

# RESTEK ADVANTAGE

## New Approaches for Increasing Analytical Sensitivity

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## 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.

### Patents and Trademarks

Restek patents and trademarks are the property of Restek Corporation. Other trademarks appearing in Restek literature or on its website are the property of their respective owners. The Restek registered trademarks used here are registered in the United States and may also be registered in other countries.

## 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

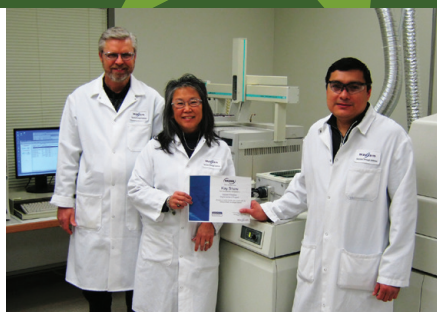
We chemists are an opinionated bunch, so the odds are good that you have some thoughts about the *Restek Advantage*. Love it? Hate it? Want to see something different in the next issue? Maybe you have a response to one of our technical articles? Whatever you have to say, let's hear it! Email your comments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



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

**“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](http://www.restek.com/dioxin2)

**Do you have a Restek success story to share?**

E-mail [advantage@restek.com](mailto: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 Premium single taper liner with wool (cat.# 23303.1) for splitless injections and a 4 mm ID Premium Precision liner with wool (cat.# 23305.1) for split injections. The thoroughly deactivated Restek Premium 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](http://www.restek.com/flipFAQ)

**-Wrestling with a question of your own?**

Call 1-800-356-1688, ext. 4, or e-mail [support@restek.com](mailto: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 Sprengle | Corporate Account Representative

Interested in joining our team?

Check out [www.restek.com/jobs](http://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  $\mu$ m) and HPLC (3 or 5  $\mu$ m) 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.*

[www.restek.com/uslc](http://www.restek.com/uslc)

## 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](http://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](http://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](http://www.restek.com/events) for more information and be sure to pay us a visit!



## More Labs Required to Source CRMs

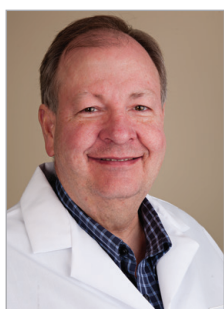
An increasing number of laboratories worldwide are being required to use certified reference materials (CRMs), which can only be manufactured and QC tested at an ISO-accredited lab. The U.S. Department of Defense insists on them, as do numerous other government agencies across North America, Europe, 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](http://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](http://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](http://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  $1 \times 10^{-6}$  cancer risk assessment level\* down to 0.35  $\mu\text{g/L}$ . As a result, the proposed minimum reporting level (MRL) for 1,4-dioxane as part of UCMR3 is 0.07  $\mu\text{g/L}$  [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](http://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 correla-

tions across a wide range ( $R^2 = 0.9998$  for 1 to 1,000  $\text{pg}/\mu\text{L}$  [10 to 10,000  $\text{pg}$  on column] and  $R^2 = 0.9996$  for 0.5 to 50  $\text{pg}/\mu\text{L}$  [5 to 500  $\text{pg}$  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  $\mu\text{L}$  CSR-LVSI in Figure 1 (approximately 5  $\text{pg}$  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  $\mu\text{L}$  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- $d_8$  that were within the expected range (Table II) and that matched published method development data very well [4].

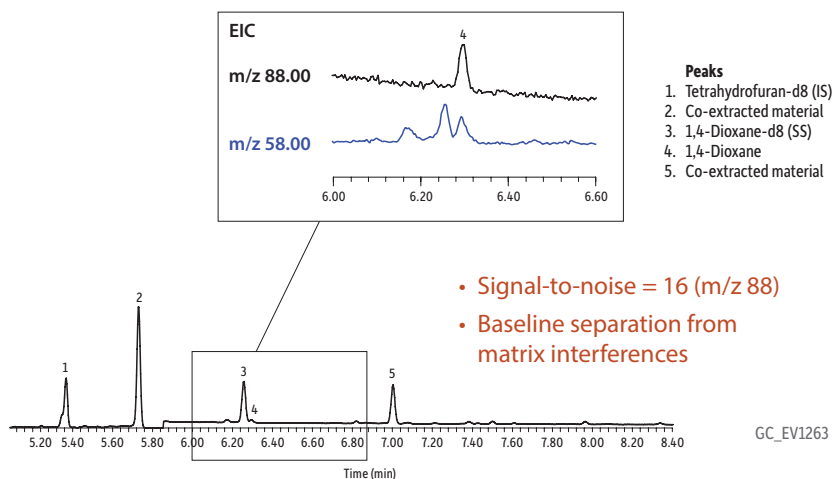
**Table I:** Calibration curve (0.5–50  $\text{pg}/\mu\text{L}$ ).

