2011.2

Our expertise, experience, and enthusiasm is your Advantage.

RESTEK ADVANTAGE

Weeding Target Analytes Out of Complex Samples

Also in this issue

Restek Connections

In This Issue

Letter from the Bench

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

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

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

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

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

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

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

Cheers!

Jack Cochran
Director of New Business & Technology

You Have Opinions... And We Want Them.

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

About Restek Corporation

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

Sitting Down With a Chromatography Icon:
W. George Fong

By Jack Cochran

W. George Fong

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

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

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

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

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

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

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

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

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

Questions From You

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

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

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

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

- **Brandon Tarr**
Product Development Engineer

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

ChromaBLOGraphy

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

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

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

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

Hot Topics

Product Spotlight

Restek Introduces Secondary Columns for GCxGC

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

www.restek.com/gcxgc

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

Have You Tried Our Reversible Inlet Seals?

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

www.restek.com/flip

Chromatography in the News

1,4-Dioxane in Your Bathwater

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

The Tar Balls Keep Rolling In

Tar ball photo courtesy of Susan Forsyth

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

Hydrofracking: Coming to a Town Near You

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

Detecting Cancer Cola via HPLC

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

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

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

Event Recap

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

HPLC 2011 | June 19–23

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

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

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

- Ty Kahler

FPRW 2011 | July 17–20

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

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

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

- Jack Cochran

Quantifying Synthetic Cannabinoid Metabolites

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

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

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

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

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

Simplified Extraction Speeds up Sample Prep

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

Analysis of Positional Isomers and Unknown Metabolites in Authentic Samples

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

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

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

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

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

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

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

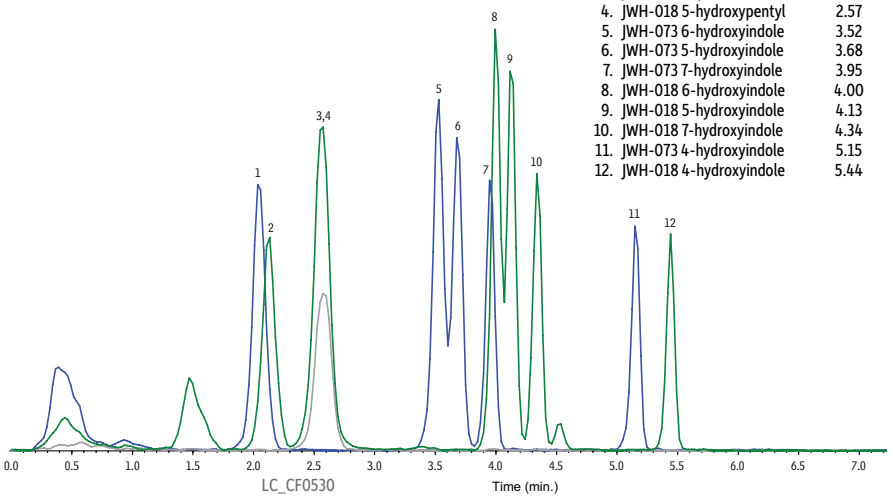
Summary

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

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

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

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



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

Sample was prepared according to the following method:

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

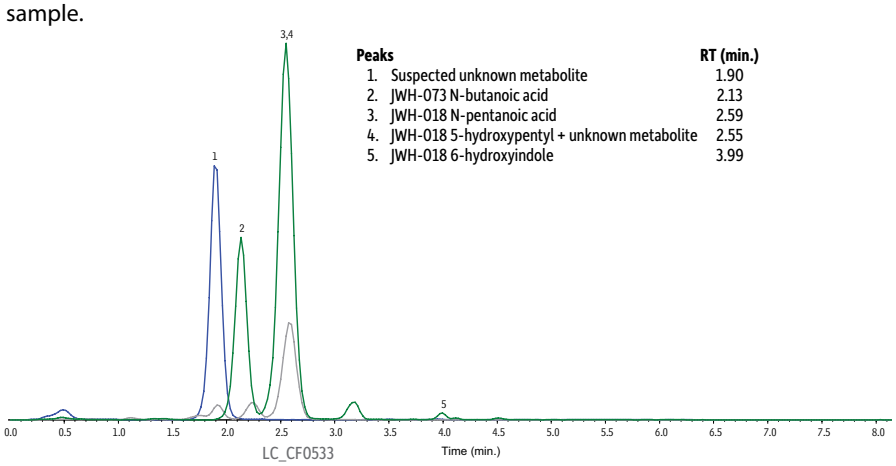
3) *Extract sample on 6 mL, 500 mg C18 high-load endcapped Resprep® SPE cartridge (cat.# 24052)*:
- Add 1 mL 5 mM ammonium acetate + 0.1% acetic acid (pH = 4.2) to sample.
- Condition cartridge with 3x 1 mL acetonitrile.
- Condition cartridge with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Apply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

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

Acknowledgement: Special thanks to Cayman Chemical for reference standards

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

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



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

Ultra Biphenyl Columns (USP L11)

Physical Characteristics:

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

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

Resprep® SPE Cartridges

(Bonded Reversed Phases)

