

# APPLICATIONS



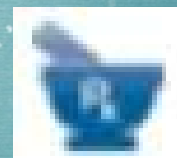
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## Pharmaceuticals

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**RESTEK**

## 8-Minute GC Analysis of Residual Solvents

Using an HP-5MS 2005 x 0.18mm (0.05mm) Column

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## Residual Solvent Analysis

Complete Solutions for Residual Solvent Testing

How to successfully implement the USP 467 method:

- Choose the right column and method
- Optimize the method for your sample
- Use the right reagents and standards
- Use the right equipment and software
- Use the right personnel and training
- Use the right documentation and reporting
- Use the right quality control and assurance
- Use the right safety and health practices
- Use the right environmental and safety practices
- Use the right waste management practices
- Use the right emergency response procedures
- Use the right incident investigation procedures
- Use the right corrective and preventive actions
- Use the right continuous improvement processes
- Use the right communication and collaboration
- Use the right leadership and management
- Use the right culture and values
- Use the right vision and mission
- Use the right strategy and goals
- Use the right resources and capabilities
- Use the right risks and opportunities
- Use the right performance and results
- Use the right feedback and improvement
- Use the right innovation and creativity
- Use the right learning and development
- Use the right change and transformation
- Use the right future and success

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK USLC™**

## Ultra Selective Liquid Chromatography™

Choose Columns Fast. Develop Methods Faster.

Key features include:

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**RESTEK**

## USLC™ Columns Put the Right Tools in Your LC Method Development Toolbox

Understanding Selectivity in Reversed Phase Separations – A Simplified Approach to RP-LC and USPLC Column Selection

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
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- High reproducibility and accuracy
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**RESTEK USLC™**

## Ultra Selective Liquid Chromatography™

Choose Columns Fast. Develop Methods Faster.

USLC™ Column Selection & Mobile Phase Adjustment Guide

Key features include:

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- Low baseline noise and drift
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- High reproducibility and accuracy

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**RESTEK**

## Two Options for Analyzing Potential Contaminants in Active Pharmaceutical Ingredients

Key features include:

- Fast, efficient, and reliable results
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- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## Separating NSADs through Anomalous Selectivity

Key features include:

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- High resolution and sensitivity
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- High reproducibility and accuracy
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- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**Rxi™**

## 3-IN-1 TECHNOLOGY

Higher Efficiency • Lower Dead Volume • Exceptional Reproducibility

### Rxi™-624SII MS Columns

Exceptionally Inert, Low Bleed Columns for Volatile Analysis

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK PHARMA**

## Innovative Pharmaceutical Solutions

### Rxi™-624SII MS

The "Go-To" GC Column for Fast, Effective Volatile Impurities Method Development

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## Revised USP 467 Residual Solvent Method

Key features include:

- Fast, efficient, and reliable results
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- Excellent peak shape and stability
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- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**Raptor™**

## Selectivity Accelerated

Key features include:

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- Low baseline noise and drift
- High reproducibility and accuracy
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- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**Raptor™**

## Stationary Phase: Biphenyl

Selectivity Accelerated

Fast, Rugged Raptor™ Columns with Time-Tested Selectivity

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## Organic Volatile Impurities: Retention Time Index

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
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- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## Optimized RP-HPLC Method for Hydrophobic Acids

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## Optimize Selectivity & Efficiency in UHPLC Separations

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## New Column Chemistry—High Impact, Low Cost Technology

Key features include:

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- Low baseline noise and drift
- High reproducibility and accuracy
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## How do small particle size columns increase sample throughput?

Key features include:

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- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy
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- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## How do intrinsically fused deactivated phases work?

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
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- High reproducibility and accuracy
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Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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**RESTEK**

## Pharmaceutical Applications

### How Column Inertness Improves the Chromatography of Basic Compounds

Key features include:

- Fast, efficient, and reliable results
- High resolution and sensitivity
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy
- Excellent peak shape and stability
- Low baseline noise and drift
- High reproducibility and accuracy

Figure 1: A typical chromatogram showing the separation of residual solvents on an HP-5MS 2005 x 0.18mm (0.05mm) column.

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## 8-Minute GC Analysis of Residual Solvents

Using an Rtx<sup>®</sup>-624 (G43) / Rtx<sup>®</sup>-WAX (G16) Column Pair

By Rick Lake, Pharmaceutical Innovations Chemist

- Dual-column detection/confirmation in 8 minutes.
- Columns produce desired selectivity and stable retention.
- Excellent peak shape and sensitivity, for reliable information.

The International Conference on Harmonization (ICH) publishes a guideline (Q3C) listing amounts of solvent residues that are acceptable in drug products and drug substances. The complete ICH list of regulated solvents, 61 compounds of differing chemical properties, is a challenge for separation on any single GC phase, as critical coelutions exist. Typically, residual solvents are identified by assaying samples and matching retention times with reference standards. If a response greater than the regulatory limit is obtained in a retention time window, a second sample is analyzed to confirm the compound's identity, using a column that has alternate selectivity. In some cases, GC/MS is employed for analyte verification. Assays for verification can be laborious and time intensive, and add unnecessary cost.

In the ICH guideline, residual solvents are grouped according to their toxicity. Class 1 compounds are carcinogens that pose a risk to both consumers and the environment. The use of these solvents is to be avoided but, if they are used, their use must be tightly controlled to ensure only trace level impurities in the final product. Class 2 compounds are non-genotoxic animal carcinogens, and concentrations of these compounds should be limited in pharmaceutical actives and products. Class 3 compounds have low toxic potential, and concentrations up to 0.5% are acceptable. Therefore, Class 3 compounds can be assayed by non-specific techniques, such as weight loss on drying. Because Class 2 compounds are the most likely prospects for GC analysis, we selected Residual Standards Class 2 Mix A and Residual Standards Class 2 Mix B (cat.#s 36271 and 36272, respectively) as the analytes for this work.

Because of advances in headspace technology — mainly dynamic sampling techniques — greater sensitivity now is achievable with this approach<sup>1</sup>, and this makes a comprehensive dual-column assay feasible. By simultaneously using two columns with differing selectivities, e.g., a G43 column (Rtx<sup>®</sup>-1301 or Rtx<sup>®</sup>-624) and a G16 column (Rtx<sup>®</sup>-Wax or Stabilwax<sup>®</sup>), a single injection can be used both to detect residual solvents and to confirm their identities. Even with two columns, however, the complexity of the sample list makes it impossible for a single temperature program to provide the flexibility needed to resolve all compounds on each column. To overcome this barrier, we used a Tekmar HT3 dynamic headspace sampler and an Agilent 6890 GC equipped with a Gerstel Modular Accelerated Column Heater (MACH) System. One of the latest advances in fast GC technology, the MACH System incorporates columns encased individually in thermally controlled bundles and heated externally from the main GC oven (Figure 1).<sup>2</sup> This independent, low thermal mass configuration allows independent, very rapid temperature ramps, upward or downward.

Collected analytes were directed to the injection port, then were split onto the two columns via a "Y" Press-Tight<sup>®</sup> connector. Independent temperature programs for each column separated the analytes for detection on dual FIDs. Using our two columns in this novel and simple-to-use setup, we resolved all compounds in the combined reference mixes in less than 8 minutes (Figure 2) — a result not possible with a conventional GC system. There was one critical co-elution on each column, but these did not involve the same compounds, and thus posed no practical problem. Also, with the low thermal mass of the MACH System modules, the cooldown and equilibration time between samples is considerably shorter than with a conventional GC oven.

Dynamic headspace sampling coupled with a Gerstel MACH column heating system makes possible rapid, comprehensive assays of residual solvents. By using other column combinations and other independent temperature programs, this system can be adapted to quickly resolve other complex mixes.