Level	Prepared Standard ( $\text{pg}/\mu\text{L}$ )	10 $\mu\text{L}$ Injection On-Column Amount ( $\text{pg}$ )	Equivalent Concentration in 500 mL Samples ( $\mu\text{g/L}$ )
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

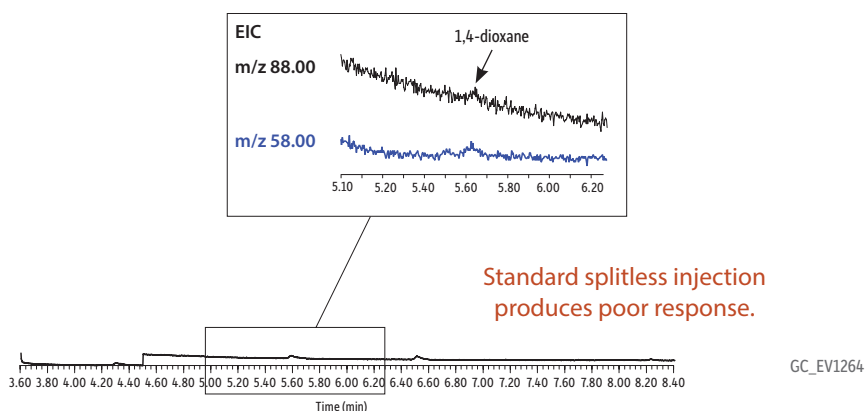
\*A  $1 \times 10^{-6}$  cancer risk assessment level corresponds to the lifetime probability of one individual in an exposed population of one million developing cancer.



**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](http://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: Restek Premium 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](http://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. Grimmer, 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.

## Restek Recommends

Our CSR-LVSI setup:

Rxi®-624Sil MS Columns &  
Rxi® Retention Gaps  
[www.restek.com/rxi](http://www.restek.com/rxi)

Press-Tight® Connectors  
[www.restek.com/presstight](http://www.restek.com/presstight)

Restek Premium Inlet Liners  
[www.restek.com/liners](http://www.restek.com/liners)



## 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 Restek Premium 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.

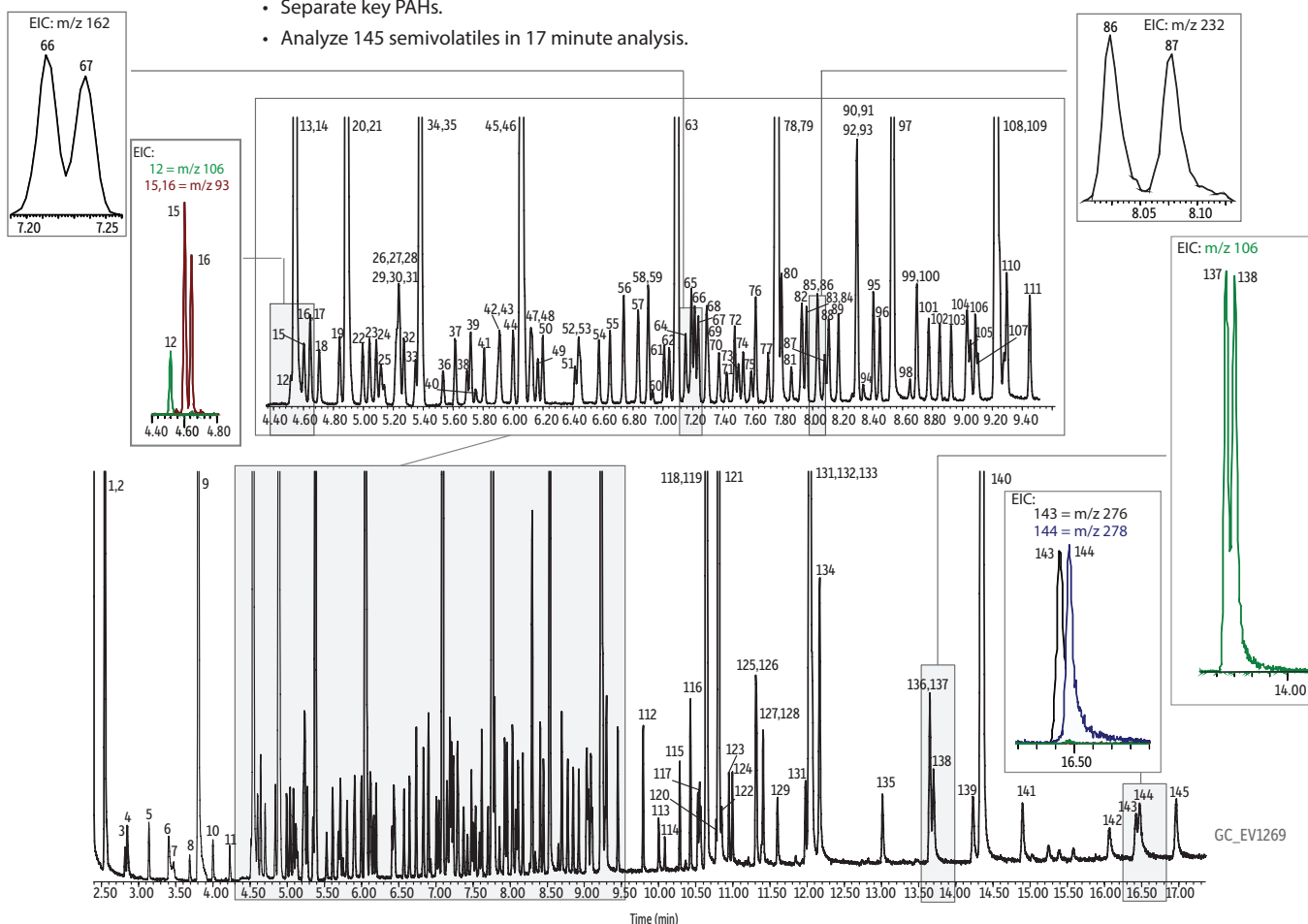
	RRF (0.5 ng)	Minimum RRF	Average RRF (0.5 – 120 ng/μL)	RRF RSD	Linear R <sup>2</sup>
2-Nitrophenol	0.710	0.100	0.770	6.9%	0.9999
2-Nitroaniline	0.204	0.010	0.226	5.4%	0.9999
3-Nitroaniline	0.218	0.010	0.226	3.5%	0.9997
2,4-Dinitrophenol	0.055	0.010	0.176	42%	0.9992
4-Nitrophenol	0.234	0.010	0.254	8.0%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