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

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

References

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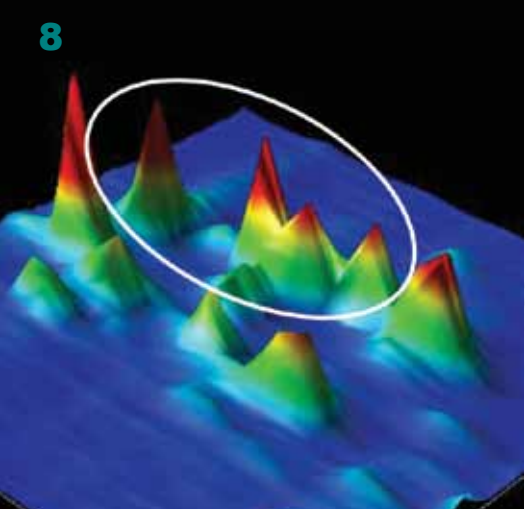
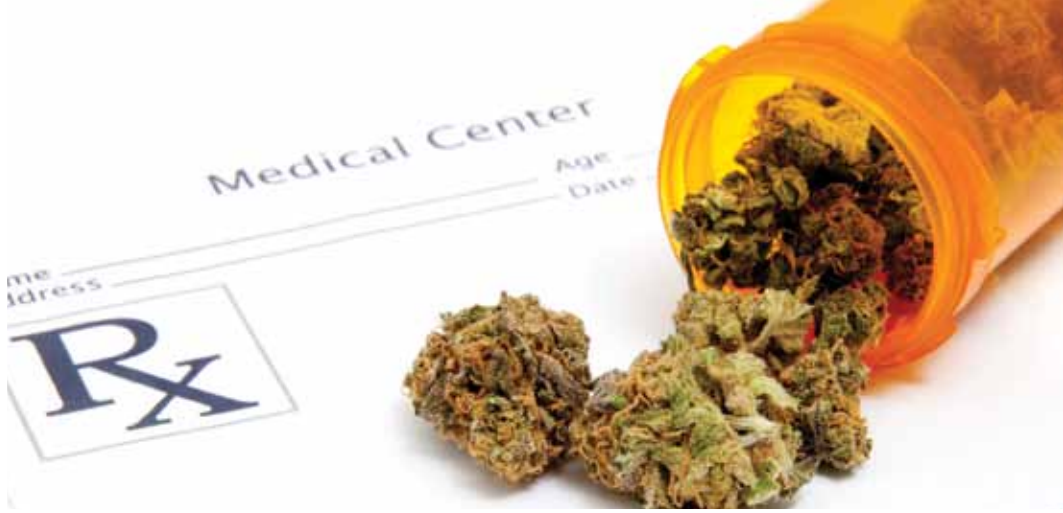
[3] B. Logan, S. Kacinko, M. McMullin, A. Xu, R. Middleberg, Technical Bulletin: *Identification of Primary JWH-018 and JWH-073 Metabolites in Human Urine*, (2011).





High Quality Analysis of Pesticides in Marijuana
Using QuEChERS, Cartridge SPE Cleanup, and GCxGC-TOFMS

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

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

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

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

QuEChERS Extraction Saves Time and Reduces Hazardous Solvent Use

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

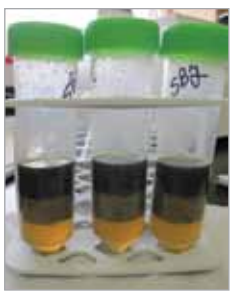
Water was added to finely ground, dry marijuana samples to increase QuEChERS extraction efficiency, especially for more polar pesticides. A vortex mixer was used to shake the solvent and sample for at least 30 minutes prior to extract partitioning. When finished, it was easy to transfer the supernatant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

Cartridge SPE Cleanup Improves GC Inlet Uptime

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

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

A. Post-centrifugation QuEChERS extracts



B. QuEChERS extracts loaded on SPE cartridge

C. Final extract

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

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

Summary

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

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

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

ND = no peak detected

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

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

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

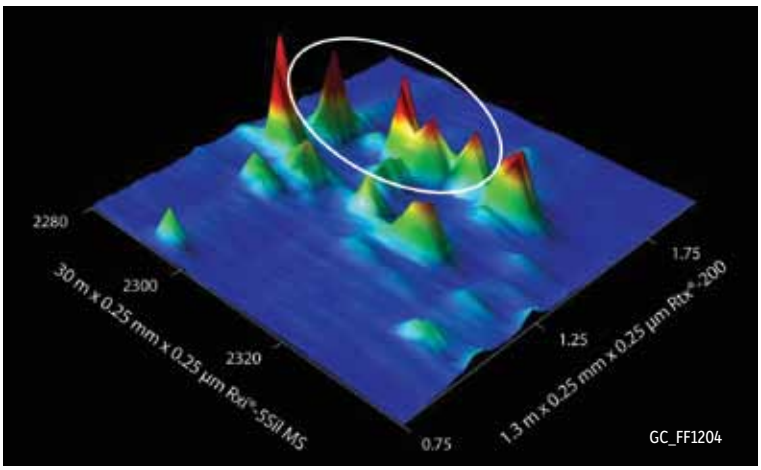
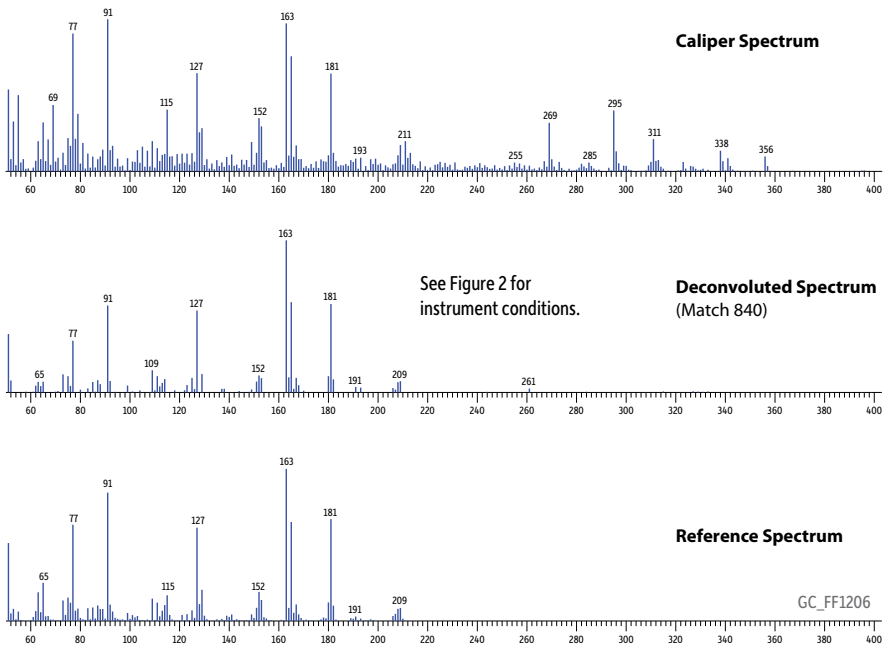


Figure 3: Positive mass spectral identification of incurred cypermethrin in illicit marijuana.