**Figure 1** Two column modules in a Gerstel MACH column heating system.

## Why do smaller particle size columns improve resolution?

### Learning Links

by Rick Lake, Pharmaceutical Innovations Chemist

Particle size ( $dp$ ), or the mean diameter of the spherical supports used to pack a column, is a physical dimension that has a significant impact on the performance of an HPLC column. Smaller particle sizes have been shown to offer higher peak efficiencies. But how does this actually affect analyte resolution? If we consider the fundamental relationship in separation science, the resolution equation (**Figure 1**), we can better understand how particle size can lead to improved separations. The resolution equation comprises three terms: selectivity, retention capacity, and efficiency. Each of these terms is affected by the specific components of an analytical method. A column's particle size, in particular, affects the efficiency term of the resolution equation.

Efficiency is ultimately derived from the *theoretical plate model of chromatography*. Conceptually, a plate refers to one complete equilibrated transfer (or partition) of a solute between the mobile and stationary phases. Efficiency is a qualitative term used to measure the *number of theoretical plates* in a given column, or the degree to which an analyte partitions between the mobile and stationary phases. In relation to particle size, efficiency is inversely proportional (**Figure 2**). Put simply, as particle size is lowered, efficiency increases, and more resolution is achieved. In contrast efficiency is directly proportional to the column length (Figure 2); therefore, an analyst can keep the same resolution and decrease the length of the column by the same factor as the particle size, shortening the analysis time. It is also beneficial that efficiency is inversely proportional to the square of the peak width—higher efficiencies produce narrower peak widths. Narrow peak widths enhance resolution by lengthening the baseline between two adjacent peaks. (An important note—this does not imply that by simply lowering the particle size we can separate all test mixes. Stationary phase selectivity is still the driving force behind resolution.)

**Figure 1** The resolution equation indicates selectivity has the greatest influence on resolution.

$$R = \underbrace{1/4 (\sqrt{N})}_{\text{Efficiency}} \times \underbrace{(k' / k' + 1)}_{\text{Retention}} \times \underbrace{(\alpha - 1)}_{\text{Selectivity}}$$

**Figure 2** Efficiency, as measured by the number of theoretical plates ( $N$ ), is inversely proportional to particle size ( $dp$ ) and directly proportional to column length ( $L$ ).

$$N \propto \frac{1}{dp} \qquad N \propto \frac{L}{dp}$$

#### RELATED SEARCHES

[particle size](#), [resolution](#), [efficiency](#)

# RESTEK USLC™

## Ultra Selective Liquid Chromatography™

*Choose Columns Fast.  
Develop Methods Faster.*

**RESTEK**

**Innovative Chromatography Products**

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# RESTEK USLC™

## Ultra Selective Liquid Chromatography™

### Ultra Selective Liquid Chromatography™ Technology

*Choose Columns Fast. Develop Methods Faster.*

What is Ultra Selective Liquid Chromatography™ (USLC™) technology? This technique is the directed application of orthogonal selectivity—the most influential factor affecting peak separation, or resolution—to provide the practicing chromatographer with the best tools for choosing columns fast and developing methods faster. Through our extensive study of reversed phase chromatography Restek created the widest range of selectivity in the industry using just 4 unique stationary phases: the USLC™ column set. We also defined a simple approach to choosing a column with the appropriate selectivity for any application.

### Selectivity Drives Separations

*Quickly and effectively resolve analytes by understanding and controlling selectivity through USLC™ technology.*

One of the most significant, yet least understood, steps of method development is finding the proper stationary phase for a particular separation. As sample complexity increases, achieving adequate resolution between matrix components and target analytes becomes more difficult. Despite recent advancements in column format, such as sub-2 micron packings and pellicular particles, resolution can still be difficult to obtain because, while these formats can increase chromatographic efficiency and analysis speed, they do not significantly influence resolution. Selectivity, as shown in Equation 1, is the single most powerful factor affecting resolution, and it is largely dependent upon stationary phase composition.

**Equation 1:** Selectivity has the greatest mathematical effect on resolution.

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

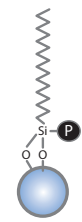
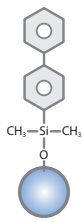
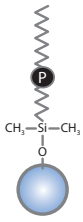
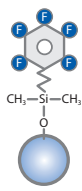
*Efficiency   Retention Factor   Selectivity*

### Real Diversity in Phase Chemistry

*A small set of defined orthogonal columns means faster separations and more robust methods.*

While numerous bonded phases are available for reversed phase chromatography, many (e.g., C8 and C18) are similar and offer only moderate changes in retention, rather than significant differences in selectivity. Method development is less laborious and time-consuming when using a full range of column selectivities, including orthogonal phase chemistries like polar embedded, phenyl, and fluorophenyl columns. Restek has led the development of the unique USLC™ column set across these phase classes to provide analysts with a more effective range of column selectivities and innovative column chemistries for method development. The USLC™ column set (Figure 1) provides the widest range of reversed phase selectivity available with just 4 columns and can be used to guide proper stationary phase selection—the least understood yet most significant part of method development.

**Figure 1:** Restek columns offer the widest range of unique and effective phase chemistries to aid the chromatographer in choosing columns fast and developing methods faster.

Restek USLC™ Phase (column class)	Aqueous C18 (alkyl)	Biphenyl (phenyl)	IBD (polar embedded)	PFP Propyl (fluorophenyl)
	 Aqueous C18	 Biphenyl	 IBD	 PFP Propyl
<b>Ligand type</b>	Proprietary polar modified and functionally bonded C18	Unique Biphenyl	Proprietary polar functional embedded alkyl	Fluorophenyl
<b>Properties</b>	<ul style="list-style-type: none"> <li>General purpose with a well-balanced retention profile.</li> <li>Compatible with 100% aqueous mobile phases.</li> <li>Ideal for multi-component LC-MS analyses.</li> </ul>	<ul style="list-style-type: none"> <li>Increased retention for dipolar, unsaturated, or conjugated solutes.</li> <li>Enhanced selectivity when used with methanolic mobile phase.</li> <li>Ideal for increasing sensitivity and selectivity in LC-MS analyses.</li> </ul>	<ul style="list-style-type: none"> <li>Increased retention for acids and water-soluble compounds.</li> <li>Compatible with 100% aqueous mobile phases.</li> <li>Capable of both reversed phase and HILIC separations.</li> </ul>	<ul style="list-style-type: none"> <li>Increased retention for both charged bases and electronegative compounds.</li> <li>Capable of both reversed phase and HILIC separations.</li> <li>Ideal for increasing sensitivity and selectivity in LC-MS analyses.</li> </ul>



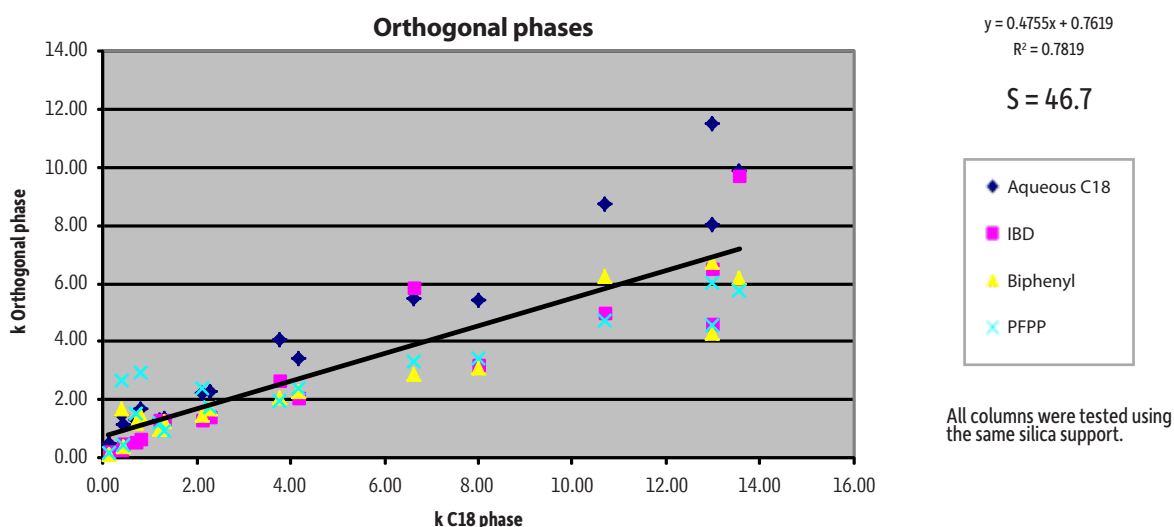
## Evaluating and Extending Selectivity

The Restek USLC™ column set offers the highest range of alternate selectivity available.