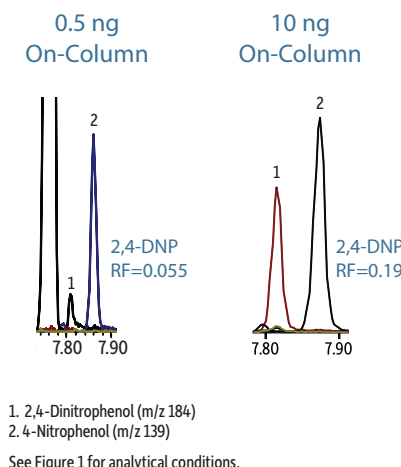


**Column:** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 318623); **Sample:** 8270 MegaMix® (cat.# 31850), 8270 Benzidines mix (cat.# 31852), Benzoic acid (cat.# 31879), Revised B/N surrogate mix (cat.# 31888), Acid surrogate mix (4/89 SOW) (cat.# 31063), Revised SV internal standard mix (cat.# 31886), 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:** Restek Premium 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](http://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.



For more environmental applications, visit [www.restek.com/enviro](http://www.restek.com/enviro)

## Restek Recommends

For ultimate sample path inertness:

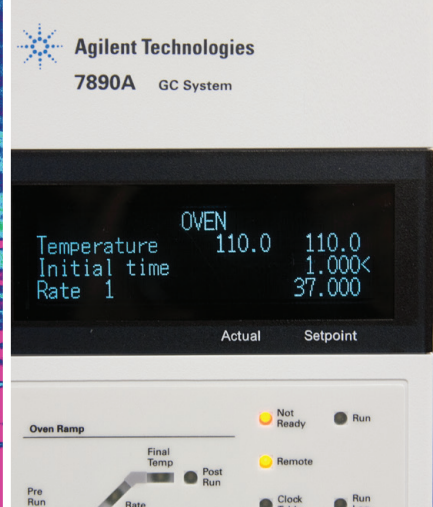
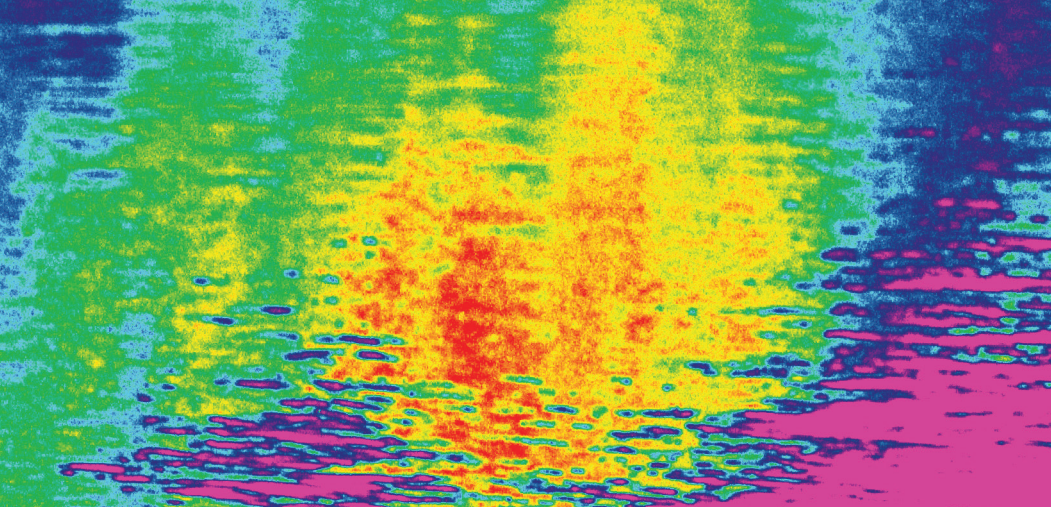
EZ Twist Top® Injection Port  
[www.restek.com/eztwist](http://www.restek.com/eztwist)

Restek Premium Inlet Liners  
[www.restek.com/liners](http://www.restek.com/liners)

Flip Seal™ Inlet Seals  
[www.restek.com/flip](http://www.restek.com/flip)

Rxi®-5Sil MS Columns  
[www.restek.com/rxi](http://www.restek.com/rxi)





# 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?

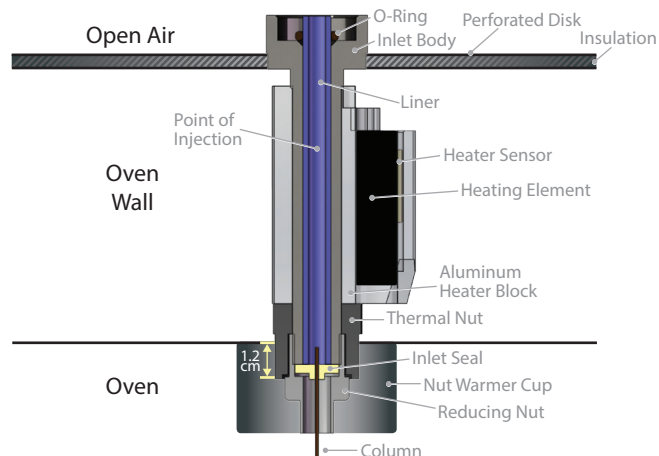
### Temperature Varies Within and Between Similar Inlets