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

ChromaBLOGraphy

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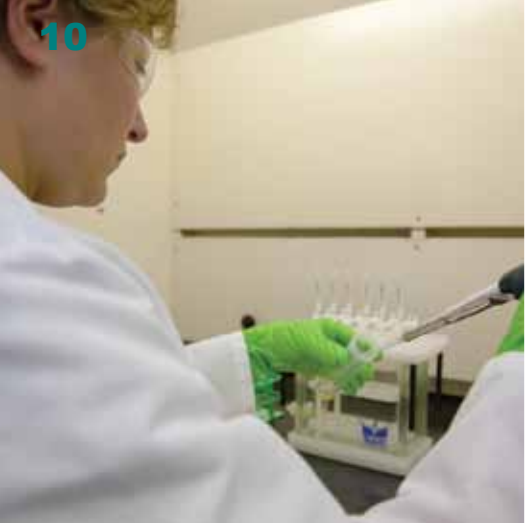
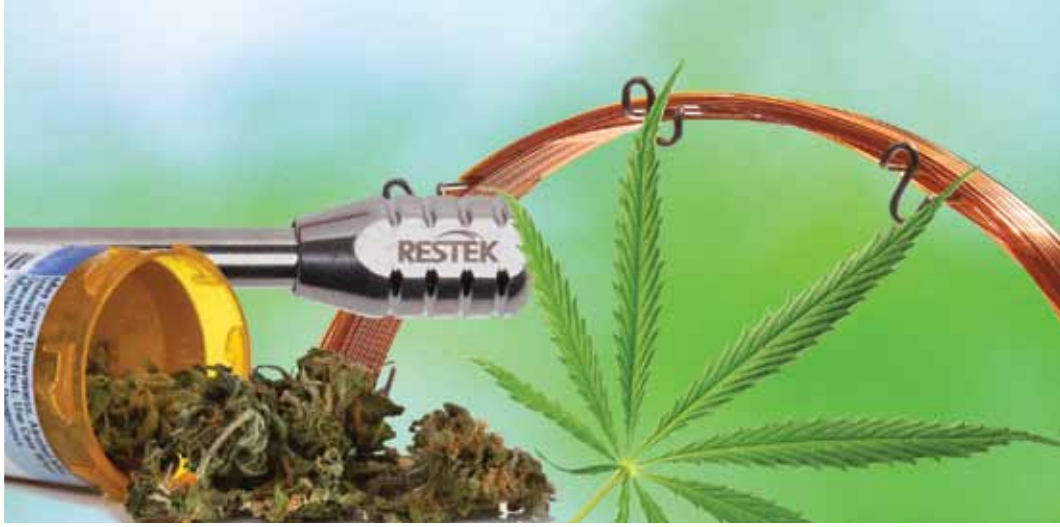




Marijuana Potency Testing—Quick and Easy by GC or LC

By Amanda Rigdon and Jack Cochran

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

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

Simple Sample Prep

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

GC Analysis

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

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

LC Analysis

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

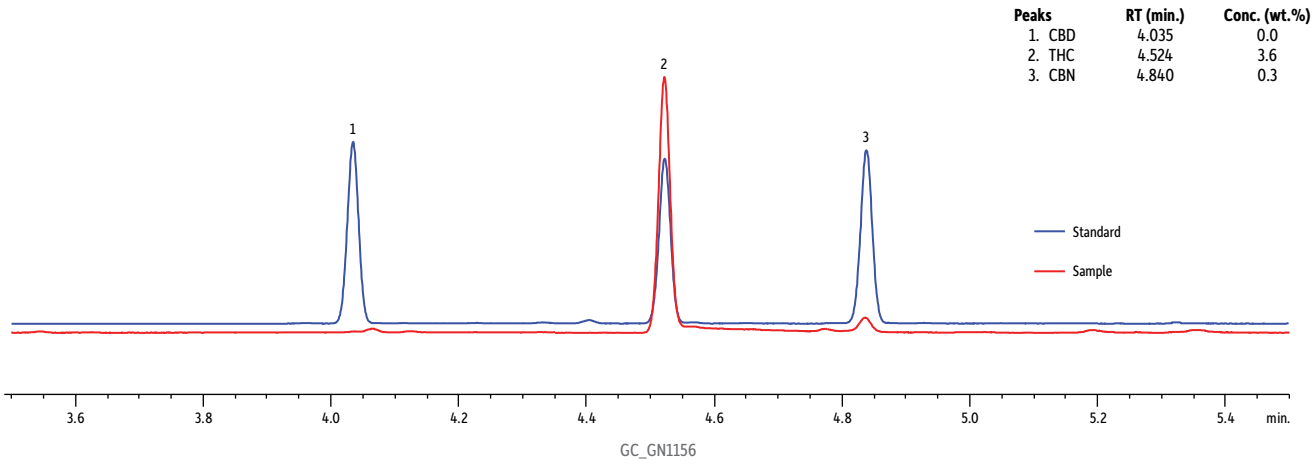
For LC potency testing, extracts were diluted 10x with isopropyl alcohol, and 10 µL of extract was injected onto a 3 µm Ultra Aqueous C18 column (cat.# 9178312). Figure 2 shows an overlay of the cannabinoid standard described above with the addition of THCA (blue trace) and a representative chromatogram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

