universal, for most common analyses	3.5 ID 5.0 OD x 95	—	20955-213.1 $44	20956-213.5 $138	20957-213.25 $485

Liners for PerkinElmer GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to PE part #	ea.	cat.#/price 5-pk.	25-pk.
 Splitless (2mm ID)	trace samples	2.0 ID 5.0 OD x 100	N6502007	20730 $29	20731 $113	20732 $475
 Auto SYS™ Splitless	headspace & purge & trap	1.0 ID 6.2 OD x 92.1	N6502006	21272 $29	21273 $120	21274 $483
 Baffle Splitter	universal, for most common analyses	3.5 ID 5.0 OD x 100	N6502008	20736 $22	20737 $85	—
 Cup Splitter	high & low MW compounds	3.5 ID 5.0 OD x 100	—	20739 $61	20740 $241	—

Liners for Thermo Finnigan TRACE™ and Focus SSL GCs

	Benefits/Uses:	ID*/OD & Length (mm)	Similar to TF part #	ea.	cat.#/price 5-pk.	25-pk.
 Splitless (3mm ID)	trace samples	3.0 ID 8.0 OD x 105	453 20032	20942 $34	20943 $130	20944 $570
 Siltek® Splitless (3mm ID)	trace samples	3.0 ID 8.0 OD x 105	—	20942-214.1 $39	20943-214.5 $150	20944-214.25 $660

*Nominal ID at syringe needle expulsion point.
†Use this liner for increased sensitivity.

All liners are **100% deactivated**

All liners are shipped intermediate polarity (IP) deactivated unless otherwise requested.

Commonly Asked GC Questions

Answered by the Restek Chromatography Information Services Group

How do I know which guard column would be best for my application?
Restek offers guard columns and transfer lines ranging from 0.025mm ID to 0.53mm ID, from 1 to 10 meters long, in fused silica or Silcosteel® treated stainless steel. Guard columns are available with nonpolar, intermediate polarity, or polar deactivation, and with several specialty deactivations.

- In most applications in which nonpolar to moderately polar solvents are used; we recommend an intermediate-polarity (IP) deactivated guard column.
- For most polar solvents except water, we generally suggest a polar deactivated guard column.
- For water-based samples, we recommend our water-resistant Hydroguard™ guard columns. This deactivation is designed to withstand the harsh "steam-cleaning" that occurs when water is rapidly vaporized in the column.
- For applications that require a highly inert surface to minimize analyte breakdown, such as pesticides analysis, we recommend a Siltek® deactivated guard column.
- For amines or other basic compounds, we offer base-deactivated guard columns.

Also, note that for many of our popular stationary phases, we offer Integra-Guard™ columns – an analytical column with an integral guard column. This eliminates the connection between the guard column and the analytical column. Much information about guard columns is presented in our free publication #59319.

What are all those different capillary column temperatures listed in your catalog?
The first temperature listed is the minimum operating temperature for the column. The two temperatures separated by a slash symbol (/) are the maximum isothermal operating temperature and the maximum temperature program temperature, respectively. The maximum temperature program temperature is the maximum temperature to which the column may be exposed briefly without causing damage. For most stationary phases, the maximum temperature program temperature is approximately 20°C above the maximum isothermal temperature. In addition to these temperatures, the polymer stability temperature sometimes is listed. This is the maximum temperature to which the polymer phase can be exposed before degradation.

I see ghost peaks when I inject a sample or standard, and my mass spectrometer identifies these peaks as a siloxane material. Is there a problem with my column?
Capillary columns can produce a varying amount of baseline noise (siloxane bleed), usually containing fragment ions at m/z 73, 207, and 281, but they will not produce any distinct peaks in an analytical run. The most common sources of distinct siloxane peaks are septum bleed and the chemicals used to deactivate the injection port liner and the glass wool packing material.

