RESTEK ADVANTAGE Newsletter

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Overview

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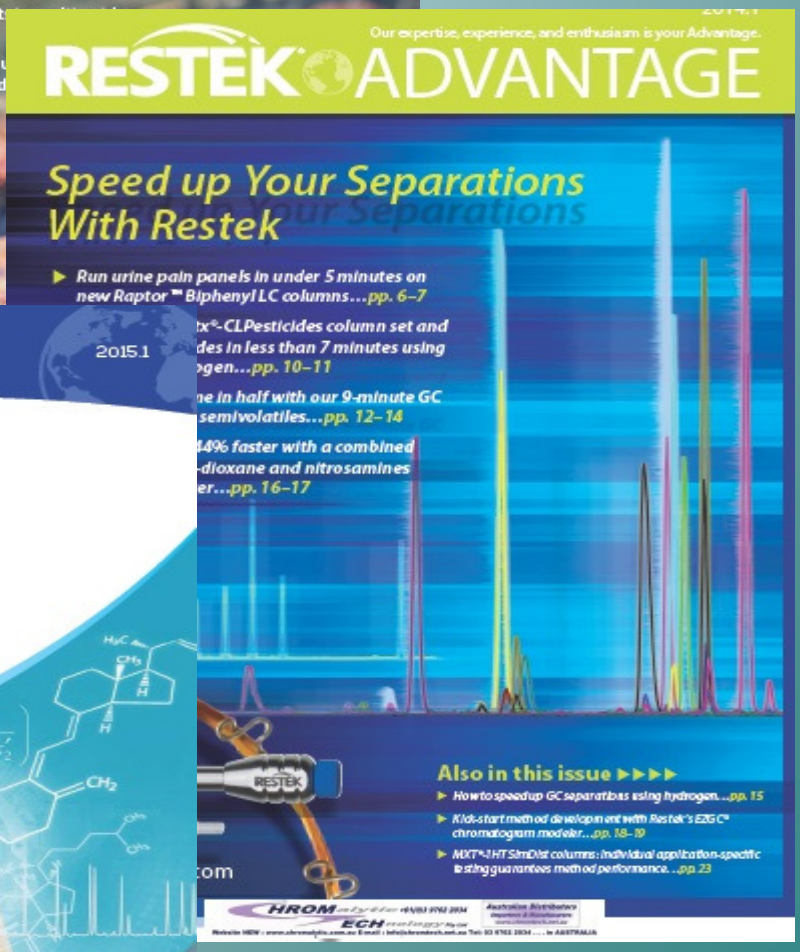
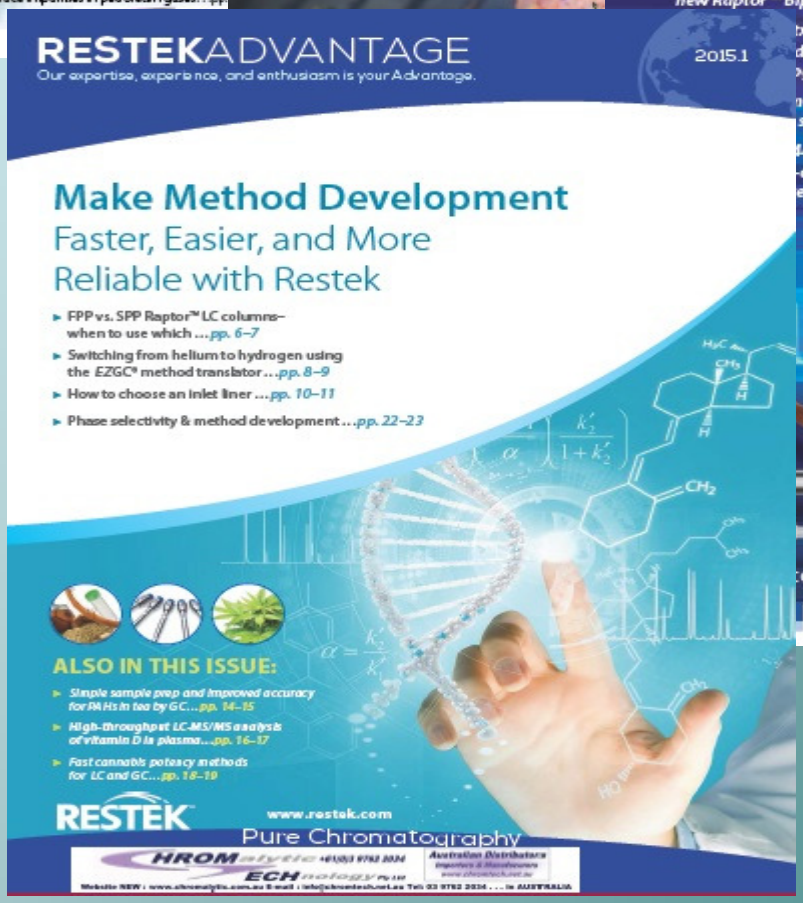




Our expertise, experience, and enthusiasm is your Advantage.

RESTEK ADVANTAGE

2012

New Approaches for Increasing Analytical Sensitivity

Also in this issue

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!

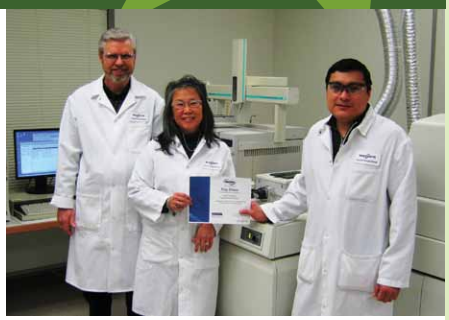
Chris English
Laboratory Manager,
Innovations Group

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

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

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

Interested in joining our team?
Check out **www.restek.com/jobs** today!

Hot Topics

All the Right Tools—All in One Box

Restek's Ultra Selective Liquid Chromatography™ (USLC®) column set represents the widest range of reversed phase selectivity available with just four stationary phases. It simplifies column choice for fast, effective method development—and the new USLC® toolbox makes things even easier!

A USLC® method development toolbox contains all four USLC® stationary phases in one convenient package. Available for UHPLC (1.9 µm) and HPLC (3 or 5 µm) in 50, 100, or 150 mm lengths, this must-have companion for method developers also includes a selection guide to help ensure that you always choose the right column the first time.

Read more about USLC® technology and order your lab a method development toolbox today by calling 1-814-353-1300, ext. 3, or contacting your Restek representative.

Restek USLC® Columns:
Choose Columns Fast. Develop Methods Faster.
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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!

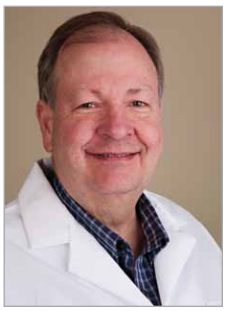
More Labs Required to Source CRMs

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.

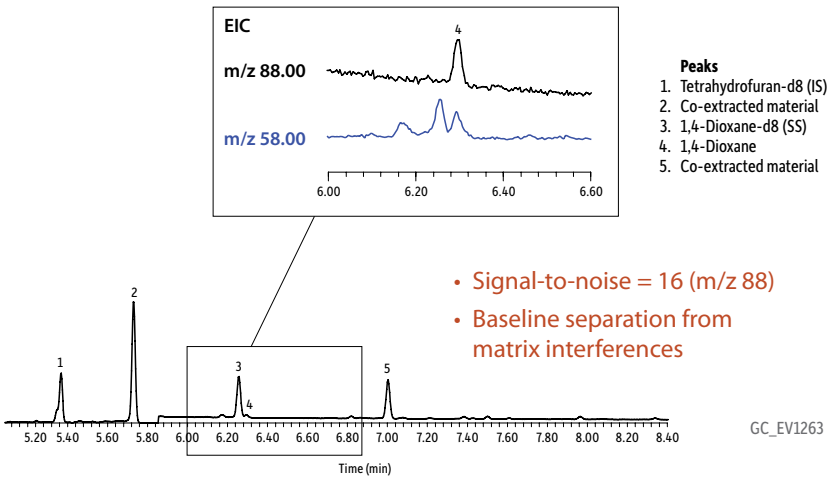


Figure 2: 1,4-Dioxane extracted ion chromatogram from a standard splitless 1 µL injection of a 0.5 pg/µL fortified drinking water extract (0.5 pg on-column). Peaks are barely distinguishable from background noise.

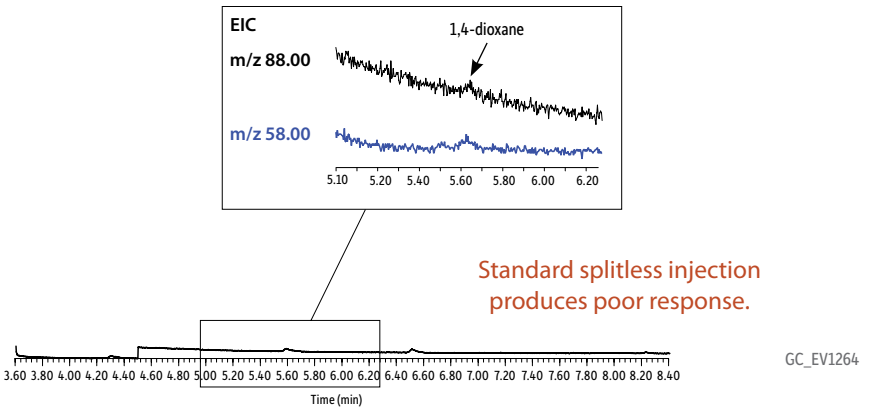


Table II: CSR-LVSI resulted in good recovery of both 1,4-dioxane and surrogate 1,4-dioxane-d8 from extracted fortified samples.

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.

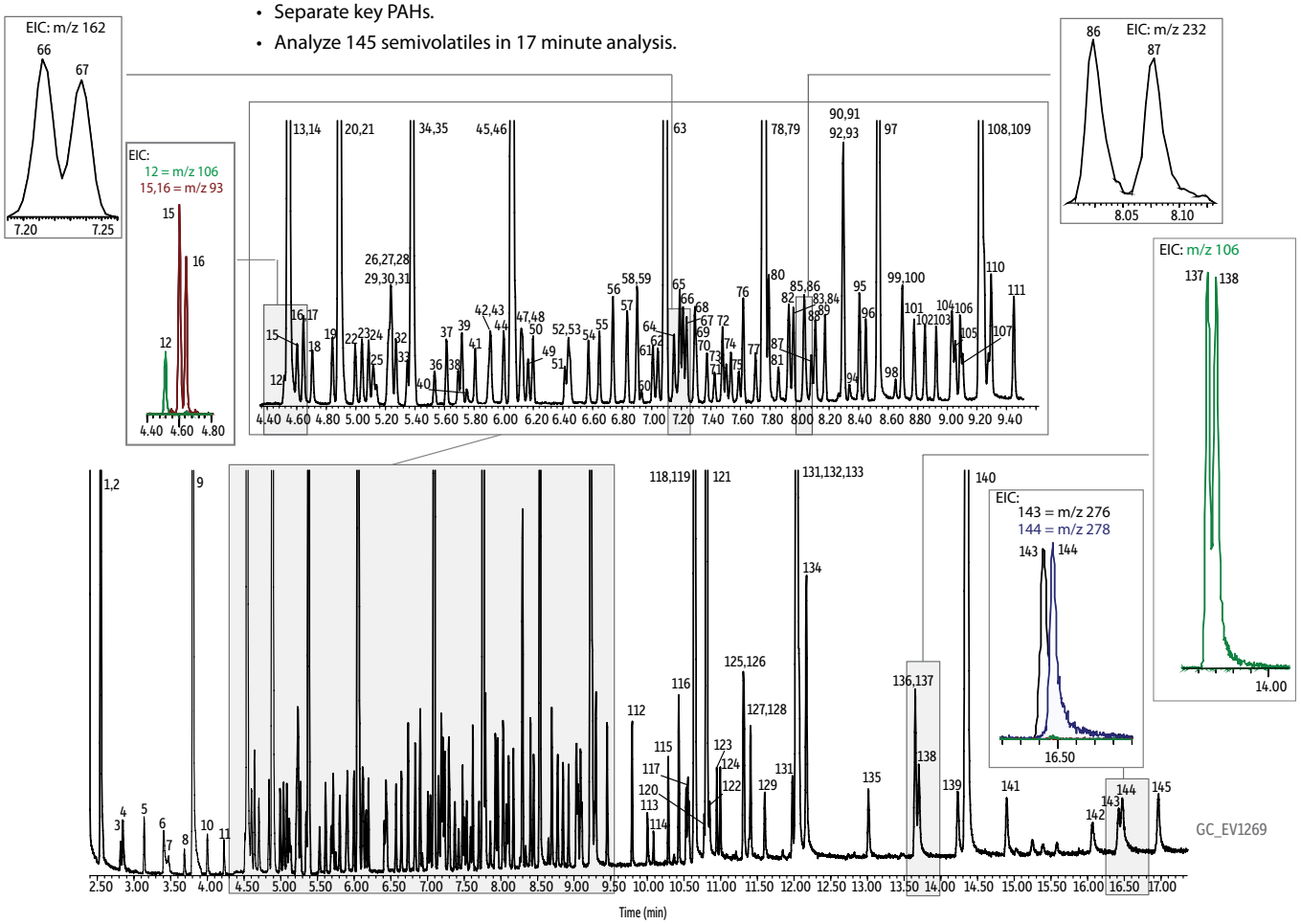
	RRF (0 .5 ng)	Minimum RF	Average RRF (0.5 – 120 ng/µL)	RRF RSD	Linear R^2
2-Nitrophenol	0.710	0.100	0.770	6.9%	0.9999
2-Nitroaniline	0.204	0.010	0.226	5.4%	0.9999
3-Nitroaniline	0.218	0.010	0.226	3.5%	0.9997
2,4-Dinitrophenol	0.055	0.010	0.176	42%	0.9992
4-Nitrophenol	0.234	0.010	0.254	8.0%	0.9914
4-Nitroaniline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

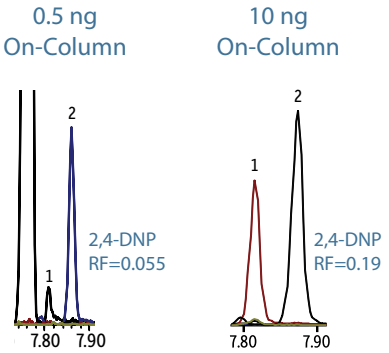


Column: Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623); **Sample**: 8270 MegaMix® (cat.# 31850), 8270 Benzidines mix (cat.# 31852), Benzoic acid (cat.# 31879), Revised B/N surrogate mix (cat.# 31888), Acid surrogate mix (4/89 SOW) (cat.# 31063), Revised SV internal standard mix (cat.# 31886), 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.

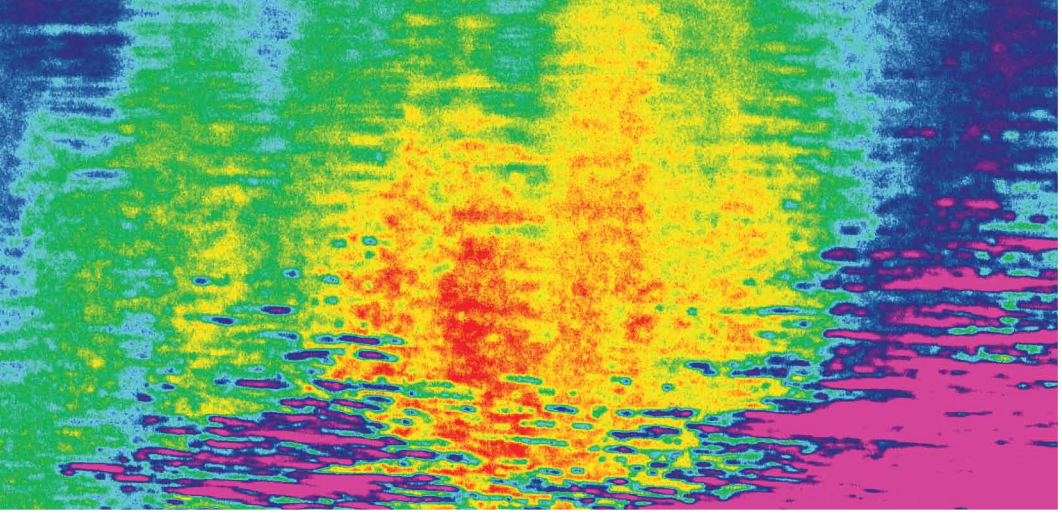
Figure 2: Response differential for 2,4-dinitrophenol and 4-nitrophenol.



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.

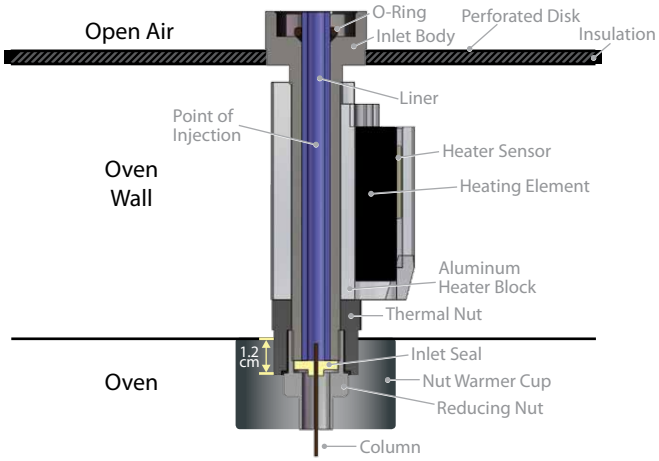
Injecting a liquid sample into a hot GC inlet is a dynamic and complex event. Of the many parameters that affect the success of an injection, inlet temperature is one of the most significant. Raising or lowering the inlet temperature setpoint can have a profound effect on how much sample is transferred 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).

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.

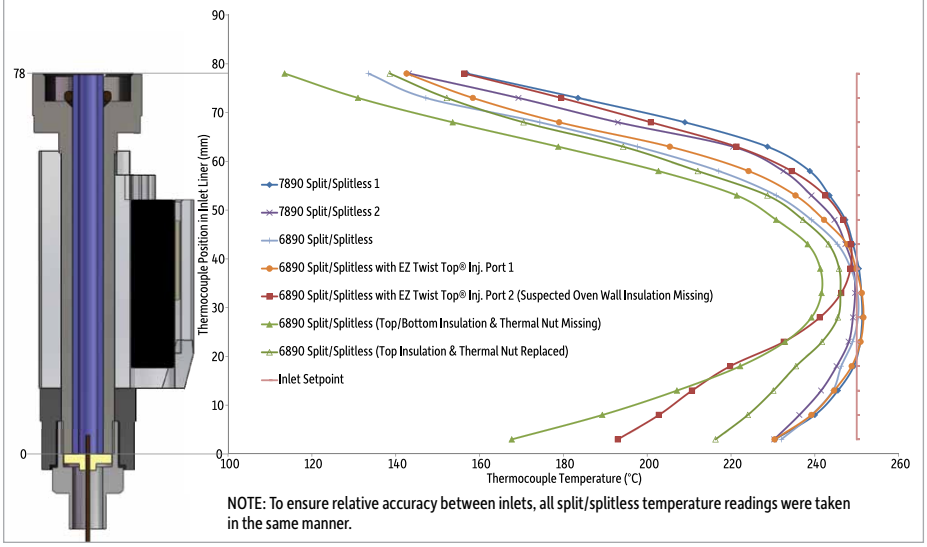


Insulation is Crucial to Minimizing Temperature Variation

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.

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

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.)

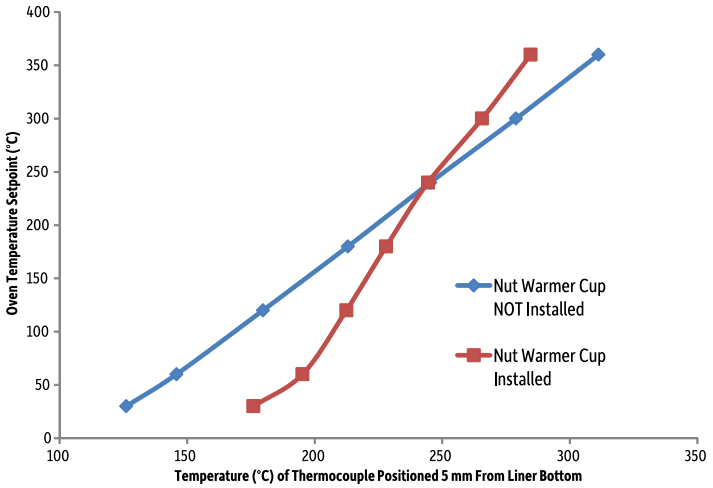
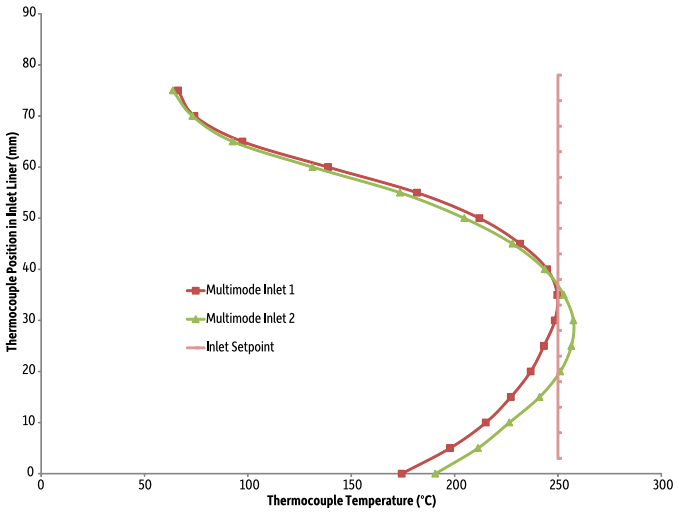


Figure 4: The Multimode Inlets (MMIs) we measured experienced almost twice the temperature drop (190 °C) of a standard split/splitless inlet between the inlet setpoint to the top of the liner.



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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*Current address: Restek Corporation, 110 Benner Circle, Bellefonte, Pennsylvania 16823, USA

- 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.

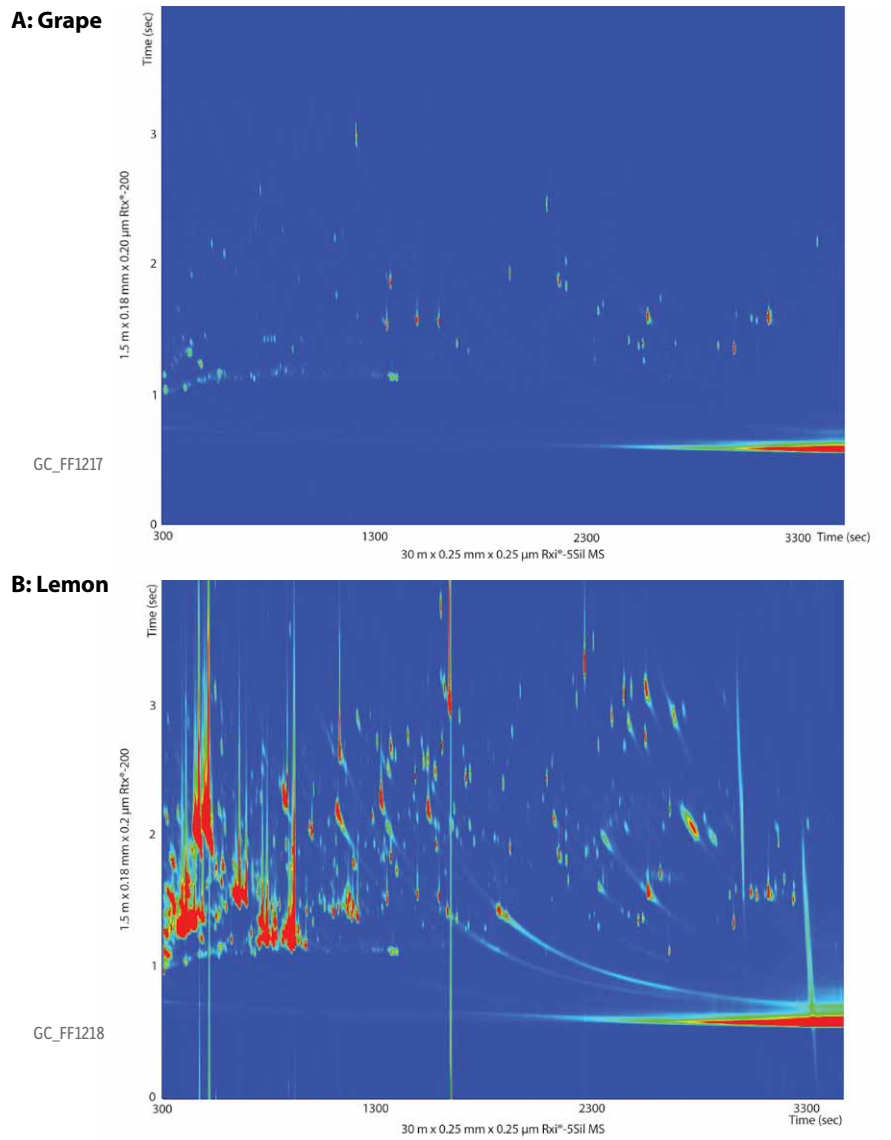
Column Selectivity and Multidimensional Techniques

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.

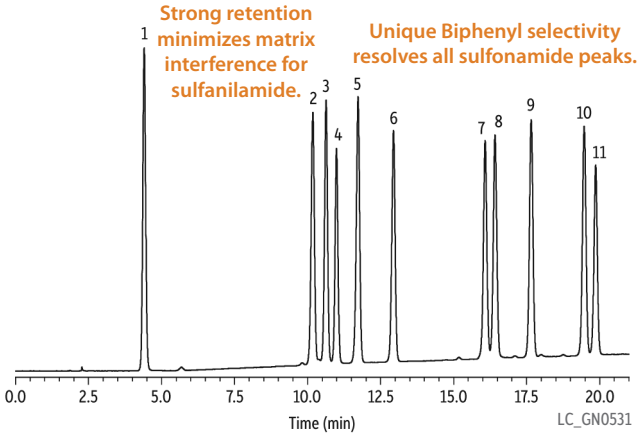
Combining USLC® Selectivity and UHPLC Speed—The Most Powerful Approach

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.

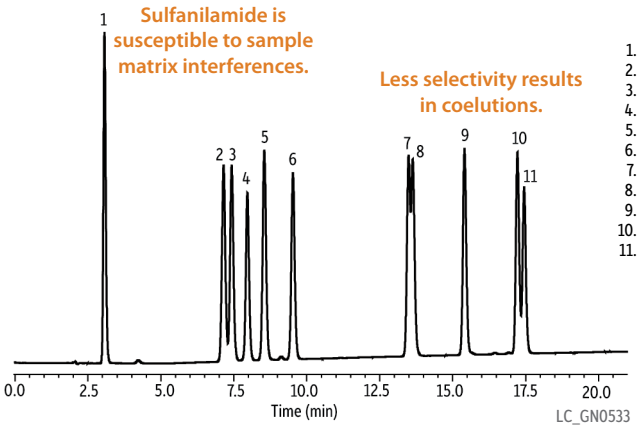
Figure 1: Due to their unique selectivity, Biphenyl columns can provide the retention and separation needed for accurate sulfonamides analysis with simple, MS-friendly mobile phases.



	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:

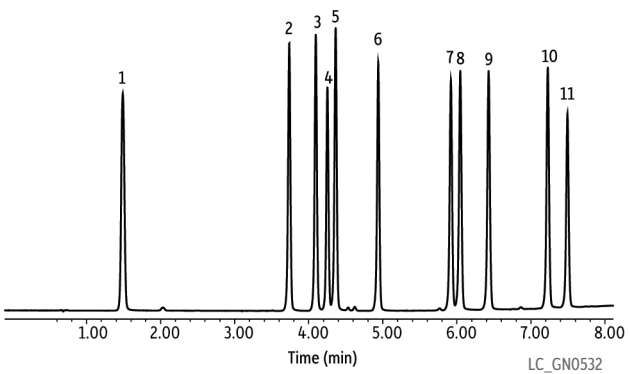
particle size: 1.9 µm, 3 µm, or 5 µm, spherical
pore size: 140 Å
carbon load: 8%
endcap: yes
pH range: 2.5 to 8
temperature limit: 80 °C

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**

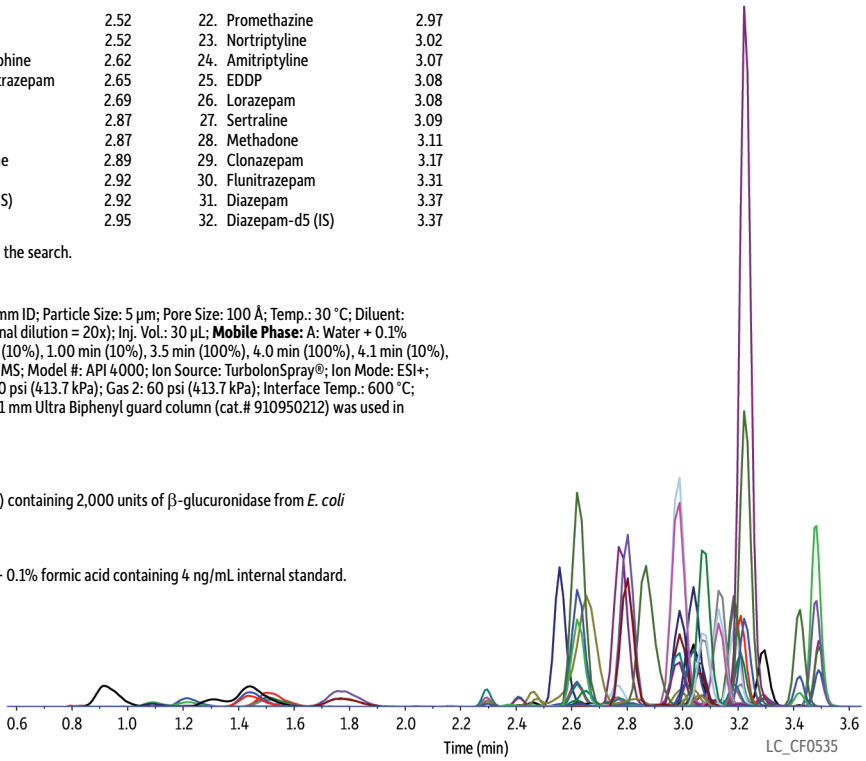
Figure 1: Analysis of 29 drug compounds and metabolites at 100 ng/mL in urine on an Ultra Biphenyl column.

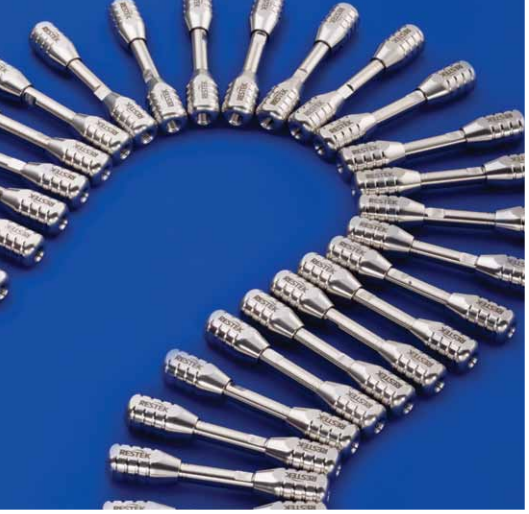
	Peaks	RT (min)						
1.	Morphine	0.95	11.	Tapentadol	2.52	22.	Promethazine	2.97
2.	Oxymorphone	1.08	12.	Zopiclone	2.52	23.	Nortriptyline	3.02
3.	Pregabalin	1.29	13.	Norbuprenorphine	2.62	24.	Amitriptyline	3.07
4.	Hydromorphone	1.34	14.	7-Aminoflunitrazepam	2.65	25.	EDDP	3.08
5.	Gabapentin	1.56	15.	Zolpidem	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.

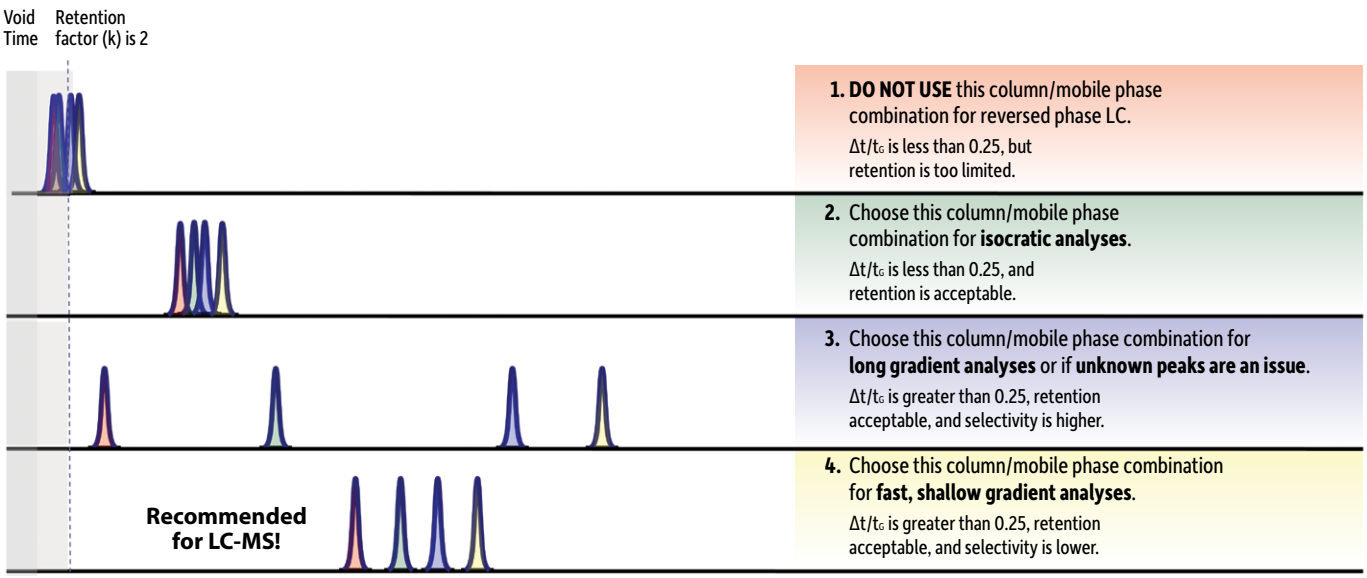
Summary

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.