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

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

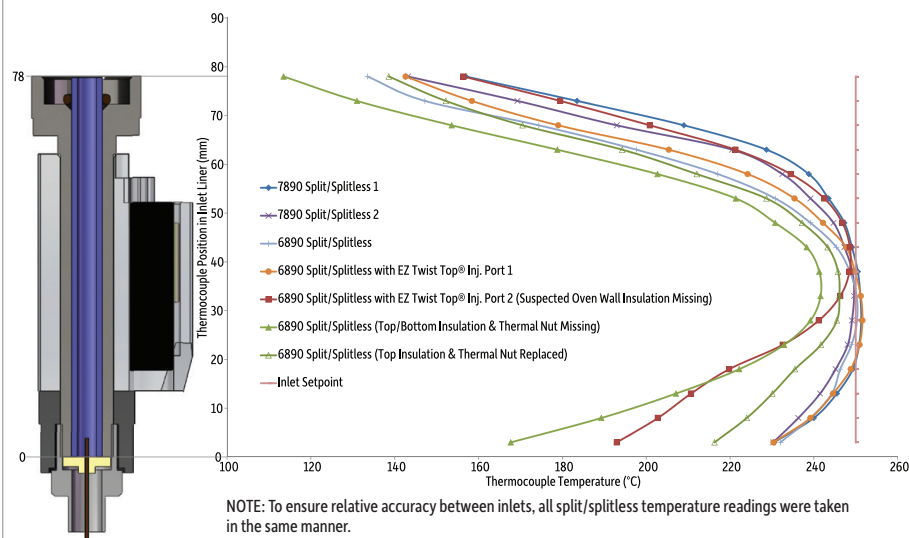
**Figure 1:** Considering how little of the GC inlet is actively heated by the heating element, it's no surprise a temperature gradient exists—especially if insulation is missing from the top or bottom.



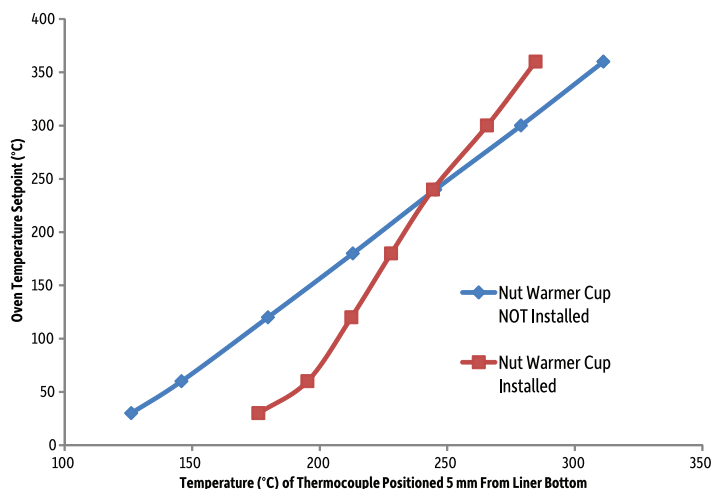
### Insulation is Crucial to Minimizing Temperature Variation

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

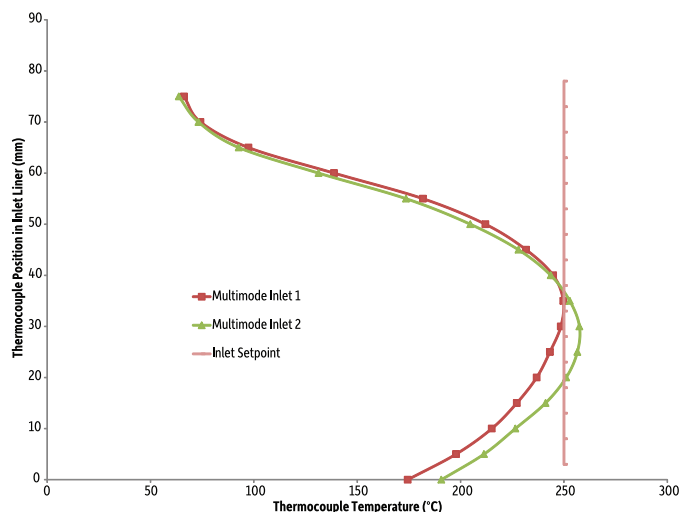
**Figure 2:** A temperature gradient exists within a GC inlet, and temperature profiles can vary between similar inlets. These variations increase dramatically with the absence of insulation.