Sometimes I experience problems when using a 1701-type column for my pesticides analysis. Are there other column choices?
On-column breakdown of chlorinated pesticides, such as endrin, methoxychlor, and DDT, are common with cyano-containing phases, such as 1701-type phases. Fortunately, there are other column choices. These include a few standard phases, such as our Rtx®-35, Rtx®-35MS, and Rtx®-50 phases. In addition, Restek has developed several specialized columns for pesticides, including Rtx®-CLPesticides & Rtx®-CLPesticides2, and Stx®-CLPesticides & Stx®-CLPesticides2 columns. These columns eliminate on-column breakdown problems, improve separation, and reduce analysis time. Information about these columns, and example chromatography, can be found on our website: www.restek.com

Can I order a fused silica column in a column cage to fit my small GC oven?
Yes. We offer several special cage options for non-standard and portable GC ovens. Please contact our **informations services** group at **800-356-1688, ext. 4**, for specifics, or **customer service (ext. 3)** for prices Please note that we cannot cage or recage columns from other manufacturers.

the Restek Chromatography Information Services Group

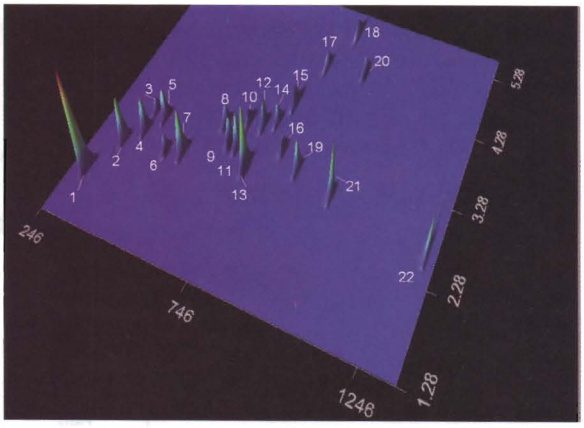
Comprehensive 2D Gas Chromatography – Making GC Separations Work Harder *Continued from page 2.*

deconvolution. GC deconvolution through real separation is a more rugged and desirable outcome – and we can still combine GC×GC with MS for further identification. It is certainly true that GC×GC demands improved performance capabilities of GC instruments, new software, and better column quality control (e.g., improved batch-to-batch column reproducibility for the 1 m lengths of 2D columns that we use). These cannot be realised without the compliance of instrument and column manufacturers. As examples of generic GC×GC applications, a low polarity (5% phenyl) 1D column coupled to a short polar (wax phase) 2D column is useful for essential oils, but recently wax-low polarity column combinations have proved equally valuable. For petrochemicals, where higher temperature operation is needed, a low polarity (5% phenyl) 1D column coupled to a short polar (50% phenyl) 2D column is often used. For environmental analysis of PCBs, a carborane phase 2D column has been reported, where selectivity towards the extent of compound planarity is sought. In this short commentary, there is no space to engage in specifics of certain GC×GC methods, but obviously there is considerable opportunity to optimise methods, and use sound principles of GC and phase selection to get the best out of GC×GC.

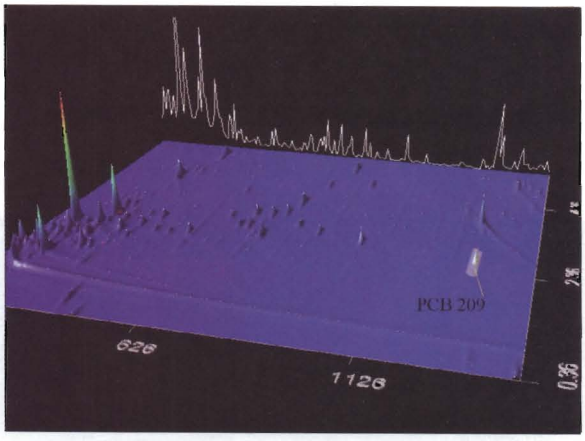
We are at the threshold of a new era in GC, and getting the best out of our GC×GC methods is a task that an increasing number of analysts will be striving for. The comments of Professor Walt Jennings (Restek Advantage, 2006.01) also ring true for GC×GC. When a method has been developed for GC×GC, and one of the columns has to be replaced, to what extent will the 2D plot faithfully reproduce our archived or master analytical result? This must be addressed in the two-dimensional experiment, to prove that the analyst can have confidence that their data interpretation protocols survive column change and routine maintenance of the system. But with the impressive capabilities of GC×GC, it is important that analytical methods and the greater information content it offers are supported by validated and reliable operation.

Editor's note: Dr. Marriott is one of the world's leading experts in 2D-GC.

An Example of 2D Gas Chromatography see *Advantage* 2005.1



GCxGC analysis of organochlorine pesticides combines primary column and confirmation column results.



Organochlorine pesticides separated from interferences in tomato extract.