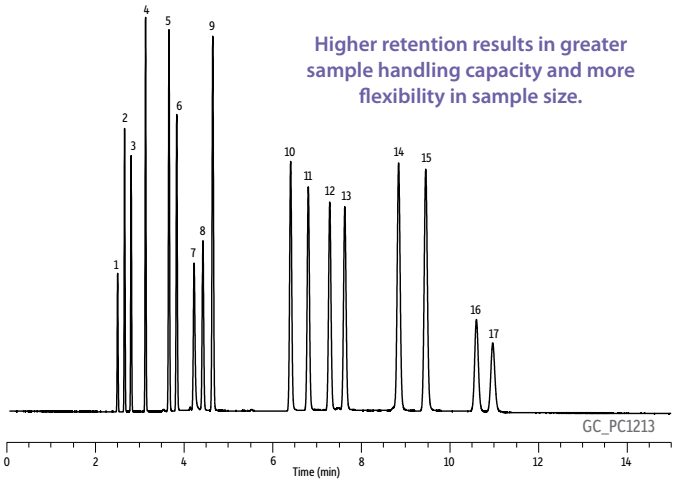
Summary

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.

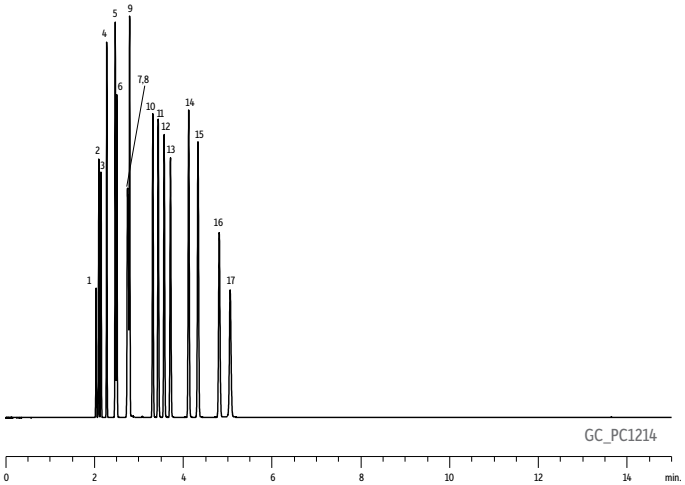
For more information on Rt®- and MXT®-Alumina BOND/MAPD PLOT columns, visit **www.restek.com/MAPD**

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



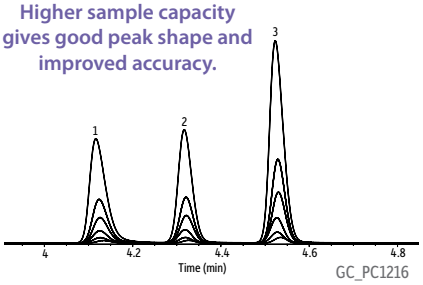
B: Select Al_2O_3 MAPD



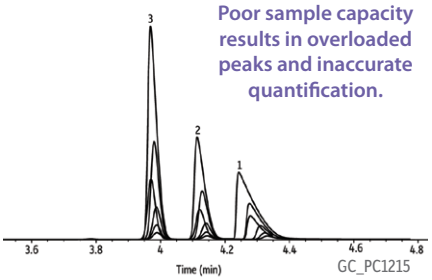
Columns: 50 m x 0.53 mm ID x 10 µm; **Sample:** PLOT column QA test mix (DCG# 547267); **Injection:** 5 µL, split, 200 °C; Split vent flow rate: 80 mL/min; **Oven:** 130 °C, isothermal; **Carrier Gas:** helium, (4.4 psi, 30 kPa); **Detector:** FID, 200 °C. **Peaks:** 1. Methane, 2. Ethane, 3. Ethylene, 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



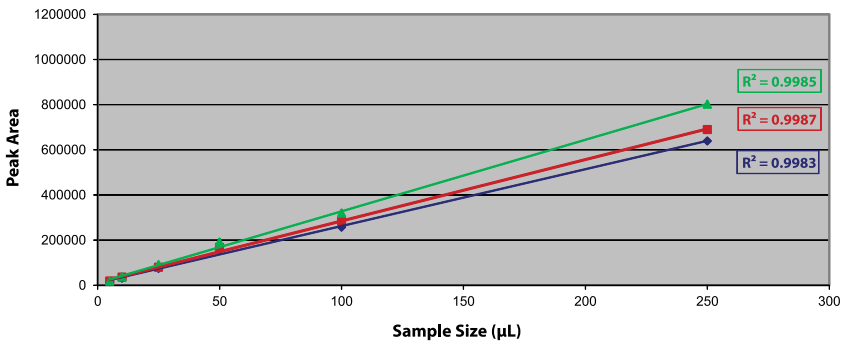
B: Select Al_2O_3 MAPD



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

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2013

Get Sizzling Separations with the Right Selectivity

Also in this issue

Restek Connections

In This Issue

Reflections from the Bench

SEE-lectivity...my colleagues Professor Frank Dorman at The Pennsylvania State University and Steve Allison at Restek always laugh at me because I put the emphasis on the first syllable, instead of selec-TIV-ity. I like to claim that it's not only my Oklahoma accent that causes this, but that I purposely stress the importance of the word up-front since I'm a chromatographer.

It used to be that gas chromatographers lived and died by stationary phase selectivity, especially in the packed column days before the efficiency of open tubular columns started conquering the separation science world. Nowadays, combine a "5-type" narrow-bore fused silica capillary GC column with a mass spectrometer and you have a very powerful "separation" system, where less emphasis is placed on high peak capacity, selective chromatography. Rightfully so, since peaks do not need to be completely separated, and, in fact, can even coelute, as long as they can be qualitatively and quantitatively determined without interference via the "selectivity" of the MS.

So do we chromatographers try and achieve separations just out of pride these days, or because of nostalgia? Maybe because we have too much time on our hands? That last one is definitely not the reason! Actually, the need for GC column selectivity still exists, and there are two key examples presented in this *Advantage*: polycyclic aromatic hydrocarbons (PAHs), and chlorinated dioxin and furan analyses. Both applications involve congener-specific determinations and, when you hear that phrase, you can assume that you will have isobaric compounds that cannot be determined without bias via MS; that is, you still NEED efficient and SELECTIVE chromatography for critical congener separations!

So amongst the other valuable articles in this issue, please enjoy our contributions describing meaningful separations done with our Rtx®-Dioxin2 column and our new Rxi®-PAH GC column. As Dave Mannus, our Restek Ireland manager, says, "They do what they say on the tin."

Finally, the icing on the Restek® *Advantage* selectivity cake is Colin Poole's excellent editorial, "Selectivity in Gas Chromatography." One of his conclusions is that there are a number of opportunities for selective stationary phase development. That sounds like a challenge and, on behalf of Restek, I accept!

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! E-mail 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 have 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. **www.restek.com**

Sitting Down With a Chromatography Icon:
Pat Sandra

By Jaap de Zeeuw

Pat Sandra

Pat Sandra is a widely known scientist who has made an indelible mark on the development of chromatography. He has written hundreds of publications in peer-reviewed journals, is a well-known speaker at events around the world, started his own chromatography group, and was the driving force behind the biannual symposium on capillary separation techniques in Riva del Garda, Italy. We spent time talking about his past, present, and future, after which I came away with a far greater appreciation of his professional accomplishments and talent. I was also struck by how fascinating a life he has led away from the lab. Here is just a taste of what we covered.

Jaap: How did you get into the world of chemistry?

Pat: I became interested in chemistry because I had a very good teacher. He could present the science in such a way that it triggered my interest. Until that time... I had a classical education, meaning... 20 hours of languages and just one hour of physics and one hour of chemistry, so I had no idea what chemistry or physics were about. Those were the base studies for becoming a doctor or pharmacist. My brother was one year older than me; he was studying medicine. So, I decided to do chemistry.

I had absolutely no intention of making a career in chemistry. By age 15 to 16, I was a semiprofessional basketball player. I was playing in the national junior team, the national military team, and training four times per week.... But you need also a job, so I could become a teacher of chemistry for 18- to 20-year-old students. At a certain time, you get bitten by the "microbes of chemistry," and you develop a real interest....

Jaap: Where did you meet your wife, Martine?

Pat: Martine was a sister of a friend of mine. Aside from basketball, I also played guitar, and we had a band called the "Balladins." We gave performances and also played in Belgium at the pre-program of Boudewijn de Groot. Martine's brother was part of the band, so Martine also travelled with us when we toured. We clicked....

Pat performs with the Balladins in 1969.

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

Behind the Scenes: Developing New Technologies

In order to cultivate superior technical expertise and generate innovative solutions, Restek has a full-spectrum, in-house Research & Development (R&D) Department whose main purpose is finding faster and easier ways for you to get more reliable results. When groundbreaking Restek products and optimized applications hit the public, they are fresh from the minds of our Innovations chemists and two applied research groups, but what you do not see directly is the foundational involvement of the R&D teams we have dubbed "Restek West" and "Discovery East."

In 2005, we started Restek West, our California-based research facility where chemists focus on capillary column phases and deactivation. Their goal is to bridge the gap between applied and basic research and to develop not only new products, but also new technologies that can be turned into myriad solutions. Discovery East is the dedicated research lab at our home office in Bellefonte, PA, tasked with seeking a better understanding of underlying scientific principles that can then be broadly applied across a wide variety of market areas. While we wish we could pull back the curtain, so to speak, we cannot tell you what they are working on now. However, we can say that there are some truly exciting breakthroughs coming your way soon that will help you solve your analytical challenges and improve your chromatography. Keep watching Restek's website, ChromaBLOGraphy, and future issues of the *Advantage* for details!

Roy Lautamo, Ashlee Gerardi, Shawn Reese and Bill Bromps from Restek West

Hot Topics

Detect and Quantify 204 Pesticides of Global Concern by LC-MS/MS

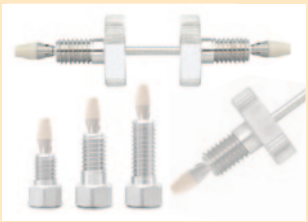
Stop spending your time and money developing methods and mixing reference standards when you could be running samples! Restek's trusted food safety experts have done the work for you—with guaranteed accuracy and lower cost.

Our new certified reference materials (CRMs) for LC-MS/MS multi-residue pesticide analysis eliminate the need to source, purchase, and store individual materials along with the need to prepare your own mixes. Choose separate ampuls for mix-and-match customization or a 204-compound kit that covers many LC-determined pesticides listed by government agencies. Designed for maximum shelf life, stability, and performance, each standard ships with documentation detailing our rigorous in-house testing. We even offer a free optimized method—including conditions and downloadable transition tables.

Set up, verify, and validate your pesticide-screening methods in less time, with proven confidence, and at a reduced cost by turning to Restek's ISO-accredited labs. For unbeatable results, also consider our internal standards, Ultra and Pinnacle® DB LC columns, and Q-sep™ QuEChERS sample prep products.

www.restek.com/LC-multi-residue

Reusable Fittings for Easy, Reliable HPLC & UHPLC Connections



Restek is pleased to offer the reusable EXP® fitting system from Optimize Technologies for the ultimate in easy, reliable LC connections.

The patented hybrid EXP® ferrule combines the durability of titanium with the sealing ability of PEEK for a swage that can be reused over and over again. And, when you choose the hand-tight fitting style, the special EXP® nut offers an effortless seal up to 8,700+ psi (600+ bar)—no tools needed! For a reliable 20,000+ psi (1,400+ bar) UHPLC connection with either fitting style, simply wrench-tighten an extra ¼ to ½ turn.

EXP® ferrules should only be used with genuine EXP® nuts. When used with an EXP® nut, the EXP® ferrule provides repeated ZDV (zero dead volume) connections to any 10-32 female threaded port, including Restek LC columns, 6-port injection valves, and more. To accommodate varying port depths, simply hold the tubing fully bottomed in the port and tighten as instructed.

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Helium Costs Soar–Turn to Hydrogen With Our Help

Known to the general public more for inflating balloons and giving high-pitched voices to partygoers than for its scientific and medical applications, helium is colorless, odorless, tasteless, inert, and even non-toxic.... Yet, it is posing a significant risk to scientists around the world. Despite efforts to increase refinery production, slow the liquidation of U.S. federal reserves, and give scientists priority access to remaining supplies, there is a severe shortage of helium on Earth, which has caused prices to skyrocket along with fears over availability and even total resource depletion. MRIs need liquid helium for their cooling systems, the aerospace industry uses the non-flammable gas to purge explosive rocket fuel, and gas telescopes use it to reduce distortion. But for Restek customers, the biggest concern is that helium has long been the lifeblood of chromatography as the king of the GC carrier gases.

For labs not willing, or not able, to pay high prices and work through source disruptions, the preferred alternative is to switch carrier gases, but switching is not as simple as plugging in a new gas line. That's why, at Restek, we have devoted considerable energy to helping our customers transition to a far less expensive, far more plentiful option that can be generated in your lab and even cut your analysis time: hydrogen.

Simply visit our website and search for "hydrogen carrier gas." You will find ample blogs, presentations, and articles by Restek's technical experts, including Jack Cochran, Jaap de Zeeuw, Jonathan Keim, Julie Kowalski, Amanda Rigdon, Alan Sensue, Chas Simons, and Jason Thomas. We solve such common issues as enhanced activity, compound hydrogenation, peak asymmetry, and column decomposition. You will also find in-depth discussions on method translation, safety, hydrogen generators, hydrogen's "scrubbing" effect on tubing, pumping capacity, solvent selection, column conditioning, and optimizing flow and heating rates, as well as detailed chromatographic analyses for organochlorine pesticides, medical cannabis potency, blood alcohol compounds (BACs), refinery gases, PAHs, and more. In short, the knowledge you need to make the move to hydrogen is at **www.restek.com**

Ready to switch?

Restek also offers a full line of hydrogen generators, including the new H2PEMPD models. Order yours today at **www.restek.com/gas**

Restek Continues Its Global Expansion

2013 has been a busy year for Restek internationally. In February, we announced the opening of a new subsidiary in the People's Republic of China. General Manager Wei Zhu (朱卫) has been using his management experience with marketing, science, and finance operations to lead Restek China, while chromatographer Zhang Shaoyu (张少玉) is providing expert technical support. This entity was established not only to serve existing in-country distributors and their customers, but also to give Chinese chemists the option of working directly with Restek for the first time.

Additionally, our branch office in Tokyo has been expanding under the leadership of Managing Director Ryosei Kanaguchi. We recently added two staff members—Masataka Okubo as Business Development Manager and Hideaki Kitami for Technical Sales and Support—to better assist analysts throughout Japan. The Restek Japan team has moved into a larger facility, and further growth is on the horizon.

On the other side of the world in Milan, Italy, Restek also became a majority owner of Superchrom S.r.l. just four months after Restek China opened its doors. Superchrom was founded in 1976 and grew into one of the most recognized and respected chromatography suppliers in Italy. Known for its passion for excellent customer service, Superchrom will be a perfect fit in the Restek global family. Mr. Carlo Ciocca, Sales and Marketing Director, will transition into the role of General Manager for this newly formed Restek enterprise.

With subsidiaries in the United Kingdom, France, Germany, Japan—and now China and Italy—as well as a robust distributor network that covers over 100 countries across six continents, our goal is to offer superior, cutting-edge chromatography solutions and world-class Plus 1 service through a local source. Wherever you live or work, you can easily find your Restek representative at **www.restek.com/distributors** Contact them 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:

- *Leak Checking Your GC: Leak Detector or Pressure Decay?*
- *Rapid Screening for Semivolatiles–144 Compounds in Less than 9 min*
- *Is There a Limit to the Volume you can Inject Into an Agilent® Split/Splitless Inlet?*

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

Event Recap

North American Chemical Residue Workshop (formerly FPRW)

In July 2013, over 300 enthusiastic attendees met in St. Pete Beach, Florida to enjoy the 50th Florida Pesticide Residue Workshop (FPRW), which today goes by the moniker "North American Chemical Residue Workshop" (NACRW). The name changed to reflect the expansion from its original topic to include other food residues and environmental contaminants (e.g., veterinary drugs, brominated flame retardants, perfluorinated compounds, etc.), but the quality of the meeting was as high as ever.

Liesl Krone, an 8th-grade student from Action School, Texas, discusses her research with Steve Lehotay, USDA (Agricultural Research Service); Lauren Bailey, AB SCIEX; and Michelangelo Anastassiades, CVUA, Stuttgart.

The technical program, organized by Kate Mastovska of Covance, was excellent and included an expanded poster session that encouraged discussions among scientists, including perhaps the youngest one ever to participate in the meeting, Action School (Texas) eighth-grader, Liesl Krone. Liesl, along with coauthor André Schreiber of AB Sciex, won a poster prize with her contribution, "What Pesticides Have You Eaten Today?". She used QuEChERS (with products from Restek!) and LC-MS/MS to study the pesticide content of organic versus non-organic fruits. Although she found low levels of pesticides in citrus and grapes, Liesl concluded that "it is still better to eat fruit than junk food."

NACRW is organized by a dedicated group of volunteers from government, academia, and industry under the guidance of a Board of Directors from FLAG Works, Inc. Restek is proud to contribute as a Platinum Sponsor for NACRW, but more importantly, our commitment extends to active roles in the organization of the meeting itself. For example, Restek's Julie Kowalski has overseen the meeting's highly successful social events, including this year's luau. The luau was a raging success culminating in an exciting fire performance! Because of her dedication to NACRW, this year Julie was elected President of the workshop for 2015.

Learn more about Restek's participation in NACRW 2013 and download copies of our technical presentations on a variety of GC and LC topics by visiting **www.restek.com/nacrw** See you next year!

Reduce Typical Analysis Times by 30% Using New Rxi®-PAH Columns for Polycyclic Aromatic Hydrocarbons

By Amanda Rigdon

- Optimized column selectivity resolves all critical PAHs.
- Faster analysis time speeds up sample throughput.
- Simple oven program ensures separations are maintained over column lifetime.

Analysis of polycyclic aromatic hydrocarbons (PAHs) in food and environmental samples is a rapidly growing area due to increasing concern about the toxicity and carcinogenicity of some isomers. PAHs are formed during incomplete combustion, making food such as grilled or smoked meats, roasted grains, and toasted cereals a major avenue of exposure for humans. Methods differ in target analytes, but the European Food Safety Authority (EFSA) PAH4, PAH8, and PAH15, as well as the EPA 16 PAH lists are among the most commonly used. Since these lists include isobaric compounds that must be separated chromatographically, column choice is an important consideration. The new Rxi®-PAH column from Restek is designed specifically for comprehensive PAH analysis in food. It has both the selectivity required to separate the most difficult PAHs, as well as optimized column dimensions (narrower bore and thinner film) that allow higher molecular weight PAHs to be analyzed without interference from column bleed.

To benchmark Rxi®-PAH column performance, analysis time and overall separation quality were compared to results obtained on an Agilent® Select PAH column. A comprehensive PAH mixture (NIST SRM 2260a) was analyzed on an Rxi®-PAH column (40 m x 0.18 mm x 0.07 µm) and an Agilent® Select PAH column (30 m x 0.25 mm x 0.15 µm) using the manufacturer's recommended conditions for each column.

Separate Critical PAHs in 30% Less Time Compared to Using an Agilent® Select PAH Column

As shown in Figures 1 and 2, similar separations are achieved on both columns. However, results were obtained on the Rxi®-PAH column in just 32 minutes, saving 13 minutes per analysis compared to the 45.5 minute run using the Agilent® Select PAH column. Due to its optimized stationary phase and dimensions, the Rxi®-PAH column has the perfect balance of selectivity and retention. This allows for resolution of critical compounds, such as the benzo [b], [k], and [j] fluoranthenes, while still eluting the heavier PAHs in a relatively short analysis time. Note that although the Agilent® Select PAH column is 10 m shorter than the Rxi®-PAH column, the analysis time on the Rxi®-PAH column is ~30% faster. In addition, the smaller internal diameter and thinner film of the Rxi®-PAH column reduce column bleed that can interfere with quantification of the higher molecular weight PAHs.

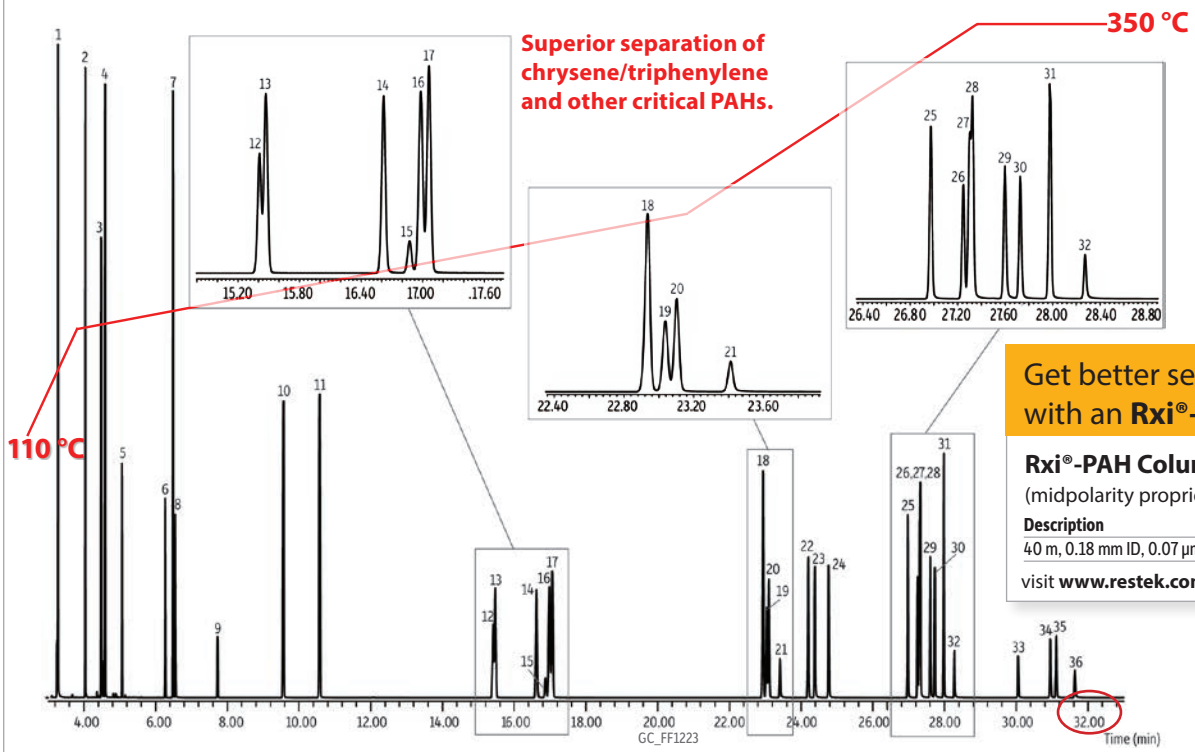
Simple Instrument Conditions Ensure Longer Method Reliability

The oven temperature overlays on the chromatograms in Figures 1 and 2 illustrate an important difference in the instrument conditions needed to achieve acceptable resolution for the most critical compounds. The recommended oven program for the Agilent® Select PAH column is extremely complicated and includes two isothermal holds in the middle of the analysis. Mid-analysis holds are problematic because they broaden peaks substantially, which reduces peak height and detectability. In contrast, the oven program needed to perform a better separation on the Rxi®-PAH column is simple and does not require isothermal holds in the middle of the analysis. This more straightforward oven program maximizes peak response and allows for more reliable performance over the lifetime of the column, even after column trimming for routine maintenance. When a column is trimmed, the oven program must be modified in order to ensure that compounds will elute at the same temperature on the shorter column as they did on the longer one, thus preserving resolution between critical compounds. To accomplish this, the oven ramp rate must increase as the column gets shorter. With the fast ramp rate used at the beginning of the analysis on the Agilent® Select PAH column, the resulting translated ramp rate for a trimmed column will quickly exceed the oven's ramping ability, compromising separation.

Summary

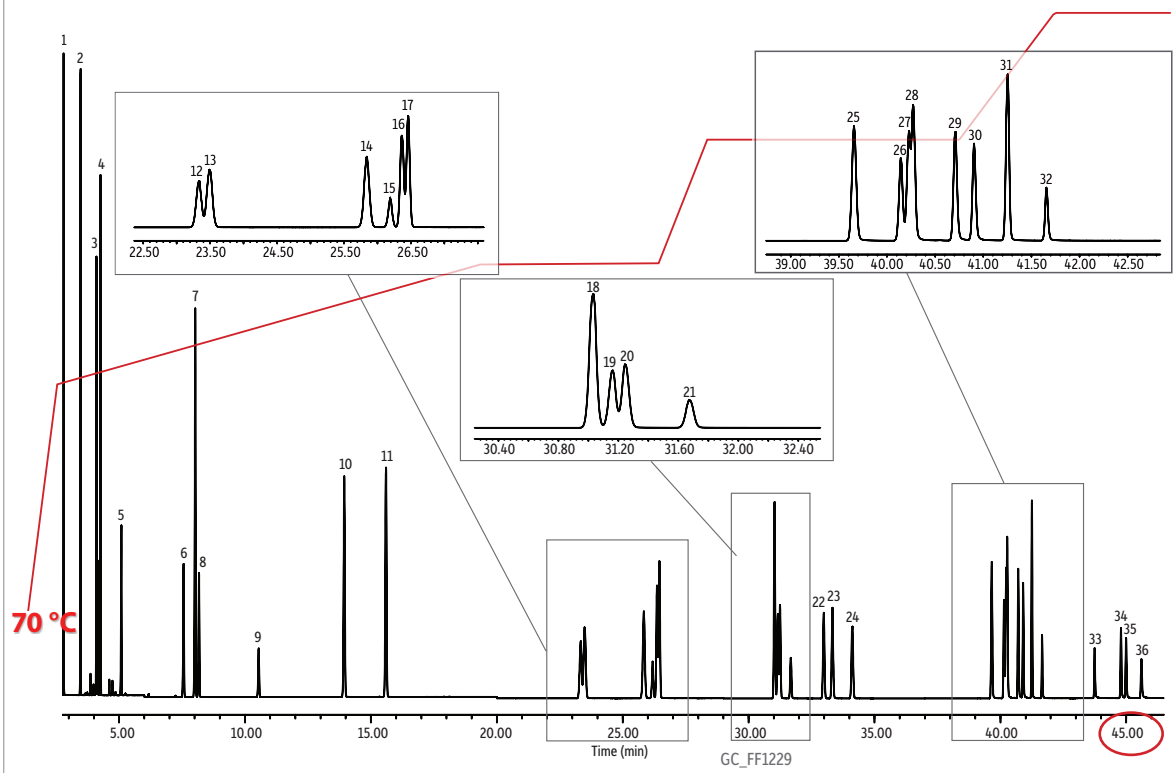
Rxi®-PAH columns from Restek are optimized specifically for PAHs in both selectivity and dimensions. Compared to the Agilent® Select PAH column, the Rxi®-PAH column offers superior performance, allowing comprehensive PAH lists to be separated quickly and reliably.

Figure 1: Separate all critical PAHs on an Rxi®-PAH column in 30% less time than when using an Agilent® Select PAH column.



Column: Rxi®-PAH, 40 m, 0.18 mm ID, 0.07 µm (cat.# 49316); **Sample:** NIST SRM 2260a PAH mix; Diluent: Toluene; Conc.: 0.2 - 2 µg/mL (SRM 2260a PAH mix was diluted 5x in toluene); **Injection:** Inj. Vol.: 0.5 µL pulsed splitless (hold 0.58 min); Liner: Sky® 2 mm single taper w/wool (cat.# 23316.1); Inj. Temp.: 275 °C; Pulse Pressure: 80 psi (551.6 kPa); Pulse Time: 0.6 min; Purge Flow: 40 mL/min; **Oven:** Oven Temp.: 110 °C (hold 1 min) to 210 °C at 37 °C/min to 260 °C at 3 °C/min to 350 °C at 11 °C/min (hold 4.5 min); **Carrier Gas:** He, constant flow; Flow Rate: 1.4 mL/min; **Detector:** MS; Mode: SIM.
For complete instrument conditions, visit www.restek.com and enter GC_FF1223 in the search. See Figure 2 for peak list. **Red line** = oven temperature program.

Figure 2: Analysis of the same PAHs on an Agilent® Select PAH column takes 45 minutes and requires a complex oven temperature program.



Peaks
1. Naphthalene
2. Biphenyl
3. Acenaphthylene
4. Acenaphthene
5. Fluorene
6. Dibenzothiophene
7. Phenanthrene
8. Anthracene
9. 4H-Cyclopenta[def] phenanthrene
10. Fluoranthene
11. Pyrene
12. Benzo[ghi]fluoranthene
13. Benzo[c]phenanthrene
14. Benz[a]anthracene
15. Cyclopenta[cd]pyrene
16. Triphenylene
17. Chrysene
18. Benzo[b]fluoranthene
19. Benzo[k]fluoranthene
20. Benzo[j]fluoranthene
21. Benzo[a]fluoranthene
22. Benzo[e]pyrene
23. Benzo[a]pyrene
24. Perylene
25. Dibenz[a,j]anthracene
26. Dibenz[a,c]anthracene
27. Indeno[1,2,3-cd]pyrene
28. Dibenz[a,h]anthracene
29. Benzo[b]chrysene
30. Picene
31. Benzo[ghi]perylene
32. Anthanthrene
33. Dibenzo[b,k]fluoranthene
34. Dibenzo[a,e]pyrene
35. Coronene
36. Dibenzo[a,h]pyrene

Conditions based on column manufacturer's recommendations. **Column:** Agilent® Select PAH, 30 m, 0.25 mm ID, 0.15 µm (cat.# CP7462); **Injection:** Inj. Vol.: 1.0 µL splitless (hold 1.0 min); **Oven:** Oven Temp.: 70 °C (hold 0.7 min) to 180 °C at 85 °C/min to 230 °C at 3 °C/min (hold 7 min) to 280 °C at 28 °C/min (hold 10 min) to 350 °C at 14 °C/min (hold 4 min); **Carrier Gas:** He, constant flow; Flow Rate: 2.0 mL/min; **Detector:** MS. For complete instrument conditions, visit www.restek.com and enter GC_FF1229 in the search.

New Rxi®-PAH GC Column Separates all EFSA PAH4 Compounds From Coeluting Congeners

By Jack Cochran, Amanda Rigdon, Roy Lautamo, and Shawn Reese

- Separate chrysene from interfering PAHs, triphenylene and cyclopenta[cd]pyrene.
- Fully resolve benzo [b], [k], [j], and [a] fluoranthenes.
- Easily distinguish benzo[e]pyrene, benzo[a]pyrene, and perylene.

Polycyclic aromatic hydrocarbons (PAHs) are a large class of semivolatile organic compounds that are made up of two or more fused aromatic rings. Naphthalene is the simplest PAH and benzo[a]pyrene is one of the most notorious, given its high carcinogenic and mutagenic potential. Although PAHs are found in fossil fuels including coal, they are primarily formed from combustion processes, such as the burning of wood and motor fuels. While PAHs can be found in almost all environmental compartments, especially air, soil, and sediment, the chief route of exposure for humans may be food. Smoked or grilled foods contain PAHs, but even cereals and cocoa beans, foods that are processed with roasting or drying at higher temperatures, can have PAHs.

Because of its high carcinogenicity, benzo[a]pyrene is often used solely to estimate the PAH carcinogenic potential of a sample. However, in a recent European Union regulation concerning maximum levels of PAHs in foodstuffs, the European Food Safety Authority (EFSA) noted that benzo[a]pyrene was not always detected in foods, while other carcinogenic and genotoxic PAHs were present, including chrysene, benz[a]anthracene, and benzo[b]fluoranthene. These compounds together with benzo[a]pyrene were termed the PAH4 by EFSA. Ultimately, EFSA decided that the PAH4 are the most suitable indicators for PAHs in food, and that determining additional PAHs would not add much value.

Selectivity Challenge: Separating all PAH4 Compounds on a Single Column

Although it may seem simple to determine only four substances in a sample using GC-MS, it is not a trivial task in the case of the EFSA PAH4 compounds. Many PAHs are isomeric and have essentially identical mass spectra, meaning that chromatographic separation is mandatory for their analysis. However, because of their structural similarity, even high efficiency GC may not chromatographically separate important isomeric congeners. For example, while only chrysene is toxic, chrysene and triphenylene both share the 228 m/z ion and are also very difficult to separate chromatographically by GC. While higher phenyl GC stationary phases, such as Rxi®-17Sil MS and Rxi®-35Sil MS columns, perform well for most PAHs, selectivity is not optimal on either column for all EFSA PAH4 compounds.

Both Rxi®-17Sil MS and Rxi®-35Sil MS columns easily resolve benzo[e] pyrene, benzo[a]pyrene, and perylene (all 252 m/z PAHs) to baseline (chromatogram not shown). However, their selectivities are not the same, resulting in different chromatographic performance for other PAHs. As shown in Figure 1, chrysene and triphenylene can be separated on high-efficiency 40 m x 0.18 mm x 0.07 µm configurations, but better resolution would be desirable.