**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](http://www.restek.com/TempEffects)





# Comprehensive Pesticide Residue Monitoring in Foods

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

By Julie Kowalski<sup>1</sup>, Jack Cochran<sup>1</sup>, Jason Thomas<sup>1</sup>, Michelle Misselwitz<sup>1</sup>, Rebecca Wittrig<sup>2\*</sup>, and André Schreiber<sup>3</sup>

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<sup>2</sup>AB SCIEX, 353 Hatch Drive, Foster City, California 94404, USA

<sup>3</sup>AB SCIEX Research and Development, 71 Four Valley Drive, Concord, Ontario, Canada L4K 4V8

\*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](http://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

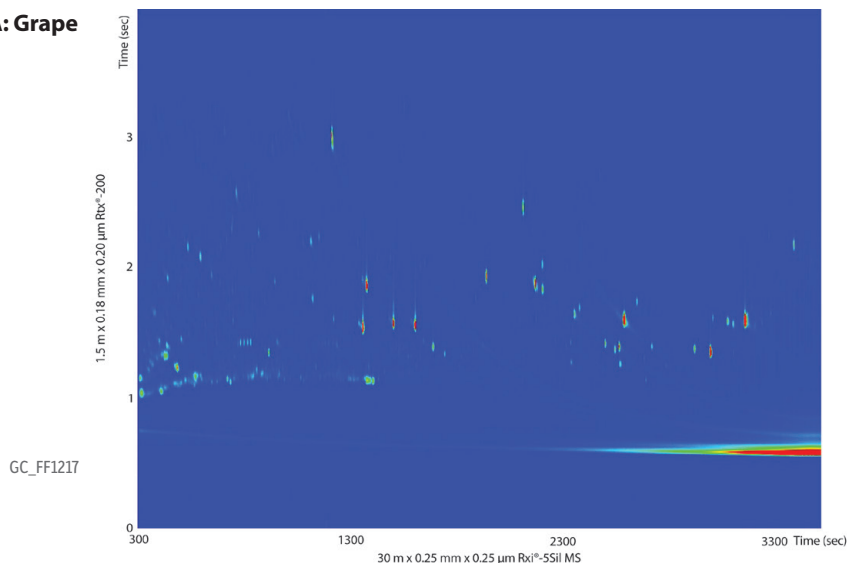
GCxGC-TOFMS. It is clear from Figure 1 that lemon contains many more coextractives than grape, as demonstrated by the large number of intense (red) signals. While it should be possible to analyze QuEChERS grape extracts for pesticides by one-dimensional GC, multidimensional techniques (e.g., GCxGC-MS, 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

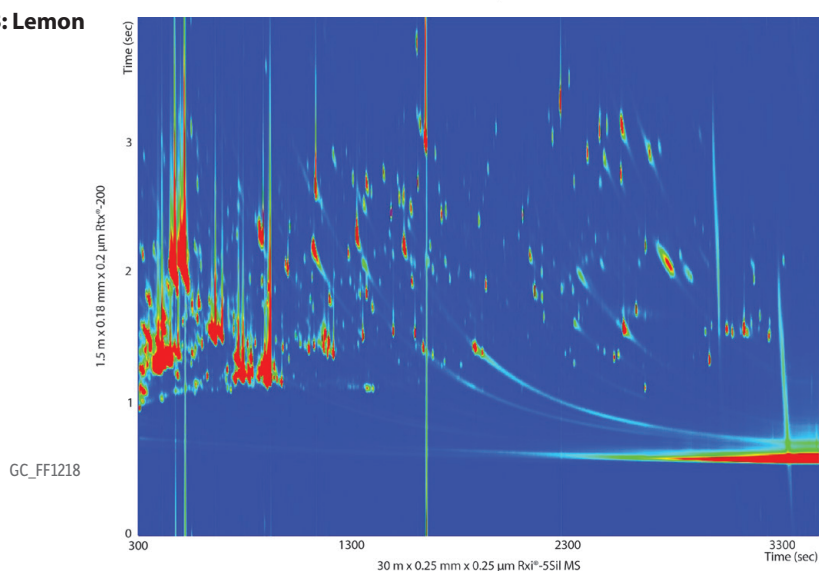
Good recoveries were obtained for most pesticides in most commodities as determined by both GCxGC-TOFMS and LC-MS/MS. As shown in Table I, quantitative results for grape were excellent, but lemon proved to be a difficult matrix as demonstrated by the fact that 11 pesticides were not detected by LC-MS/MS 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.

#### A: Grape



#### B: Lemon



**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](http://www.restek.com) and enter chromatograms GC\_FF1217 and GC\_FF1218 in the search.

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](http://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.

**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
Carfentrazon 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

## Restek Recommends

Comprehensive solutions:

Q-sep™ QuEChERS Sample Prep Products  
[www.restek.com/quenchers](http://www.restek.com/quenchers)

GCxGC Columns and Resources  
[www.restek.com/gcxcgc](http://www.restek.com/gcxcgc)

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## 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.

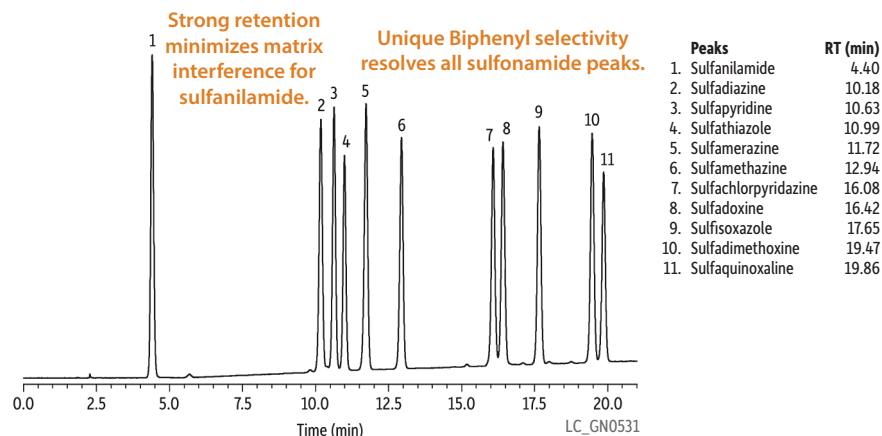
### Combining USLC® Selectivity and UHPLC Speed—The Most Powerful Approach

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  $\mu$ m 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.