Summary

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

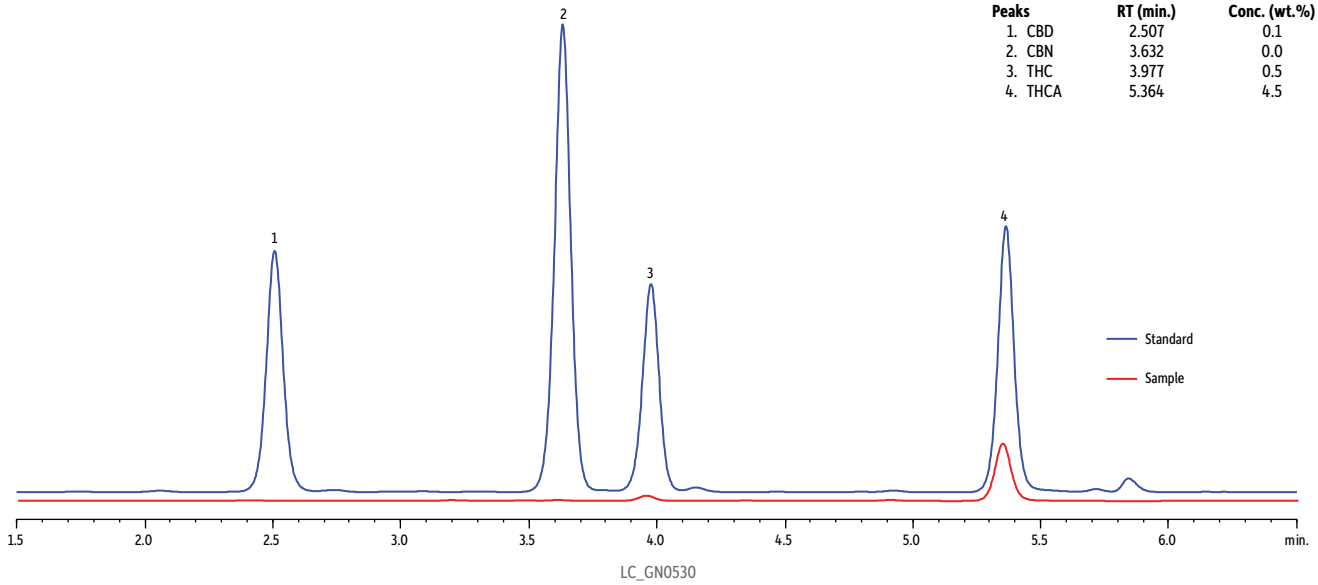
For further details, visit our technical blog at
www.restek.com/potpotency

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



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

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



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

Rxi®-5Sil MS Columns (fused silica)

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

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

similar **phases**

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

Ultra Aqueous C18 Columns (USP L1)

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

similar **phases**

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

Acknowledgment

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

Simplify HPLC and UHPLC Method Development

With the Restek USLC™ Column Set

By Rick Lake and Ty Kahler

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

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

Change Your Habits—and Your Columns—to Optimize Resolution

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

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

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

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

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

Efficiency *Retention Factor* *Selectivity*

The Highest Range of Alternate Selectivity

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

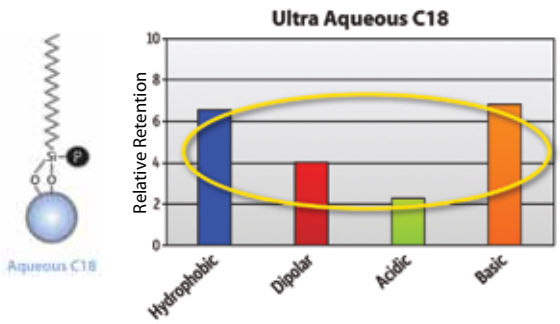
Figure 1 illustrates the retention profile of a C18 compared with those of the 4 Restek USLC™ columns. USLC™ phases are highly selective and exhibit significantly different retention profiles based on specific solute chemical properties, so you can match USLC™ columns to specific analytes and accelerate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

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

Summary

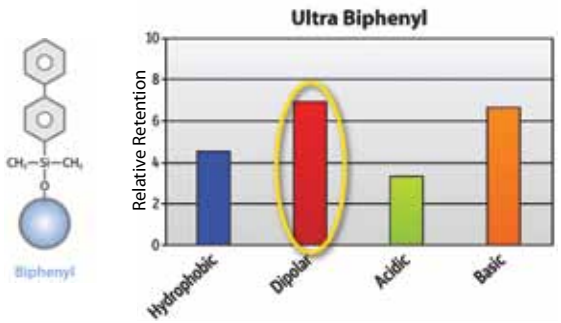
The Restek USLC™ column set has a profile that encompasses the widest range of reversed phase selectivity available today. Instead of manually altering mobile phases, operational parameters, or instrument settings—often with minimal effect on resolution—take advantage of the Restek USLC™ column set.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