In addition, while both Rxi®-17Sil MS and Rxi®-35Sil MS columns can be used to analyze benzo fluoranthenes there are differences in their performance (Figure 2). Although the most important benzo fluoranthene--benzo[b]fluoranthene--is easily separated on both columns, benzo [k] and [j] fluoranthenes are incompletely resolved on Rxi®-35Sil MS columns, which could be unfavorable for chemists who want to determine additional PAH congeners.

Rxi®-PAH Columns--Best Overall Performance for all EFSA PAH4 Compounds

Based on the work above, we theorized a stationary phase with selectivity between the Rxi®-17Sil MS and Rxi®-35Sil MS columns would offer the best separations for both chrysene and triphenylene, as well as the benzo fluoranthenes. The new Rxi®-PAH GC column was designed with intermediate selectivity specifically for separating the important EFSA PAH4 compounds from potential interferences. The data in Figure 3 demonstrate that this was achieved, as both the chrysene/triphenylene and benzo fluoranthene separations are optimized. In addition, benz[a]anthracene is also easily separated from any potential interfering PAHs. As with the Rxi®-17Sil MS and Rxi®-35Sil MS columns, the new Rxi®-PAH column had no trouble pro-

Figure 1: Chrysene and triphenylene can be separated on both Rxi®-17Sil MS and Rxi®-35Sil MS columns, but resolution is not optimal on the Rxi®-17Sil MS column.

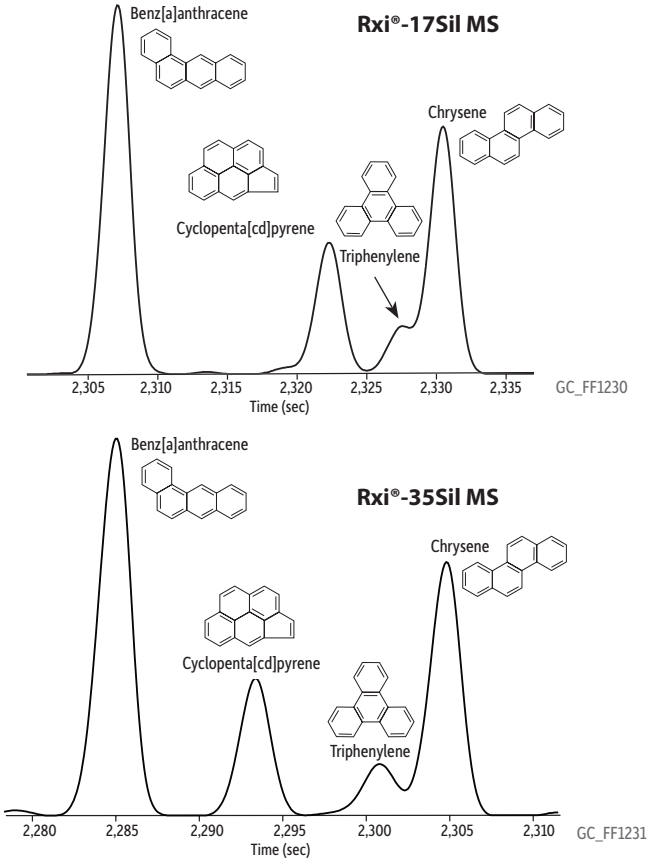
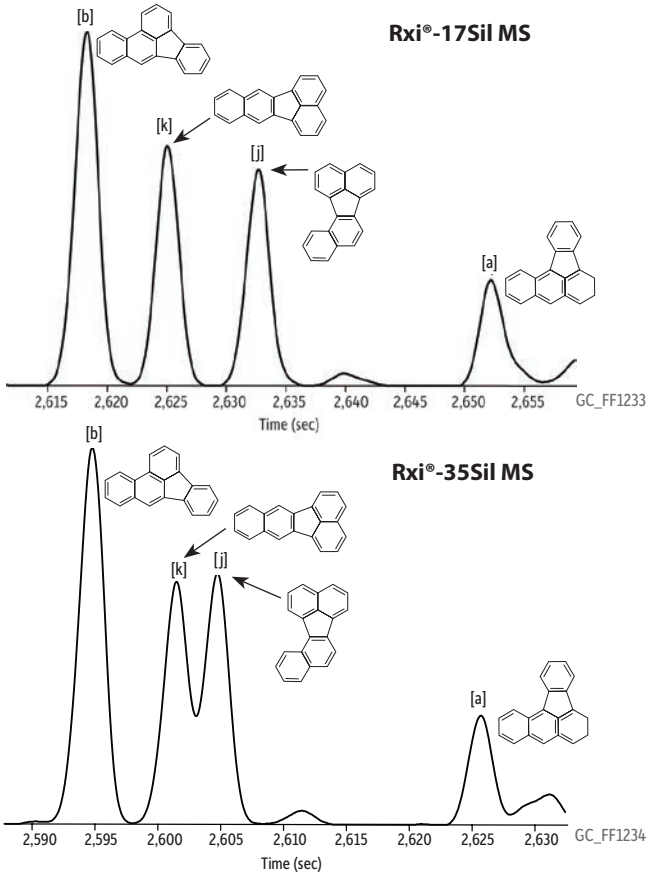


Figure 2: Benzo fluoranthenes are baseline resolved on Rxi®-17Sil MS columns, but not completely separated on Rxi®-35Sil MS columns.



viding better than baseline separation of benzo[e]pyrene, benzo[a]pyrene, and perylene (data not shown). Given its superior performance for all the EFSA PAH4 compounds, the Rxi®-PAH column is recommended for accurate determinations of these toxic PAHs in food samples.

GC-FID Conditions:
Pulsed splitless injection, 275 °C, 1 µL coal tar extract
4 mm single taper Sky® inlet liner with wool
Pulse pressure 80 psi, pulse time 1.5 min, purge valve time 88 sec

All columns 40 m x 0.18 mm x 0.07 µm
He, constant flow 1.2 mL/min (~32 cm/sec at 80 °C)
80 °C (1.5 min), 4.9 °C/min to 340 °C (10.44 min)
65 min run time

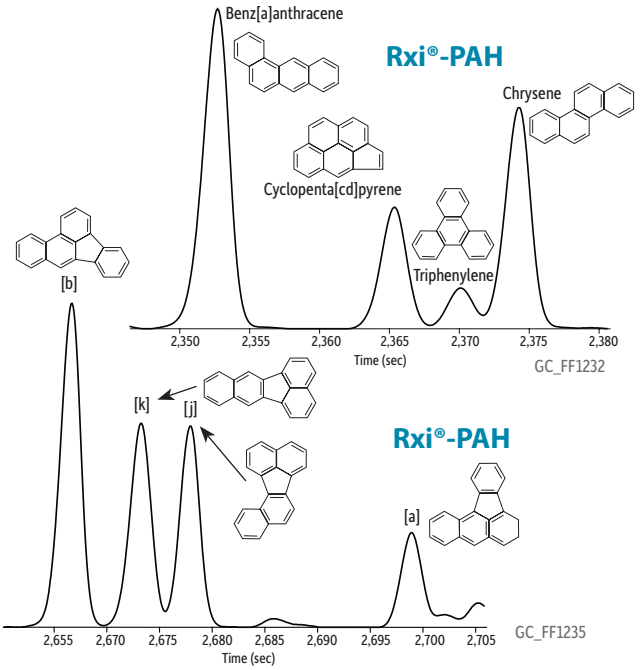
Flame ionization detector at 350 °C
50 mL/min nitrogen makeup (+ column flow)
40 mL/min hydrogen and 450 mL/min air
10 Hz data collection rate

Note that these simple linear GC oven programming conditions were used only to map elution orders and make direct column comparisons easier. The GC oven program for an optimized 33 min analysis that separates the EFSA PAH4 on a 40 m x 0.18 mm x 0.07 µm Rxi®-PAH GC column is available at **www.restek.com/rxi-pah**

Rxi®-PAH Columns (fused silica)
(midpolarity proprietary phase)

Description	temp. limits	qty.	cat.#
40 m, 0.18 mm ID, 0.07 µm	to 360 °C	ea.	49316
30 m, 0.25 mm ID, 0.10 µm	to 360 °C	ea.	49318
60 m, 0.25 mm ID, 0.10 µm	to 360 °C	ea.	49317

Figure 3: Only Rxi®-PAH GC columns can simultaneously separate chrysene and triphenylene, as well as all the benzo fluoranthenes. Optimized selectivity ensures accurate quantification of all EFSA PAH4 compounds, including benzo[a]pyrene (not shown) and benz[a]anthracene.



Mitigating Matrix Effects: Sample Prep and Calibration

Strategies for Multiresidue Pesticide LC-MS/MS Analysis of Foods

By Julie Kowalski, Ph.D.; Sharon Lupo; and Jack Cochran

The increased selectivity and sensitivity of LC-MS/MS have made it a popular technique for monitoring pesticide residues in food and, in some cases, have even decreased the need for rigorous sample preparation. However, if sample preparation isn't adequate for a given commodity, matrix effects can cause poor data quality and difficult quantification. Matrix effects can be mitigated by sample preparation procedures that reduce levels of coextracted matrix material and/or by experimental strategies like matrix-matched calibration that compensate for matrix effects. Since food commodities differ significantly in complexity, we tested a range of food types and evaluated both sample preparation and calibration strategies, in order to determine recommendations that balanced data quality with the convenience of a simple sample dilution. Here, we compared, QuEChERS sample preparation to a dilution method, using both matrix-matched and solvent calibration.

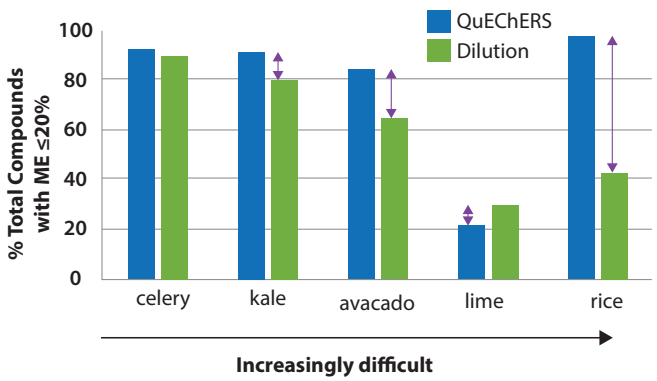
For this work, we evaluated matrix effects and recoveries for over 100 pesticides commonly included in multiresidue methods. We used a variety of food types that differed in characteristics (high water [celery], high pigment [kale], high fat [avocado], citrus [lime], and dry [brown rice flour]) and tested two fortification levels (10 and 500 µg/kg), with subsequent pesticide determinations by LC-MS/MS. Preparation varied by commodity and procedures as well as final results are detailed at **www.restek.com/matrix-effects** in the full application note. In brief, sample preparation entailed an initial QuEChERS extraction for all samples, followed by 20x dilution for dilution method samples; for QuEChERS samples, extracts were further processed by QuEChERS dSPE and then diluted 10x.

Matrix Challenges Differ by Commodity

Matrix effects were calculated by dividing the slope of a calibration curve generated using matrix-matched standards by the slope of the solvent-only calibration curve. Values of 80-120% indicate minor matrix effects and are considered acceptable. We evaluated the four testing categories by comparing the percent of compounds that fell within ± 20% of the solvent curve values (Figure 1).

Celery has high water content, intermediate color, and is low in fat. Results from QuEChERS and dilution methods are almost identical and both strategies are successful. Kale contained more pigment than celery and was slightly more challenging, but similar results were also seen in this high-water matrix. For both, the dilution method saved time and eliminated the potential loss of analytes by sample cleanup.

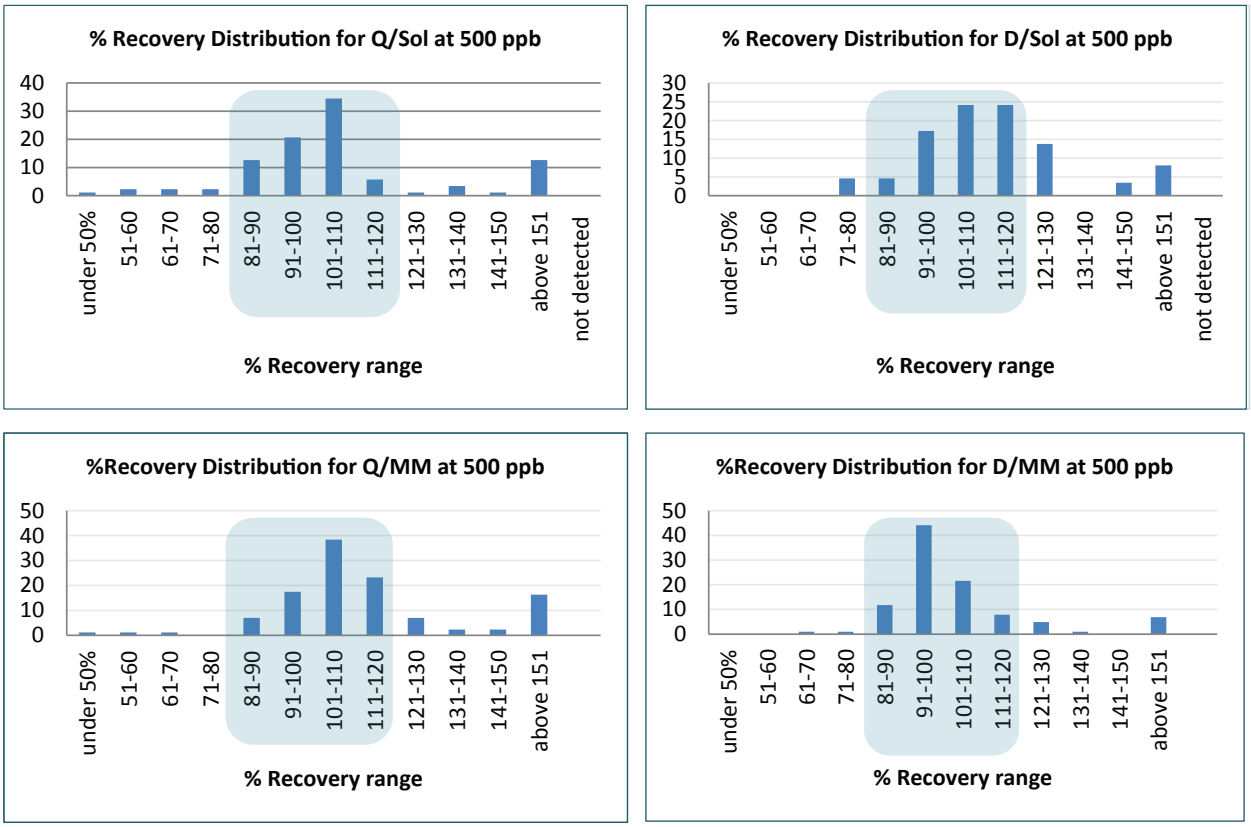
Figure 1: Comparison of the percent of analytes that have matrix effects within 80-120% by commodity and sample prep method.



> Matrix complexity determines the true value of dilute-and-shoot LC-MS/MS

Other matrices, such as avocado, citrus, and grains are typically more difficult. Avocado is more challenging chiefly because of its high fat content. Both the QuEChERS and dilution methods were less effective at minimizing matrix effects in avocado than they were in easier commodities like celery and kale. While the QuEChERS method produced low, but acceptable, matrix effects for 84% of analytes, the dilution method produced low matrix effects for just 64% of analytes, indicating that it was not as effective as the QuEChERS method for this matrix. The poor performance of both methods for lime is not surprising as citrus fruits are known to be difficult to analyze by LC-MS/MS methods.

Figure 2: Percent recovery distribution comparing sample preparation and calibration strategy combinations for celery samples.



Q = QuEChERS, D = Dilution, Sol = Solvent calibration, MM = Matrix-matched calibration

Grains present challenges for sample preparation because they are dry and contain high levels of coextracted material. For brown rice flour, the performance of the methods is significantly different. The QuEChERS method shows low matrix effects for 98% of analytes, while the dilution method did so for only 42%. This demonstrates that dilution alone was not able to reduce the amount of coextractives to an acceptable level. In contrast, the QuEChERS dSPE cleanup step was able to remove carbohydrates and fatty acids that are commonly found in high levels in grains.

Evaluation of Sample Prep/Calibration Strategy

We also evaluated the performance of the sample processing strategies with different calibration methods to determine the best combinations for different commodity types. To determine when the additional time and expense of sample preparation or matrix-matched calibration may be warranted, percent recovery values for all analytes, excluding incurred pesticides, were calculated for each commodity. Recoveries were determined for each sample preparation approach using both solvent and matrix-matched calibrations and the distributions were plotted as shown for celery in Figure 2. These plots were used to compare biasing of recovery values among sample preparation/calibration strategies. Since the 80-120% recovery range is considered satisfactory for quantitative work, further evaluations focused on that range (see full application note for data and discussion).

Using the recovery data, we determined that with the easiest commodities, the dilution method and solvent-only calibration gave acceptable recovery values. However, for other commodities either a matrix-matched curve or cleanup was needed to obtain good recovery values. The high carbohydrate and citrus commodities proved to be too difficult with the specific methods we tested here. In almost every case, use of a matrix-matched calibration provided improvement.

Summary

The dilution technique has been heavily promoted in recent years and is sometimes endorsed as a universal method. However, pesticide residue testing involves a wide variety of food types as well as a large number of pesticides that vary in physiochemical properties and a single method is generally not adequate to address this complexity. In our study, both QuEChERS and dilution methods performed well for high water commodities, offering time savings in both sample processing and also in standard preparation because solvent standards can be used. However, as commodities become more challenging with higher concentrations of coextractives, especially fat and carbohydrates, successful methods will involve sample cleanup in combination with dilution and matrix-matched calibration.

To access the complete application note, visit **www.restek.com/matrix-effects**

Rtx®-Dioxin2 Column Provides First Non-Cyano Separation of 2,3,4,7,8-PeCDF While Also Separating 2,3,7,8-TCDF and 2,3,7,8-TCDD

By Jack Cochran

- Isomer specificity for 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, and 2,3,7,8-TCDF in one GC column.
- Better thermal stability than cyano columns; use up to 340 °C with confidence.
- Unique selectivity for toxic dioxin and furan congeners allows use as a confirmation GC column.

Polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) are halogenated organic pollutants that persist in the environment and bioaccumulate through the food chain. While not all PCDFs and PCDDs have known health effects, several key congeners have been shown to be mutagenic or carcinogenic in human or animal models. These pollutants form when chlorinated compounds, such as polyvinyl chloride (PVC) or polychlorinated biphenyls (PCBs), burn at 400–700 °C, conditions that are common when garbage is burned using an open fire (e.g., backyard burning) or when an inefficient solid waste incinerator is used. PCDFs and PCDDs have relatively poor water solubility, so once released into the environment they adsorb to lipids. When ingested they are stored in adipose tissue and bioaccumulate rather than being eliminated. In fact, one of the primary routes of exposure for humans is the consumption of fatty animal tissue.

Analytical Approaches for Dioxins and Furans

Of the 136 tetra- through octa-PCDFs and PCDDs, only 17 are considered toxic; however, their analysis is complicated by coelutions with nontoxic congeners. Among the most difficult to resolve are 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD), 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF), and 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF). Analysis of PCDFs and PCDDs usually employs GC-MS methods, such as EPA Methods 1613 and 8290a, which specify the use of a 60 m 5% diphenyl/95% dimethyl polysiloxane GC column (e.g., DB-5, Rxi®-5ms columns) for primary analysis. These columns have specificity for the 2,3,7,8-TCDD isomer, but they do not have the selectivity required to separate the most critical furans, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF. In order to accomplish this, these methods suggest the use of confirmation columns with high cyano content stationary phases. Unfortunately, cyano-based columns have very low maximum operating temperatures (240-275 °C) , which is not ideal for chlorinated dioxin/furan analysis, especially if you want to keep them in the same GC oven as the 5% phenyl primary column that has a maximum operating temperature of 350 °C.

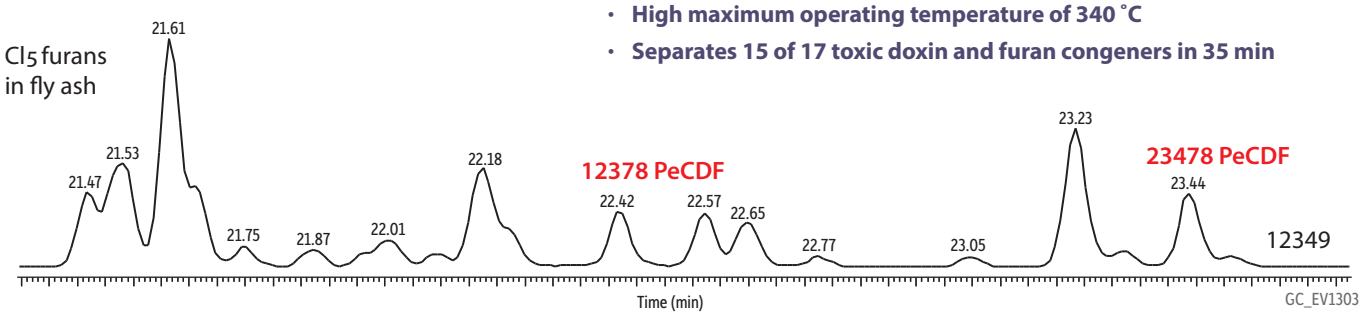
Compared to cyano-based columns, the Rtx®-Dioxin2 column is a much better alternative because it can resolve 2,3,7,8-TCDD as well as 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF. This column is an excellent confirmatory column for EPA Methods 1613 and 8290a since it has isomer specificity and 340 °C thermal stability. In addition, the Rtx®-Dioxin2 column is the only non-cyano based column that can separate 2,3,4,7,8-PeCDF from potentially interfering congeners. Although 2,3,7,8-TCDD and 2,3,7,8-TCDF get a great deal of attention due to their toxicity and presence in biota samples, 2,3,4,7,8-PeCDF also has high toxicity (toxic equivalence factor [TEF] = 0.3) and can dominate the overall toxicity of some samples, especially those where the contamination is from particular chloralkali processes.

Selectivity for the Most Critical Toxic Congeners

The chromatogram in Figure 1 shows the separation of 2,3,4,7,8-PeCDF on a 60 m x 0.18 mm x 0.10 µm Rtx®-Dioxin2 column. Note that the selectivity of the Rtx®-Dioxin2 column allows 2,3,4,7,8-PeCDF to be separated from potential interferences, whereas on a 5% diphenyl column it will coelute with 1,2,3,6,9-, 1,2,4,8,9-, and 1,2,6,7,9-PeCDF congeners, leading to high quantification bias and false toxicity for the sample. In addition, 1,2,3,7,8-PeCDF is completely separated without bias. The column used here is a custom configuration that separates 15 of 17 2,3,7,8-chlorinated dioxins and furans, including 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF), which contributes greatly to the overall toxicity of certain samples, including those from chloralkali processes, and is also not separated on 5% diphenyl columns. As noted earlier and shown in Figure 2, both 2,3,7,8-TCDF and 2,3,7,8-TCDD are also easily separated from potentially interfering congeners.

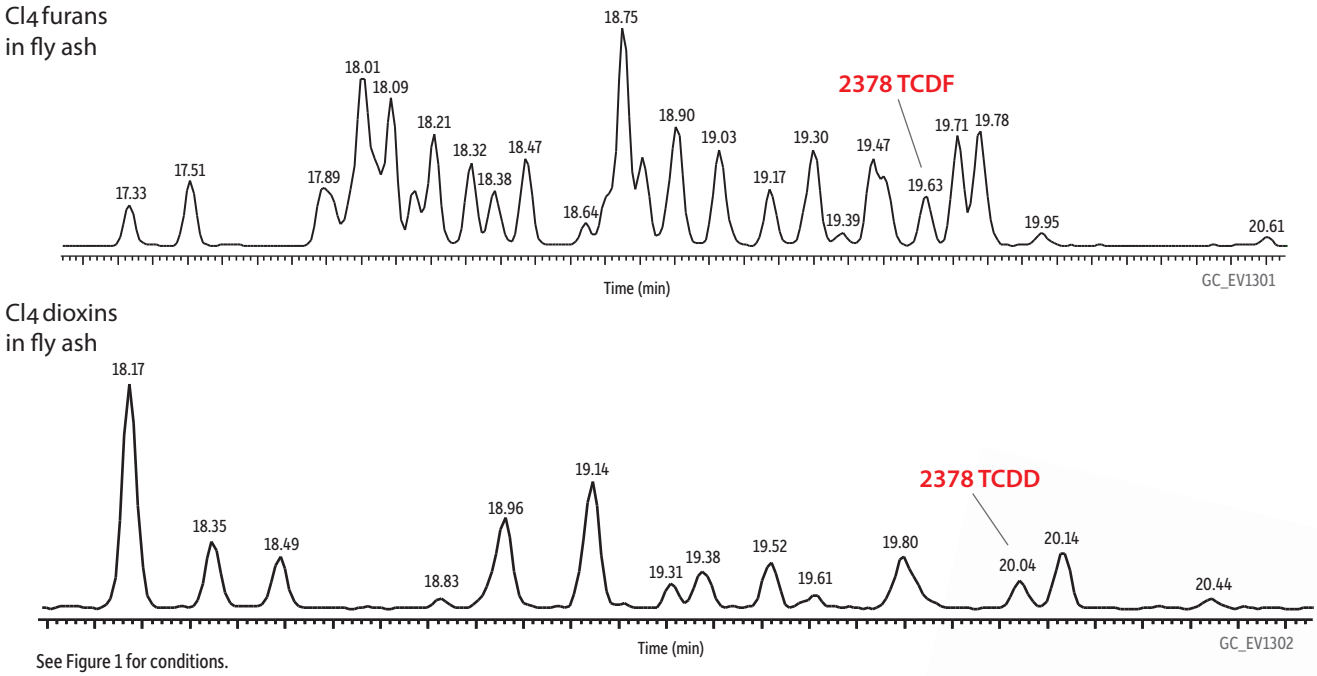
Figure 1: Toxic pentachlorodibenzofurans are easily separated on an Rtx®-Dioxin2 column. These compounds are not resolved from interferences on 5% phenyl columns.

- **Good selectivity for furans not separated on DB-5**
- **High maximum operating temperature of 340 °C**
- **Separates 15 of 17 toxic doxin and furan congeners in 35 min**



Column: Rtx®-Dioxin2, 60 m, 0.18 mm ID, 0.10 µm (cat.# CC1212 [custom]); **Sample**: Fly ash extract; Diluent: Nonane; Conc.: This is a "real world" sample extract; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: 2 mm Sky® single taper w/wool (cat.# 23316.1); Inj. Temp.: 280 °C; Purge Flow: 40 mL/min; **Oven:** Oven Temp.: 120 °C (hold 1 min) to 200 °C at 35 °C/min to 280 °C at 4.5 °C/min to 330 °C at 20 °C/min (hold 4.8 min); **Carrier Gas**: He, constant flow; Flow Rate: 1 mL/min; **Detector**: HRMS; Mode: Scan; Transfer Line Temp.: 300 °C; Analyzer Type: Magnetic Sector; Source Temp.: 280 °C; Tune Type: PFK; Electron Energy: 40 eV; Resolving Power: 10,000; **Instrument:** Waters AutoSpec Ultima Mass Spectrometer; **Acknowledgement:** Terry Kolic, Ontario Ministry of Environment.

Figure 2: The selectivity of the Rtx®-Dioxin2 column separates toxic TCDF and TCDD congeners from potential interferences, allowing accurate reporting for sample toxicity.



See Figure 1 for conditions.

Summary

The Rtx®-Dioxin2 column is a better choice than a cyano-based column for confirmatory analysis of dioxins and furans. The Rtx®-Dioxin2 column has higher thermal stability and equivalent or superior selectivity for critical toxic congeners, making it the ideal column for PCDF and PCDD analysis.

Acknowledgements
Restek thanks Terry Kolic of the Ontario Ministry of the Environment for her collaboration on this project.

For the latest ChromaBLOGraphy posts on dioxin and furan analysis, visit blog.restek.com

Rtx®-Dioxin2 Columns (fused silica)
(proprietary Crossbond® phase)

Description	temp. limits	qty.	cat.#
40 m, 0.18 mm ID, 0.18 µm	20 to 320/340 °C	ea.	10759
60 m, 0.25 mm ID, 0.25 µm	20 to 320/340 °C	ea.	10758
60 m, 0.18 mm ID, 0.10 µm	20 to 320/340 °C	ea.	CC1212

Shorter GC Column Speeds up Analysis of TO-15 VOCs in Air While Still Meeting Method Requirements

By Jason S. Herrington, Ph.D.

- Increase productivity by analyzing samples in nearly half the time.
- Reliably meet Method TO-15 performance requirements.
- Ensure clean blanks with highly inert SilcoCan® canisters and a TO-Clean canister cleaning system.

Testing levels of volatile organic compounds (VOCs) in air is of great importance due to their ubiquitous presence in indoor, outdoor, and personal air, and also because VOCs and their atmospheric reaction products have well known adverse environmental impacts and detrimental human health effects. EPA Method TO-15 is a performance-based guidance document that provides sampling and analytical procedures for the measurement of a subset of VOCs that have been identified as hazardous air pollutants (HAPs) [1]. Although 60 m GC columns are typically used for TO-15 VOC analysis, a shorter 30 m column was used in this investigation to determine if method criteria could be met with faster analysis times. A subset of key results and experimental details are summarized here; the complete study can be accessed at **www.restek.com/rapidTO-15**

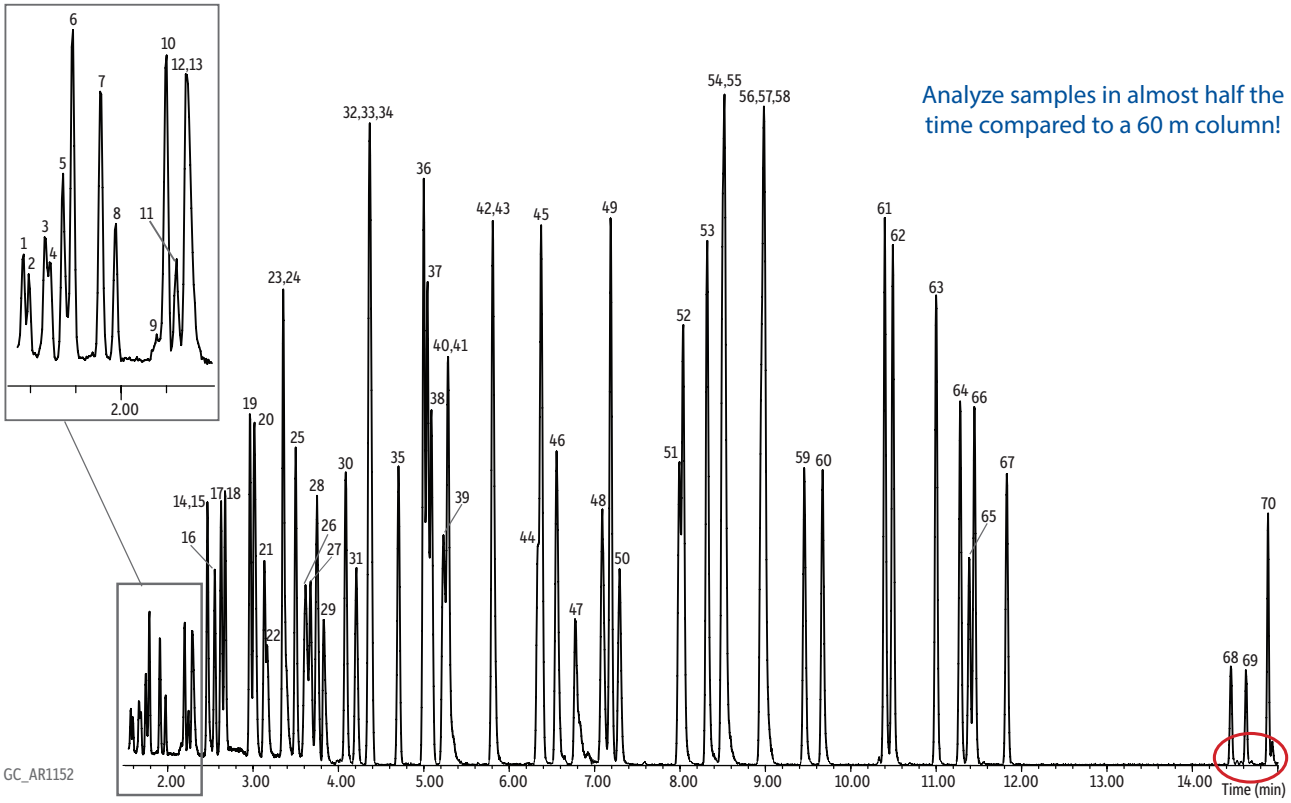
The data shown in Table I demonstrate method performance requirements were easily met using a 30 m x 0.32 mm x 1.00 µm Rxi®-5Sil MS analytical column along with a Nutech preconcentrator. Canister blank concentrations were <0.2 ppbv; the average relative standard deviation (RSD) of calibration relative response factors (RRFs) was 9.31%; average scan and selected ion monitoring (SIM) method detection limits (MDLs) were 0.06 ppbv and 35.9 pptv, respectively; average replicate precision was 6.86 %RSD; and average analytical accuracy for all 65 targeted TO-15 VOCs was 103%. Furthermore, these results were achieved in a fast 16.5 minute GC analysis time (Figure 1) and total sample cycle time was ~30 min. Use of the shorter column provided significant time savings compared to 60 m columns that typically require cycle times of 45–60 min.

Labs interested in faster analyses and increased sample throughput can access the complete study at **www.restek.com/rapidTO-15**

Acknowledgements
Nutech Instruments, Wasson-ECE Instrumentation, EST Analytical

References
[1] U.S. Environmental Protection Agency, Compendium Method TO-15, *Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared Canisters and Analyzed by Gas Chromatography-Mass Spectrometry (GC-MS)*, 1999.