**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 UFLCxx.

For more about the advantages of USLC® Biphenyl columns, visit [www.restek.com/uslc](http://www.restek.com/uslc)

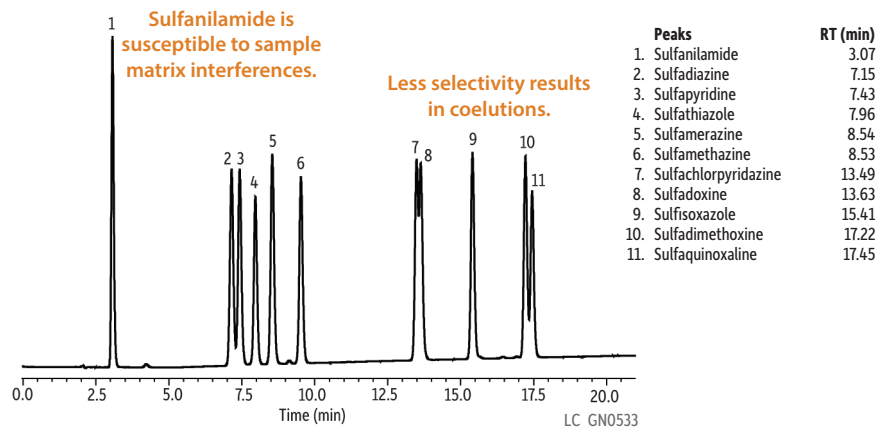
## Ultra Biphenyl Columns (USP L11)

### Physical Characteristics:

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

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

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



**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 UFLCxx.

## Pinnacle® DB Biphenyl Columns (USP L11)

### Physical Characteristics:

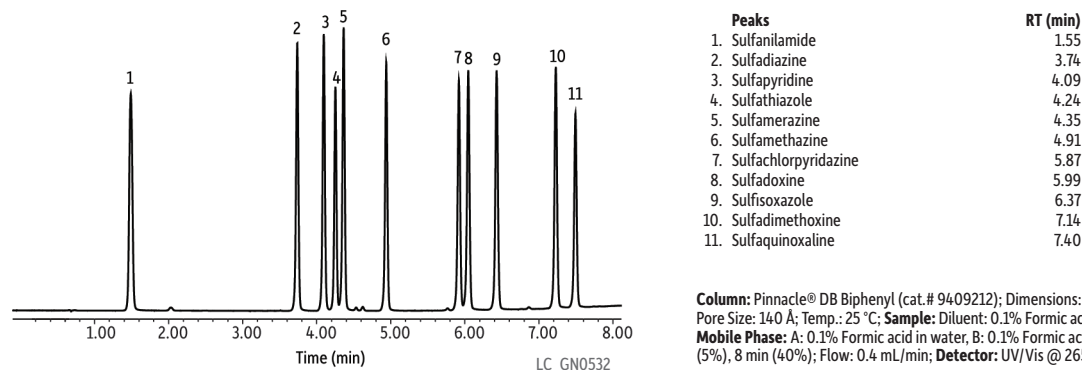
particle size: 1.9 µm, 3 µm, or 5 µm, spherical endcap: yes  
pore size: 140 Å pH range: 2.5 to 8  
carbon load: 8% temperature limit: 80 °C

Description	cat.#
<b>1.9 µm Columns</b>	
100 mm, 2.1 mm ID	9409212

## ordering note

For guard cartridges for these columns, visit our website at [www.restek.com](http://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.



**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 UFLCxx.





## 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  $\mu$ m 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  $\geq 10$  at the LOQ, and each qualifier ion had a signal-to-noise ratio of  $\geq 3$ . 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  $\leq 15\%$  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  $\leq 15\%$  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  $\mu\text{g/mL}$  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  $\mu\text{m}$  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](http://www.restek.com/cft)

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### Ultra Biphenyl Columns (USP L11)

#### Physical Characteristics:

particle size: 3  $\mu\text{m}$  or 5  $\mu\text{m}$ , spherical  
pore size: 100 Å  
carbon load: 15%  
endcap: fully endcapped  
pH range: 2.5 to 8  
temperature limit: 80 °C