The diverse selectivity provided by USLC™ columns can be demonstrated empirically using the hydrophobic-subtraction model [1]. This model is a novel procedure for characterizing selectivity that uses test probes to define the solute and stationary phase interactions in reversed phase separations. Restek is leading the commercial application of this model by implementing it in the development of USLC™ bonded phases. To evaluate phase selectivity using the hydrophobic-subtraction model, the retention characteristics of the solute probes are compared across different phases relative to a C18 benchmark with all columns using the same silica base.

The resulting scatter plot is an excellent way to visualize selectivity. Stationary phases with similar selectivity show high linearity when graphed. However, stationary phases with alternate selectivity—even orthogonality—produce significant scatter around the regression line. The high degree of scatter shown in Figure 2 shows just how diverse the phases in the USLC™ column set are. When we quantify column selectivity based on this correlation by calculating the selectivity (S) statistic [2], the resulting value of 46.7 shows that the USLC™ column set truly has the highest range of selectivity available.

**Figure 2:** Restek has extended the selectivity range for commercially available columns and defined a column set—the 4 USLC™ phases—that is ideal for fast column choice and faster method development.



## USLC™ Columns Making the Right Selectivity Choices Accelerate Method Development Separations

Restek USLC™ columns offer the widest range of selectivity available and are an integral part of successful method development (Figure 3). Ideal for column switching systems, these columns provide the orthogonal separations needed to create optimal resolution and robust methods—all in a 4-column set. Combining USLC™ phases with a suitable column format (Figure 4) gives practicing chromatographers the most powerful tools available for successful method development.

**Figure 3:** Restek offers the widest range of phase selectivities for HPLC and UHPLC.

Column Type	Column Line							
	Restek		Waters			Phenomenex	Agilent	
	Pinnacle DB	Ultra	Acquity CSH	Acquity HSS	Acquity BEH	Kinetex	Zorbax RRHD	Poroshell 120
Alkyl (C18 and C8)	●	●	●	●	●	●	●	●
Phenyl	●	●	●		●			
Polar Embedded Alkyl	●	●						
Fluorophenyl	●	●	●			●		

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



**Figure 4:** There's a USLC™ Column for Nearly Every Instrument Platform, Scale, and Application.

Column Line*	Particle Diameter	Use
Pinnacle DB	1.9 µm	UHPLC
Ultra	3 and 5 µm	HPLC

\* In addition to USLC™ stationary phases, Restek also offers C8, C18, and silica.

Column Class	Column ID
Capillary	<1.0 mm
Microbore	1.0 mm
Narrow bore	2.1–3.0 mm
Standard bore	3.2–4.6 mm
Semi-prep	10–21.2 mm
Prep	30–50 mm

For detailed analysis of USLC™ column selectivity data, visit [www.restek.com/uslcarticle](http://www.restek.com/uslcarticle)

#### References

- [1] L.R. Snyder, J.W. Dolan, P.W. Carr, The Hydrophobic-Subtraction Model of Reversed-Phase Column Selectivity, *J. Chromatogr. A* 1060 (2004) 77.
- [2] U.D. Neue, J.E. O'Gara, A. Mendez, Selectivity in Reversed-Phase Separations influence of the Stationary Phase, *J. Chromatogr. A* 1127 (2006) 161.

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General Interest

## USLC™ Columns Put the Right Tools in Your LC Method Development Toolbox

### Understanding Selectivity in Reversed Phase Separations – A Simplified Approach to HPLC and UHPLC Column Selection

By Rick Lake and Ty Kahler

*The most significant influence on chromatographic peak separation, or resolution, is column selectivity. Unfortunately, column selectivity is also the least understood and most underutilized parameter. To improve selectivity, method developers often concentrate on manually altering mobile phases, operational parameters, and instrumentation. But because stationary phases offer more significant selectivity differences, you can drastically speed up HPLC and UHPLC method development by instead focusing on column choice. In this article, we discuss column selection for reversed phase separations and, using the hydrophobic-subtraction model (H-S model), identify a set of just 4 stationary phases—Restek's USLC™ column set—that encompasses the widest selectivity range available on the market.*

#### The Role of Selectivity in Liquid Separations

When performing a liquid separation, we generally focus on choosing the right instrumentation—especially since the recent advent of UHPLC—and end up choosing columns rather hastily, either by proximity (using the column that is already on the instrument or in the closest drawer) or by habit (using a column that has offered problem-free service in the past). While never optimal, this practice should be particularly concerning for a method developer because improper column choice can lead to needlessly labor- and time-intensive method development. If we consider the impact of column selectivity on peak separation, or resolution, we can see why choosing the right column can be so advantageous.

Resolution is the result of 3 cumulative terms: efficiency (N), retention capacity (k), and selectivity (α). How well we resolve our analytes, and how quickly we do so, depends upon our ability to control these 3 factors. Of the 3, the selectivity term mathematically affects resolution to the greatest degree (Equation 1). Put another way, resolution is largely a function of selectivity.

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

$$R = \frac{1}{4} \sqrt{N} \times \left( \frac{k}{k+1} \right) \times (\alpha - 1)$$

Efficiency      Retention Factor      Selectivity

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## Changing Columns to Create Significant Changes in Selectivity

Since resolution is largely a function of selectivity, any discussion about improving resolution should focus primarily on altering selectivity. It has often been taught in HPLC method development that one can effectively alter selectivity by adjusting mobile phases to reach a desired separation. This, of course, is true. However, mobile phase adjustment can be laborious—often involving many preparation adjustments and column equilibration times—and typically creates only marginal selectivity differences. In addition, some elution profiles are not practical with certain mobile phases and detection modes, including mass spectrometry (MS) and refractive index (RI).

On the other hand, changing stationary phases (i.e., columns) can be much easier and can also result in more significant selectivity differences because stationary phases can offer alternate and even orthogonal separations. These alternate separations can also be scouted very quickly using precise scouting gradients.

With the number of columns commercially available today, choosing the right one can be difficult, even overwhelming. By quantifying stationary phase selectivity, we can create new guidelines for effectively and easily choosing columns to help reduce method development time and increase method ruggedness.

## Quantifying Column Selectivity Using the Hydrophobic-Subtraction Model (H-S Model)

Many models exist for choosing solvents and mobile phase additives, but not until recently has stationary phase characterization received much attention. Column selectivity has been largely overlooked due, in part, to its complexity, particularly for liquid separations. But now that Snyder et al. have proposed their popular hydrophobic-subtraction model (H-S model) [1], we can begin to compare and quantify stationary phase selectivity in reversed phase separations and determine (often through orthogonal separations) which stationary phases produce the greatest degree and range of selectivity differences. Only then can we identify a small set of columns that will form the contents of an efficient and effective method development toolbox.

The H-S model is a novel treatment that empirically defines reversed phase selectivity by analyzing a varied collection of solute test probes and then utilizing 5 established selectivity parameters—hydrophobicity (H), steric hindrance (S\*), hydrogen bond acidity (A), hydrogen bond basicity (B), and cation exchange activity (C)—to identify the contributions of silica sorbents and stationary phases on selectivity. This model has been used by many organizations, including United States Pharmacopeia (USP), to find column equivalency.