Figure 1: Analysis of 65 EPA Method TO-15 compounds in 16.5 minutes on a 30 m x 0.32 mm x 1.00 µm Rxi®-5Sil MS column.



Column: Rxi®-5Sil MS, 30 m, 0.32 mm ID, 1.00 µm (cat.# 13654); **Sample:** TO-15 65 component mix (cat.# 34436), TO-14A internal standard/tuning mix (cat.# 34408); Diluent: Nitrogen; Conc.: 10.0 ppbv, 400 mL injection; **Injection:** Direct; **Oven:** Temp: 32 °C (hold 1 min) to 150 °C at 9 °C/min to 230 °C at 33 °C/min; **Carrier Gas:** He, constant flow; Flow Rate: 1.5 mL/min; Linear Velocity: 44 cm/sec @ 32 °C; **Detector:** MS; Mode: Scan; Transfer Line Temp.: 230 °C; Analyzer Type: Quadrupole; Source Temp.: 230 °C; Quad Temp.: 150 °C; Electron Energy: 69.9 eV; Solvent Delay Time: 1.0 min; Tune Type: BFB Ionization Mode: EI; Scan Range: 35 - 250 amu; Scan Rate: 3.32 scans/sec; **Preconcentrator:** Nutech 8900DS; **Trap 1 Settings:** Type/Sorbent : Glass beads; Cooling temp: -155 °C; Preheat temp: 5 °C; Preheat time: 0 sec; Desorb temp: 20 °C; Desorb flow: 5 mL/min; Desorb time: 360 sec; Bakeout temp: 200 °C; Flush flow: 120 mL/min; Flush time: 60 sec; Sweep flow: 120 mL/min; Sweep time: 60 sec; **Trap 2 Settings:** Type/Sorbent: Tenax®; Cooling temp: -35 °C; Desorb temp: 190 °C; Desorb time: 30 sec; Bakeout temp: 200 °C; Bakeout time: 10 sec; **Cryofocuser:** Cooling temp: -160 °C; Inject time: 140 sec; **Internal Standard:** Purge flow: 100 mL/min; Purge time: 6 sec; Vol.: 100 mL; ISTD flow: 100 mL/min; Standard: Size: 200 mL; Purge flow: 100 mL/min; Purge time: 6 sec; Sample flow: 100 mL/min; **Instrument:** HP6890 GC & 5973 MSD; **Acknowledgement:** Nutech Instruments. For peak identifications, visit **www.restek.com** and enter GC_AR1152 in the search.

Table I: Results from blank, calibration, MDL, precision, and accuracy experiments demonstrate Method TO-15 criteria were met by the analytical system. Results for the full list of 65 TO-15 components may be found at **www.restek.com/rapidTO-15**.

Analyte	Average Blank Concentration (pptv)[1]	Calibration (%RSD)[2]	Scan MDL (ppbv)[3]	SIM MDL (pptv)[4]	Precision (%RSD)[5]	Analytical Accuracy (%)[6]
Propylene	BDL	8.51	0.10	66.9	9.08	87.2
1,2-Dichlorotetrafluoroethane (Freon 114)	BDL	18.9	0.08	65.3	7.71	102
Ethanol	160	21.4	0.19	94.6	9.01	104
Acrolein	BDL	9.96	0.09	31.0	6.70	111
Acetone	BDL	10.8	0.14	45.1	5.55	98.8
Isopropyl alcohol	BDL	13.2	0.05	50.9	10.2	94.2
Methylene chloride	BDL	12.7	0.05	56.3	5.68	97.9
2-Butanone (MEK)	ND	7.47	0.06	39.9	7.34	99.1
Tetrahydrofuran	ND	7.97	0.08	41.6	9.72	94.0
Benzene	BDL	8.92	0.02	61.2	6.60	101
1,4-Dioxane	ND	11.5	0.08	19.6	7.10	99.1
Toluene	BDL	4.98	0.03	17.0	5.67	102
Ethylbenzene	BDL	20.3	0.03	34.3	6.10	112
o-Xylene	ND	6.38	0.02	24.7	7.50	118
1,2,4-Trimethylbenzene	ND	1.86	0.07	68.2	4.92	114
1,2-Dichlorobenzene	BDL	6.26	0.07	36.4	7.72	112
1,2,4-Trichlorobenzene	ND	15.9	0.24	39.0	6.42	89.0
Naphthalene	ND	17.7	0.15	70.3	6.82	84.9

[1]Determined by SIM analysis of six SilcoCan® air monitoring canisters (cat. # 24142-650) filled with (50% RH) nitrogen to 30 psig and stored for 3 days.
[2]RRF from five-point calibration curve in scan mode.
[3]Calculated as the standard deviation of seven replicate analyses of a 0.20 ppbv standard and the Student's *t* test value for 99% confidence.
[4]Calculated as the standard deviation of seven replicate analyses of a 75.0 pptv standard and the Student's *t* test value for 99% confidence.
[5]The average %RSD obtained from seven replicate analyses in scan and seven replicate analyses in SIM.
[6]Determined from a 10.0 ppbv audit standard.
BDL – Below detection limit
ND – Not detected

Improve Results for Chlorinated Pesticides Analysis
by GC-ECD With Florisil® SPE Cleanup

By Jason Thomas and Jonathan Keim

- Remove coextracted polar contaminants with Florisil® SPE cartridge cleanup.
- Improve quantification with low background levels and high peak responses.
- Obtain excellent recoveries for chlorinated pesticides analysis.

Many chlorinated pesticides have been banned for use because of their short- and long-term toxicity, carcinogenicity, and environmental persistence. Despite the fact that most of these chlorinated pesticides are now illegal to use, manufacture, and transport in many areas, organochlorines are a common source of pesticide poisoning that results in reportable illness. Most chlorinated pesticides have limited water solubility and mobility, but they bioaccumulate and persist in the environment. Since there is an ongoing risk of exposure from a number of sources, it is essential to test soils, wastewater, and sediments for their presence.

Standard methods for the preparation and analysis of pesticide-containing hazardous wastes require initial liquid/liquid extraction with dichloromethane, gel permeation chromatography (GPC) fractionation of higher molecular weight interferences, and a final cleanup of polar contaminants (like trichlorophenol) with Florisil® columns or Florisil® solid phase extraction (SPE) tubes before analysis with GC electron capture detection (ECD). Many labs have found that these sample cleanup precautions reduce high background levels that result in difficult quantitation and frequent GC and detector maintenance.

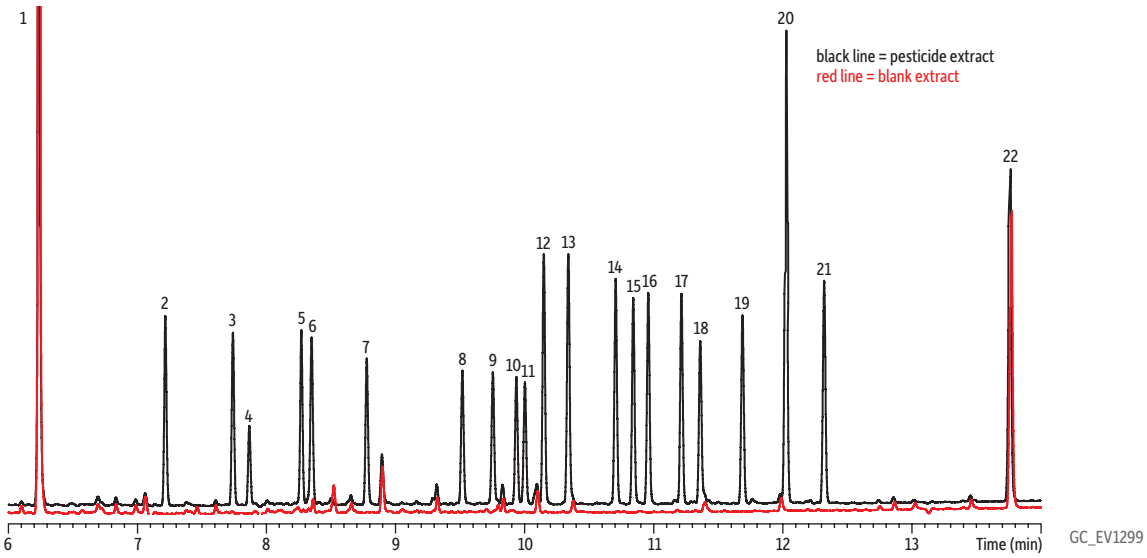
With Florisil® cleanup, extracts have lower backgrounds, producing less interference and better recoveries. Results in Table I show that recovery levels are excellent for chlorinated pesticides when following the method described in Figure 1. In addition, recovery for the breakthrough indicator compound trichlorophenol was just 3.7%, demonstrating that breakthrough was minimal. Try Resprep® Florisil® cleanup cartridges from Restek for improved data quality and reduced maintenance when analyzing chlorinated pesticides by GC-ECD.

Order today at **www.restek.com/florisil**

Table I: Florisil® SPE tubes (n=6) yield excellent recoveries for chlorinated pesticides.

Compound	tR (min)	% Recovery
2,4,5,6-Tetrachloro-m-xylene	6.23	85.2
α-BHC	7.21	86.5
γ-BHC	7.74	88.4
β-BHC	7.86	88.9
δ-BHC	8.27	93.1
Heptachlor	8.35	97.0
Aldrin	8.78	88.0
Heptachlor epoxide	9.52	93.4
trans-Chlordane	9.75	94.9
cis-Chlordane	9.94	89.5
Endosulfan I	10.00	90.2
4,4'-DDE	10.15	89.4
Dieldrin	10.34	101.2
Endrin	10.71	96.5
4,4'-DDD	10.84	93.7
Endosulfan II	10.96	92.8
4,4'-DDT	11.22	94.5
Endrin aldehyde	11.37	94.1
Endosulfan sulfate	11.69	89.9
Methoxychlor	12.03	108.4
Endrin ketone	12.32	91.5
Decachlorobiphenyl	13.76	108.4

Figure 1: Florisil® SPE cleanup reduces background levels, ensuring good responses and recoveries for target pesticides.



Column	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 universal "Y" Press-Tight® connector (cat.# 20406-261)
Sample	2,4,5,6-Tetrachloro-*m*-xylene (cat.# 32027) Decachlorobiphenyl (BZ #209) (cat.# 32029) Organochlorine pesticide mix AB #2 (cat.# 32292)
Injection	
Inj. Vol.:	2 µL splitless (hold 0.75 min)
Liner:	Sky® 4 mm single taper w/wool (cat.# 23303.5)
Inj. Temp.:	250 °C
Purge Flow:	50 mL/min
Oven	
Oven Temp.:	110 °C (hold 0.5 min) to 320 °C at 15 °C/min (hold 5 min)
Carrier Gas	He, constant flow
Flow Rate:	3.5 mL/min
Detector	µ-ECD @ 330 °C
Make-up Gas Flow Rate:	50 mL/min
Make-up Gas Type:	N2
Data Rate:	50 Hz
Instrument	Agilent/HP6890 GC
Notes	A mixed standard was prepared in 1 mL hexane (see peak list for nominal concentration of each component). For cleanup, a Florisil® tube (cat.# 24034) was first conditioned with 6 mL hexane. The 1 mL standard was then loaded on the tube and eluted with hexane:acetone (90:10), collecting 10 mL of eluent. The eluent was then concentrated down to 1 mL and analyzed.

Peaks	Conc. (ng/mL)
1. 2,4,5,6-Tetrachloro-*m*-xylene (SS)	20
2. α-BHC	5
3. γ-BHC	5
4. β-BHC	5
5. δ-BHC	5
6. Heptachlor	5
7. Aldrin	5
8. Heptachlor epoxide	5
9. *trans*-Chlordane*	5
10. *cis*-Chlordane*	5
11. Endosulfan I	5
12. 4,4'-DDE	10
13. Dieldrin	10
14. Endrin	10
15. 4,4'-DDD	10
16. Endosulfan II	10
17. 4,4'-DDT	10
18. Endrin aldehyde	10
19. Endosulfan sulfate	10
20. Methoxychlor	50
21. Endrin ketone	10
22. Decachlorobiphenyl (SS)	20

* For information regarding the nomenclature used for *cis*-chlordane and *trans*-chlordane, visit www.restek.com/chlordane-notice

Resprep® SPE Cartridges (Normal Phase)

	3 mL/500 mg (50-pk.)	6 mL/500 mg (30-pk.)	6 mL/1,000 mg (30-pk.)	15 mL/2 g (15-pk.)
Florisil (EPA SW 846 methods and CLP protocols)	24031 24032*	26086**	24034 26085**	26228

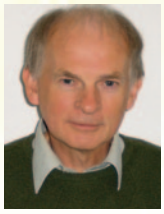
24031

All cartridges are manufactured using high density polypropylene and have polyethylene frits unless otherwise noted. Cartridges may be processed by any one or all of these techniques: positive pressure, sidearm flask, centrifuge, or vacuum manifold.

*PTFE frits **Glass tubes with PTFE frits

Selectivity in Gas Chromatography

By Dr. Colin Poole



Dr. Colin Poole *is a polychromatographer with broad interests in the separation and detection of small molecules in biological, environmental, and food samples. He is the coauthor of over 400 papers and eight books, an editor of Journal of Chromatography A and three scientific encyclopedias, and a member of the editorial boards of five other analytical chemistry journals. Dr. Poole is a professor at Wayne State University and has served in several capacities as a Science Advisor to the U.S. Food and Drug Administration.*

All chromatographers need a working knowledge of selectivity to facilitate the development of separation methods with the desirable properties of adequate resolution in a reasonable time. Selectivity is usually measured by the separation factor (the ratio of the retention factors for any two peaks in the chromatogram). The separation factor is an observation. Why two peaks are separated to different extents on different columns requires some basic understanding of the interactions between the two compounds and the separation system. This knowledge is not widespread, and many practicing chromatographers pride themselves on their ability to achieve a separation without ever unearthing how it was achieved. This is possible because resolution is the observed result from the contribution of kinetic and thermodynamic parameters on a separation. In gas chromatography the smallest useful separation factor is about 1.005 and requires a column with a plate count of slightly more than one million. A difficult task but just achievable with existing columns. With a separation factor of 1.05 a plate count of about 12,000 is all that would be required and this represents a very easy separation. Thus, large separation factors are never needed for gas chromatography and the range of separation factors required for any peak pair in the chromatogram is in the range 1.005 to 1.05. So long as mixtures are not too challenging, success seems almost guaranteed because of the high kinetic performance of the columns in gas chromatography. Or put another way, you don't have to be very lucky to take any column from the shelf and find that it can separate moderately complex mixtures by identifying a suitable temperature. If success and expertise are seen as related, then even the newest member of staff is suddenly an expert.

The how of separations, to me a true measure of expertise, requires an intimate knowledge of stationary phase chemistry and an understanding of how compounds interact with and become distinguished by the stationary phase. Over quite a lengthy career in gas chromatography, I have participated in the development and abandonment of many one-time exciting and ultimately disappointing approaches to describing and modeling selectivity in chromatography. The selectivity of a stationary phase can be defined as its relative capacity to enter into specific intermolecular interactions represented by dispersion, induction, orientation, and hydrogen bonding. The transfer of a compound in the gas to the stationary phase requires that initially a cavity is formed in the stationary phase of the same size as the solute; this is accompanied by reorganization of the stationary phase molecules to minimize the disruption of cavity formation and to establish a more favorable orientation for intermolecular interactions. Lastly the solute is inserted in the cavity and establishes solute-stationary phase interactions that vary in magnitude depending on the complementary capabilities of the solute and stationary phase to interact with each other. Cavity formation is the penalty extorted to gain entry to the stationary phase and only depends on the properties of the stationary phase.

Polar stationary phases have stronger stationary phase internal interactions and cavity formation is more costly in free energy currency. Thus, the spacing between adjacent members of a homologous series is smaller on polar phases than on low polarity phases because of the higher cost of cavity formation extorted for an increase in cavity size. To make the payment for cavity formation, the solute-stationary phase interactions set up when the solute fills the cavity need to exceed the cost of cavity formation. The complementary nature of these interactions is where selectivity comes in. All solutes will establish dispersion interactions that scale approximately with size, but interactions of a dipole-type (induction and orientation) and hydrogen bonding depend on the dipolarity, polarizability, and proton donor/acceptor properties of the solute and stationary phase. The different combinations of these interactions are why stationary phase chemistry is important in separations and why different columns are required for optimized separations of mixtures of varied compounds.

The roles of temperature in optimizing separations in gas chromatography are, first, that a minimum temperature is required to create a gas phase mixture (achieve an adequate vapor pressure to have a presence in the gas phase) and, second, that cavity formation and solute-stationary phase interactions are temperature dependent. For the latter, there are two temperature contributions: one that affects only the stationary phase (cavity formation) and the other that involves both the stationary phase and the solute (the intermolecular interactions). An important recent observation is that for moderately polar stationary phases that are stable to high temperatures (in this case, 300 °C) polar interactions endure, although they are weaker than at lower temperatures. In addition, selectivity differences between stationary phases also endure, but are generally not as great as at lower temperatures. This means that selectivity endures to high temperatures and that stationary phase selection remains important. Thus, the myth that all columns behave the same at high enough temperatures is not true for the range of typical temperatures used in gas chromatography (at least up to 300 °C). It is also possible to demonstrate that individual differences in selectivity between stationary phases are not preserved as temperature is varied and, therefore, single temperature scales of selectivity are of limited use for column selection.

There are now quite large databases for open-tubular columns that include quantitative information on selectivity and its variation with temperature. For polysiloxane stationary phases it is clear that what might be referred to as the selectivity space is not uniformly populated. There are no stationary phases that are hydrogen-bond acids and so this interaction is not exploited, although many compounds are hydrogen-bond bases. The reliance on a limited number of monomers for the synthesis of stationary phases results in islands in the selectivity space surrounded by empty space inaccessible with current stationary phases. In reality, therefore, there are plenty of opportunities for stationary phase development, and we should not become complacent with the status quo, or try to solve all problems as the new staff member alluded to earlier, by overexploitation of column efficiency.

Now Online!

Our *EZ*GC™ Web App Will Kick-Start Your GC Method Development

- ***EZ* to Register** – If you have a Restek login, you're already done! (And if you don't, you can get one at no charge and with no hassle.)
- ***EZ* to Get Started** – A quick, 5-minute video will show you everything you need to know.
- ***EZ* to Use** – Just enter your target compounds, and in seconds, the *EZ*GC™ system gives you a customized method, including column, conditions, and model chromatogram.
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- ***EZ* to Save** – Print your chromatogram and custom settings, or save them for future reference.

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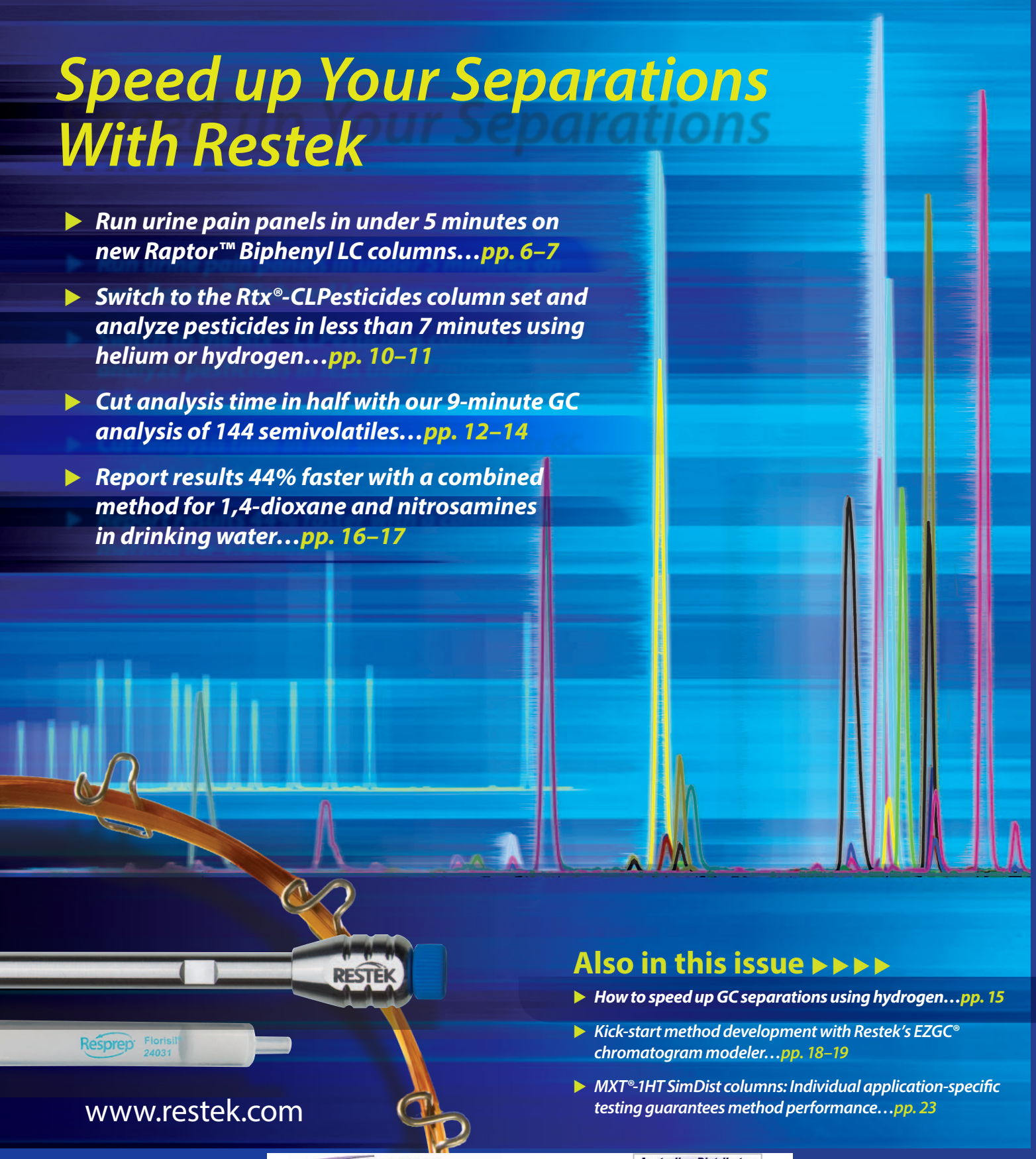
Our expertise, experience, and enthusiasm is your Advantage.

RESTEK® ADVANTAGE

Speed up Your Separations With Restek

- Run urine pain panels in under 5 minutes on new Raptor™ Biphenyl LC columns…pp. 6–7
- Switch to the Rtx®-CLPesticides column set and analyze pesticides in less than 7 minutes using helium or hydrogen…pp. 10–11
- Cut analysis time in half with our 9-minute GC analysis of 144 semivolatiles…pp. 12–14
- Report results 44% faster with a combined method for 1,4-dioxane and nitrosamines in drinking water…pp. 16–17



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Also in this issue ▶▶▶▶

- How to speed up GC separations using hydrogen…pp. 15
- Kick-start method development with Restek's EZGC® chromatogram modeler…pp. 18–19
- MXT®-1HT SimDist columns: Individual application-specific testing guarantees method performance…pp. 23

Restek® Connections

In This Issue

About Restek Corporation

For over 25 years, Restek has been a leader in the development and manufacturing of GC and LC columns, reference standards, sample preparation materials, accessories, and more. Our reputation for unbeatable Plus 1 customer service and top-quality products is well known throughout the international chromatography community, and we are proud to provide analysts around the world with products and services to monitor the quality and safety of air, water, soil, food, pharmaceuticals, chemicals, and petroleum.
www.restek.com

Reflections from the Bench

Thinking back on my days of working in a contract research lab, I can still picture our COO pacing the floor, demanding more revenue. "We need more samples!" he was known to shout. "We need more throughput! Work harder, work faster!"

This edition of the *Restek® Advantage* is full of information to help chromatographers reduce their run times, increase efficiencies, and cut costs. If we would have had this information back in the day, our COO's shouts may just have been, "Great work! Bonuses for all!"

A fairly quick and easy way to speed up GC separations is to switch your carrier gas to hydrogen. The linear velocity of hydrogen compared to helium is much faster and can potentially cut a run time in half. Throw in a hydrogen generator, and you end up with a nearly endless supply of cost-effective carrier gas. Along with general information on switching to hydrogen, this *Advantage* provides an example for reducing analysis time and cost with organochlorine pesticides on the Rtx®-CLPesticides columns.

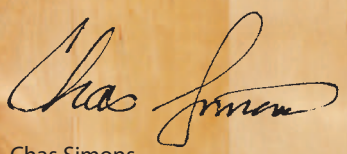
Switching column types is another way to reduce analysis times. Restek's new SPP LC column, the Raptor™ Biphenyl, permits fast separations without having to pay for a UHPLC system. A 5-minute pain panel application demonstrates the durability and speed that can be obtained on this innovative column.

Shorter GC columns with smaller internal diameters and thinner films can also increase efficiencies. A typical 25-minute semivolatile assay was cut down to 9 minutes by simply changing column dimensions. Not only will you analyze more samples in a shift, but shorter columns typically cost less, so you'll save your lab money, too.

When developing methods, wouldn't it be nice if you could just give someone a list of compounds and have them tell you the column and analytical conditions to use? Think of how many days of development time you would save per method.... Well, Restek's *EZ*GC® chromatogram modeler can do exactly that. Try it out online!

So relax, work smarter, but not necessarily harder. Turn the page to get started letting your carrier gas, column choice, and instrument pick up the speed for you.

Cheers!



Chas Simons
Technical Service Manager

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. Today's featured topic is leak detection.

Q: Why should I pay for an electronic leak detector when I can just "SNOOP"?

A: Leak detection can be done with many different tools and techniques. For example, to check the inner tube of my bicycle, I pressurize the tube, push it underwater, and look for bubbles. Using water does not negatively impact the tube's effectiveness.

Made by Swagelok Company, "Snoop®" is one brand of soap-based liquid leak detector, and it is a great product for many applications like compressor or natural gas lines. The challenge is that when a leak is present, the soap solution can enter the gas lines. In many applications, this is not an issue....

But, if the gas will be used for a high-precision analytical technique like GC or GC-MS, it is a major problem. Using liquid leak detectors on your GC lines can introduce water and soap impurities into your system.

- Water can hydrolyze deactivated silica—glass wool and stationary phases—at high temps. Polar compounds will tail and liner transfer will be more difficult. Catalytic activity will impact sensitive compounds.
- Organic impurities result in highly unstable baselines, ghost peaks, and reduced sensitivity.

Soap-based liquid leak detectors should not be used on gas chromatography systems. Instead, a Restek® electronic leak detector helps you avoid contamination and allows a safe, fast, clean check of any GC fitting for hassle-free troubleshooting. It also helps you plan preventative maintenance before a serious leak develops and turns into lost sensitivity or a costly repair.

Yes, a bottle of liquid leak detector is cheap, but the Restek® electronic leak detector helps provide security that your GC setup is correct and your results will be repeatable for the longest possible time. It will repeatedly pay for itself by protecting your instrument, minimizing downtime, and improving data quality.

Redesigned and better than ever, Restek's new electronic leak detector is highly sensitive to helium, hydrogen, and nitrogen along with argon and carbon dioxide. Learn more at **www.restek.com/leakdetector**

- **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!

Author Spotlight: Jaap de Zeeuw

International GC Specialist

An accomplished author and speaker, Jaap is a 35-year industry veteran known as "The Chrom Doctor" by *Separation Science* readers. He is currently producing a series for them on the *Impact of GC Parameters on the Separation* as a follow-up to last year's *Ghost Peaks in Gas Chromatography* feature articles. He has also published his recent work in *The Analytical Scientist, Petro Industry News, Chromatography Today, American Laboratory, the Journal of Separation Science,* and of course, through Restek. You can find links to published works by Jaap and Restek's other chromatography experts at **www.restek.com/news**

To contact Jaap directly or learn more about him, visit **www.restek.com/jaap-dezeeuw**

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.

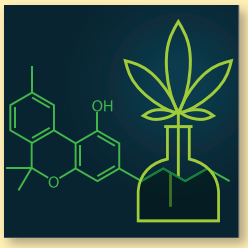
Here's a look at some of our latest posts:

- *Important Medical Marijuana Cannabinoids Analyzed by GC-FID on Rxi®-35Sil MS and Rtx®-35 Columns*
- *I'll Stop the Autosampler and Melt the LVI With You….*
- *The Raptor™ Biphenyl Blows Away a Conventional C18 in This Gunpowder Assay*
- *The International Network of Environmental Forensics 2014 Conference*

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

Hot Topics

Growing Analytical Solutions for Medical Cannabis Labs



Whether you are an experienced potency testing chemist or a manager starting a new lab, Restek has the products and expertise you need for success. From analyzing potency and pesticides by LC, to testing for terpenes and residual solvents by GC, Restek offers the chromatography columns, accessories, certified reference materials (CRMs), and sample preparation supplies that you need for accurate, reliable medical marijuana analyses.

We're proud to have helped medical cannabis labs establish sound analytical practices from the beginning, and we will continue to be there for you every step of the way as the testing landscape changes. Most recently, Restek has expanded the most comprehensive selection of cannabinoid-related reference standards by adding cannabidiolic acid (CBDA, cat.# 34094). This diluted, DEA-exempt standard is the first and only CRM on the market and is a must for accurate potency testing and strain ID.

See how Restek can help your lab grow at
www.restek.com/medical-cannabis

Raptor™ ARC-18 Columns Are Born for LC-MS/MS

The birth of the new Raptor™ SPP LC column line began with Restek's time-tested Biphenyl phase, and it has now grown to include a new Restek® phase: the ARC-18.

Designed and intended specifically for use on LC-MS/MS systems, the Raptor™ ARC-18 column offers a well-balanced retention profile without the drawbacks of using an ordinary C18 in the harsh, acidic mobile phases needed for mass spectrometry (MS). After extended use in these low-pH (≤ 2.0) conditions, the sterically protected ARC-18 offers consistent retention, peak shape, and response for charged bases, neutral acids, small polar compounds, and more. For the rapid analysis of large, multiclass assays by LC-MS/MS, the Raptor™ ARC-18 truly is *ahead of the curve*.

Superficially porous particles (SPP or "core-shell" particles) changed LC by boosting column efficiency and reducing analysis times, but they were only the beginning. Raptor™ LC columns combine the speed of SPP with the resolution of highly selective USLC® technology. Experience *Selectivity Accelerated* with this new species of chromatographic column at **www.restek.com/raptor**

Food Safety Labs: Simplify Your QuEChERS Method Evaluation

Restek's new QuEChERS performance standards kit was specifically designed to provide low-cost, "entry-level" mixes to food safety and contract labs evaluating the QuEChERS extraction approach for determining pesticides in fruits and vegetables. Our performance standards kit helps simplify standard preparation and ensure data quality, so you can set up and validate GC and LC pesticide residue screening methods faster, with greater confidence, and at significantly less cost.

Produced and tested in accordance with our ISO Guide 34 and 17025 accreditations, the Restek® QuEChERS performance standards kit is ideal for broad class work because it provides a wide range of commonly used organochlorine, organonitrogen, organophosphorus, and carbamate pesticides. Volatile, polar, active, base-sensitive, and nonvolatile compounds are included to allow comprehensive evaluation of QuEChERS extraction and cleanup efficiencies as well as optimization of instrument conditions. Analytes are divided into three ampuls for maximum stability and shelf life.

Restek offers complete solutions for labs running QuEChERS methods. Visit **www.restek.com/quechers** for additional reference materials, extraction salts, dispersive SPE cleanup tubes, GC and LC columns, accessories, and more.

EXP® Guards Help Raptor™ Columns Last Even Longer

To help further extend the life of already-rugged Raptor™ LC columns, Restek has mated our innovative superficially porous particles (SPP) with patent-pending guard column hardware developed by Optimize Technologies. The unique EXP® direct connect holder features an auto-adjusting design that provides ZDV (zero dead volume) connections to any 10-32 female port. Patented titanium hybrid ferrules can be installed repeatedly without compromising the high-pressure seal, and cartridges can be changed without breaking inlet/outlet fluid connections—and without tools!

Sold separately and available in three different IDs, a Raptor™ LC guard column cartridge in an EXP® direct connect holder is the ultimate in column protection. Raptor™ guard cartridges are available in Restek's Biphenyl and ARC-18 phases—with 5 µm particles and additional Raptor™ stationary phases coming soon.

www.restek.com/raptor

Optimal Performance and Reliable Results for Active, Unsaturated Hydrocarbons

New Rt®-Silica BOND columns are robust, selective PLOT columns that offer excellent inertness and loadability. These versatile columns are ideal for the analysis of light hydrocarbons, sulfur gases, and halocarbons above ambient temperature (up to 260 °C). Carbon dioxide and other gases can also be retained at ambient temperature.

Our unique QC testing protocols ensure consistent column-to-column performance to minimize downtime. Only Restek measures the selectivity of every column with methyl acetylene and 1,3-butadiene, unsaturated C4 hydrocarbons that are more sensitive selectivity probes than the unsaturated C3 hydrocarbon probes used by other manufacturers. In addition, Rt®-Silica BOND columns are tested to confirm efficiency and inertness in order to provide optimal peak shape and response for active analytes.

As with all Restek® PLOT columns, our proprietary manufacturing process minimizes particle generation, reducing common problems such as signal spikes, valve damage, and clogged FID jets. Rt®-Silica BOND columns also display outstanding stability in the presence of water due to their unique bonded-silica stationary phase. Find the Restek® PLOT column that's best for you at **www.restek.com/plot**

Increased Availability and 100% Guaranteed

Effective immediately, Restek Corporation has unveiled a new 100% satisfaction guarantee for all Sky® inlet liners. These exceptionally inert liners are now produced with new proprietary Restek® processes and apply some of the tightest testing specifications in the industry. The Sky® liners analysts want most are in stock now and are guaranteed to deliver outstanding performance that exceeds your expectations.