Description	cat.#
5 $\mu\text{m}$ Columns	
100 mm, 2.1 mm ID	9109512

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

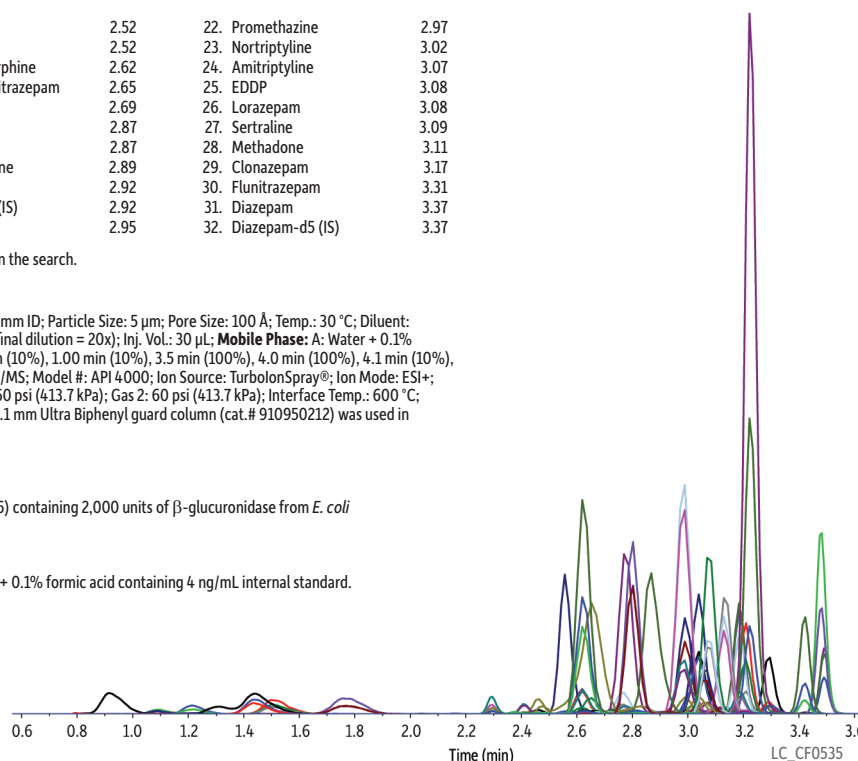
Peaks	RT (min)	11. Tapentadol	2.52	22. Promethazine	2.97
1. Morphine	0.95	12. Zopiclone	2.52	23. Nortriptyline	3.02
2. Oxymorphone	1.08	13. Norbuprenorphine	2.62	24. Amitriptyline	3.07
3. Pregabalin	1.29	14. 7-Aminoflunitrazepam	2.65	25. EDDP	3.08
4. Hydromorphone	1.34	15. Zolpidem	2.69	26. Lorazepam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

For MRM transitions, visit [www.restek.com](http://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  $\mu\text{m}$ ; 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  $\mu\text{L}$ ; **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: TurbolonSpray®; 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  $\mu\text{m}$ , 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  $\beta$ -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  $\mu\text{L}$  of sample with 900  $\mu\text{L}$  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 ( $\Delta t$ ) 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](http://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](http://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](http://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 <sub>g</sub> )	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

**RESTEK USLC®**  
Ultra Selective Liquid Chromatography™

## All the Right Tools— All in One Box

Introducing the USLC® Method Development Toolbox



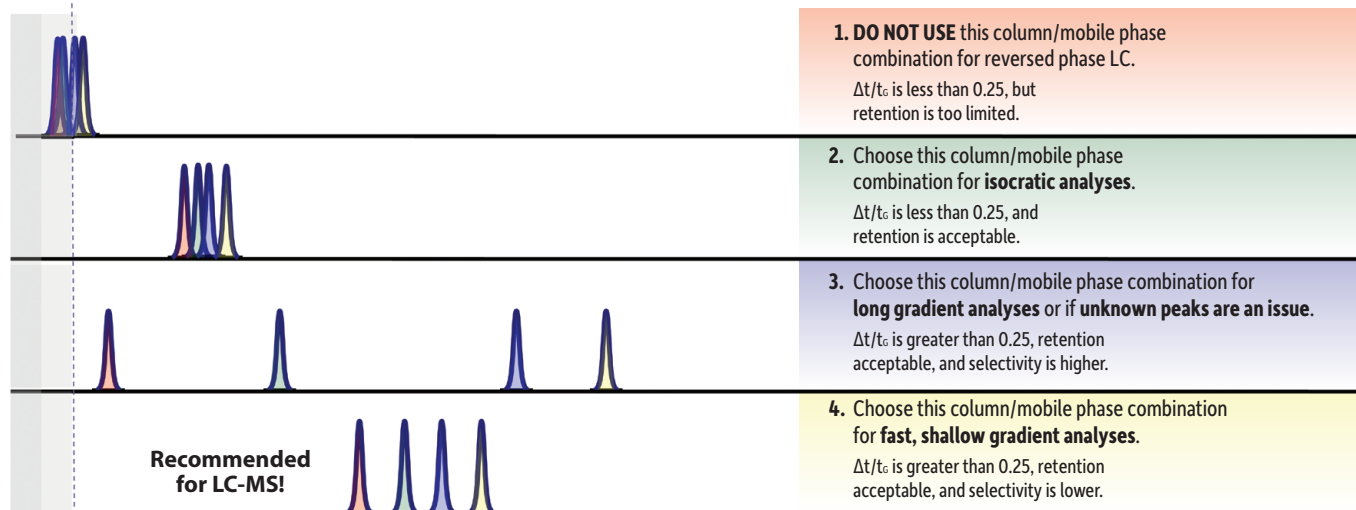
- USLC® method development toolbox contains all four USLC® stationary phases in one convenient package.
- Available for UHPLC (1.9 µm) and HPLC (3 or 5 µm) in 50, 100, or 150 mm lengths.
- Included selection guide makes it even easier to pick the right column the first time.

[www.restek.com/toolbox](http://www.restek.com/toolbox)

*Choose Columns Fast.  
Develop Methods Faster.*

**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.

Void Time Retention factor (k) is 2







## 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 tempera-

ture 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.