Columns:	Rtx®-5 9m, 0.18mm ID, 0.20µm (10m column, cat.# 40201, with 1m removed) Rtx®-200 1m, 0.18mm ID, 0.20µm (1m of 10m column, cat.# 45001)
Inj.:	1µL, split, 250°C, split ratio 50:1
Oven:	Primary: 50°C (0.2 min.), 30°C/min. to 140° (no hold), 5°C/min. to 250°C (no hold) Secondary: 50°C offset from primary oven
Instrument:	LECO GCxGC/ECD
Modulator:	Temperature offset: 30°C Modulation time: 6 sec
Det.:	ECD, 325°C, 150mL/min. nitrogen makeup gas, 50Hz

Tradeshow Schedule

We'd be happy to talk with you at any of the following meetings or shows. We'll post our booth numbers as they become available to us.

September, 2006

Date September 17-21
Show 120th AOAC Annual Meeting & Exposition
Location Hyatt Regency, Minneapolis, MN

October, 2006

Date October 3-7
Show SOFT 2006 Annual Meeting
Location Hilton Austin, Austin, TX

Date October 7-10
Show ACIL 69th Annual Meeting
Location San Antonio Marriott River Center, San Antonio, TX

Date October 11
Show Chromatography Society Triad Symposia
Location AstraZeneca, Charnwood, England

Date October 11-12
Show Midwestern Association of Forensic Scientists (MAFS)
Location Hyatt downtown, Indianapolis, IN

Date October 17-19
Show Gulf Coast Conference
Location Moody Gardens Convention Center & Hotel, Galveston Island, TX

Date October 24-25
Show Chromatography Society Triad Symposia
Location Pfizer, Sandwich, England

Date October 31 - November 3
Show SEMA Show
Location Las Vegas Convention Center, Las Vegas, NV

November, 2006

Date November 1-2
Show WWEM Water, Wastewater and Environmental Monitoring Conference Exhibition & Workshops
Location Telford International Centre, Telford, Shropshire, England

Date November 1-4
Show 32nd Annual NEAFS Meeting
Location Tarrytown DoubleTree Hotel, Tarrytown, NY

Date November 6-7
Show California Association of Toxicologists
Location Miramonte Resort and Spa, Palm Springs, CA

Date November 6-10
Show SWAFS/NWAFS Joint Meeting and Training Conference
Location Doubletree Hotel, Colorado Springs, CO

Date November 9
Show 2006 Anachem Symposium
Location Burton Manor, 27777 Schoolcraft Rd., Livonia, MI

Date November 13-16
Show 2006 Eastern Analytical Symposium
Location Garden State Exhibit Center, Somerset, NJ

Date November 21-22
Show Chromatography Society Triad Symposia
Location GlaxoSmithKline, Stevenage, England

For latest updates, see our Tradeshow Calendar at **www.restek.com/ontheroad**.

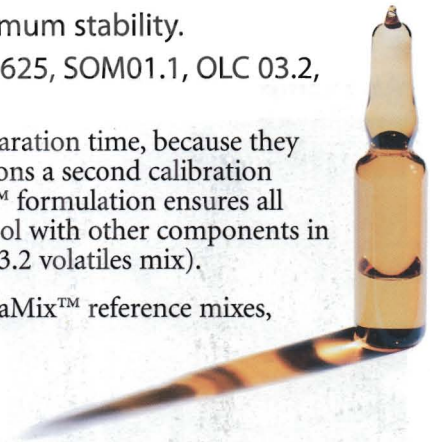
GET YOUR MIX

Time-Saving MegaMix™ Environmental Reference Mixes.

- Largest number of target analytes in one mix, formulated for maximum stability.
- Available for US EPA methods 8260, 8270, 502.2, 524.2, 525.2, 624, 625, SOM01.1, OLC 03.2, OLM 04.2, Skinner List volatiles, Skinner List semivolatiles.

MegaMix™ mixes simplify preparation of calibration mixes, and shorten preparation time, because they include a maximum numbers of compatible target analytes. In some applications a second calibration analysis has been required for coeluting target compounds, but the MegaMix™ formulation ensures all included analytes can be calibrated in one analysis (e.g., 3- and 4- methylphenol with other components in OLC 03.2 semivolatiles mix; *m*- & *p*- xylene with other components in OLC 03.2 volatiles mix).

Save time, save effort, minimize potential for preparation problems – use MegaMix™ reference mixes, only from Restek or authorized distributors.



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