Sky® inlet liners are available in 1-, 5-, and 25-packs for most common GCs, including Agilent, Bruker/Varian, PerkinElmer, Shimadzu, and Thermo Scientific. *For details on our 100% satisfaction guarantee and to view our entire product line of robust Sky® liners, visit **www.restek.com/sky**

Put True Blue Performance to work in your GC today!

Restek at *analytica* and Beyond!

In April 2014, Munich hosted the International Trade Fair for Laboratory Technology, Analysis, and Biotechnology—better known to most as *analytica*. Championed by our German subsidiary, Restek GmbH (**www.restekgmbh.de**), Restek was there to greet this year's record 34,000+ attendees, taking the opportunity to meet with many of you face-to-face, share our latest innovations, and answer your questions about Restek® products and services.

Of course, it wasn't all business over the show's four days. Clad in our World Cup chromatography jerseys, we offered chances to win big at the "Catch Me If You Can" game while Herr Branicki treated visitors to delicious waffles on a stick!

We are looking forward to returning to Munich for *analytica 2016*, but why wait until then? Restek has a packed events calendar for the remainder of 2014. See below for a few highlights or visit **www.restek.com/events** for a full list.

See you on the road!

2014 Events Calendar

ASMS | June 15–19 | Baltimore, MD, U.S.
BFR | June 22–24 | Indianapolis, IN, U.S.
A&WMA | June 24–27 | Long Beach, CA, U.S.
EPRW | June 30–July 3 | Dublin, Ireland

NACRW | July 20–23 | St. Pete Beach, FL, U.S.
PRChem | July 29–Aug 3 | San Juan, Puerto Rico

NEMC | Aug 4–8 | Washington, DC, U.S.
INEF | Aug 4–6 | Cambridge, UK
Dioxin | Aug 31–Sept 5 | Madrid, Spain

JASIS | Sept 3–5 | Makuhari, Chiba, Japan
AOAC | Sept 7–10 | Boca Raton, FL, U.S.
analytica China | Sept 24–26 | Shanghai, China
COLACRO | Sept 29–Oct 3 | Cartagena, Colombia

Turn to Raptor™ SPP Biphenyl LC Columns for Fast, Rugged Pain Panels

By Sharon Lupo, Ty Kahler, and Paul Connolly

- Retain and separate isobaric compounds for more definitive results.
- Increase sample throughput with fast, 5-minute analysis times.
- Run longer before replacing your column.

The industry-leading Biphenyl is one of Restek's most popular LC stationary phases because it is particularly adept at separating compounds that are challenging to resolve or that elute early on other phenyl and C18 chemistries. By combining this innovative ligand with the speed of superficially porous particles (commonly referred to as SPP or "core-shell" particles), Restek's R&D chemists created the robust Raptor™ Biphenyl—a column that is extremely useful for fast separations in bioanalytical testing applications like pain panels or other drug and metabolite analyses, especially those that require a mass spectrometer (MS).

More Aromatic Selectivity for Bioanalytical Analyses

SPP core-shell columns commonly employ traditional phenyl-hexyl stationary phases, but the Biphenyl is the next generation of phenyl column chemistry. It provides greater aromatic selectivity than commercially available phenyl-hexyl columns [1] and a higher degree of dispersion than conventional phenyls. As a result, the Raptor™ Biphenyl allows you to more easily separate bioanalytical compounds, such as aromatics (Figure 1).

Rugged Pain Panels From Urine in Under 5 Minutes

Pain panels can be difficult to optimize and reproduce due to the limited selectivity of C18 and phenyl-hexyl phases, but not on the Raptor™ Biphenyl. Pain panel analyses using a Raptor™ Biphenyl column offer full peak elution in under 3.5 minutes with complete isobaric resolution and a completed cycle time of less than 5 minutes (Figure 2). Widely used competitor columns give you tailing peaks, longer run times, and coelutions; but the Raptor™ Biphenyl exhibits the selectivity and performance needed for this critical analysis.

Clinically Proven to Optimize Your Bioanalytical Workflows

For nearly a decade, the Restek® Biphenyl has been the column of choice for clinical testing because of its ability to produce highly retentive, selective, and rugged (see page 8) reversed-phase separations of drugs and metabolites. By bringing the speed of SPP to the Biphenyl family, the Raptor™ Biphenyl column presents clinical labs with an even faster option for pain panels in urine and many other important clinical assays—without the expensive UHPLC instrumentation.

To see how the Raptor™ Biphenyl can help you improve your bioanalytical run times and results, visit **www.restek.com/ADV1411**

Figure 1: Raptor™ Biphenyl columns exhibit the highest aromatic selectivity compared to other SPP phenyl columns.

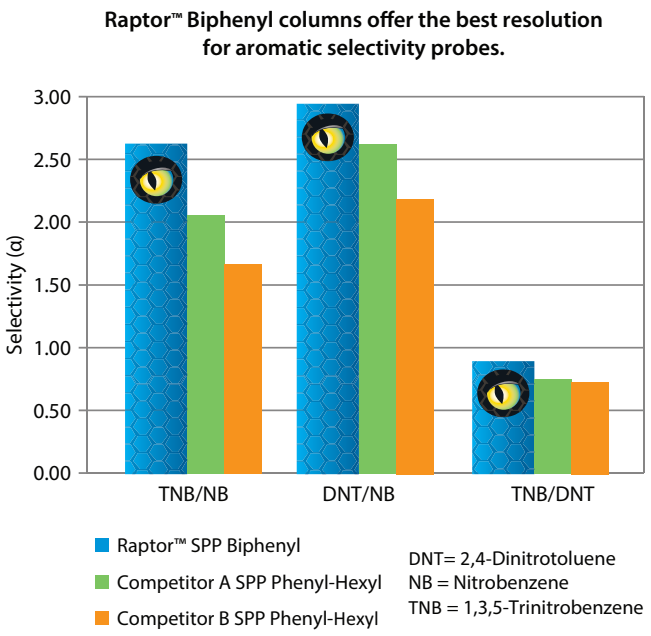
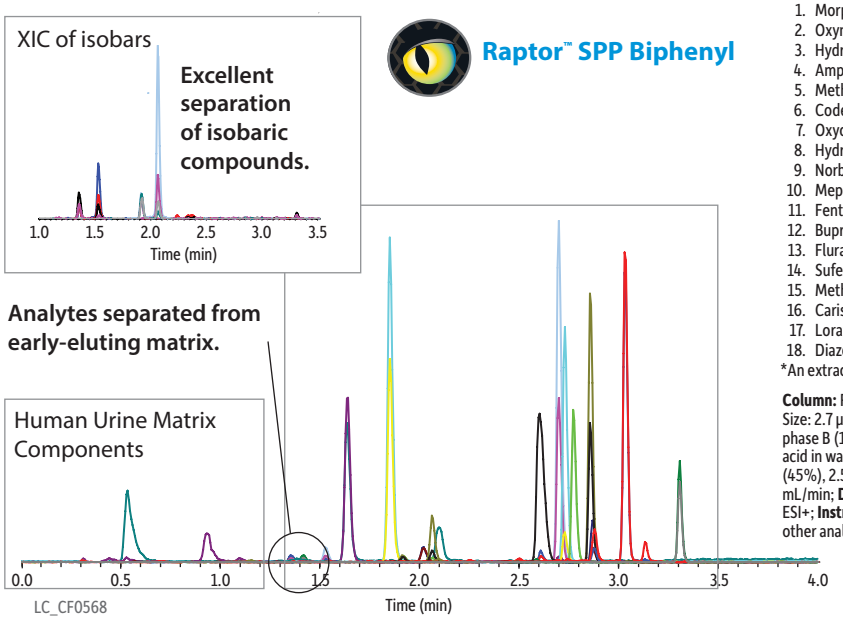


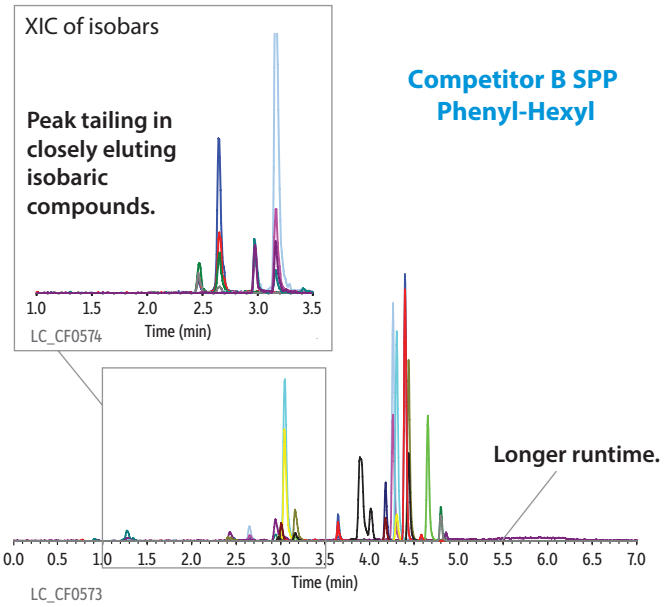
Figure 2: Get complete isobaric resolution and sub-5-minute pain panel runs with the Raptor™ Biphenyl column.

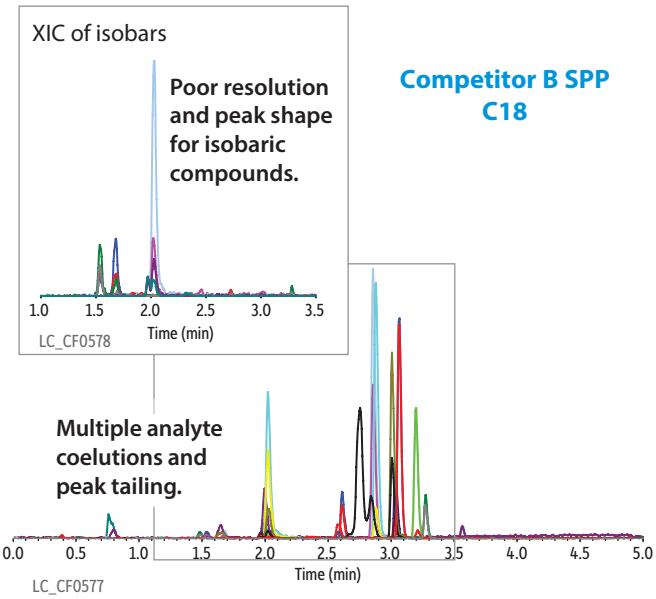


	Peaks	t_R (min)	Precursor ion	Product ion 1	Product ion 2
1.	Morphine*	1.34	286.2	152.3	165.3
2.	Oxymorphone	1.40	302.1	227.3	198.2
3.	Hydromorphone*	1.52	286.1	185.3	128.2
4.	Amphetamine	1.62	136.0	91.3	119.2
5.	Methamphetamine	1.84	150.0	91.2	119.3
6.	Codeine*	1.91	300.2	165.4	153.2
7.	Oxycodone	2.02	316.1	241.3	256.4
8.	Hydrocodone*	2.06	300.1	199.3	128.3
9.	Norbuprenorphine	2.59	414.1	83.4	101.0
10.	Meprobamate	2.61	219.0	158.4	97.2
11.	Fentanyl	2.70	337.2	188.4	105.2
12.	Buprenorphine	2.70	468.3	396.4	414.5
13.	Flurazepam	2.73	388.2	315.2	288.3
14.	Sufentanil	2.77	387.2	238.5	111.3
15.	Methadone	2.86	310.2	265.3	105.3
16.	Carisoprodol	2.87	261.2	176.3	158.1
17.	Lorazepam	3.03	321.0	275.4	303.1
18.	Diazepam	3.31	285.1	193.2	153.9

*An extracted ion chromatogram (XIC) of the isobars is presented in the inset.

Column: Raptor™ Biphenyl (cat.# 9309A5E); Dimensions: 50 mm x 3.0 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 30 °C; **Sample:** Diluent: urine:mobile phase A:mobile phase B (17:76:7); Conc.: 10-100 ng/mL; Inj. Vol.: 10 µL **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in methanol; **Gradient (%B):** 0.00 min (10%), 1.50 min (45%), 2.50 min (100%), 3.70 min (100%), 3.71 min (10%) 5.00 min (10%); **Flow:** 0.6 mL/min; **Detector:** AB SCIEX API 4000™ MS/MS; Ion Source: TurboIonSpray®; Ion Mode: ESI+; **Instrument:** API LC-MS/MS; **Notes:** Lorazepam was prepared at 100 ng/mL; all other analytes are 10 ng/mL.





Column: Competitor B SPP Phenyl-Hexyl; Dimensions: 50 mm x 4.6 mm ID; Particle Size: 2.6 µm; Pore Size: 100 Å; Temp.: 22 °C; **Sample:** Diluent: urine:mobile phase A:mobile phase B (17:76:7); Conc.: 10-100 ng/mL; Inj. Vol.: 10 µL **Mobile Phase:** A: 10 mM ammonium formate in water, B: 0.1% formic acid in methanol; **Gradient (%B):** 0.00 min (5%), 4.00 min (100%), 5.00 min (100%), 5.10 min (5%), 7.00 min (5%); **Flow:** 0.6 mL/min; **Detector:** AB SCIEX API 4000™ MS/MS; Ion Source: TurboIonSpray®; Ion Mode: ESI+; **Instrument:** API LC-MS/MS; **Notes:** Lorazepam was prepared at 100 ng/mL; all other analytes are 10 ng/mL.

Column: Competitor B SPP C18; Dimensions: 50 mm x 3.0 mm ID; Particle Size: 2.6 µm; Pore Size: 100 Å; Temp.: 25 °C; **Sample:** Diluent: urine:mobile phase A:mobile phase B (17:76:7); Conc.: 10-100 ng/mL; Inj. Vol.: 10 µL **Mobile Phase:** A: 10 mM ammonium formate in water, B: 0.1% formic acid in methanol; **Gradient (%B):** 0.00 min (5%), 3.00 min (100%), 4.00 min (100%), 4.10 min (5%), 5.00 min (5%); **Flow:** 0.5 mL/min; **Detector:** AB SCIEX API 4000™ MS/MS; Ion Source: TurboIonSpray®; Ion Mode: ESI+; **Instrument:** API LC-MS/MS; **Notes:** Lorazepam was prepared at 100 ng/mL; all other analytes are 10 ng/mL.

[1] In-house testing based on: M. R. Euerby, P. Petersson, W. Campbell, W. Roe, *Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing phenyl moieties using principal component analysis,* J. Chromatogr. A 1154 (2007) 138–151.

Raptor™ Biphenyl LC Columns (USP L11)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
30 mm	9309A32	9309A3E	9309A35
50 mm	9309A52	9309A5E	9309A55
100 mm	9309A12	9309A1E	9309A15
150 mm	9309A62	9309A6E	9309A65

Guard cartridges and Raptor™ phases also available.

Raptor™ LC Columns: The New Standard for Performance and Durability in SPP Core-Shell Columns

By Paul Connolly

Superficially porous particles (commonly referred to as SPP or "core-shell" particles) have been proven to provide fast separations without the need for expensive ultra high performance liquid chromatography (UHPLC) instruments, thereby increasing sample throughput without capital investment. These particles feature a solid, impermeable core enveloped by a thin, porous layer of silica that decreases the diffusion path and reduces peak dispersion. As a result, they offer significantly higher efficiency than traditional fully porous particles of similar dimensions—often rivaling the efficiency of smaller particles. However, fast analyses and high efficiency are of little value if the column cannot repeatedly withstand aggressive, high-pressure conditions.

Stable Efficiencies Under Elevated Back Pressures

One of the greatest advantages of an SPP column is the ability to achieve fast, efficient separations by operating at higher linear velocities than are possible with a conventional fully porous particle column. However, these higher velocities also result in higher back pressures. Raptor™ columns were designed to handle the increased pressures needed to achieve *Selectivity Accelerated*, and handle them far better than other SPP columns.

Restek's R&D team exposed Raptor™ columns, as well as similar columns from other manufacturers, to high linear velocities using a mobile phase of 55% acetonitrile in water. We measured the efficiency of these columns using a common reverse phase test mix and found that within 300–1,100 injections, competition columns started exhibiting a drastic loss of efficiency compared to their pretest value. Raptor™ columns, however, showed no signs of loss, maintaining their efficiency to within ± 3% of the pretest value over the course of 3,000 injections (Figure 1). Due to Restek's proprietary packing procedures, Raptor™ columns feature this exemplary robustness regardless of internal dimension (Figure 2).

Column-to-Column and Lot-to-Lot Reproducibility

To help keep your productivity high and your lab expenses low, an LC column must produce exceptional selectivity and fast analysis times not just once, but every time. Ruggedness and repeatability are essential, which is why from the silica and the bonding technique, to the packing process and upgraded hardware, every decision that went into creating new Raptor™ LC columns was made to ensure superlative retention time reproducibility, from injection to injection (Figure 3) and from lot to lot (Figure 4). We also adopted new quality control (QC) specifications to guarantee the retention time stability you need for worry-free MRM analyses.

> Outstanding stability and reproducibility mean consistent, reliable results.

Experience *Selectivity Accelerated*

Due in part to their outstanding stability and reproducibility, Raptor™ LC columns provide the practicing analyst with the most powerful tools available for fast, reliable data and efficient method development. And because they are from Restek, they are backed by the manufacturing and quality systems you've come to trust along with the best Plus 1 service in the industry. Choose Raptor™ columns for all of your valued assays to experience *Selectivity Accelerated*.

Learn more about Raptor™ SPP LC columns at
www.restek.com/ADV1412

Figure 1: At high pressures, competitor columns experience a quick and sharp drop in efficiency, but Raptor™ columns are unaffected to at least 3,000 injections.

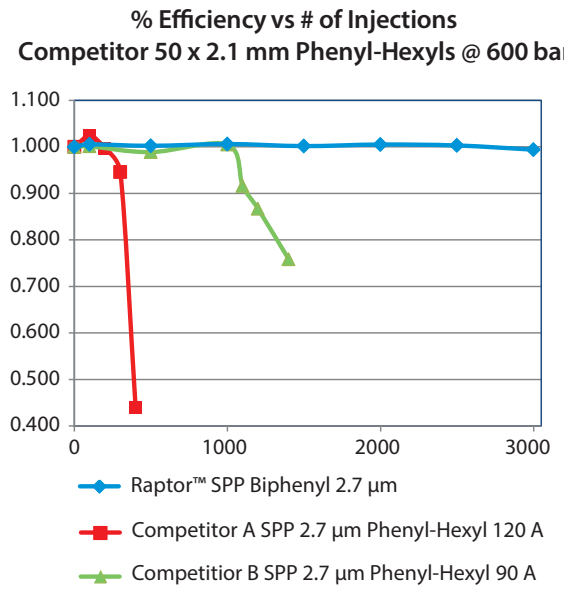


Figure 2: Regardless of diameter, Raptor™ columns exhibit the same ability to maintain excellent efficiency.

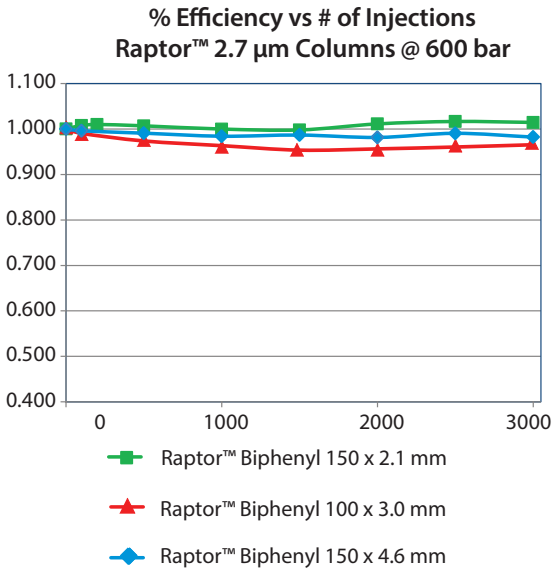
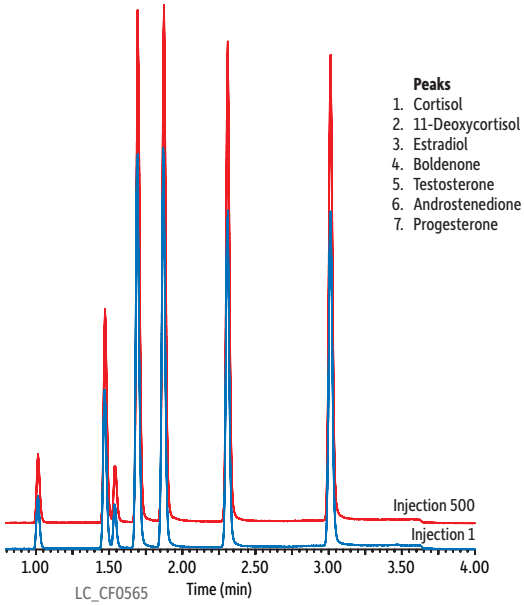
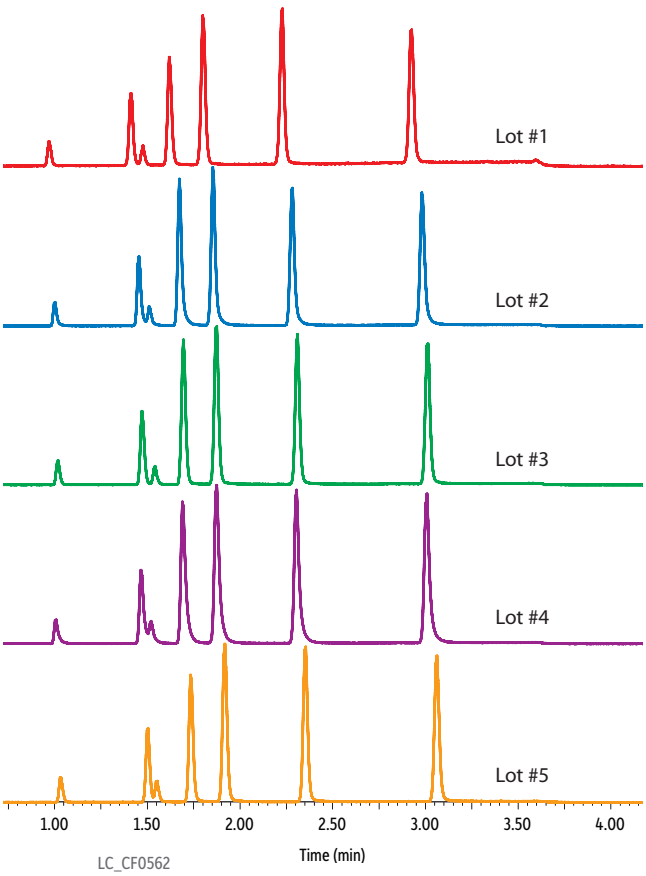


Figure 3: Even after hundreds of injections, a Raptor™ column will provide consistent, reliable data.



Column: Raptor™ Biphenyl (cat.# 9309A1E); Dimensions: 100 mm x 3.0 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Temp.: 30 °C; **Sample:** Diluent: initial mobile phase; Conc.: 50 ng/mL; Inj. Vol.: 5 µL; **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (40%), 3.00 min (80%), 3.01 min (40%), 5.00 min (40%); **Flow:** 0.700 mL/min; **Detector:** Waters Xevo TQ-S; Ion Mode: ESI+; **Instrument:** Waters.

Figure 4: From one lot to the next, every Raptor™ Biphenyl column you purchase will perform the same.



See Figure 3 for compound list and conditions.

Raptor™ ARC-18 LC Columns (USP L1)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
30 mm	9314A32	9314A3E	9314A35
50 mm	9314A52	9314A5E	9314A55
100 mm	9314A12	9314A1E	9314A15
150 mm	9314A62	9314A6E	9314A65

See page 7 for Raptor™ Biphenyl columns.
Additional phases coming soon!

Speed up Organochlorine Pesticides GC-Micro-ECD Analysis and Reduce Costs by Switching to Hydrogen Carrier Gas and Rtx®-CLPesticides Columns

By Jason Thomas, Chris English, Jack Cochran, and Gary Stidsen

Over the past few years, the increasing cost and uncertain availability of helium have prompted many labs to explore the use of hydrogen as an alternate carrier gas. While switching from helium to hydrogen can be problematic for methods using a mass spectrometer, methods that employ an electron capture detector (ECD) are generally good candidates for transfer. For commonly used ECD methods that use helium, such as organochlorine pesticides analysis following EPA Method 8081, conversion to hydrogen provides a significant cost savings opportunity. Since Rtx®-CLPesticides and Rtx®-CLPesticides2 columns were developed specifically for organochlorine pesticides analysis by GC-ECD, their unique column selectivities provide optimal peak resolution and fast analysis times whether operated using helium or hydrogen. In addition, since the Rtx®-CLPesticides columns provide good separation between critical peaks, they can be trimmed for maintenance without sacrificing resolution which results in longer column lifetimes.

Get Results Fast With Helium and Rtx®-CLPesticides Columns

Despite cost and availability concerns, helium continues to be commonly used as a carrier gas because it provides excellent separations in a reasonable analysis time. The Rtx®-CLPesticides column set can be operated using helium under an accelerated multi-ramp oven program to obtain sub-7 minute run times (Figure 1), the fastest analysis times in the industry. In our evaluation of a competitor's CLP column set, the Rtx®-CLPesticides column pair provided faster separations regardless of the carrier gas that was used (visit **www.restek.com/ADV1413** for comparative data). These columns are robust enough to maintain resolution after heavy use and aggressive conditions. Their unique selectivity ensures all compounds are adequately separated for accurate integration, and fast analysis times allow maximum sample throughput using helium. Note that in order to achieve the separation shown here, it may be necessary to use an oven pillow or 220 V instrument, otherwise acceptable resolution may not be obtained because the actual oven temperatures and ramp rates may be lower and slower than the instrument settings.

Save Money by Converting to Hydrogen Carrier Gas

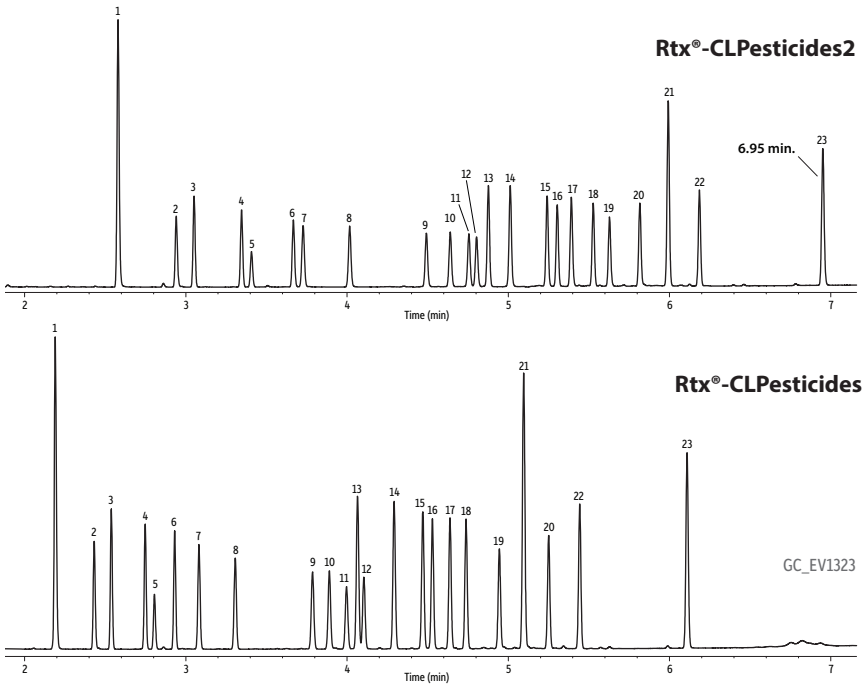
Hydrogen is a popular alternative to helium carrier gas because it is less expensive and its higher optimal linear velocity means faster flow rates can be used. Faster flow rates result in shorter analysis times and increased sample throughput. To convert from helium to hydrogen carrier gas, we adjusted the flow rate such that the linear velocity increased and kept the same aggressive oven program. As shown in Figure 2, the Rtx®-CLPesticides columns provided good separations and the analysis time was reduced to just 6.75 min. While analysis times are usually much faster when switching from helium to hydrogen, they are comparable in this case because the optimized selectivity of the Rtx®-CLPesticides column pair allowed helium to be used at an extremely fast flow rate well above its optimal linear velocity.

> Whether you stick with helium or save money by converting to hydrogen, Rtx®-CLPesticides columns give you ideal separations in less than 7 minutes!

Since hydrogen is explosive, safety must be considered when switching carrier gases. Fortunately, hydrogen generators minimize much of the risk. Hydrogen generators produce hydrogen gas on demand, so much smaller quantities are stored compared to high-pressure gas cylinders. In addition, generators use controlled flow rates, built-in sensors, and automatic shut-off features that turn the unit off in the event a leak is detected. With these safety features engineered into the unit, hydrogen generators reduce much of the risk associated with using hydrogen carrier gas.

One issue with using hydrogen that receives less attention than safety concerns is the potential for chemical reactions that create active sites and decreased peak response. This can be caused by metal

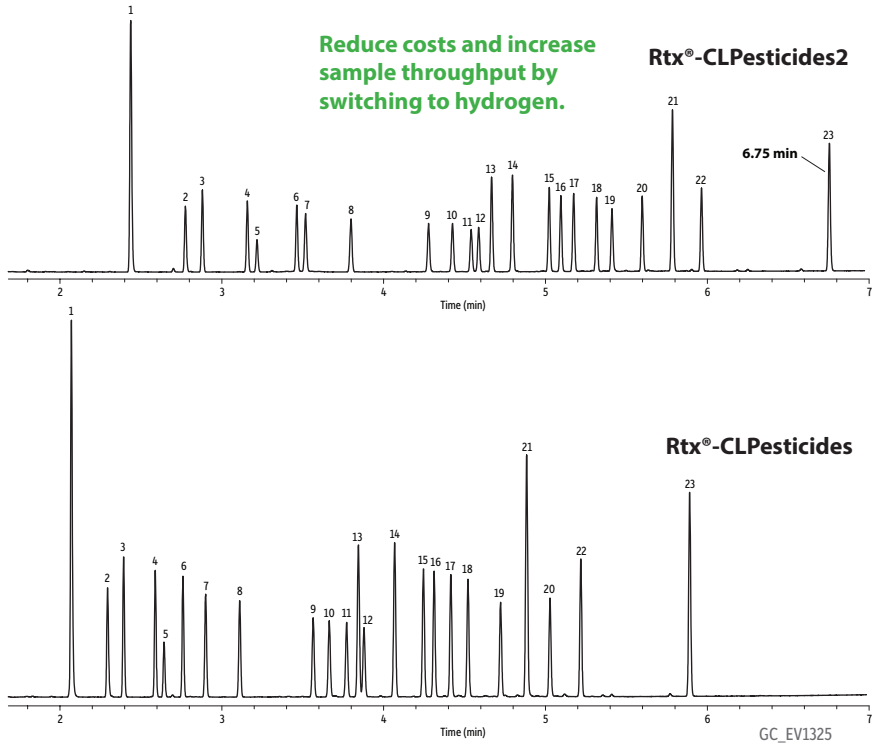
Figure 1: Rtx®-CLPesticides columns provide fast analysis times using helium, allowing sample throughput to be maximized.



Peaks	Conc. (µg/mL)	Peaks	Conc. (µg/mL)
1. Tetrachloro-*m*-xylene	20	13. 4,4'-DDE	10
2. Hexachlorobenzene	5	14. Dieldrin	10
3. α-BHC	5	15. Endrin	10
4. γ-BHC	5	16. 4,4'-DDD	10
5. β-BHC	5	17. Endosulfan II	10
6. δ-BHC	5	18. 4,4'-DDT	10
7. Heptachlor	5	19. Endrin aldehyde	10
8. Aldrin	5	20. Endosulfan sulfate	10
9. Heptachlor epoxide	5	21. Methoxychlor	50
10. *trans*-Chlordane	5	22. Endrin ketone	10
11. *cis*-Chlordane	5	23. Decachlorobiphenyl	20
12. Endosulfan I	5		

Columns: Rtx®-CLPesticides2 30 m, 0.32 mm ID, 0.25 µm (cat.# 11324) and Rtx®-CLPesticides 30 m, 0.32 mm ID, 0.32 µm (cat.# 11141) using Rxi® guard column 5 m, 0.32 mm ID (cat.# 10039) with universal "Y" Press-Tight® connector (cat.# 20406-261); **Sample:** Organochlorine pesticide mix AB #2 (cat.# 32292); 2,4,5,6-Tetrachloro-*m*-xylene (cat.# 32027); Decachlorobiphenyl (BZ #209) (cat.# 32029); Hexachlorobenzene (cat.# 32231); Diluent: *n*-Hexane; **Injection:** Inj. Vol.: 2 µL splitless (hold 0.3 min); Liner: Sky® 4 mm single taper w/wool (cat.# 23303.5); Inj. Temp.: 250 °C; Purge Flow: 50 mL/min; **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, constant flow; Linear Velocity: 71 cm/sec; **Detector:** Micro-ECD @ 340 °C; Make-up Gas Flow Rate: 50 mL/min: Make-up Gas Type: N_2; Data Rate: 50 Hz; **Instrument:** Agilent/HP6890 GC.

Figure 2: Using hydrogen carrier gas saves money, reduces helium dependence, and speeds up analysis times for organochlorine pesticides analysis.