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



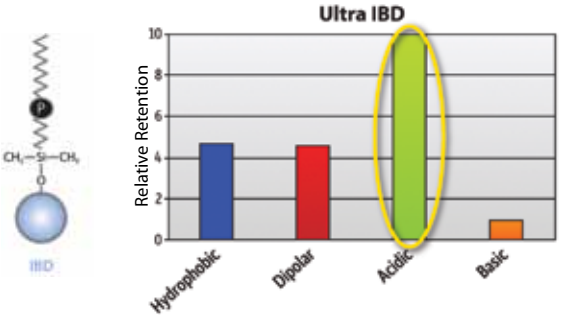
Restek USLC™ Phase: **Aqueous C18**

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



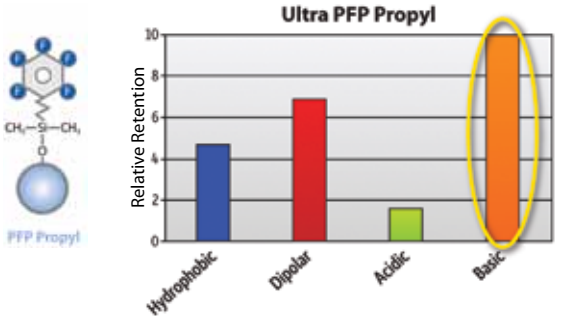
Restek USLC™ Phase: **Biphenyl**

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



Restek USLC™ Phase: **IBD**

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

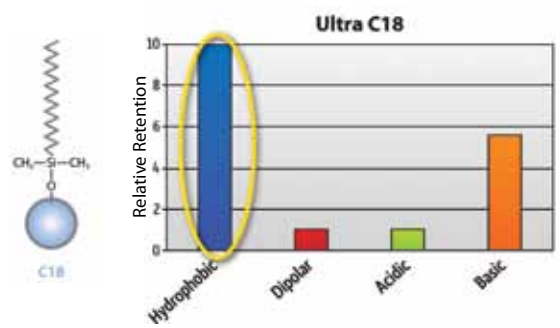


Restek USLC™ Phase: **PFP Propyl**

Properties:

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

C18 BENCHMARK

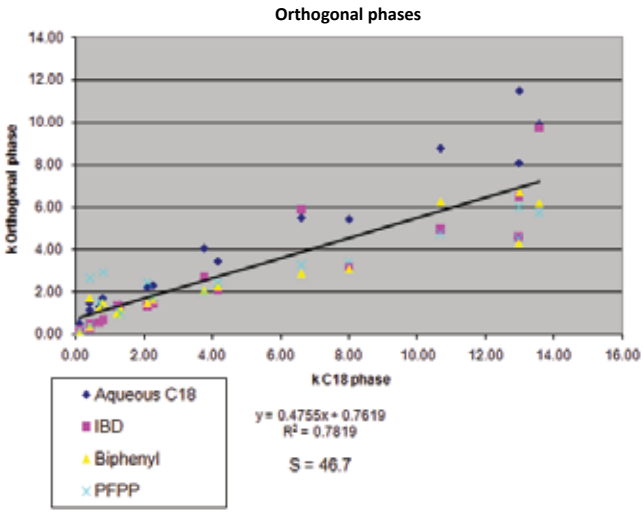


Restek Phase: **C18 Benchmark**

- General purpose.
- Strong hydrophobic retention.

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

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



References

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

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

Acknowledgements

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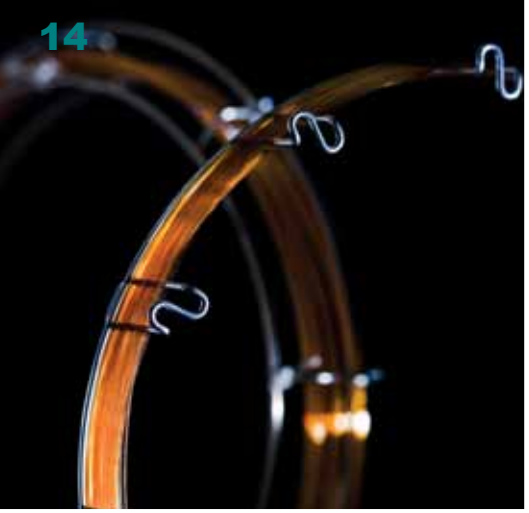
For a detailed analysis of USLC™ column selectivity data, visit **www.restek.com/USLCarticle**



7 EPA Methods on 1 Column Pair

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

By Jason Thomas

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

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

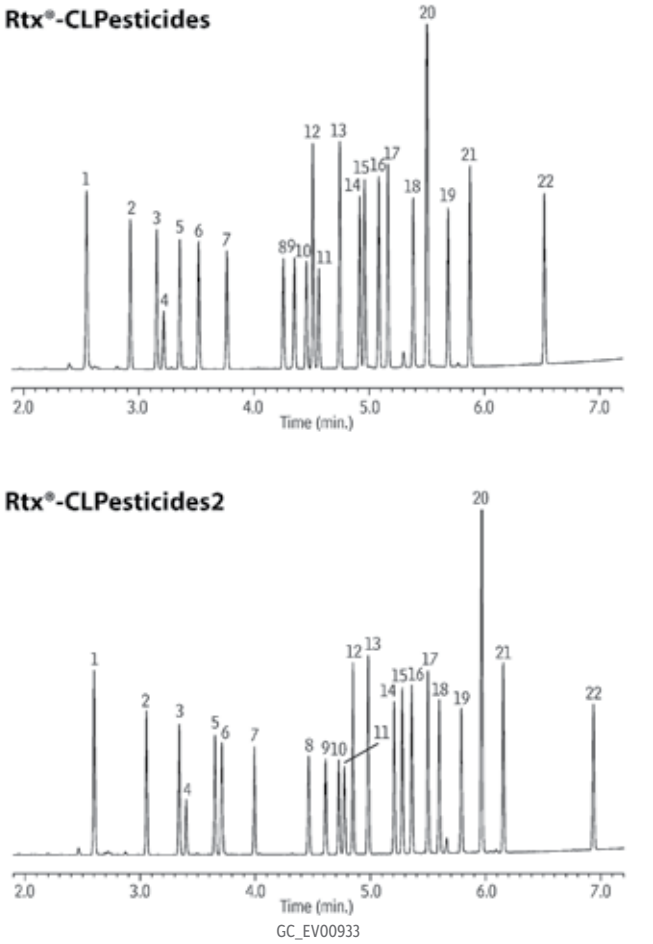
Numerous environmental contaminants are halogenated, and many tend to be quite toxic. Although some of these, like dioxins, are analyzed using HRMS for increased specificity, many EPA methods have been developed for pesticides, PCBs, DBPs, and other similar compounds using the ECD. These methods tend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