The selectivity value (Fs) of the H-S model is normally used to find the similarity between columns, but it can conversely be used to find column **dissimilarity**, even orthogonality, to highlight selectivity differences and simplify column selection. Table I compares a variety of stationary phases and reveals which phases offer increased selectivity. (Because the H-S model evaluates the contributions of both stationary phase and silica support on selectivity, we intentionally kept the silica support constant throughout our experiments to isolate the effect of stationary phases on selectivity.) Each value was calculated relative to a C18 benchmark. The columns showing high Fs values—like the 4 Restek USLC™ phases shown in **blue**—exhibit the greatest dissimilarity in selectivity relative to the C18, so they are excellent choices when a C18 does not provide the selectivity needed.

**Table I:** The Fs term of the hydrophobic-subtraction model (H-S model) can numerically determine which stationary phases are most dissimilar to a C18, illustrating the phases needed to extend the selectivity range in reversed phase chromatography. The 4 Restek USLC™ phases are shown in **blue**.

Terms Calculated from the Hydrophobic-Subtraction Model (H-S Model)							
Stationary Phase Type	Hydrophobicity	Steric Hindrance	Hydrogen Bond Acidity	Hydrogen Bond Basicity	Cation Exchange Activity	Selectivity Function	Rank Dissimilarity
	H	S*	A	B	C	Fs	
Ultra C18 (control)	1.051	0.033	-0.032	-0.023	0.057	0.0	—
Ultra C8	0.0871	0.013	-0.0199	0.019	-0.032	11.2	8
Ultra C4	0.0738	-0.010	-0.276	0.019	0.032	11.3	7
Ultra C1	0.613	-0.054	-0.408	0.016	-0.032	17.9	6
Ultra Aqueous C18	0.808	-0.128	0.378	0.013	0.0229	25.4	5
Ultra Biphenyl	0.661	-0.189	-0.283	0.042	0.204	28.4	4
Ultra Cyano†	0.409	-0.041	-0.801	-0.011	-0.110	29.1	3
Ultra PFP Propyl	0.671	-0.092	-0.213	-0.007	0.658	52.0	2
Ultra IBD	0.672	-0.035	-0.052	0.233	-0.564	63.7	1

All columns were tested using the same silica support.

† NOTE: The cyano phase also ranks high in terms of dissimilarity, but the more rugged PFP Propyl phase was ultimately chosen for the USLC™ column set because it better withstands the low pH levels required for mass spectrometry while offering equally heightened retention of basic compounds.

## Characterizing Selectivity at the Molecular Level

Often during method development, after we have made our initial column choice, we still find ourselves struggling to resolve compounds as we try to find a “better” column. This difficulty is often due to an inability to find a column with *alternate* selectivity. Quantifying stationary phase selectivity (Table I) is a very important step in identifying a small and effective column set for method development, but we must further define selectivity at a molecular level to ensure that the columns in our method development toolbox exhibit not just *high* selectivity, but also *alternate* selectivity based on potential analyte types.

Selectivity ( $\alpha$ ) is practically determined from the difference in retention factors ( $k$ ) of 2 peaks. Therefore, to produce alternate selectivity, we must alter the retention of one peak relative to the other. (Increasing the retention of both peaks equally results in higher retention capacity, but no change in selectivity because the difference between the 2 peaks does not change.) If we focus column selection on intermolecular interactions, we can see how specific phases create selectivity by altering the retention profile of specific solutes in relation to others—true selectivity.

So before we can confirm alternate selectivity, we first need to characterize the types of intermolecular interactions commonly encountered in reversed phase chromatography (RPC). In our experiments, we measured 4 major types of interactions—dispersion, polarizability, hydrogen bonding, and cation exchange. To further simplify things and more easily define a guideline, we can relate these measured interactions to chemical properties as noted below:

- **Dispersion** is the term for the van der Waals interactions that exist to some extent in all organic molecules, including polar molecules. It is the major driver for RPC and is a major retention mechanism for alkyl phases (i.e., C1 through C18). Since the retention is proportionate to the hydrophobicity of the molecule, we can call these interactions *hydrophobic retention*.
- **Polarizability** is the ability of a stationary phase to change its electron distribution in the presence of an analyte and induce a dipole interaction. It is commonly seen in phenyl-based columns and is the main reason we often switch from a C18 to a phenyl to find alternate selectivity. The Restek Biphenyl column has 2 phenyl rings to enhance polarizability. These interactions are most commonly seen in dipolar, unsaturated, or conjugated compounds and fused-ring compounds with electron withdrawing groups (like nitro groups). For our purposes, we will define these interactions simply as *dipolar retention*.
- **Hydrogen bonding** is used in RPC when a solute and a stationary phase form a chemical bond in which a hydrogen atom of one molecule is attracted to an electronegative atom, especially a nitrogen, oxygen, or fluorine, of another molecule. Although hydrogen bonding results in retention of other solute types, we will focus on its ability to increase retention for acidic compounds and will call it *acidic retention*.
- **Cation exchange** is an electrostatic interaction between a cationic solute and an anion within the stationary phase. Cation exchange, or electrostatic interaction, is most commonly employed in RPC for the retention of protonated bases. Therefore, for simplicity, we will call it *basic retention*.

Table II outlines the common solute retention profiles for the specific interactions we measured in our experimentation. With these intermolecular interactions defined, we can now use their retention profiles to determine which highly selective columns produce alternate selectivity for specific compound types, thereby radically simplifying column selection.

**Table II:** Common retention profiles measured for modern reversed phase columns as they relate to molecular interactions.

Solute Interaction	Type of Solute Retained	Common Phase Category	H-S Model Term	Probes Measured
Dispersion	Hydrophobic	C18	H	Toluene, Ethylbenzene
Polarizability	Dipolar	Biphenyl	n/a*	Anisole, Benzonitrile
Hydrogen Bonding	Acidic	Polar Embedded	B	4-Butylbenzoic Acid, Mefenamic Acid
Cation Exchange	Basic	Fluorinated Phenyl	C	Berberine, Amitriptyline, Nortriptyline

\* Because polarizability is not measured by the H-S model, Restek used anisole and benzonitrile probes to mathematically determine the degree of polarizability of each stationary phase.

## Extending the H-S Model to Simplify Column Choice

To determine a simplified guideline for column selection, Restek has extended the H-S model by analyzing empirical selectivity data of our stationary phases (Table I) against the RPC molecular interactions described in Table II. Through matching stationary phases to specific solute types based on these measured intermolecular attractions, we can aid method development in 2 significant ways: First, we can find a small set of columns with a wide range of alternate selectivity for use in method development. Second, we can define a process for selecting columns based on the chemical properties of our analytes when scouting column selectivity.

Extrapolating the retention data for the solute probes in the H-S model allows us to correlate the retention characteristics of specific solutes to stationary phase types. Ultimately, this correlation has enabled us to match column type to the selective retention of our analytes' chemical properties, making column selection truly definable by the chemical composition of our analytes.

Figure 1 illustrates the retention profile of a C18 compared with the profiles of the 4 Restek Ultra Selective Liquid Chromatography™ (USLC™) columns. We can see changes in selectivity across these columns as illustrated by the circled areas showing heightened retention for particular solute types. (Selectivity is the retention of one solute relative to another.) The 4 USLC™ columns exhibit varied retention profiles based upon solute type and, therefore, will exhibit alternate selectivity relative to one another. Because we have a small, quantified column set—4 Restek USLC™ phases—that is highly selective **and** exhibits significantly different retention profiles based on specific solute chemical properties, we can now match columns to specific analytes and, thus, simplify method development.

**Figure 1:** Stationary phase selectivity can be determined 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.

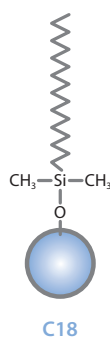
Restek Phase:  
**C18**

Stationary Phase Category:  
C18 (L1)

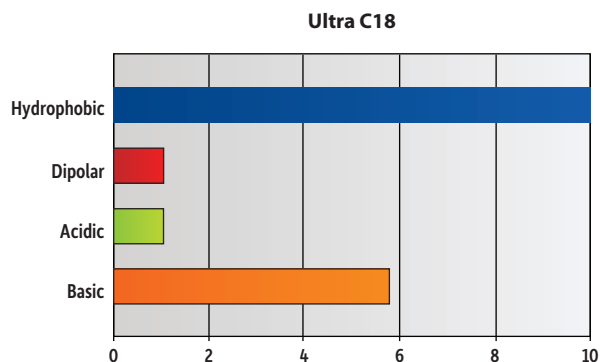
Ligand Type:  
Densely bonded and fully end-capped octadecyl silane

Properties:

- General purpose.
- Strong hydrophobic retention.