See Figure 1 for peak identification. **Columns:** Rtx®-CLPesticides2 30 m, 0.32 mm ID, 0.25 µm (cat.# 11324) and Rtx®-CLPesticides 30 m, 0.32 mm ID, 0.32 µm (cat.# 11141) using Rxi® guard column 5 m, 0.32 mm ID (cat.# 10039) with universal "Y" Press-Tight® connector (cat.# 20406-261); **Sample:** Organochlorine pesticide mix AB #2 (cat.# 32292); Hexachlorobenzene (cat.# 32231); 2,4,5,6-Tetrachloro-*m*-xylene (cat.# 32027); Decachlorobiphenyl (BZ #209) (cat.# 32029); **Injection:** Inj. Vol.: 2 µL splitless (hold 0.3 min); Liner: Sky® 4 mm single taper w/wool (cat.# 23303.5); Inj. Temp.: 250 °C; Purge Flow: 50 mL/min; **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**: H_2, constant flow; Linear Velocity: 81 cm/sec; **Detector**: Micro-ECD @ 340 °C; Make-up Gas Flow Rate: 50 mL/min; Make-up Gas Type: N_2; Data Rate: 50 Hz; Instrument: Agilent/HP6890 GC.

did you **know**?

Rtx®-CLPesticides are ideal for more than just pesticides. You can analyze pesticides, PCBs, herbicides and more with this unique column set. Visit **www.restek.com/CLP7** for chromatograms, sample prep supplies, and reference standards for these and other applications.

shavings in the liner catalyzing a hydrogenation reaction between the hydrogen carrier gas and the pesticides. Since metal shavings are generated by the needle scraping a metal needle guide, using a Merlin Microseal septum and a 23-gauge needle will eliminate this problem and is recommended when using hydrogen carrier gas. Merlin Microseal septa are made from a wear-resistant fluorocarbon elastomer. The elastomer construction of the Merlin Microseal, in combination with its unique two-stage sealing mechanism and rounded needle, greatly reduce the generation of metal particles in the liner.

Regardless of the carrier gas you choose, the success of your analysis depends in large part on which analytical columns are used. Whether you stick with helium or explore using hydrogen, Restek's Rtx®-CLPesticides column set provides optimal peak resolution and fast analytical run times.

Visit **www.restek.com/ADV1413** for the complete method evaluation.

Restek Recommends

Set up for Success

- **Rtx®-CLPesticides column kit**
  www.restek.com/CLPkit
- **Hydrogen generators**
  www.restek.com/Gas-Generators
- **Merlin Microseal septa and 23-gauge needle**
  www.restek.com/Merlin
- **MXT® "Y"-Union connector kits**
  www.restek.com/MXTconnector

Cut Analysis Time in Half for Semivolatiles Using Split Injection and Fast GC

By Chris Rattray, Michelle Misselwitz, and Jack Cochran

- Analyze 144 semivolatiles in just 9 minutes with fast GC.
- Maintain critical separations with an Rxi®-5ms GC column.
- Reduce downtime for maintenance with split injection and guard column.

Semivolatile compounds are usually analyzed by GC using splitless injection because they must be accurately detected at very low levels. For example, reporting limits are 10 ng/L in waters and 0.33 mg/kg in soils and sludges for EPA Method 8270D. Generally speaking, splitless injection is used instead of split injection when such low-level detection is required, because more of the sample is introduced onto the column. The tradeoff is that with splitless injection, total analysis times for semivolatiles are relatively long and typically range from 22 to 28 minutes on 30 m x 0.25 mm x 0.25 µm or 30 m x 0.25 mm x 0.50 µm 5% phenyl-type columns. However, recent hardware advances enable the performance of fast GC analysis using short, narrow bore, high efficiency columns. Using split injection and fast GC with a 10 m segment of a 0.18 mm x 0.18 µm Rxi®-5ms column, analysis of 144 semivolatiles can be completed in less than 9 minutes.

Setting up for Speed

In order to achieve the fast analysis shown in Figure 1, it is critical that you measure the effective length of the composite column, which in all GC columns varies from segment to segment due to internal diameter variation of the fused silica tubing. This is important because there are some critical separations which are highly dependent on elution temperature. This is most accurately done by measuring the dead time (holdup time) of an unretained peak and then adjusting the instrument's pressure/flow calculator until the calculated holdup time matches the experimentally determined time. For more on the importance of using an accurate column length, visit Restek's blog (ChromaBLOGraphy) at **www.restek.com/ADV1414** for further discussion and detailed instructions.

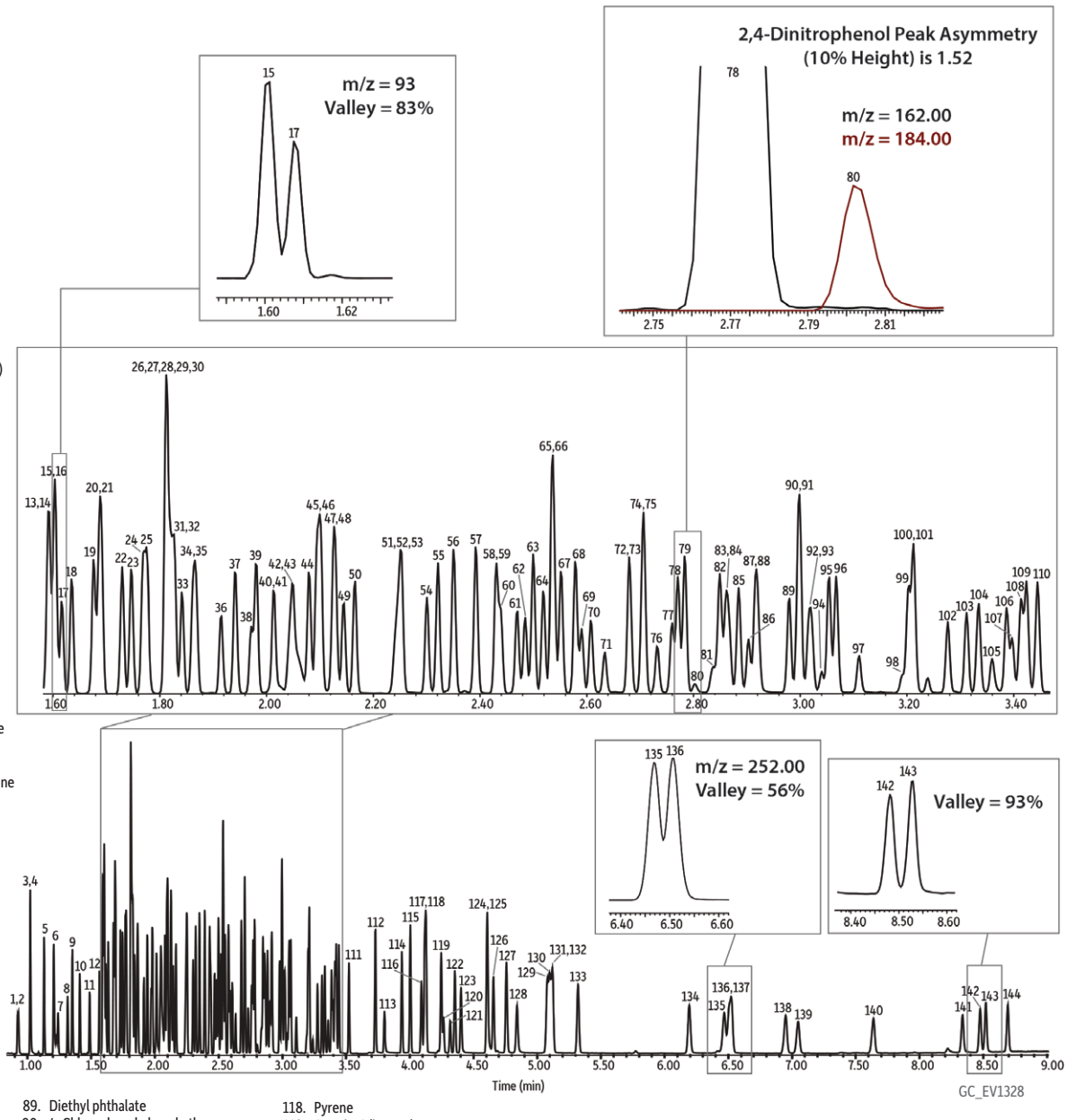
Because this is a fast analysis using a narrow-bore, high efficiency column, the widths of the early eluting peaks are extremely narrow (approximately 1.2 seconds at the baseline). Therefore, a high acquisition rate is required to properly define the peak. For this work, a speed of 11.5 scans per second was used to minimize the spectral tilt that plagues scanning quadrupole instruments when acquisition rates are too low. The instrument setup and additional operating conditions are detailed in Figure 1. Note that a retentive guard column (Rxi®-5ms) was used to further minimize the amount of non-volatile extracted material introduced on to the head of the analytical column. In conjunction with split injection, using a guard column protects the analytical column and results in reduced downtime for maintenance and less frequent mass spec venting for analytical column replacement.

Meet Calibration and Resolution Requirements

In order to assess the viability of split injection and fast GC for semivolatiles analysis, calibration and chromatographic performance were assessed. First, a 7-point calibration curve was prepared at 2.0 to 120 µg/mL using Restek® 8270 reference standards, establishing an on column calibration range of 0.1 to 6.0 ng. Calibration response factors (RF) and minimum criteria are presented in Table I for a select group of compounds. The average %RSD for the entire calibration was 11%, with very few compounds exceeding method criteria. Additionally, only two compounds failed to meet minimum response factor criteria at the lowest calibration level (0.1 ng on column), pentachlorophenol (min RF 0.050) and 2,4-dinitrophenol (min RF 0.010); however, both compounds met method linearity requirements when evaluated from 5.0 to 120 µg/mL (0.25 to 6.0 ng on column).

It is important to note that resolution is not compromised for any of the critical separations. The benzo[b]fluoranthene–benzo[k]fluoranthene separation is shown at the expected calibration verification level (CCV) of 20 µg/mL (1.0 ng on column), where it is evaluated as 56% valley. This is close to the 50% valley criteria specified in the method, and testing showed it remained stable even after more than a meter of guard was removed as long as the composite column length is calibrated in the software. In addition, the aniline–bis(2-choroethyl)ether separation is another critical isobaric separation that is extremely sensitive to elution temperature. While split injection normally allows you to increase the starting temperature

Figure 1: All key semivolatile compounds were resolved in just 9 minutes using split injection and the fast GC setup shown here.



Peaks

1. 1,4-Dioxane-d8 (IS)
2. 1,4-Dioxane
3. N-Nitrosodimethylamine
4. Pyridine
5. Ethyl methacrylate
6. 2-Picoline
7. N-Nitrosomethylethylamine
8. Methyl methanesulfonate
9. 2-Fluorophenol (SS)
10. N-Nitrosodiethylamine
11. Ethyl methanesulfonate
12. Benzaldehyde
13. Phenol-d6 (SS)
14. Phenol
15. Aniline
16. Pentachloroethane
17. Bis(2-chloroethyl) ether
18. 2-Chlorophenol
19. 1,3-Dichlorobenzene
20. 1,4-Dichlorobenzene-d4 (IS)
21. 1,4-Dichlorobenzene
22. Benzyl alcohol
23. 1,2-Dichlorobenzene
24. 2-Methylphenol
25. Bis(2-Chloroisopropyl)ether
26. *p*-Cresol (4-methylphenol)
27. *m*-Cresol (3-methylphenol)
28. Acetophenone
29. N-Nitrosopyrrolidine
30. N-Nitrosomorpholine
31. N-Nitrosodi-*n*-propylamine
32. *o*-Toluidine
33. Hexachloroethane
34. Nitrobenzene-d5 (SS)
35. Nitrobenzene
36. N-Nitrosopiperidine
37. Isophorone
38. 2-Nitrophenol
39. 2,4-Dimethylphenol
40. Bis(2-chloroethoxy)methane
41. Benzoic acid
42. 2,4-Dichlorophenol
43. α,α-Dimethylphenethylamine (Phentermine)
44. 1,2,4-Trichlorobenzene
45. Naphthalene-d8 (IS)
46. Naphthalene
47. 4-Chloroaniline
48. 2,6-Dichlorophenol
49. Hexachloropropene
50. Hexachlorobutadiene
51. Caprolactam
52. N-Nitroso-*n*-butylamine
53. 1,4-Phenylenediamine
54. 4-Chloro-3-methylphenol
55. Isosafrole I (isomer)
56. 2-Methylnaphthalene
57. 1-Methylnaphthalene
58. Isosafrole II (isomer)
59. 1,2,4,5-Tetrachlorobenzene
60. Hexachlorocyclopentadiene
61. 2,4,6-Trichlorophenol
62. 2,4,5-Trichlorophenol
63. 2-Fluorobiphenyl (SS)
64. Safrole
65. Biphenyl
66. 2-Chloronaphthalene
67. 1-Chloronaphthalene
68. Diphenyl ether
69. 2-Nitroaniline
70. 1,4-Naphthoquinone
71. 1,4-Dinitrobenzene
72. Dimethyl phthalate
73. 1,3-Dinitrobenzene
74. 2,6-Dinitrotoluene
75. Acenaphthylene
76. 1,2-Dinitrobenzene
77. 3-Nitroaniline
78. Acenaphthene-d10 (IS)
79. Acenaphthene
80. 2,4-Dinitrophenol
81. 4-Nitrophenol
82. Dibenzofuran
83. Pentachlorobenzene
84. 2,4-Dinitrotoluene
85. 1-Naphthalamine
86. 2,3,5,6-Tetrachlorophenol
87. 2-Naphthalamine
88. 2,3,4,6-Tetrachlorophenol
89. Diethyl phthalate
90. 4-Chlorophenyl phenyl ether
91. Fluorene
92. 2-Methyl-5-nitroaniline
93. 4-Nitroaniline
94. 4,6-Dinitro-2-methylphenol
95. N-Nitrosodiphenylamine
96. 1,2-Diphenylhydrazine (as Azobenzene)
97. 2,4,6-Tribromophenol (SS)
98. 1,3,5-Trinitrobenzene
99. Diallate
100. Phenacetin
101. 4-Bromophenyl phenyl ether
102. Hexachlorobenzene
103. Atrazine
104. 4-Aminobiphenyl
105. Pentachlorophenol
106. Propyzamide
107. Pentachloronitrobenzene
108. Phenanthrene-d10 (IS)
109. Phenanthrene
110. Anthracene
111. Carbazole
112. di-*n*-Butyl phthalate
113. 4-Nitroquinoline oxide
114. Isodrin
115. Fluoranthene
116. Benzidine
117. Pyrene-d10 (SS)
118. Pyrene
119. Aramite I (isomer)
120. *p*-Terphenyl-d14 (SS)
121. Aramite II (isomer)
122. Dimethylaminoazobenzene
123. Chlorobenzilate
124. 3,3'-Dimethylbenzidine
125. Kepone
126. *n*-Butyl benzyl phthalate
127. Bis(2-ethylhexyl) adipate
128. 2-Acetylaminofluorine
129. 3,3'-Dichlorobenzidine
130. Chrysene-d12 (IS)
131. Benz[a]anthracene
132. Chrysene
133. Bis(2-ethylhexyl) phthalate
134. Di-*n*-octyl phthalate
135. Benzo[b]fluoranthene
136. Benzo[k]fluoranthene
137. 7,12-Dimethylbenzo[a] anthracene
138. Benzo[a]pyrene
139. Perylene-d12 (IS)
140. 3-Methylcholanthrene
141. Dibenz[a,j]acridine
142. Indeno[1,2,3-cd]pyrene
143. Dibenz[a,h]anthracene
144. Benzo[ghi]perylene

Column: Rxi®-5ms, 10 m, 0.18 mm ID, 0.18 µm using Rxi®-5ms 2 m, 0.18 mm ID, with 0.4 mm OD matched press-fit, BGB (cat.# 2525LD); **Sample:** Revised SV internal standard mix (cat.# 31886), Revised B/N surrogate mix (cat.# 31888), Acid surrogate mix (4/89 SOW) (cat.# 31063), 8270 MegaMix® (cat.# 31850), 8270 Benzidines mix (cat.# 31852), Benzoic acid (cat.# 31879), Appendix IX mix #1, revised (cat.# 32459), Appendix IX mix #2 (cat.# 31806); Diluent: Dichloromethane; Conc.: 20 µg/mL (1.0 ng on column); **Injection:** Inj. Vol.: 0.5 µL split (split ratio 10:1); Liner: 2 mm Split Precision® liner w/deact. wool (cat.# 20824); Inj. Temp.: 275 °C; Split Vent Flow Rate: 9 mL/min; **Oven:** Oven Temp.: 35 °C (hold 0.5 min) to 115 °C at 95 °C/min to 240 °C at 65 °C/min to 265 °C at 6 °C/min to 330 °C at 35 °C/min (hold 1 min); **Carrier Gas:** He, constant flow; Flow Rate: 0.9 mL/min; **Detector**: MS; Mode: Scan; Transfer Line Temp.: 320 °C; Analyzer Type: Quadrupole; Source Type: Inert; Drawout Plate: 3mm ID; Source Temp.: 330 °C; Quad Temp.: 180 °C; Electron Energy: 70 eV; Solvent Delay Time: 0.75 min; Tune Type: BFB; Ionization Mode: EI; **Instrument:** Agilent 7890A GC & 5975C MSD; **Notes:** The 10 m analytical and 2 m guard columns used in this application are segments of a 20 m x 0.18 mm ID x 0.18 µm df Rxi®-5ms column (cat.# 13402). The 20 m column was cut to yield one 10 m analytical column and five 2 m guard columns.

Scan Program:

Group	Start Time (min)	Scan Range (amu)	Scan Rate (scans/sec)
1	0.75	35-450	11.47
2	3.67	35-450	6.48

Table I: Evaluation of response factors across the full calibration range for select compounds demonstrates that excellent linearity and precision results were obtained for semivolatiles using split injection and fast GC.

		2.0 µg/mL (n=3)	5.0 µg/mL (n=3)	10 µg/mL (n=3)	20 µg/mL (n=3)	40 µg/mL (n=3)	80 µg/mL (n=3)	120 µg/mL (n=3)	ICAL Eval		Precision (20 µg/mL)
		0.1 ng on-column	0.25 ng on-column	0.5 ng on-column	1 ng on-column	2 ng on-column	4 ng on-column	6 ng on-column			
Name	Min 8270D RRF	RRF							RRF RSD%	R^2	RRF RSD% (n=7)
1,4-Dichlorobenzene-D4 (IS)	--	--	--	--	--	--	--	--	--	--	--
Phenol	0.800	2.180	1.839	1.773	1.729	1.655	1.697	1.666	9	--	1
2-Chlorophenol	0.800	1.627	1.500	1.416	1.377	1.286	1.260	1.261	9	--	2
Naphthalene-D8 (IS)	--	--	--	--	--	--	--	--	--	--	--
Nitrobenzene	0.200	0.516	0.460	0.438	0.426	0.405	0.418	0.408	8	--	1
2-Nitrophenol	0.100	0.122	0.129	0.133	0.138	0.140	0.147	0.134	6	--	5
2,4-Dichlorophenol	0.200	0.304	0.300	0.288	0.281	0.268	0.278	0.271	4	--	2
4-Chloroaniline	0.010	0.506	0.460	0.450	0.430	0.396	0.404	0.388	9	--	2
Hexachlorobutadiene	0.010	0.282	0.255	0.247	0.246	0.239	0.256	0.263	5	--	2
4-Chloro-3-methylphenol	0.200	0.328	0.288	0.290	0.280	0.264	0.273	0.272	7	--	2
Acenaphthene-D10 (IS)	--	--	--	--	--	--	--	--	--	--	--
Hexachlorocyclopentadiene	0.050	0.256	0.285	0.298	0.327	0.335	0.349	0.331	10	--	2
2,4,6-Trichlorophenol	0.200	0.405	0.384	0.396	0.396	0.395	0.409	0.409	2	--	3
2-Nitroaniline	0.010	0.220	0.242	0.259	0.281	0.279	0.302	0.291	10	--	2
2,6-Dinitrotoluene	0.200	0.204	0.219	0.229	0.240	0.237	0.250	0.251	7	--	2
3-Nitroaniline	0.010	0.423	0.428	0.418	0.437	0.425	0.451	0.457	3	--	1
2,4-Dinitrophenol	0.010	0.000	0.013	0.029	0.046	0.060	0.076	0.075	55	0.997	8
4-Nitrophenol	0.010	0.138	0.157	0.182	0.208	0.214	0.242	0.248	19	--	4
2,4-Dinitrotoluene	0.200	0.203	0.231	0.261	0.285	0.289	0.316	0.321	15	--	2
2,3,5,6-Tetrachlorophenol	0.010	0.218	0.234	0.254	0.262	0.254	0.275	0.275	8	--	3
2,3,4,6-Tetrachlorophenol	0.010	0.226	0.253	0.270	0.289	0.273	0.298	0.299	9	--	2
4-Nitroaniline	0.010	0.276	0.298	0.320	0.339	0.348	0.368	0.379	10	--	3
Phenanthrene-D10 (IS)	--	--	--	--	--	--	--	--	--	--	--
4,6-Dinitro-2-methylphenol	0.010	0.012	0.026	0.038	0.050	0.059	0.069	0.068	44	0.998	8
Atrazine	0.010	0.191	0.183	0.198	0.198	0.202	0.195	0.189	3	--	3
Pentachlorophenol	0.050	0.036	0.074	0.090	0.105	0.109	0.122	0.124	28	0.999	6
Carbazole	0.010	1.070	1.047	1.007	0.990	0.962	0.980	1.005	3	--	2
Chrysene-D12 (IS)	--	--	--	--	--	--	--	--	--	--	--
3,3'-Dichlorobenzidine	0.010	0.294	0.340	0.363	0.387	0.379	0.400	0.403	10	--	3
Perylene-D12 (IS)	--	--	--	--	--	--	--	--	--	--	--
Benzo[b]fluoranthene	0.700	1.191	1.220	1.234	1.286	1.274	1.371	1.493	7	--	1
Benzo[k]fluoranthene	0.700	1.483	1.443	1.456	1.459	1.395	1.373	1.345	3	--	1

and decrease the time of the initial oven hold time (speeding up your analysis, and reducing post analysis cool-down times), this separation does require the half minute for analyte focusing in order to maintain this resolution. The indeno[1,2,3-cd]pyrene–dibenz[a,h]anthracene peaks are also separated nearly to baseline (valley = 93%).

ChromaBLOGraphy

For expanded discussion of this work, visit our technical blog.

Rapid Screening for Semivolatile Compounds—144 Compounds in Less Than 9 Minutes **www.restek.com/ADV1414**

Speed up Separations With Hydrogen Carrier Gas for Fast GC

By Jaap de Zeeuw

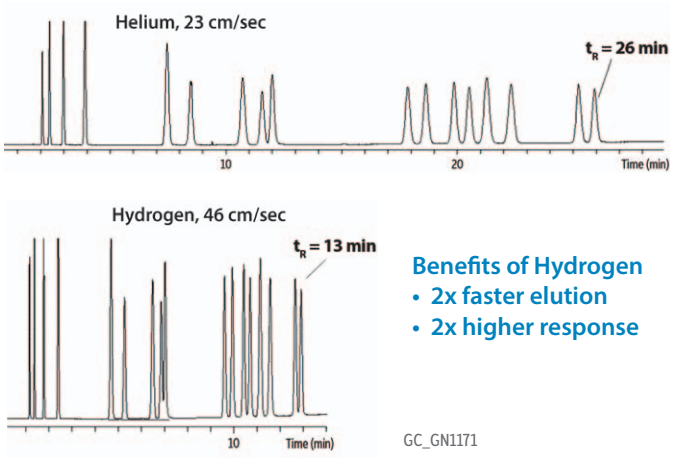
Reducing analysis time with fast GC offers labs an effective way to run more samples in less time. In some situations, when there is excess separation between peaks, you can reduce analysis time by removing "extra" resolution through the use of a shorter column, increased column flow, and faster oven programming. But, what can you do when you only have "just enough" resolution between peaks and you do not want to use a different column? In this case, using hydrogen carrier gas is the best option. With hydrogen, analysis time can be greatly reduced while maintaining the original separation.

Since the optimal linear velocity for hydrogen is approximately twice that of helium, switching to hydrogen can cut analysis times nearly in half (Figure 1). By changing to hydrogen, not only do we get faster analysis times, but we also benefit from higher peak response when using FID. Since the peaks elute twice as fast, they will be twice as high, so we can inject just half the sample and still get the same signal-to-noise ratio. This allows us to save time in another way as well; since less sample is injected there will be less contamination of the inlet liner and less downtime for maintenance.

> Switching to hydrogen carrier gas gives you the same separation in about half the time.

For isothermal analysis, the conversion from helium to hydrogen carrier gas is relatively straightforward as the linear velocity will be set to roughly double that of helium and the amount of sample injected onto the column should be cut in half. Note that when changing the linear velocity from the optimum for helium to the optimum for hydrogen, the actual factor (accounting for gas compressibility) is about 1.7 rather than 2. To be as accurate as possible, use of a method translator is recommended. Converting to hydrogen is somewhat more complex for temperature-programmed analysis as the oven program must be adjusted in order to maintain the same separation. To help maintain the same elution temperatures for the analytes, the isothermal times must be cut in half and the temperature program rate must be multiplied by a factor of two in order to obtain the same separation in half the time.

Figure 1: Analytes can be separated in half the time using hydrogen carrier gas, significantly improving lab productivity.



Column: Rt®-Alumina BOND/MAPD; **Sample:** Hydrocarbon mixture; **Injection:** split; **Oven: Detector:** FID; **Notes:** Using hydrogen as the carrier gas allows the linear velocity to be doubled so compounds elute in half the time. Helium: 23 cm/sec; Hydrogen: 46 cm/sec.

Of course, when using hydrogen, safety must also be considered. Fortunately, today's hydrogen generators minimize much of the risk. Features such as small storage volumes, controlled flow, built-in leak sensors, and automatic shut-off allow labs to safely reap the benefits of using hydrogen for fast GC.

Large Volume Splitless Injection GC-MS for 1,4-Dioxane and Nitrosamines in Water Using One SPE Method Instead of Two

By Chris Rattray and Jack Cochran

- Reduce overall time by 44% compared to separate methods for 1,4-dioxane and nitrosamines.
- Optimize sample prep with Resprep® SPE tubes specifically tested for target compounds.
- Quantify at ppt levels without interferences using an Rxi®-5Sil MS GC column and CSR-LVSI GC-MS.

Global concern over the carcinogenic potential of 1,4-dioxane and several nitrosamines has resulted in increased interest in the development of more efficient testing methods. 1,4-Dioxane is listed as a Group 2B compound by the World Health Organization's International Agency for Research on Cancer (IARC) and the latest edition of the Report on Carcinogens (RoC) published by the National Toxicology program lists 15 nitrosamines as likely human carcinogens. N-nitrosodimethylamine (NDMA) is the primary nitrosamine of concern and, like 1,4-dioxane, its high water solubility limits the efficiency of purge-and-trap and liquid-liquid extraction when determining low ppt concentrations. In drinking water, 1,4-dioxane can be analyzed using a simple electrospray ionization (EI) GC-MS method (e.g., EPA Method 522), whereas nitrosamines are generally analyzed by positive chemical ionization (CI) using liquid methanol or acetonitrile reagent gas along with GC-MS/MS (e.g., EPA Method 521). Run separately, these methods are quite time-consuming, with a typical 20-sample batch taking nearly 32 hours for sample preparation and analysis (Table I). In an effort to help labs reduce the time and expense associated with testing 1,4-dioxane and nitrosamines separately, we recently developed a combined method that reduced the total time required by 44%.

The benefits of a combination method include fewer samples to collect, ship, and extract, as well as much higher sample throughput. The method summarized here uses Resprep® SPE tubes (cat.# 26032), which are specifically tested for use with 1,4-dioxane and nitrosamines, and dichloromethane eluent to concentrate 0.50 L water samples to 10 mL extracts. Because the final SPE extract cannot be concentrated via evaporation due to volatile compound loss, we employed the concurrent solvent recondensation–large volume splitless injection (CSR-LVSI) technique developed by chemists at Thermo Scientific [1,2]. The CSR-LVSI setup uses a standard splitless injector to deliver 50 µL injections of extract to a pre-column connected to an analytical column followed by MS analysis. A thick film Rxi®-5Sil MS column was chosen for this application because its high thermal stability allows the use of an oven program that reaches 330 °C. This ensures that all the co-extracted material is removed from the analytical column between runs, which is critical because both the analytes and co-extracted materials have small m/z ions, increasing the chance of interferences.

Figure 1 illustrates the viability of using 50 µL CSR-LVSI when combined with standard EI GC-MS equipment for the combined analysis of 1,4-dioxane and various nitrosamines. Although EI is not currently allowable under Method 521, it is advantageous because it is less complex than CI and does not require additional capital investment for equipment. Using a high column flow (5 mL/min) during the solvent focusing step allowed us to shorten the analysis time while maintaining the resolution of analytes from the solvent peak and potential interferences. While operating in selected ion mode, especially when dealing with low molecular weight ions, it is critical that the peaks of interest be separated from interferences. While not the primary focus of this work, tetrahydrofuran (THF) was also included since it is a common unregulated contaminant ideally suited to this technique. Unfortunately, significant blank contamination raised the quantitation limit for THF to 0.34 ppb.

To evaluate the method for 1,4-dioxane and nitrosamines, linearity and recovery at two fortification levels were assessed (Table II). Linear responses were obtained for the ranges shown, but in some cases lower levels were injected and then dropped from the final calibration due to poor signal-to-noise ratios. Since even low levels of contamination are a concern for low ppt-level analysis, bottled drinking water was used for blanks and fortified samples. Some compounds were still detected in the blanks, so quantification limits (QLs) were set at twice the blank levels instead of the planned lowest calibration level for each compound. Recoveries for lab fortified samples were generally excellent, although in some instances the higher QL set

based on the blank levels was greater than the spiked concentration. While the QLs are generally near or below health risk assessment concentrations, additional cleanup of laboratory reagent (or blank) water is recommended so the lower QLs can be achieved. Overall, this combined method for 1,4-dioxane and nitrosamines in drinking water provides a means of saving time and increasing sample throughput, and it also offers a good starting point for modifying similar methods for these compounds.

Visit **www.restek.com/ADV1415** for the complete method evaluation.

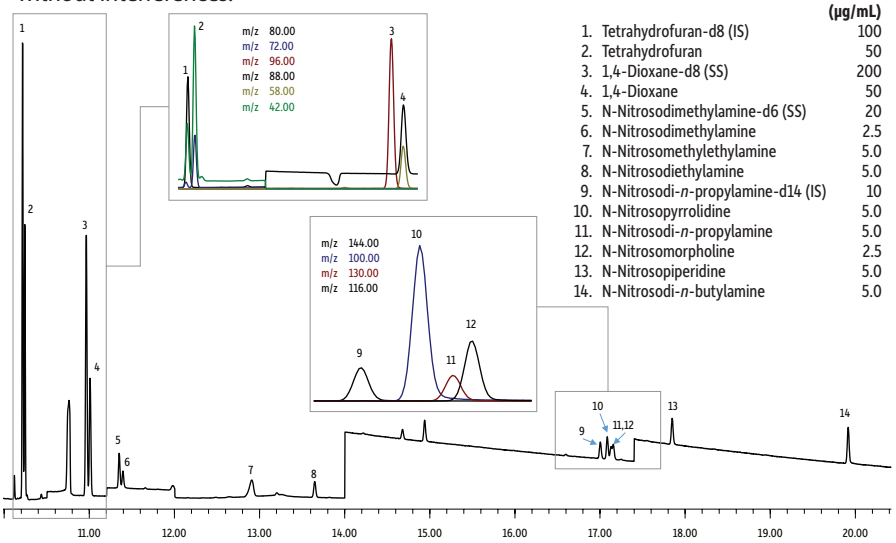
References

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

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

[3] U.S. Environmental Protection Agency, Integrated Risk Information System (IRIS), 2013. www.epa.gov/iris/index.html (accessed November 25, 2013).

Figure 1: Combined SIM analysis of 1,4-dioxane and nitrosamines using CSR-LVSI and an Rxi®-5Sil MS column allows all target analytes to be determined from a single injection without interferences.



Peaks	Conc. (µg/mL)
1. Tetrahydrofuran-d8 (IS)	100
2. Tetrahydrofuran	50
3. 1,4-Dioxane-d8 (SS)	200
4. 1,4-Dioxane	50
5. N-Nitrosodimethylamine-d6 (SS)	20
6. N-Nitrosodimethylamine	2.5
7. N-Nitrosomethylethylamine	5.0
8. N-Nitrosodiethylamine	5.0
9. N-Nitrosodi-*n*-propylamine-d14 (IS)	10
10. N-Nitrosopyrrolidine	5.0
11. N-Nitrosodi-*n*-propylamine	5.0
12. N-Nitromorpholine	2.5
13. N-Nitrosopiperidine	5.0
14. N-Nitrosodi-*n*-butylamine	5.0

GC_EV1334

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 SGE® µ-union; **Sample:** N-Nitrosodimethylamine-d6 (cat.# 33910), 1,4-Dioxane-d8 (cat.# 30614), N-Nitrosodi-*n*-propylamine-d14 (cat.# 33911), Tetrahydrofuran-d8 (cat.# 30112), Nitrosamine calibration mix, Method 521 (cat.# 31898), Appendix IX mix #1, revised (cat.# 32459); Diluent: Dichloromethane; For full list of conditions search for chromatogram# **GC_EV1334** at **www.restek.com**

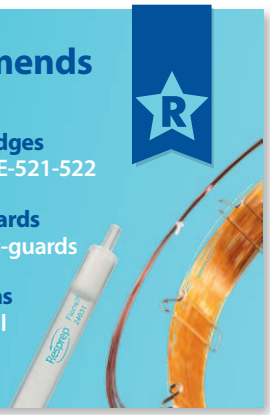


Table I: Combining the extraction and analysis of 1,4-dioxane and nitrosamines into a single method allows a time savings of approximately 14 hours per 20-sample batch, compared to running both methods separately.