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

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

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

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



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

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

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

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

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

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

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

Optimal Performance for 7 ECD Methods

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

Cut Analysis Time in Half for Method 8081

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

Summary

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

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

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

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

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

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

Large Volume Splitless Injection With an Unmodified GC Inlet

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

By Michelle Misselwitz and Jack Cochran

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

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

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

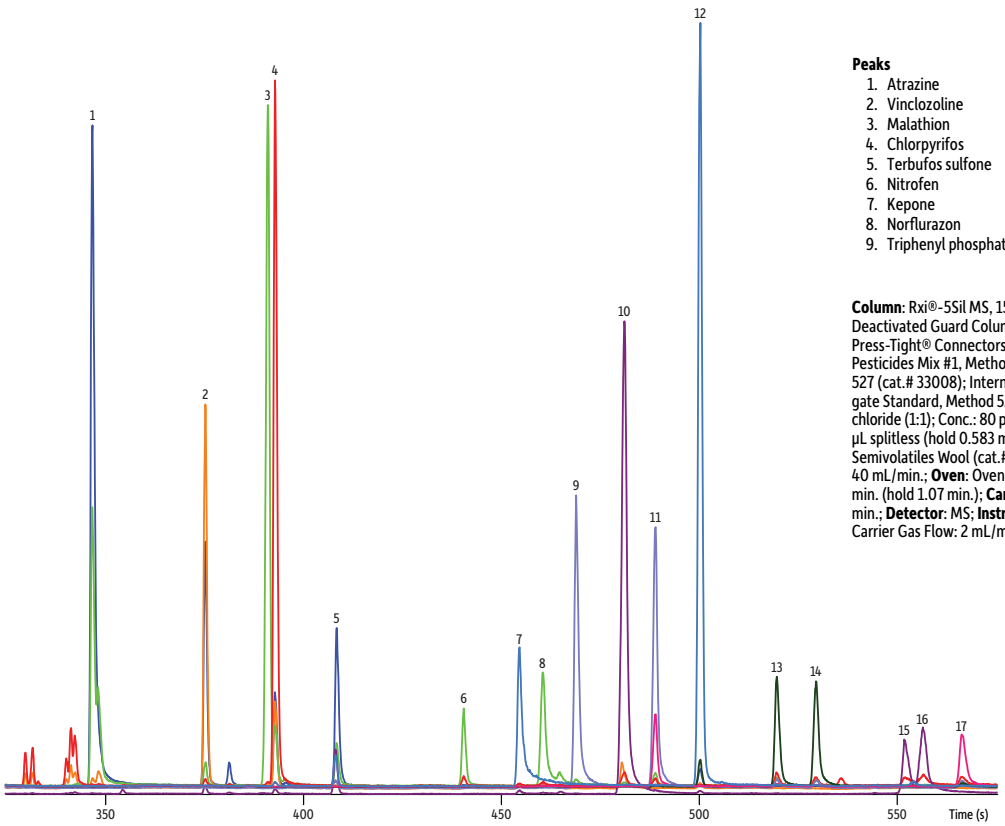
Table I: Calibration standards and concentration equivalents.

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

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

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

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



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

Linear Responses for Challenging Compounds Using CSR-LVSI

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

Determine Sub-ppb Levels Without Extract Concentration

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

Summary

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

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

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

Rxi®-5Sil MS Columns (fused silica)

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

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

Resprep® Resin SPE Disks

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

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

By Jaap de Zeeuw, Rick Morehead, and Tom Vezza

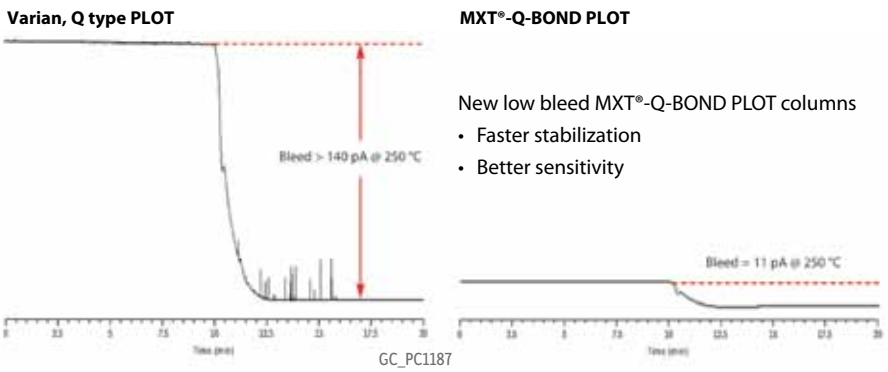
- New technology ensures consistent flows and predictable retention times.
- Rugged metal MXT® tubing stands up to process GC analyzer conditions.
- Available with all major adsorbents in 3.5" coils or on 7" 11-pin cages.