**C18**  
C18 Benchmark



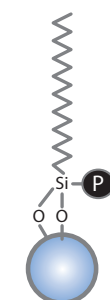
Restek USLC™ Phase:  
**Aqueous C18**

Stationary Phase Category:  
Modified C18 (L1)

Ligand Type:  
Proprietary polar modified and functionally bonded C18

Properties:

- General purpose with a well-balanced retention profile.
- Compatible with 100% aqueous mobile phases.
- Ideal for multi-component LC-MS analyses.



**Aqueous C18**

Well-balanced retention profile.

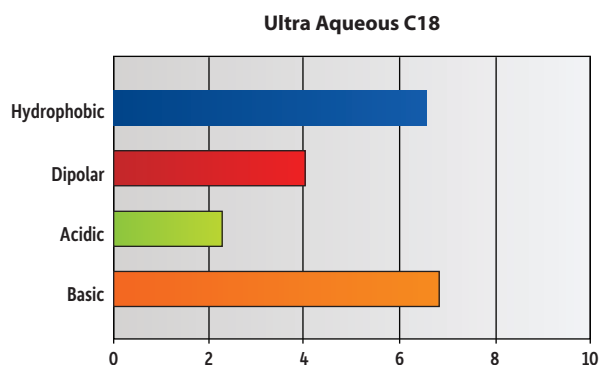


Figure 1, continued

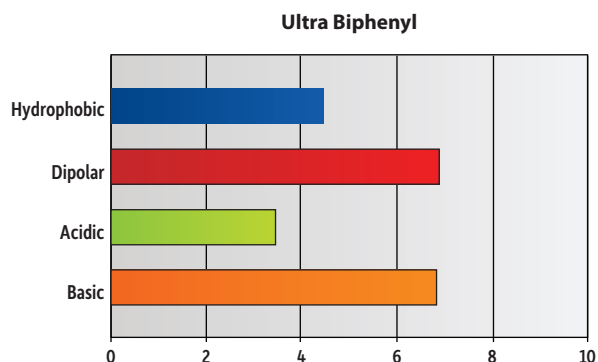
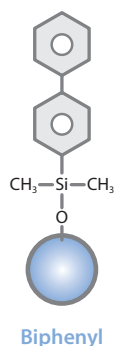
Restek USLC™ Phase:  
**Biphenyl**

Stationary Phase Category:  
Phenyl (L11)

Ligand Type:  
Unique Biphenyl

Properties:

- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.



Heightened retention for dipolar compounds.

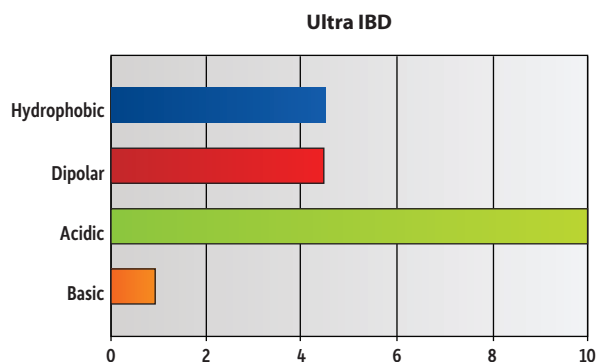
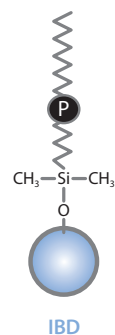
Restek USLC™ Phase:  
**IBD**

Stationary Phase Category:  
Polar Embedded Alkyl (L68)

Ligand Type:  
Proprietary polar functional embedded alkyl

Properties:

- Increased retention for acids and water-soluble compounds.
- Compatible with 100% aqueous mobile phases.
- Capable of reversed phase and HILIC separations.



Heightened retention for acidic compounds.

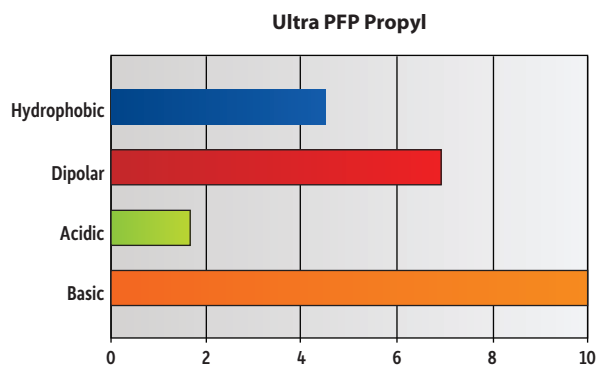
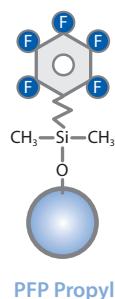
Restek USLC™ Phase:  
**PFP Propyl**

Stationary Phase Category:  
Proprietary end-capped pentafluorophenyl propyl (L43)

Ligand Type:  
Fluorophenyl

Properties:

- Increased retention for charged bases and electronegative compounds.
- Capable of reversed phase and HILIC separations.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.



Heightened retention for basic compounds.

All columns were tested using the same silica support.

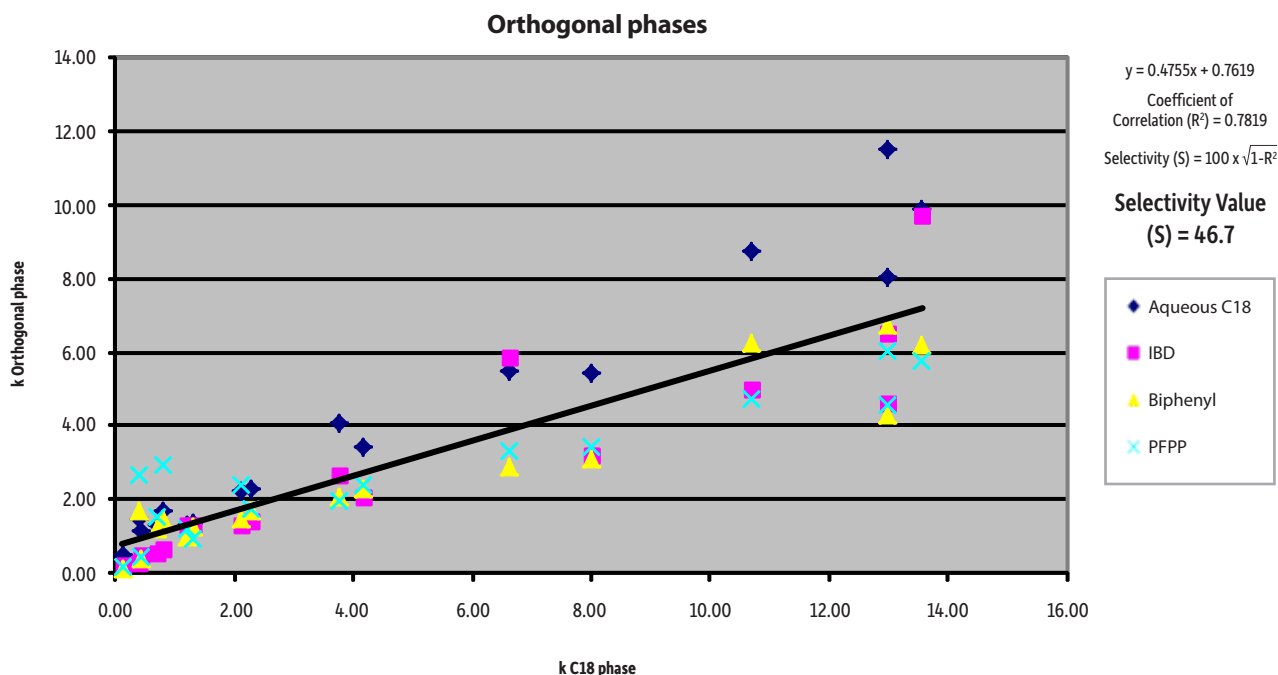
### Confirming the Alternate Selectivity of the USLC™ Column Set

To further confirm that each USLC™ column provides alternate selectivity—not only when compared to the C18 benchmark, but also when compared to the other columns in the set—we quantified the column set's range of selectivity (S) as described by Neue et al. [2]. Looking at the retention characteristics of the H-S model solute probes, we can define selectivity as the degree of scatter along the regression line when comparing stationary phases to the conventional C18 benchmark (Figure 2).