	Hours Required for Extraction and Analysis of a 20-Sample Batch				
	Nitrosamines (Method 521)	1,4-Dioxane (Method 522)	Methods 521 and 522 Run Separately	Combined Method	Time Saved Using Combined Method
Sample Prep Time	8*	4	12*	4	8
GC Analysis Time	12.6	7.1	19.7	13.7	6
Total Time	20.6	11.1	31.7	17.7	14 44% savings!

Note: Sample prep times were estimated. Analysis times were determined based on calculated oven program times listed in the methods. Oven cool down, equilibration, and injection sequence times were approximated based on comparison of the oven program times and actual times.
*Includes a required extract concentration step.

Table II: Method evaluation results demonstrate 1,4-dioxane and nitrosamines can be reported at trace levels when using a time-saving combined approach for sample prep and analysis.

			Linearity		Fortification Level 1 (N=3)				Fortification Level 2 (N = 3)			
Analyte	Quantitation Limit (ng/L)	IRIS 1x10^{-6} Cancer Conc. (ng/L)*	Equivalent Sample Conc. Range (ng/L)	R	Spiked Amount (ng/L)	Avg. Recovery (ng/L)	Avg. % Recovery	% RSD	Spiked Amount (ng/L)	Avg. Recovery (ng/L)	Avg. % Recovery	% RSD
1,4-Dioxane	36	350	10–1,000	0.999	50	72	140	1.9	500	510	100	19
N-nitrosodimethylamine	0.5	0.7	0.5–50	0.998	2.5	2.3	92	9.3	25	17	68	26
N-nitrosomethylethylamine	2.0	2.0	2.0–100	0.998	5.0	3.9	78	22	50	48	96	4.8
N-nitrosodiethylamine	0.56	0.2	0.2–100	0.998	5.0	4.7	94	4.7	50	48	96	1.2
N-nitrosopyrrolidine	2.8	20	0.2–100	0.997	5.0	5.3	106	1.9	50	46	92	5.1
N-nitrosodi-*n*-propylamine	6.8	5.0	1.0–100	0.997	5.0	<6.8	--	--	50	43	86	3.8
N-nitrosomorpholine	1.1	--	0.2–50	0.994	2.5	2.5	100	3.6	25	23	92	4.0
N-nitrosopiperidine	1.4	--	0.2–100	0.997	5.0	4.5	90	1.6	50	46	92	2.4
N-nitrosodi-*n*-butylamine	14	6.0	0.4–100	0.997	5.0	<14	--	--	50	49	98	4.9

* The IRIS 1x10^{-6} concentration is the one in a million cancer risk assessment value based on consumption of two liters of contaminated water a day for a lifetime [3].

Accurately Determine Trace-Level Pesticide Residues in Complex Tea Samples Using QuEChERS and GCxGC-TOFMS

By Michelle Misselwitz and Jack Cochran

- Restek® GCxGC columns separate analytes that cannot be determined by 1D GC-MS.
- Simple QuEChERS extraction and dSPE cleanup—no need for additional sample prep.
- Good separation and sensitivity assure accurate recoveries at 10 pg on-column.

Herbal tea has been valued throughout history for its potential health benefits. Used frequently in traditional Chinese medicine, teas are formulated using different blends of herbs, plants, and spices depending on the desired medicinal properties. As with any plant-based commodity, there is a potential for pesticide residues to remain in the final product. The dried plant material found in herbal tea poses a significant challenge for trace-level pesticide residue analysis. The extract, even after an extensive cleanup, can contain a large amount of coextracted material that can overwhelm the target pesticides, making trace detection very difficult. Furthermore, any nonvolatile material that is not removed during extract cleanup is deposited on the inlet and column, which means maintenance must be performed more often. In this study, we assessed the potential of pairing the EN 15662 QuEChERS extraction method with a fast dispersive solid phase extraction (dSPE) cleanup to prepare herbal tea samples for analysis using GCxGC-TOFMS.

GCxGC With Orthogonal Columns Can Reduce Sample Prep

Herbal teas vary significantly in ingredients, so in order to assess the impact of matrix complexity more broadly we used several types of tea that varied in composition. Teas were fortified at 1,000 ng/g (100 pg on-column) and 100 ng/g (10 pg on-column) using Restek's new QuEChERS performance standards and an organohalide pesticides mix. The fortification solution contained 53 pesticides that ranged in volatility and included many of the GC-amenable pesticides found in tea or in the other botanicals used in tea products. The sample extracts were first evaluated by one dimensional GC-TOFMS; however, the sample matrix obscured the target pesticides and made recovery determination difficult even at the high fortification level. When a sample is too complex for 1D GC-MS determination, further cleanup with cartridge SPE is often needed, but this adds time and expense. Instead, we used GCxGC-TOFMS to chromatographically separate the matrix components from the target pesticides. This allows us to use the less intensive dSPE procedure without additional cleanup steps. As shown in Figure 1, the GCxGC-TOFMS setup using an Rxi®-5ms column in the first dimension and an Rtx®-200 column in the second dimension provided good separation for both types of tea. The differing (orthogonal) selectivities of the analytical columns allowed even potential isobaric interferences that would have coeluted in 1D GC-MS to be resolved in the second dimension (Figure 2).

Accurately Recover Trace-Level Pesticide Residues in Complex Teas

The QuEChERS extraction combined with dSPE cleanup and GCxGC-TOFMS determination provided excellent separation and sensitivity. The dSPE cleanup formulation contained 7.5 mg of graphitized carbon black (GCB) and 50 mg each of primary secondary amine (PSA) and C18. While the use of GCB is important to reduce pigments, like chlorophyll, that can quickly degrade GC performance, it can also negatively impact the recoveries of planar pesticides like hexachlorobenzene and chlorothalonil. The moderate amount of GCB used here provided a good balance between removing some pigment to reduce nonvolatile residues and also yielding good recoveries of planar pesticides. When nonvolatile residue accumulation did become a concern, replacing the inlet liner restored GC performance. Note that in this case a single taper liner without wool was used to improve response for active compounds. In order to improve early eluting analyte peak shapes, which improves detectability, we installed a deactivated 1 m Rxi® guard column (retention gap) prior to the first analytical column.

Individual recoveries for selected pesticides are presented in Table I, along with the average percent recovery of all 53 pesticides for each matrix and fortification level. The orthogonal selectivity of the analytical columns and sensitivity of the GCxGC-TOFMS allowed all target analytes to be separated and quantified even at the low fortification level of 100 ng/g (10 pg on-column). The GCxGC system uses modulation, a quick cryogenic trapping, and injection of analyte bands onto the secondary column to focus peaks near the detector

Figure 1: Fast and easy to use, the *EZ*GC® chromatogram modeler delivers recommended columns and conditions for 233 compounds in just 25 seconds. Simply copy and paste in your analyte list to get a customized separation!

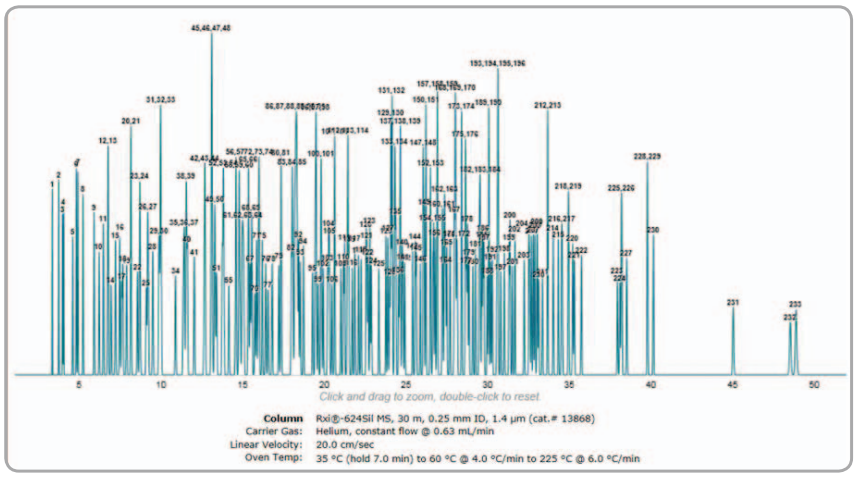


Figure 2: Zoom into areas of interest and hover over peaks to view compound names and retention times.

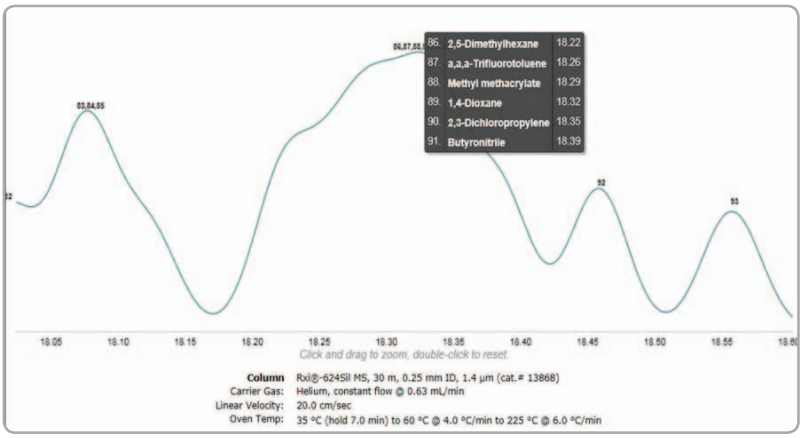
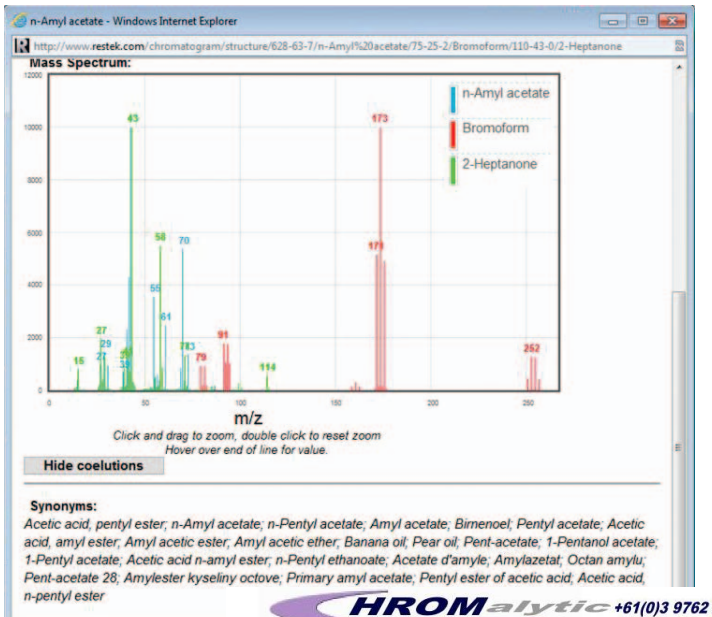


Figure 3: By clicking on a specific compound its mass spectrum is displayed. This also allows the user to click on the coelutions bar and show an overlay of the different spectra. In this example, three compounds coelute, but they are resolved by their mass spectra.



EZGC® Tips & Tricks

- Looking for isomers? Make sure to enter them as individual compounds, not as a combined term. For example, a search for "*m/p*-xylene" yields no results; whereas, entering "*m*-xylene" and "*p*-xylene" separately will produce recommended GC columns and conditions.
- The best way to find your compound is by using the CAS#.
- Not sure of the correct spelling? The *EZ*GC® software will suggest compounds if the spelling of an analyte is not quite right.
- Keep life simple. You can copy and paste lists directly from our reference standards product pages.

Here's What Others Say

"The EZGC® chromatogram modeler saves a lot of time for new method development. It is also a great teaching tool for new chromatographers."

- Egwuatu Ikpeama, Manager—Laboratory Operations, Paragon Laboratories, Inc.

"The process of finding a column that is compatible with your specific target analytes is considerably easier with the EZGC® chromatogram modeler. It saves quite a bit of time and money."

- Mitch Spicer, Chemical Analyst MicroMethods Laboratory

"The EZGC® chromatogram modeler is a fantastic program for column selection. It's extremely easy to use, even for entry-level chromatographers."

- Jennifer Whitaker, Lab Supervisor

"Great tool to help get started on many GC separations! The EZGC® chromatogram modeler is fast and very easy to use."

- Brian Hefner, Scientist

Accurately Determine Trace-Level Pesticide Residues in Complex Tea Samples Using QuEChERS and GCxGC-TOFMS

By Michelle Misselwitz and Jack Cochran

- Restek® GCxGC columns separate analytes that cannot be determined by 1D GC-MS.
- Simple QuEChERS extraction and dSPE cleanup—no need for additional sample prep.
- Good separation and sensitivity assure accurate recoveries at 10 pg on-column.

Herbal tea has been valued throughout history for its potential health benefits. Used frequently in traditional Chinese medicine, teas are formulated using different blends of herbs, plants, and spices depending on the desired medicinal properties. As with any plant-based commodity, there is a potential for pesticide residues to remain in the final product. The dried plant material found in herbal tea poses a significant challenge for trace-level pesticide residue analysis. The extract, even after an extensive cleanup, can contain a large amount of coextracted material that can overwhelm the target pesticides, making trace detection very difficult. Furthermore, any nonvolatile material that is not removed during extract cleanup is deposited on the inlet and column, which means maintenance must be performed more often. In this study, we assessed the potential of pairing the EN 15662 QuEChERS extraction method with a fast dispersive solid phase extraction (dSPE) cleanup to prepare herbal tea samples for analysis using GCxGC-TOFMS.

GCxGC With Orthogonal Columns Can Reduce Sample Prep

Herbal teas vary significantly in ingredients, so in order to assess the impact of matrix complexity more broadly we used several types of tea that varied in composition. Teas were fortified at 1,000 ng/g (100 pg on-column) and 100 ng/g (10 pg on-column) using Restek's new QuEChERS performance standards and an organohalide pesticides mix. The fortification solution contained 53 pesticides that ranged in volatility and included many of the GC-amenable pesticides found in tea or in the other botanicals used in tea products. The sample extracts were first evaluated by one dimensional GC-TOFMS; however, the sample matrix obscured the target pesticides and made recovery determination difficult even at the high fortification level. When a sample is too complex for 1D GC-MS determination, further cleanup with cartridge SPE is often needed, but this adds time and expense. Instead, we used GCxGC-TOFMS to chromatographically separate the matrix components from the target pesticides. This allows us to use the less intensive dSPE procedure without additional cleanup steps. As shown in Figure 1, the GCxGC-TOFMS setup using an Rxi®-5ms column in the first dimension and an Rtx®-200 column in the second dimension provided good separation for both types of tea. The differing (orthogonal) selectivities of the analytical columns allowed even potential isobaric interferences that would have coeluted in 1D GC-MS to be resolved in the second dimension (Figure 2).

Accurately Recover Trace-Level Pesticide Residues in Complex Teas

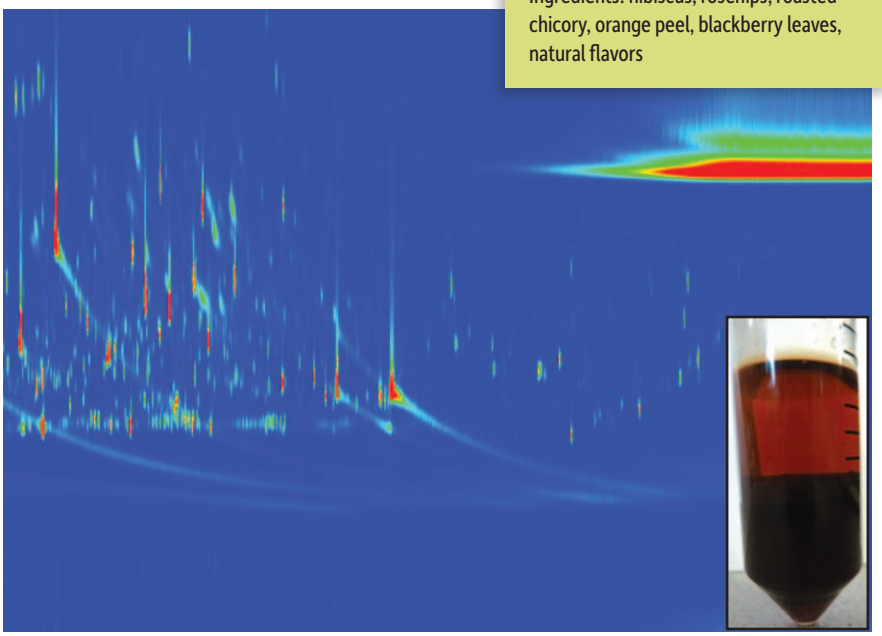
The QuEChERS extraction combined with dSPE cleanup and GCxGC-TOFMS determination provided excellent separation and sensitivity. The dSPE cleanup formulation contained 7.5 mg of graphitized carbon black (GCB) and 50 mg each of primary secondary amine (PSA) and C18. While the use of GCB is important to reduce pigments, like chlorophyll, that can quickly degrade GC performance, it can also negatively impact the recoveries of planar pesticides like hexachlorobenzene and chlorothalonil. The moderate amount of GCB used here provided a good balance between removing some pigment to reduce nonvolatile residues and also yielding good recoveries of planar pesticides. When nonvolatile residue accumulation did become a concern, replacing the inlet liner restored GC performance. Note that in this case a single taper liner without wool was used to improve response for active compounds. In order to improve early eluting analyte peak shapes, which improves detectability, we installed a deactivated 1 m Rxi® guard column (retention gap) prior to the first analytical column.

Individual recoveries for selected pesticides are presented in Table I, along with the average percent recovery of all 53 pesticides for each matrix and fortification level. The orthogonal selectivity of the analytical columns and sensitivity of the GCxGC-TOFMS allowed all target analytes to be separated and quantified even at the low fortification level of 100 ng/g (10 pg on-column). The GCxGC system uses modulation, a quick cryogenic trapping, and injection of analyte bands onto the secondary column to focus peaks near the detector

Figure 1: GCxGC-TOFMS contour plots of two herbal teas that varied in complexity. In both cases, the different selectivities of Rxi®-5ms and Rtx®-200 columns separated the matrix components from the analytes of interest.

A. Unfortified Berry Tea

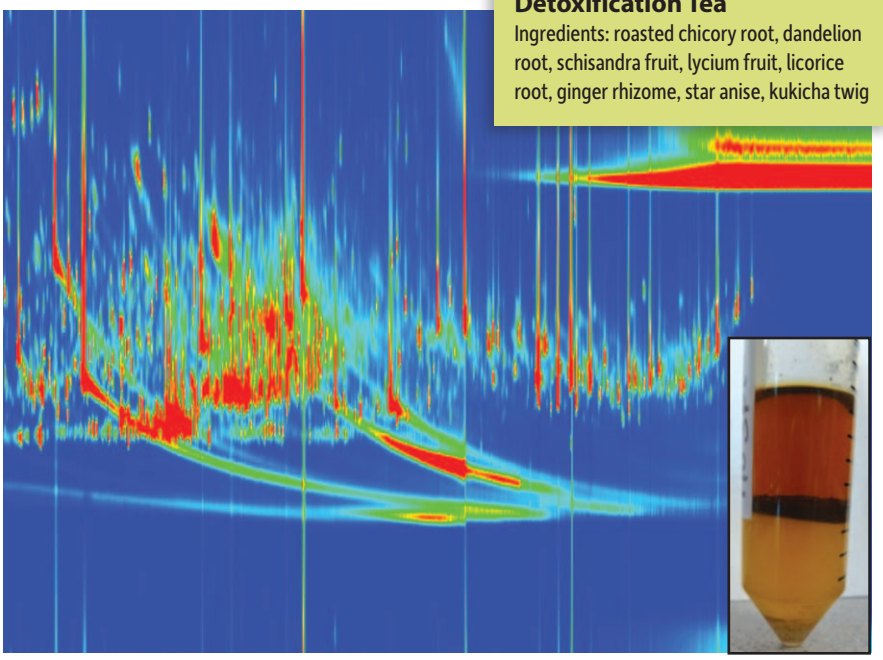
Berry Tea
Ingredients: hibiscus, rosehips, roasted chicory, orange peel, blackberry leaves, natural flavors



GC_FS0502

B. Unfortified Detoxification Tea

Detoxification Tea
Ingredients: roasted chicory root, dandelion root, schisandra fruit, lycium fruit, licorice root, ginger rhizome, star anise, kukicha twig



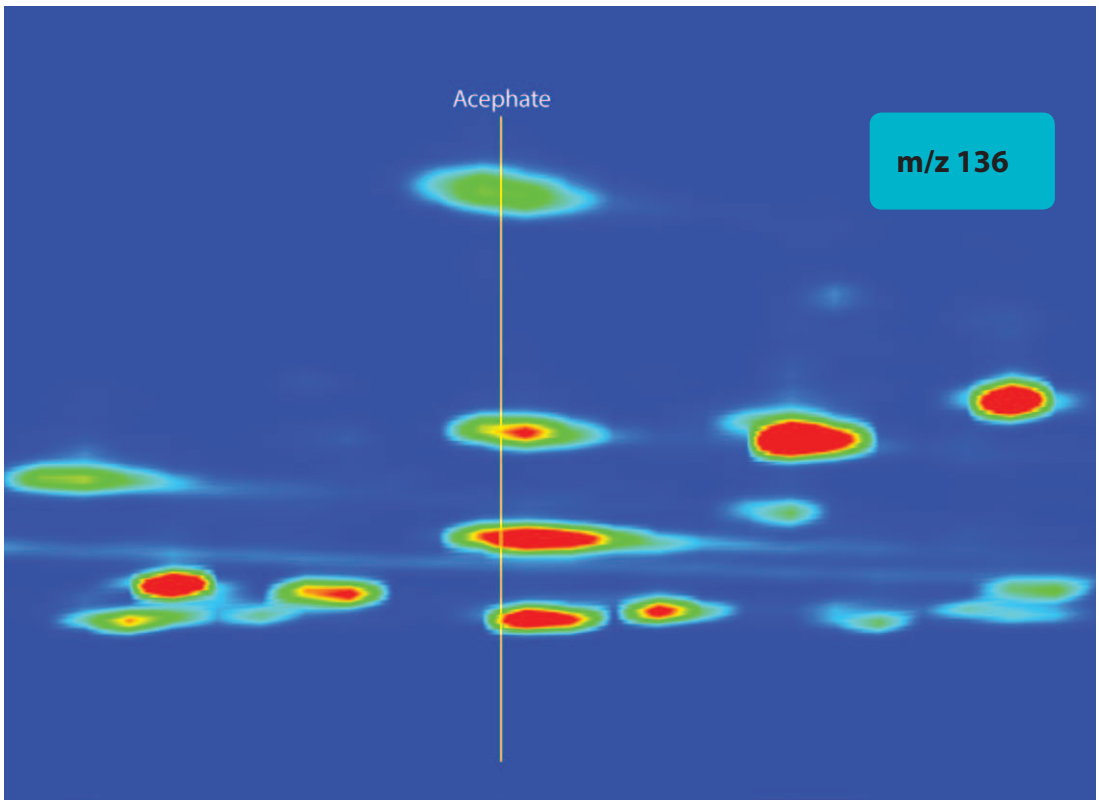
GC_FS0503

Column: Rxi ®-5ms 30 m, 0.25 mm ID, 0.25 µm (cat.# 13423) with Rxi® guard column 1.0 m, 0.25 mm ID (cat.# 10029); Rtx®-200 1.0 m, 0.25 mm ID, 0.25 µm (cat.# 15124); **Sample A:** Berry tea, QuEChERS internal standard mix for GC-MS analysis (cat.# 33267); **Sample B:** Detoxification tea, QuEChERS internal standard mix for GC-MS analysis (cat.# 33267); **Injection:** Inj. Vol.: 1.0 µL splitless (hold 1.0 min); Liner: 4 mm Sky® single taper (cat.# 23302.1); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min; **Oven:** Oven Temp.: Rxi ®-5ms: 70 °C (hold 1.0 min) to 330 °C at 8.0 °C/min (hold 6.5 min); Rtx®-200: 75 °C (hold 1.0 min) to 335 °C at 8.0 °C/min (hold 6.5 min); **Carrier Gas:** He, corrected constant flow (1.4 mL/min); **Modulation:** Modulator Temp.; Offset: 20 °C; Second Dimension: Separation Time: 2.00 sec; Hot Pulse Time: 0.60 sec; Cool Time between Stages: 0.40 sec; **Detector:** MS; Mode: Transfer Line Temp.: 320 °C; Analyzer Type: TOF; Source Temp.: 225 °C; Electron Energy: 70 eV; Mass Defect: -20 mu/100 u; Ionization Mode: EI; Acquisition Range: 45-550 amu; Spectral Acquisition: Rate: 100 spectra/sec; **Instrument:** LECO Pegasus® 4D GCxGC-TOFMS; **Notes:** All column connections were made with an SGE® SilTite® µ-union.

in order to increase signal-to-noise ratios. This also allowed the full mass range determination of pesticides of interest in incurred samples at low pg on-column levels, amounts that would be below the typical sensitivity of a 1D GC-MS full scan analysis. Method evaluation results clearly demonstrated that determination of pesticide residues in herbal tea can be routinely and accurately accomplished using QuEChERS extraction, dSPE cleanup, and GCxGC-TOFMS. The use of Rxi® columns of different selectivity chromatographically separated coextracted matrix material so that a more laborious cleanup was not necessary.

Visit **www.restek.com/ADV1416** for further discussion of this work in our technical blog.

Figure 2: Acephate is fully resolved on the Rtx®-200 second dimension column, allowing accurate quantification. It would have coeluted with isobaric interferences in 1D GC.



GC_FS0504

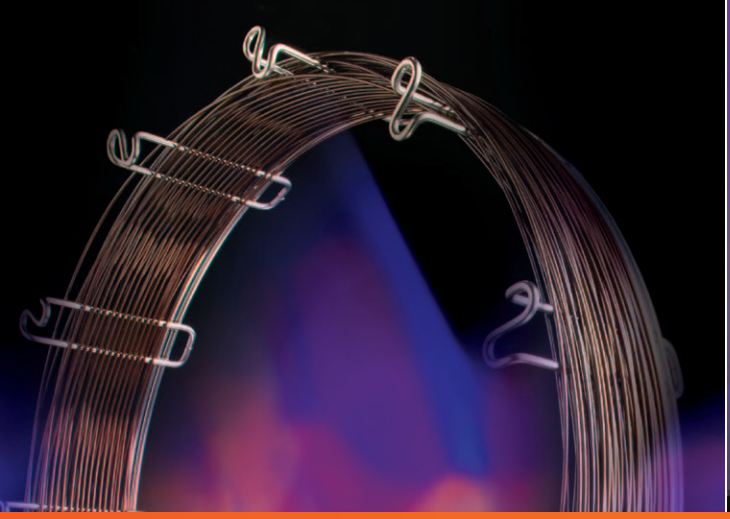
See Figure 1 for conditions

Table I: The combination of QuEChERS extraction, dSPE cleanup, and GCxGC-TOFMS resulted in good recoveries for most pesticides.

	%Recovery from "Berry" Tea		%Recovery from "Detoxification" Tea	
	1,000 ng/g	100 ng/g	1,000 ng/g	100 ng/g
Acephate	88	81	88	89
***o*-Phenylphenol**	92	95	88	101
Omethoate	78	88	85	93
Hexachlorobenzene	62	62	61	71
Dimethoate	94	96	92	108
Simazine	90	106	90	96
Atrazine	92	96	98	99
Diazinone	93	95	88	96
Chlorothalonil	79	100	74	99
Vinclozoline	99	91	93	107
Carbaryl	94	91	97	103
Metalaxyl	96	90	100	88
PCB 52 (IS)	–	–	–	–
Methiocarb	90	89	96	115
Malathion	95	94	98	67
Fenthion	88	98	98	101

	%Recovery from "Berry" Tea		%Recovery from "Detoxification" Tea	
	1,000 ng/g	100 ng/g	1,000 ng/g	100 ng/g
Chlorpyrifos	93	88	88	109
Cyprodinil	71	73	83	81
Thiabendazole	62	77	75	86
Captan	99	176	103	100
Imazalil	91	91	75	72
Endosulfan sulfate	95	104	99	102
Propargite	96	105	90	83
Iprodione	99	74	90	101
Bifenthrin	86	82	85	84
Fenpropathrin	93	93	90	104
Azinphos-methyl	85	102	98	81
cis-Permethrin	88	70	87	97
Deltamethrin	88	78	90	93
Average recovery of all 53 pesticides	90% (±11)	94% (±33)	89% (±10)	97% (±24)

IS = internal standard



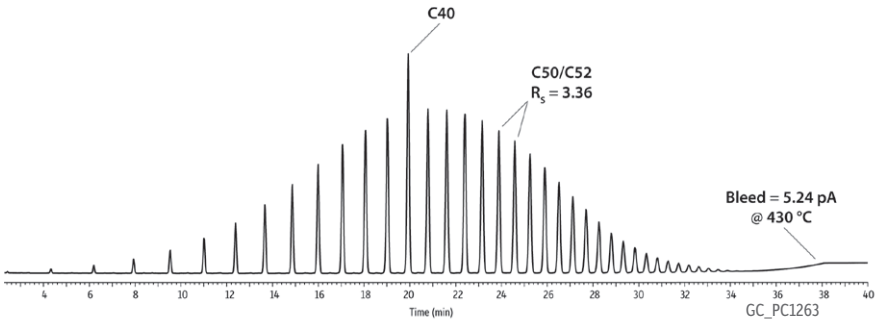
Only MXT®-1HT SimDist Columns are Individually Application Tested up to 430 °C for Guaranteed Method Performance

By Barry Burger and Katarina Oden

- Reduce downtime and increase confidence in your data.
- No batch testing—each 5 m x 0.53 mm x 0.10 µm column is individually QC-tested up to 430 °C.
- Guaranteed to meet ASTM Method D6352-12, D7169-11, and D7500-12 requirements.

Accurate determination of the boiling range distribution of medium and heavy fractions using GC simulated distillation provides critical information regarding the composition of crude oil. ASTM Methods D6352-12, D7169-11, D7500-12 are often used to characterize petroleum distillates in the boiling range of 174 °C to 735 °C, but these analyses present many challenges to refineries and contract laboratories. Both the column construction and the polydimethylsiloxane (PDMS) polymer must be robust enough to withstand the high method temperatures without significant degradation. In addition, columns must yield a C50/C52 resolution value between 2 and 4, exhibit acceptable peak symmetry, and reliably produce accurate boiling point data.

Figure 1: Every 5 m x 0.53 mm x 0.10 µm MXT®-1HT SimDist column (cat.# 70112) is individually QC tested to 430 °C against D6352-12 requirements and is also guaranteed to meet ASTM D7169-11 and D7500-12 method requirements!



Column: MXT®-1HT SimDist, 5 m, 0.53 mm ID, 0.10 µm (cat.# 70112); **Sample:** Polywax® 655; **Oven**: Oven Temp.: 50 °C to 430 °C at 10 °C/min (hold 5 min); **Carrier Gas:** He, constant flow; Flow Rate: 18 mL/min; **Detector:** FID @ 430 °C; Make-up Gas; Flow Rate: 24 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 358 mL/min; **Instrument:** Agilent/HP6890 GC.

While other columns on the market are isothermally tested for performance, only Restek provides application-specific testing for all 5 m x 0.53 mm x 0.10 µm MXT®-1HT SimDist columns (cat.# 70112). Since each one is tested up to 430 °C, performance is guaranteed to meet ASTM Method D6352-12, D7169-11, and D7500-12 requirements. The 1HT PDMS phase is designed to achieve bleed of less than 15 pA at 430 °C and has a maximum operating temperature of 450 °C. In addition, the rugged Siltek®-treated MXT® tubing eliminates breakage while ensuring column inertness. Unlike competitor columns, each and every 5 m x 0.53 mm x 0.10 µm column is tested against ASTM Method D6352-12 requirements up to 430 °C with Polywax® 655 (C18–C90 hydrocarbons). QC acceptance criteria include C50/C52 resolution, bleed, and C50 asymmetry (skew). Each cat.# 70112 column you purchase will arrive with a chromatogram like the one above, your assurance that the column will perform as required. Increase lab productivity and confidence in your data with an application-tested MXT®-1HT SimDist column from Restek!

Try one today! **www.restek.com/SimDist**

Polywax® Standards

Description	Conc. in Solvent and Volume	cat.#
Polywax 500	Neat, 1 g	36224
Polywax 655	Neat, 1 g	36225
Polywax 850	Neat, 1 g	36226
Polywax 1,000	Neat, 1 g	36227

MXT®-1HT SimDist Column

(Siltek®-treated stainless steel)

Description	temp. limits	qty	cat#
5 m, 0.53 mm ID, 0.10 µm	-60 to 430/450 °C	ea.	70112

Dynamic Duo

Restek's Leak Detector and ProFLOW 6000 Flowmeter

An Unbeatable Combination!

Protect your instrument and improve data quality with this powerful pair from Restek. Checking for leaks and verifying flows before you start helps you avoid costly problems later.

Description	qty.	cat.#
Dynamic Duo Combo Pack (Restek Leak Detector and ProFLOW 6000 Flowmeter)	kit	22654
Related Products and Accessories		
Leak Detector With Hard-Sided Carrying Case and Universal Charger Set (U.S., UK, European, Australian)	ea.	22655
Small Probe Adaptor for Leak Detector	ea.	22658
Restek ProFLOW 6000 Electronic Flowmeter With Hard-Sided Carrying Case	ea.	22656
Soft-Sided Storage Case for Leak Detector or ProFLOW 6000 Flowmeter	ea.	22657

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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.

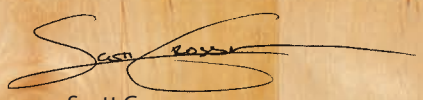
However you look at it, method development comes with a great deal of responsibility. Many of us are developing methods for very important applications in our own industries. So, building an accurate, precise, and robust method that doesn't require a third arm and a lucky rabbit's foot is vital. I appreciate 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.