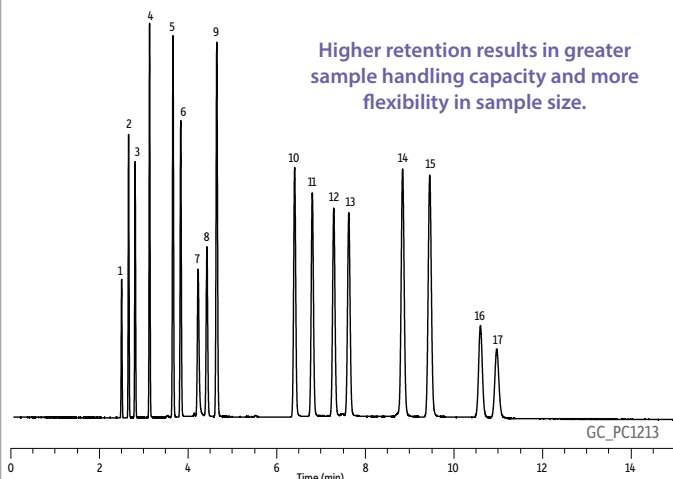
### Summary

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.

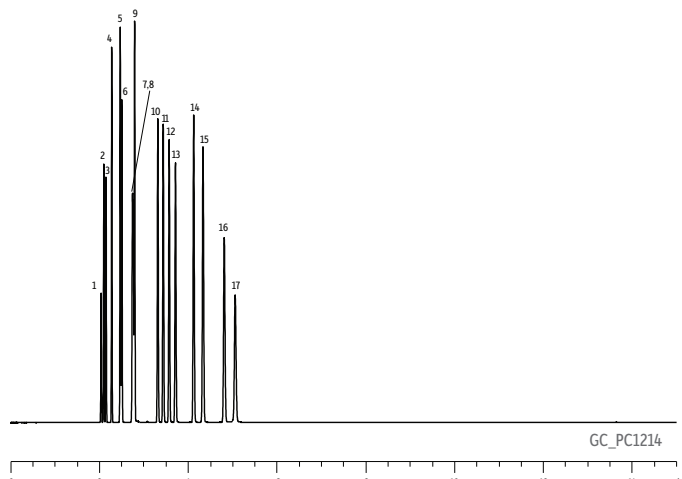
For more information on Rt®- and MXT®-Alumina BOND/MAPD PLOT columns, visit [www.restek.com/MAPD](http://www.restek.com/MAPD)

**Figure 1:** Rt®-Alumina BOND/MAPD columns have greater absolute retention than Select Al<sub>2</sub>O<sub>3</sub> MAPD columns, resulting in greater sample handling capacity through increased resolution.

**A: Rt®-Alumina BOND/MAPD**



**B: Select Al<sub>2</sub>O<sub>3</sub> MAPD**

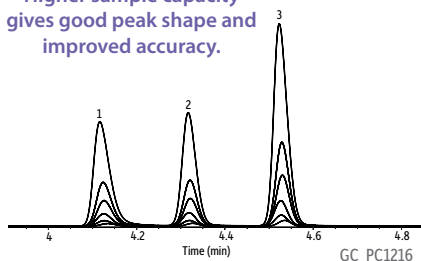


**Columns:** 50 m x 0.53 mm ID x 10 µm; **Sample:** PLOT column QA test mix (DCG# 547267); **Injection:** 5 µL, split, 200 °C; **Split vent flow rate:** 80 mL/min; **Oven:** 130 °C, isothermal; **Carrier Gas:** helium, (4.4 psi, 30 kPa); **Detector:** FID, 200 °C. **Peaks:** 1. Methane, 2. Ethane, 3.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

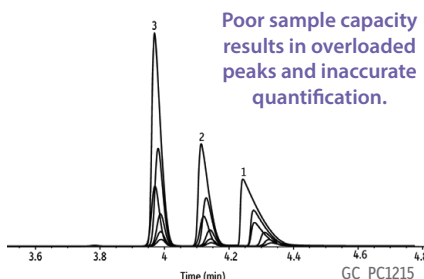
**A: Rt®-Alumina BOND/MAPD**

Higher sample capacity gives good peak shape and improved accuracy.



**B: Select Al<sub>2</sub>O<sub>3</sub> MAPD**

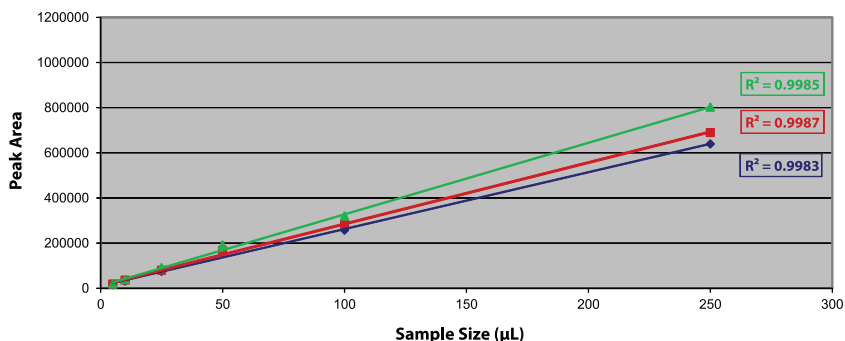
Poor sample capacity results in overloaded peaks and inaccurate quantification.



**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<sub>2</sub>O<sub>3</sub> 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.



# 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 identifi-

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.

cation 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.

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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Lit. Cat.# GNAD1535-UNV

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