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

New Technology Improves Column Stability

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

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



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

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

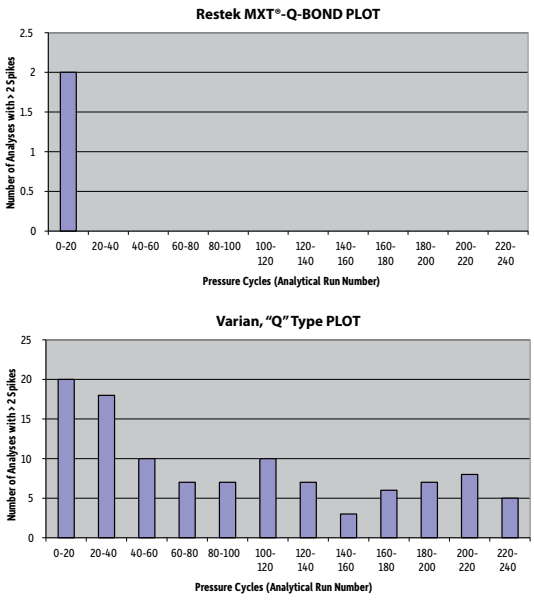
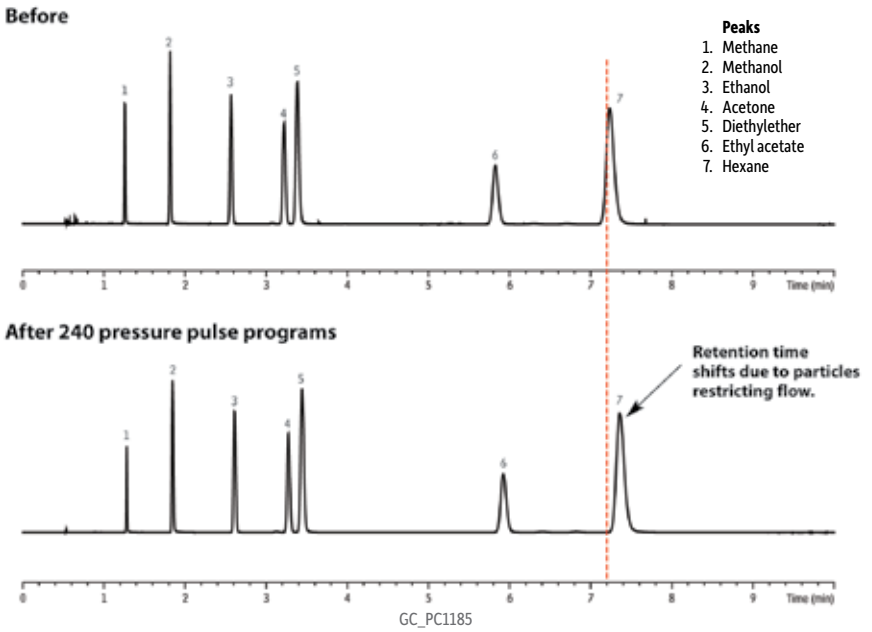
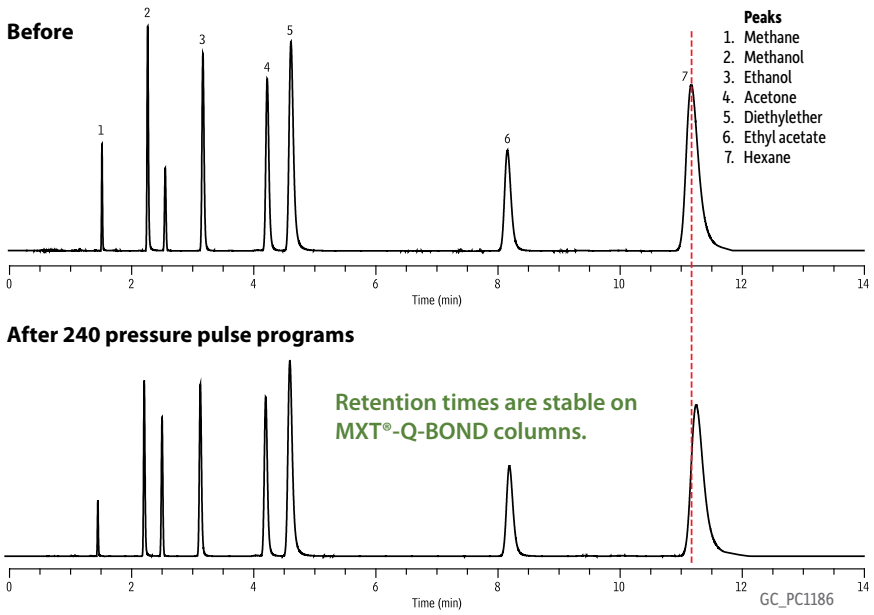


Figure 3: A conventional PLOT column releases particles following pressure pulsing, forming restrictions in the column that affect flow behavior and change retention time.



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

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



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

Stable Flow Ensures Predictable Retention Times

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

Key Phases Available for Optimized Separations

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

Summary

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

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

MXT®-Q-BOND Columns

(Siltek®-treated stainless steel PLOT)

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

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

Rethinking the Use of Wool With Splitless GC

By Scott Grossman

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

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

Should Splitless Mean Wool-Free?