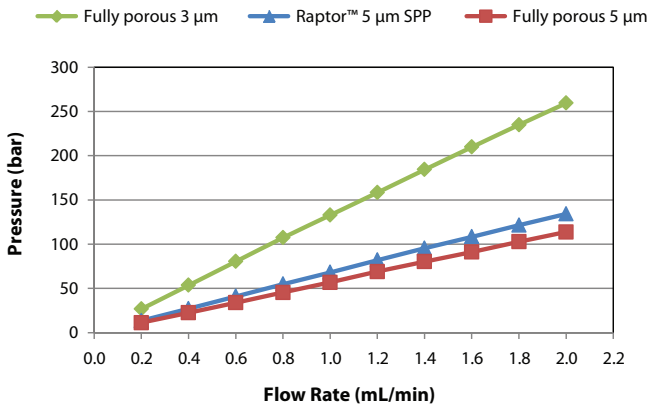
How to Choose between Raptor™ 2.7 vs. 5 µm SPP LC Columns

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.

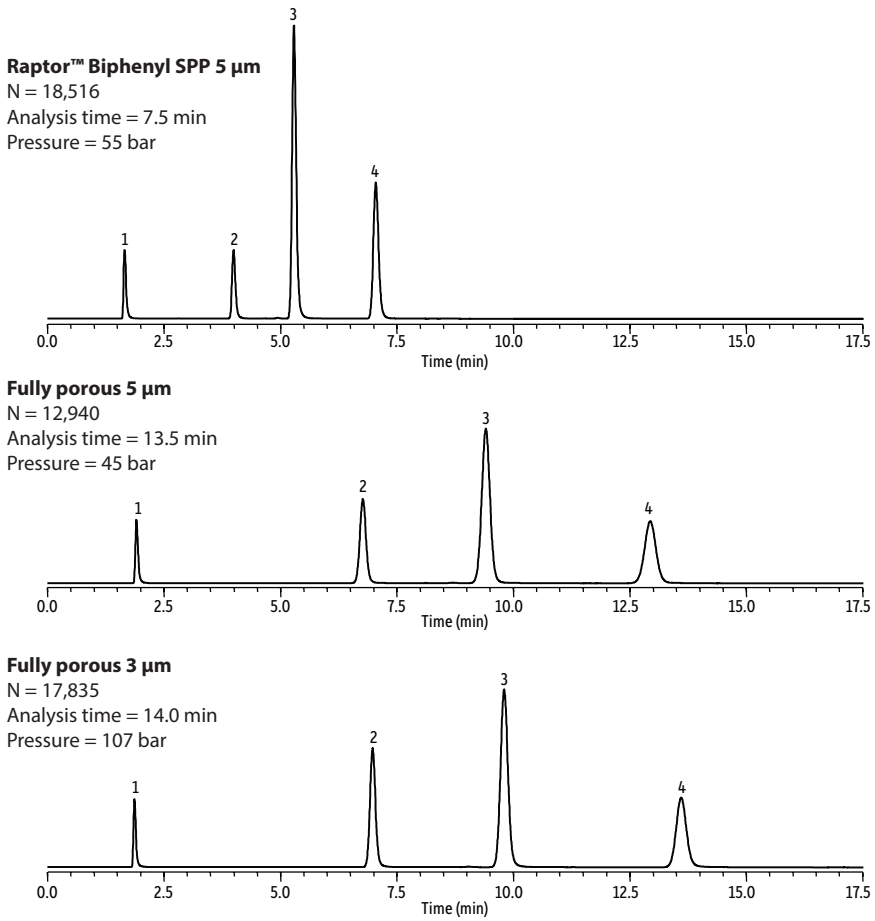
Raptor™ 2.7 µm diameter particle columns exhibit greater efficiency and sensitivity than the 5 µm, but the operating pressures are somewhat higher. Since extra-column peak broadening is most pronounced with short, small-diameter columns packed with small particles, 2.7 µm columns are best suited for 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	LC Reversed Phase Test Mix #1 (cat.# 35005)
Diluent:	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

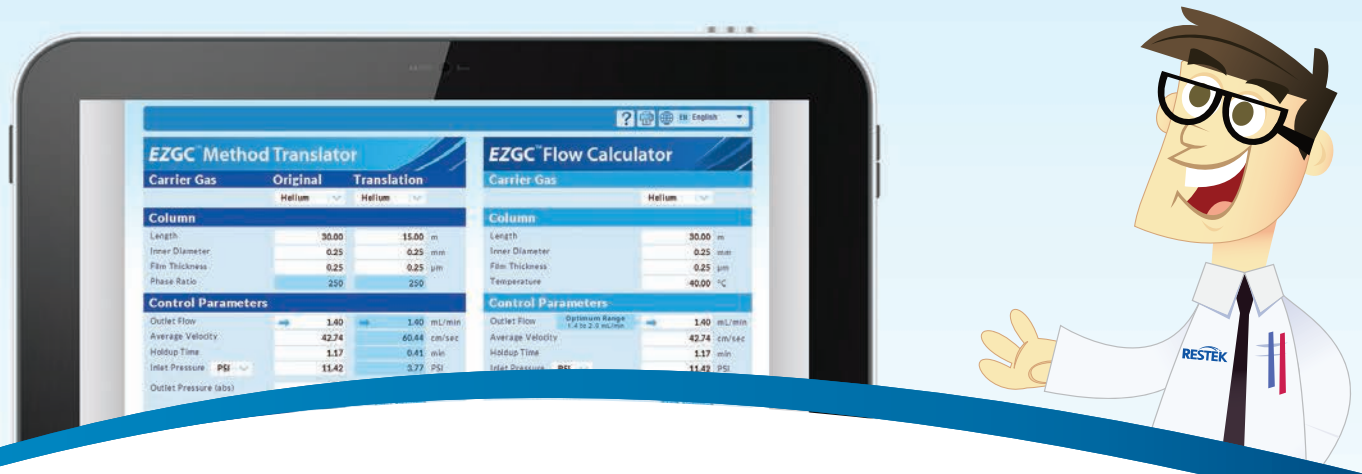
Experience *Selectivity Accelerated*

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

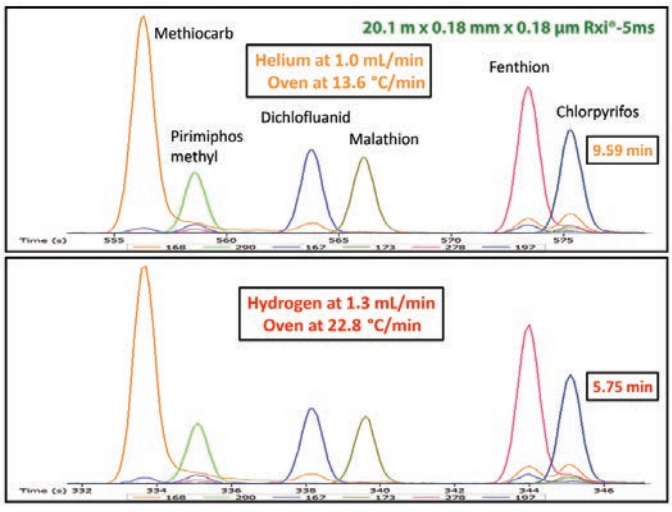
- Improve throughput by translating your GC method from slower helium to faster hydrogen carrier gas.
- Substitute expensive helium GC carrier gas with hydrogen and get the same chromatogram with translation.
- Improve MS detectability by using hydrogen at a lower flow rate without sacrificing separations.

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.

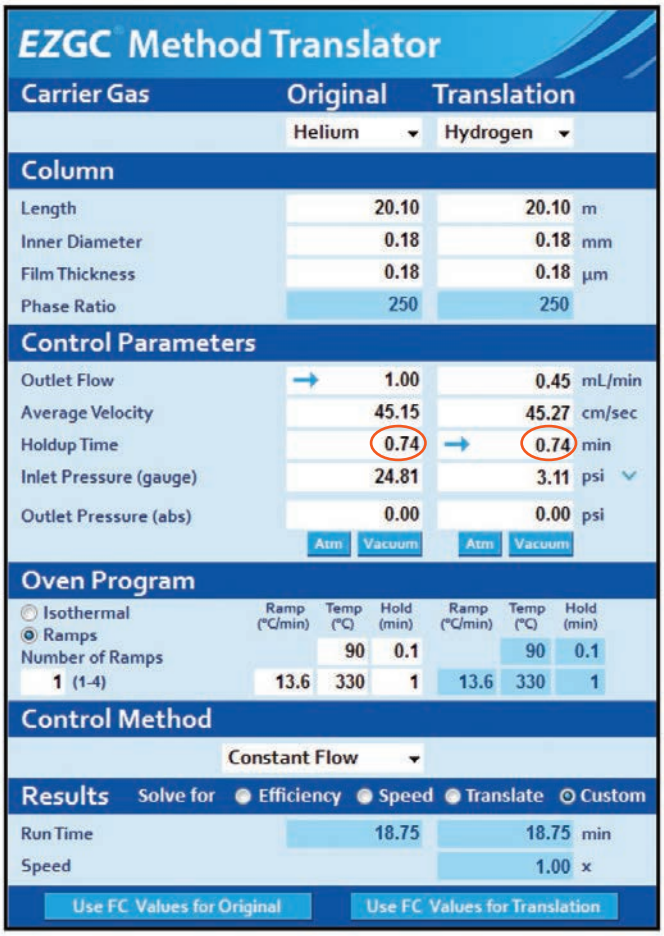
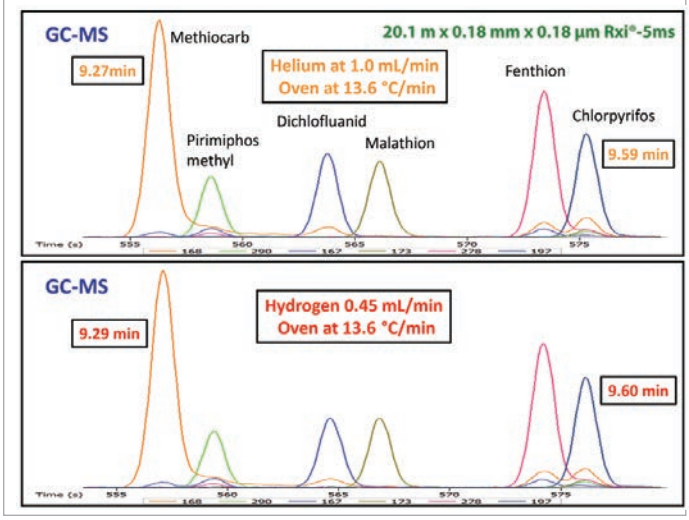


Figure 3: Get the advantage of switching to hydrogen, without having to reset retention time windows. Use the *EZ*GC® method translator/flow calculator to establish conditions that give the same retention times as your original method.



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.

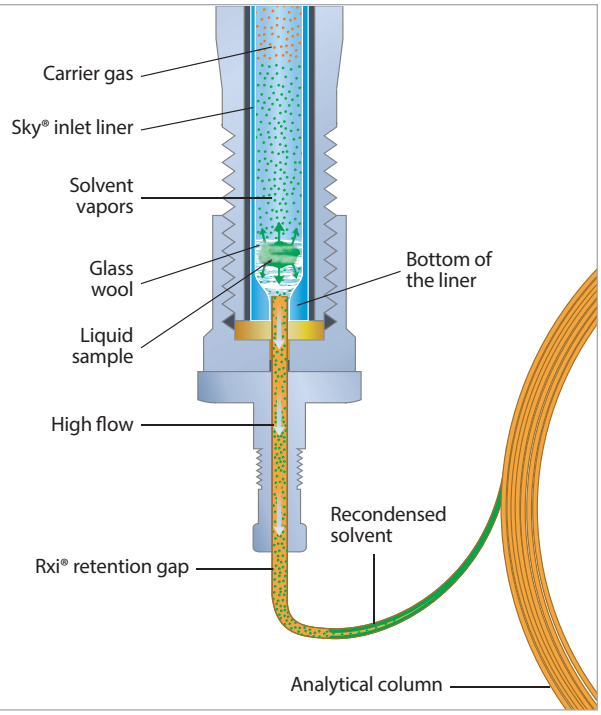


Table I: Starting points for CSR-LVSI method optimization.

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.

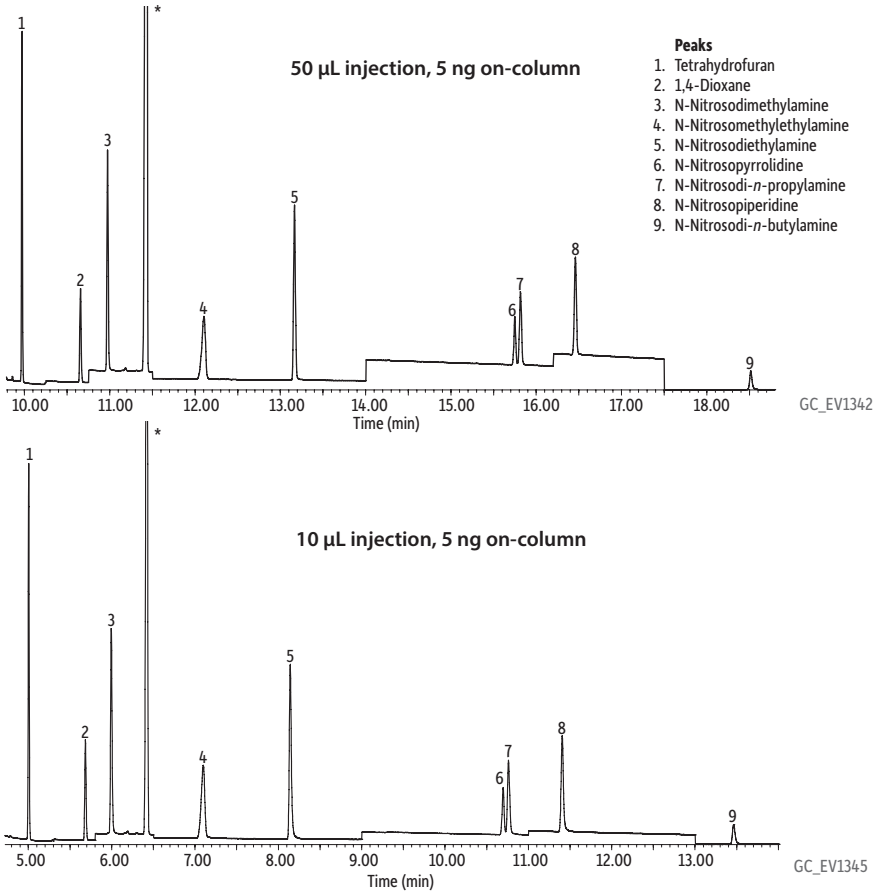
[3] J. Cochran, *The Solvent Effect in Concurrent Solvent Recondensation Large Volume Splitless Injection with Methylene Chloride – EPA Method 8270 Semivolatiles*, ChromaBLOGraphy, Restek Corporation, 2011 http://blog.restek.com/?p=1902 (accessed March 2, 2012).

[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.

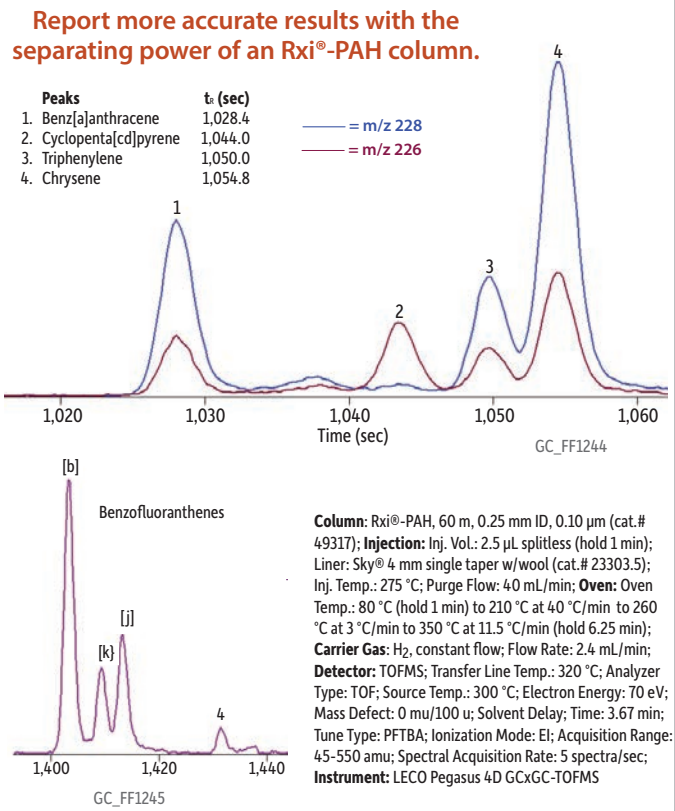
References

[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.

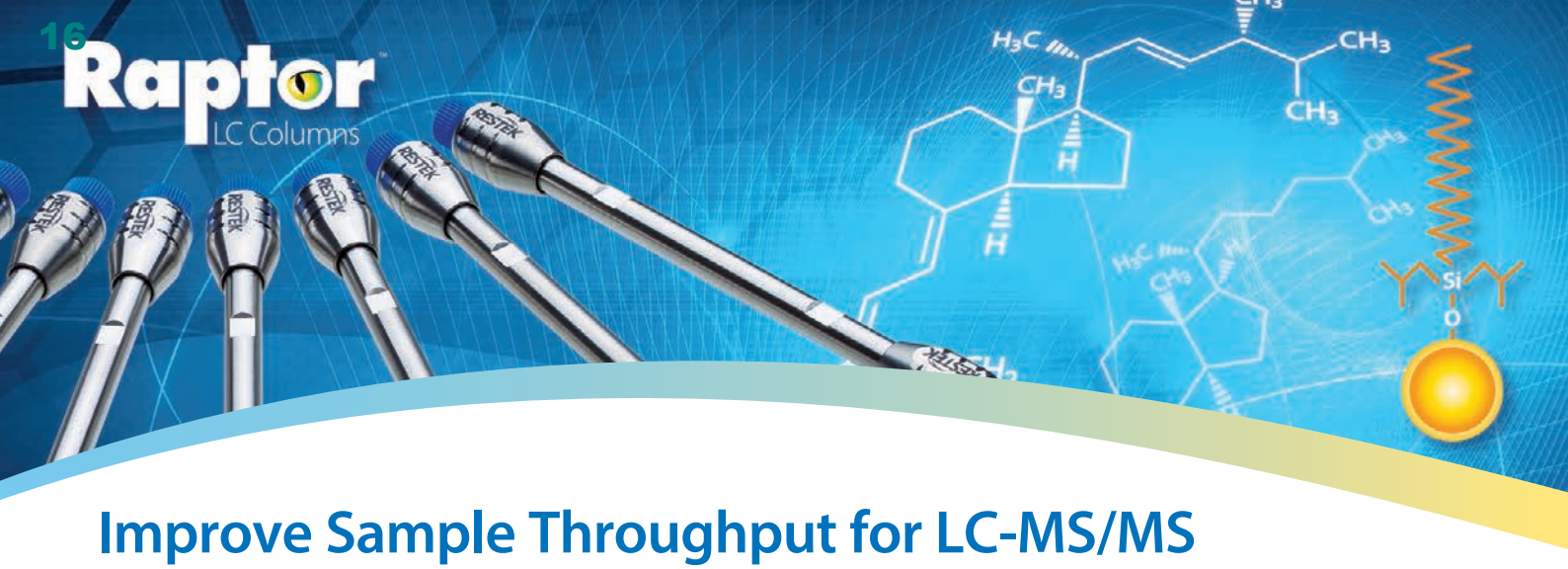
Silica SPE Cleanup

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.

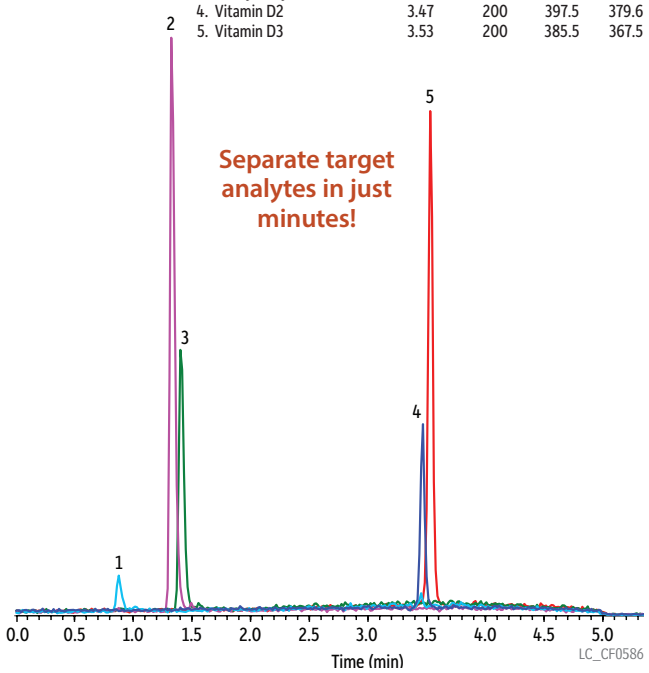
Separating fat-soluble vitamins by LC can be quite time-consuming, taking up to 20 minutes or longer by some methods. However, the new Raptor™ ARC-18 LC column can analyze these difficult compounds using reversed-phase chromatography (RPC) in less time than traditional columns, which helps increase sample 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.

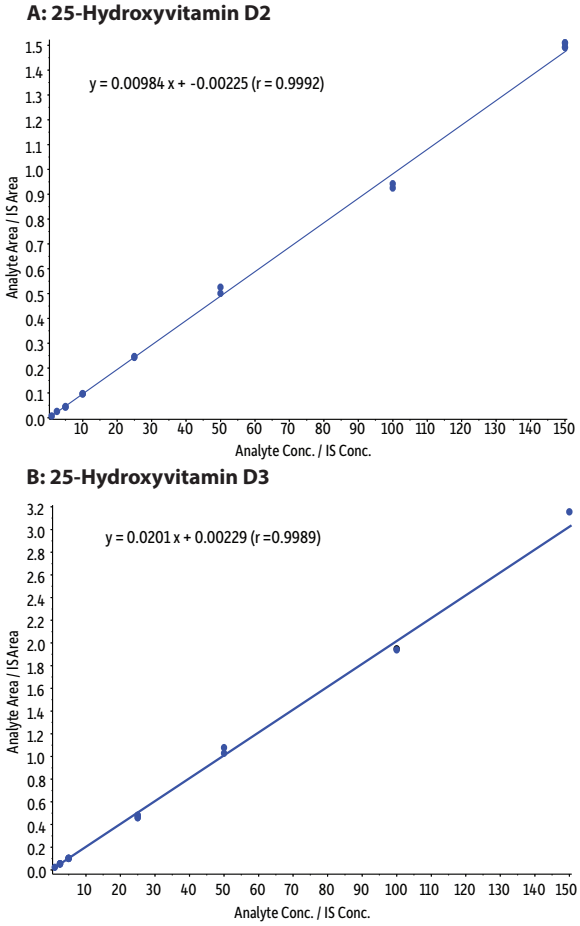
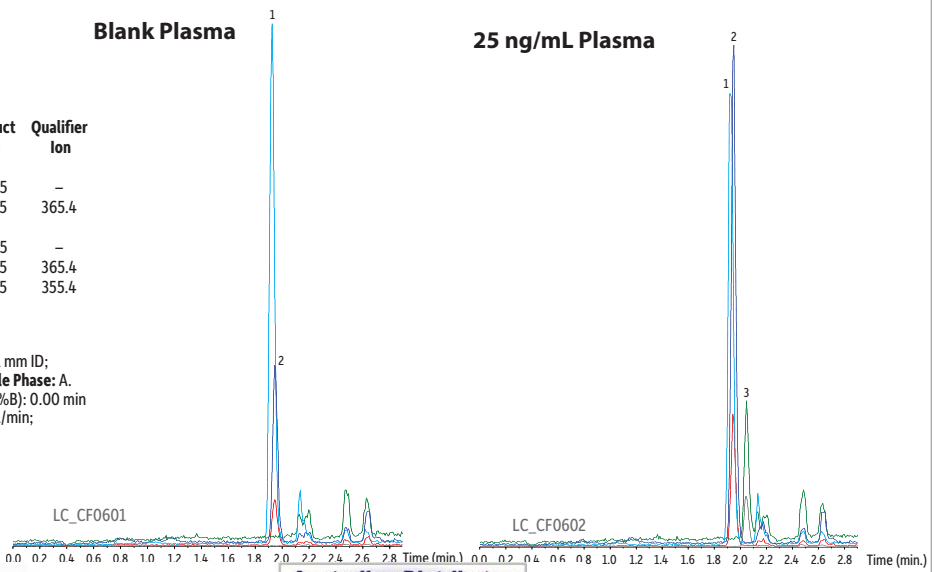
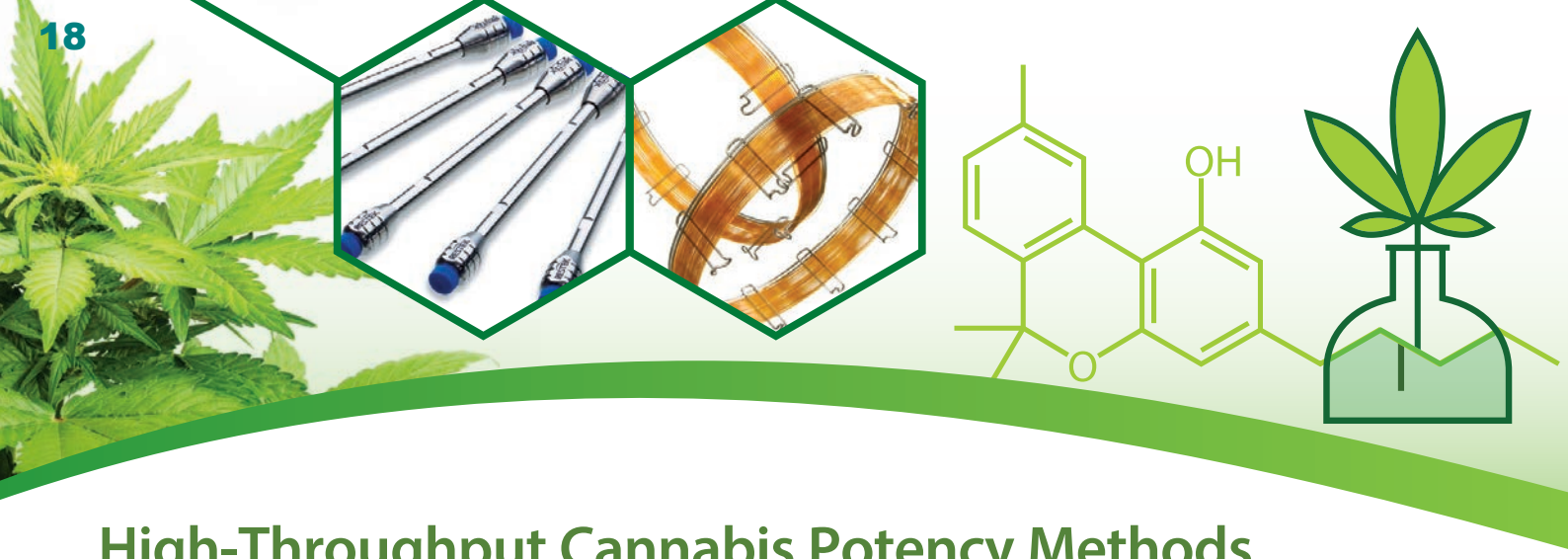


Figure 3: Good separation of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 from matrix components ensures more accurate results.

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.

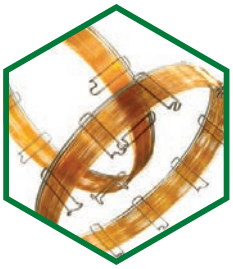
As shown in Figure 1, Restek has developed a fast analysis (3.8 min analysis [7 min total cycle time]) of cannabinoids that can be performed on any LC instrument. By utilizing Raptor™ column technology, you can obtain UHPLC speed without the capital investment. Also, we specifically chose simple, fast, 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.

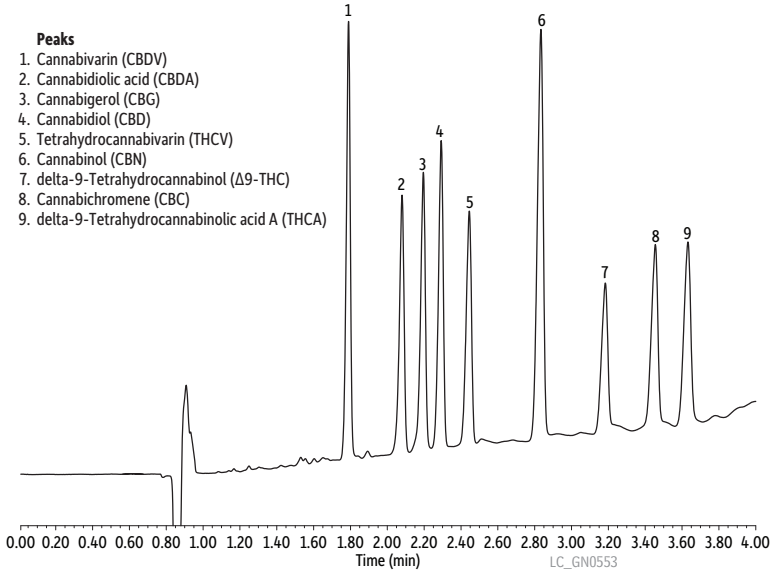
Whether you are using LC or GC for cannabis potency analysis, Restek can provide the products and expertise you need to obtain accurate results quickly. Use the methods shown here for analyzing the full spectrum of acid and neutral cannabinoids using LC with minimal capital investment, or get extremely 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.

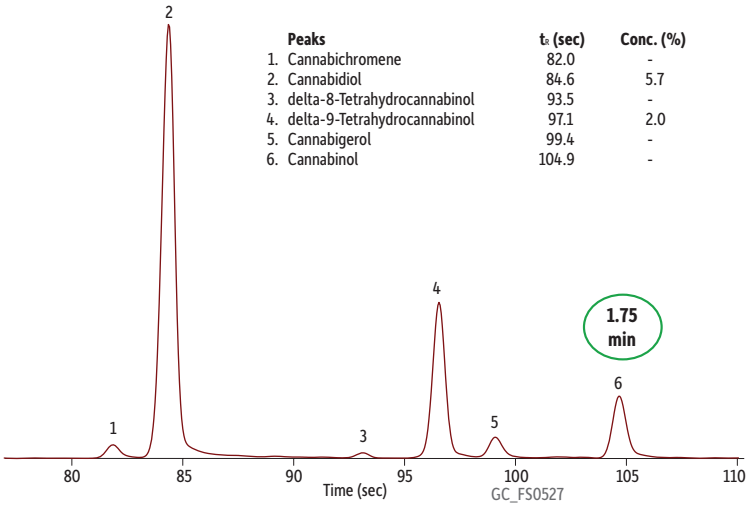


Column	Raptor™ ARC-18 (cat.# 9314A65)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	2.7 µm
Temp.:	50 °C
Sample	Cannabidiolic acid (cat.# 34094) Cannabigerol (cat.# 34091) Cannabidiol (cat.# 34011) Cannabinol (cat.# 34010) delta-9-Tetrahydrocannabinol (THC) (cat.# 34067) Cannabichromene (cat.# 34092) 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).

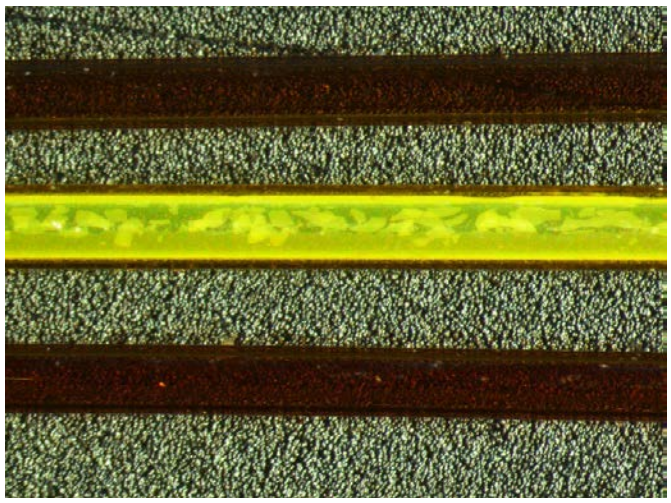


Figure 2: The Rt®-Silica BOND PLOT column shows no large particle spikes, even with temperature and pressure variation.

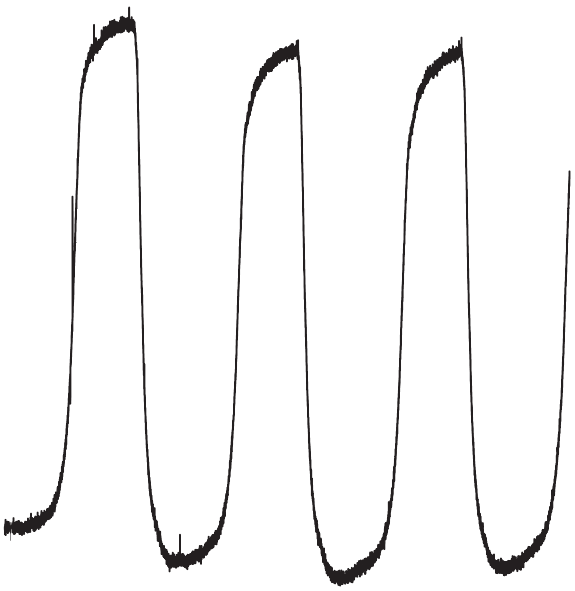


Figure 3: Sulfur Compounds in Butane.

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.