Two very similar stationary phases will produce similar retention for the solute probes and, when graphed, will show high linearity and high correlation. Two very *dissimilar*, or alternately selective, stationary phases that differ in the retention of the solute probes will show a high degree of scatter around the regression line. More scatter reveals that columns are more different, or orthogonal, from one another because it shows larger differences in selectivity. To measure this difference and use it as a means of comparing stationary phases, we can calculate a selectivity (S) value for the columns in the USLC™ column set. Note that because silica and mobile phase contributions could also alter the retention of the test probes, it is important to use identical silica supports and mobile phase compositions as to not bias the results and to allow focus only on the stationary phase contributions to selectivity.

With a selectivity value (S) of 46.7, Restek USLC™ phases produce an incredible range of alternate selectivity —using only 4 columns.

**Figure 2:** Restek has extended the selectivity range for commercially available columns and defined a column set—the 4 USLC™ phases—that is ideal for simplified column selection and ease of method development.



All columns were tested using the same silica support.

## Conclusion: The Right Tools for Maximum Selectivity

The H-S model offers the chromatographic method developer a practical approach to column selection. With a simplified model described above, we can now easily create predictable and alternate selectivity, effectively influencing the most significant factor contributing to resolution. Now that we have identified the small USLC™ column set with a wide range of quantified selectivity, we can quickly determine the best column for nearly any instrument platform and reversed phase or HILIC application by referencing predefined retention profiles. This column set can also be used to get the most out of column switching by providing a functional column set.

The Restek USLC™ column set, consisting of a balanced Aqueous C18, a Biphenyl, a fluorinated PFP Propyl, and a polar embedded IBD, has a profile that encompasses the widest range of reversed phase selectivity available today. Putting the right tools—like the USLC™ column set—in your method development toolbox means maximum alternate selectivity and peak separation with minimal effort.

## Acknowledgements

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- [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.

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# RESTEK USLC™

Ultra Selective Liquid Chromatography™

*Choose Columns Fast. Develop Methods Faster.*

## USLC™ Column Selection & Mobile Phase Adjustment Guide



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# RESTEK USLC™

## Ultra Selective Liquid Chromatography™

### Ultra Selective Liquid Chromatography™ (USLC™)

technology is the directed application of selectivity—the most influential factor affecting peak separation, or resolution—to provide the practicing chromatographer with the best tools available for choosing columns fast and developing methods faster.

Each USLC™ stationary phase is optimized for a different chemical interaction and solute type. More importantly for you, this optimization means that each phase also provides different (or “orthogonal”) selectivity. In fact, the well-defined USLC™ 4-column set offers the widest range of selectivity in the market, so you can simply and effectively separate almost any combination of organic analytes.

This guide will help you easily choose the right USLC™ column for nearly any reversed phase or HILIC application. It will also help you properly fine tune your mobile phase based on column choice and analyte type to further improve your results without guesswork or wasted time. When you have USLC™ columns and this guide, you always have the right tools in your method development toolbox!



# Using This Guide

To quickly and easily choose your USLC™ column and fine tune your mobile phase, follow these 5 simple steps.

## 1) Classify Your Target Analyte(s)

It may seem obvious, but in order to choose a column to target specific analytes, you must first define them. Classify your analytes into the following 4 functional groups\*:

- **Hydrophobic:** These molecules are often regarded as “water fearing,” as they are non-polar and prefer neutral stationary phases and solvents. Hydrocarbons are the ideal example of hydrophobic molecules.  
  
Note: A simple guideline can be applied here to define the hydrophobicity of the molecule—a molecule with a carbon-to-heteroatom (any atom other than carbon or hydrogen) ratio of 3:1 or higher is often amenable to the hydrophobic interactions of reversed phase analysis. If your molecule contains less than a 3:1 ratio or has limited retention on a C18, focus instead on other applicable function groups.
- **Dipolar:** These molecules are capable of dipole moments or non-uniform distributions of electrons causing positive and negative charges. They can contain either permanent dipoles (polar molecules) or induced dipoles (polarizable molecules).
- **Acidic\*\*:** We can define acids as molecules capable of either donating protons (Brønsted-Lowry definition) or accepting electrons (Lewis definition).
- **Basic\*\*:** We can define bases as molecules capable of either accepting protons (Brønsted-Lowry definition) or donating electrons (Lewis definition).

\* If your analyte fits into more than one functional group, consider routine scouting (e.g., column switching) with all 4 columns to determine your best overall selectivity.

\*\* For acidic and basic compounds, you will also need to identify the pKa of your target analyte(s).



# Using This Guide

## 2) Choose Your Column

Each USLC™ column is optimized for a different chemical interaction and solute type. After you have classified your analyte(s), use the column interaction and solute retention profiles on pages 8–15 to choose the best one for your application.

### Column Interaction Profile

Put simply, selectivity is the retention of one compound relative to another. Therefore, because solutes will be retained to different degrees by different molecular interactions, we can fundamentally define a column's selectivity based on the molecular interactions it delivers.

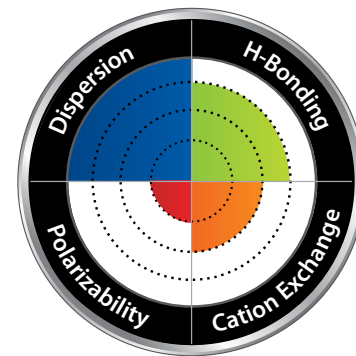
The pie chart provided for each USLC™ stationary phase in this guide (Figure 1) identifies the same 4 molecular interactions (color coded to correspond to the retention of a different solute type). The more rings shown for a given interaction, the more significant a role it plays in defining solute retention. These defining interactions and their less-prominent complements are also listed below the chart.

If you know what type of column interaction you need for your analysis, use these charts to select your USLC™ column.

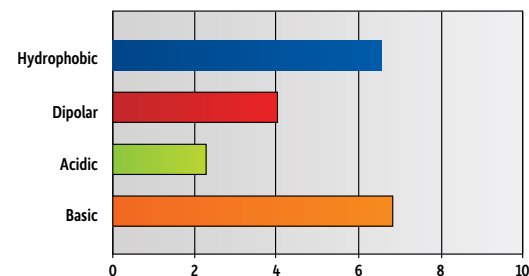
### Solute Retention Profile

Because each USLC™ column has a different interaction profile, it will preferentially retain different solutes. Use the column retention profiles in this guide (Figure 2) to quickly find the column you need based on the type of analyte you want to retain—hydrophobic, dipolar, acidic, or basic. The longer the bar in the graph, the more significant the solute retention. Simply choose the column that delivers heightened selectivity for your analyte type!

**Figure 1.** Sample Column Interaction Profile



**Figure 2.** Sample Solute Retention Profile



### 3) Scout Your Mobile Phase

Once you've chosen a column, you need to fine tune your mobile phase, but before you can, you must first ensure that your starting mobile phase will yield acceptable data. Choosing a highly customized mobile phase may prove to be unnecessary or even detrimental to data quality, so instead, scout your mobile phase *using a 4–mobile phase system* and the following recommendations:

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)

To scout your mobile phase, run scouting gradients using all A/B combinations above (e.g., A1/B1, A1/B2, A2/B1, A2/B2), then proceed with the combination that yields the best results.