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

Putting Wool Through the Wringer

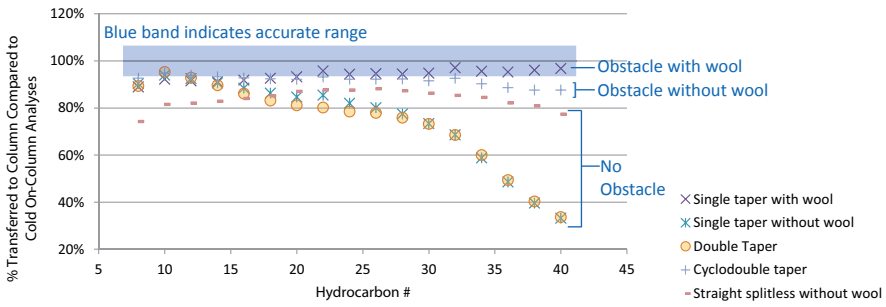
Since the integral question is whether you lose sample when performing splitless injections without wool, I opted to benchmark with cold on-column injections to force 100% of the sample onto the column. My sample was a 17-component mixture of straight-chain hydrocarbons spanning a molecular weight range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

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

Use Wool With Confidence

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

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

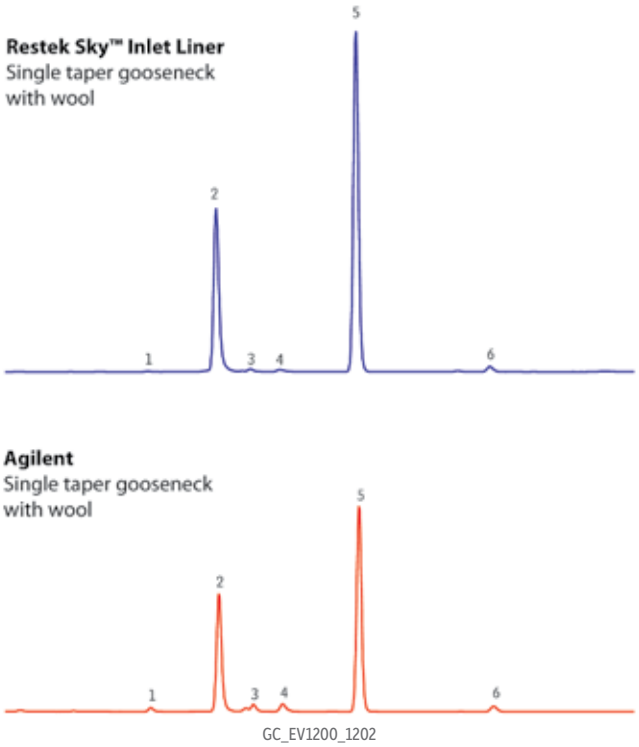


References

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

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

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



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

	% Breakdown	
	Endrin	DDT
Restek	4.8	1.3
Agilent	12	5.2

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

*breakdown products

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

Innovators in Chromatography

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

Analysis of Brominated Flame Retardants by Liquid Chromatography Mass Spectrometry

By Dr. Chris Marvin, Environment Canada

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

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

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

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

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

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

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

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

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

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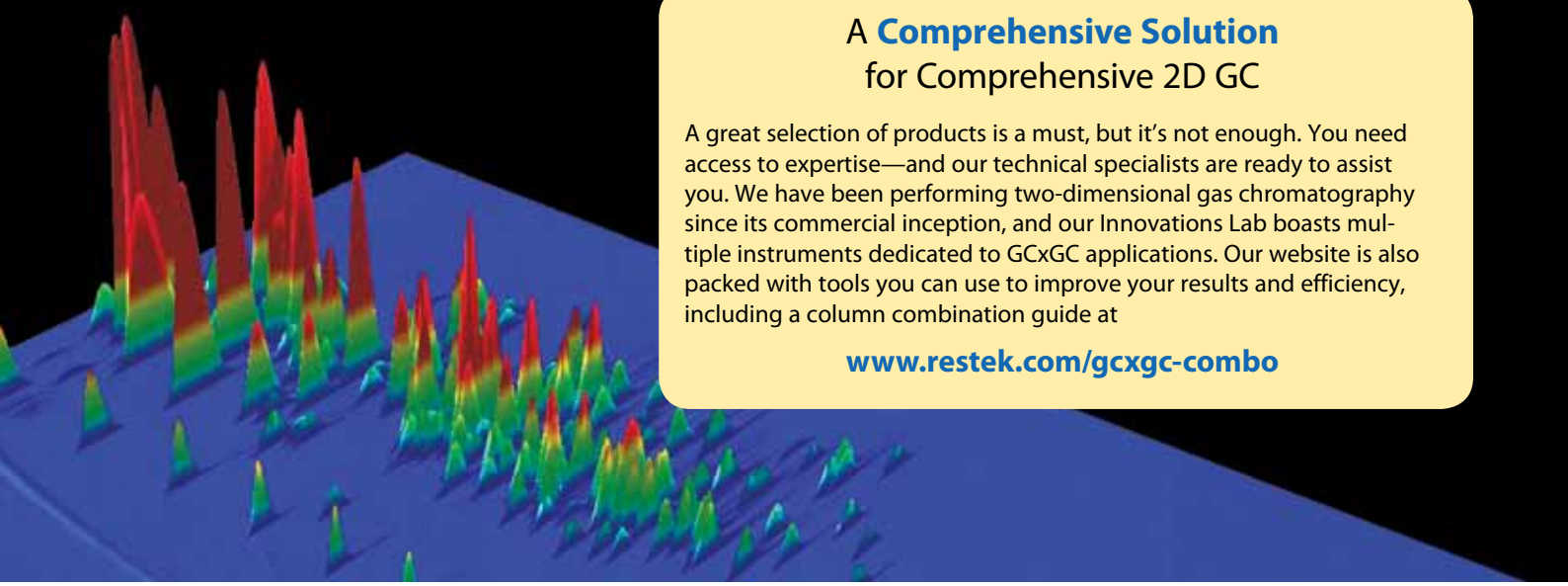

A **Comprehensive Solution** for Comprehensive 2D GC

A great selection of products is a must, but it's not enough. You need access to expertise—and our technical specialists are ready to assist you. We have been performing two-dimensional gas chromatography since its commercial inception, and our Innovations Lab boasts multiple instruments dedicated to GCxGC applications. Our website is also packed with tools you can use to improve your results and efficiency, including a column combination guide at

www.restek.com/gcxgc-combo

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