For more information on running a scouting gradient, contact

**Restek Technical Service ([support@restek.com](mailto:support@restek.com)).**

#### Tech Tip

Many detectors, including mass spectrometers, are not amenable to traditional mobile phase additives like phosphate buffers and ion-pairing agents. That's why we designed the USLC™ column set to work with simple, mass spectrometer-compatible mobile phases (i.e., volatile and acidic).

### 4) Adjust Your Mobile Phase

If the data quality from your scouting test is unacceptable because of asymmetrical peaks, low retention, etc., you will need to choose an alternate buffer or otherwise change the pH of your mobile phase to correct the problem. Here are some helpful guidelines for changing your mobile phase pH when using a USLC™ column:

- The target mobile phase pH for a USLC™ column is between 2 and 4, but a pH between 2 and 8 is acceptable. A pH above 8 will reduce the lifetime of your column and is not recommended.
- For acidic and basic solutes, mobile phase pH should ideally be at least 1.5 units below your analyte's pKa (which will shift equilibrium to where USLC™ technology is most effective—charged form for bases and neutral form for acids).
- If using an IBD or PFP Propyl column in HILIC mode, increasing the organic percentage in your mobile phase can lead to heightened retention of ionic or very polar analytes. (If you classified your analyte as having less than a 3:1 carbon-to-heteroatom ratio in step 1, we recommend you explore this option.)
- Keep buffer concentrations low (between 5 and 20 mM).
- TFA should be avoided because it reduces sensitivity with electrospray ionization.

For additional help adjusting the pH for your mobile phase, contact

**Restek Technical Service ([support@restek.com](mailto:support@restek.com)).**



## 5) Fine Tune Your Mobile Phase

Mobile phases have a greater effect on selectivity when they work in conjunction with a column's interaction and retention profiles. Once you have adjusted your mobile phase pH enough to ensure acceptable chromatography, increase selectivity for your target analytes by fine tuning your mobile phase based on your column choice using the charts on pages 8–15.

**Figure 3.** Sample mobile phase fine-tuning chart.

If your target analyte is...	Consider...
A Basic or Acidic Moiety	<ul style="list-style-type: none"> <li>• First, increasing pH to increase retention of bases.</li> <li>• Second, decreasing pH to increase retention of acids.</li> <li>• Third, altering or mixing protic and aprotic solvents to adjust retention and selectivity.</li> </ul>
Ionic or Charged	<ul style="list-style-type: none"> <li>• Increasing buffer strength to decrease retention.</li> </ul>
An Aromatic or Alkyl Isomer	<ul style="list-style-type: none"> <li>• Decreasing temperature to increase selectivity.</li> </ul>



### Tech Tip

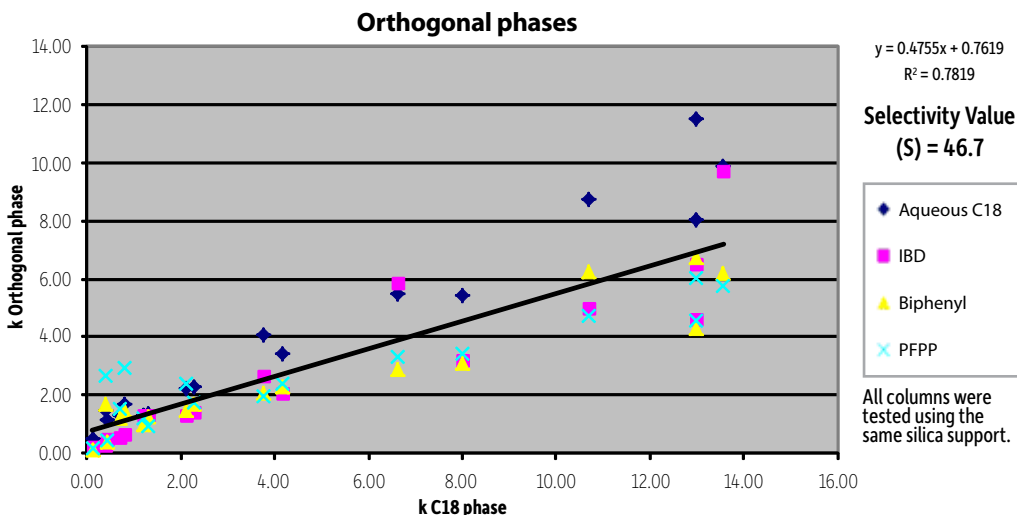
USLC™ columns are a perfect fit with electrospray ionization mass spectrometry because they:

- Retain ionized molecules.
- Create retention that alleviates matrix-induced ion suppression and reduces charge competition.
- Improve ionization and sensitivity with low-surface tension mobile phases.



# Defining Selectivity

The hydrophobic-subtraction (H-S) model is a novel procedure for characterizing selectivity [1]. The retention characteristics of a set of solute probes are compared across different stationary phases relative to a C18 benchmark with all columns using the same silica base. In the resulting scatter plot, stationary phases with similar selectivity show high linearity when graphed, but stationary phases with alternate selectivity—even orthogonality—produce significant scatter [2]. The high degree of scatter and resulting selectivity value (S) of 46.7 shows that the USLC™ column set truly has the highest range of selectivity available!



You can learn more about the H-S model and how Restek used it to create the USLC™ column set at [www.restek.com/USLCarticle](http://www.restek.com/USLCarticle)

## References

- [1] L.R. Snyder, J.W. Dolan, P.W. Carr, The Hydrophobic-Subtraction Model of Reversed-Phase Column Selectivity, *J. Chromatogr. A* 1060 (2004) 77.
- [2] U.D. Neue, J.E. O'Gara, A. Mendez, Selectivity in Reversed-Phase Separations Influence of the Stationary Phase, *J. Chromatogr. A* 1127 (2006) 161.



# Stationary Phase: Aqueous C18

No practical column set is complete without a C18, but this Restek phase far outperforms your run-of-the-mill C18 column. Our rugged Aqueous C18 has a well-balanced retention profile. It can effectively retain more types of solutes than a conventional C18 and is ideal for multi-component LC-MS analyses. The Aqueous C18 boasts high reproducibility and compatibility with many mobile phase conditions—even 100% aqueous and acidic. And when used with a gradient, it eliminates the all-too-common issue of multiple compounds coeluting near the column void time.

## Column Description:

### Stationary Phase Category:

C18 (L1)

### Ligand Type:

Proprietary polar modified and functionally bonded C18

### Properties:

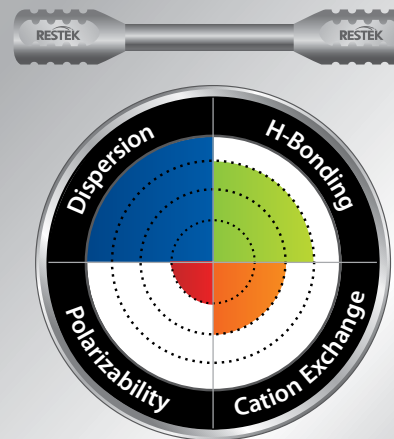
- General purpose with a well-balanced retention profile.
- Compatible with 100% aqueous mobile phases.
- Ideal for multi-component LC-MS analyses.

### Switch to an Aqueous C18 when:

- Limited retention or selectivity for polar compounds is observed on a C18.



## Column Interaction Profile:



### Defining Solute Interaction:

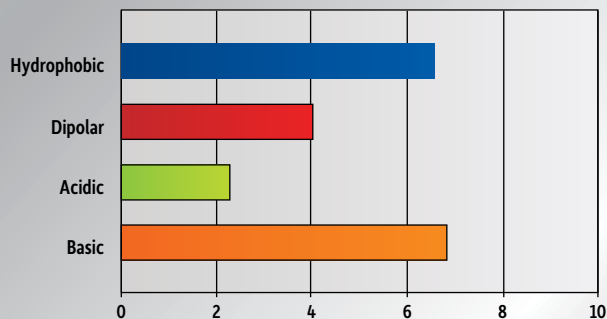
- Dispersion

### Complementary Solute Interaction:

- Hydrogen bonding



### Solute Retention Profile:

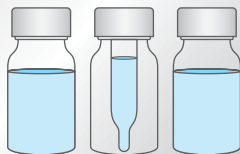


#### Target Analyte Structure:

- Hydrocarbons

#### Target Analyte Functionalities:

- Organic acids
- Ketones
- Isomeric species



### Fine Tuning Your Mobile Phase:

#### If your target analyte is...

A Basic or Acidic Moiety

#### Consider...

- First, increasing pH to increase retention of bases.
- Second, decreasing pH to increase retention of acids.
- Third, altering or mixing protic and aprotic solvents to adjust retention and selectivity.

Ionic or Charged

- Increasing buffer strength to decrease retention.

An Aromatic or Alkyl Isomer

- Decreasing temperature to increase selectivity.

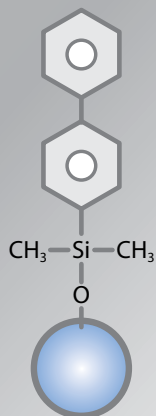


For step-by-step instructions on choosing your USLC™ column and fine tuning your mobile phase, see page 3.



# Stationary Phase: Biphenyl

Welcome to the next generation of phenyl columns. The Restek Biphenyl offers a greater degree of dispersion than conventional phenyls and a greater degree of polarizability than phenyl hexyls, creating higher selectivity and a greater range of usability. Because of these heightened interactions, this column shows substantial increases in retention and orthogonal selectivity when using methanol mobile phases.



## Column Description:

### Stationary Phase Category:

Phenyl (L11)

### Ligand Type:

Unique Biphenyl

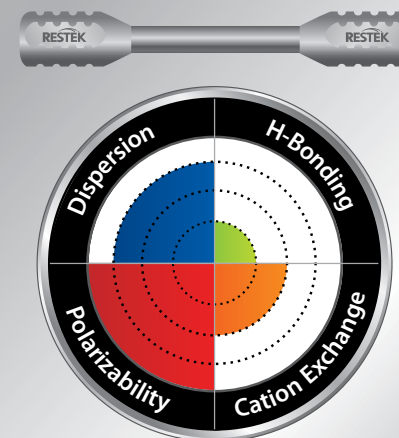
### Properties:

- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

### Switch to a Biphenyl when:

- Limited selectivity is observed on a C18.
- You need to increase retention of hydrophilic aromatics.

## Column Interaction Profile:



### Defining Solute Interactions:

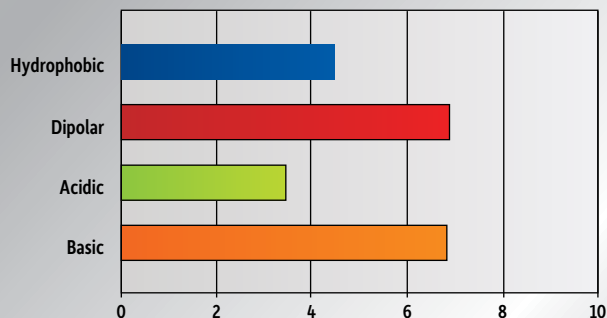
- Polarizability
- Dispersion

### Complementary Solute Interaction:

- Cation exchange

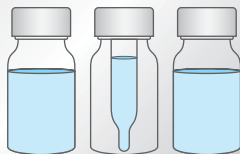


### Solute Retention Profile:



#### Target Analyte Structures:

- Aromatic
- Dipolar



#### Target Analyte Functionalities:

- Hydrophilic aromatics
- Strong dipoles
- Lewis acids
- Dipolar, unsaturated, or conjugated compounds
- Fused-ring compounds with electron withdrawing groups

### Fine Tuning Your Mobile Phase:

If your target analyte is...	Consider...
Conjugated or Aromatic	• Increasing methanol percentage to alter retention and selectivity.
Dipolar	• Decreasing acid strength and/or concentration to increase retention.
Ionic or Charged	• Decreasing buffer strength to increase retention relative to neutrals.
A Basic or Acidic Moiety	• First, increasing pH to increase retention of bases. • Second, decreasing pH to increase retention of acids.

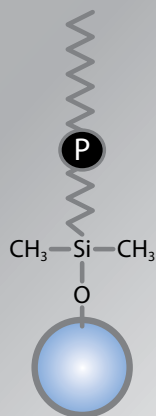


For step-by-step instructions on choosing your USLC™ column and fine tuning your mobile phase, see page 3.



# Stationary Phase: IBD

The IBD is a polar embedded column that acts as a strong hydrogen bonder and may be the most versatile column available today. With a unique polar group, this column is very retentive and selective for acids. It also provides symmetrical peak shape for strong bases. Restek's IBD is compatible with 100% aqueous mobile phases and can be used under HILIC conditions to retain very polar, ionic compounds in highly organic mobile phases.



## Column Description:

### Stationary Phase Category:

Polar Embedded Alkyl (L68)

### Ligand Type:

Proprietary polar functional embedded alkyl

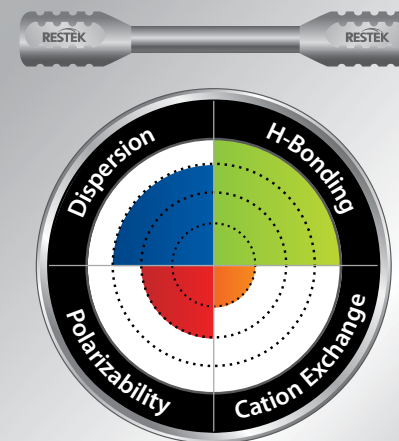
### Properties:

- Increased retention for acids and water-soluble compounds.
- Compatible with 100% aqueous mobile phases.
- Capable of reversed phase and HILIC separations.

### Switch to an IBD when:

- You need improved retention or selectivity of acidic compounds or compounds capable of hydrogen bonding.
- You need improved symmetry for strong bases.

## Column Interaction Profile:



### Defining Solute Interactions:

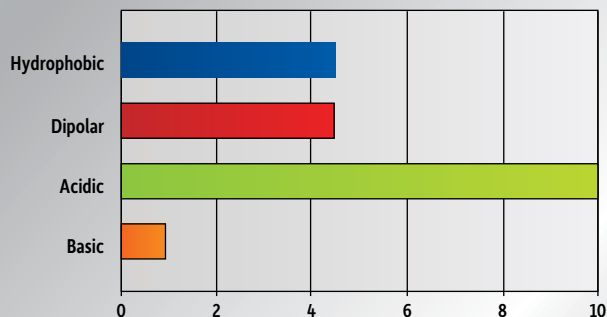
- Hydrogen bonding
- Dispersion

### Complementary Solute Interaction:

- Polarizability



### Solute Retention Profile:

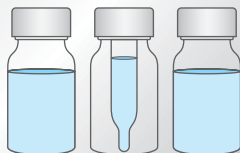


#### Target Analyte Structure:

- Oxygenated species

#### Target Analyte Functionalities:

- Alcohols
- Carboxylic acids
- Quaternary ammonium compounds



### Fine Tuning Your Mobile Phase:

If your target analyte is...	Consider...
Acidic	<ul style="list-style-type: none"> <li>• Decreasing acid strength and/or concentration to increase retention relative to neutrals and bases.</li> </ul>
Ionic or Charged	<ul style="list-style-type: none"> <li>• Increasing buffer strength to decrease retention.</li> </ul>
A Basic or Acidic Moiety	<ul style="list-style-type: none"> <li>• Altering or mixing protic and aprotic solvents to adjust retention and selectivity.</li> </ul>



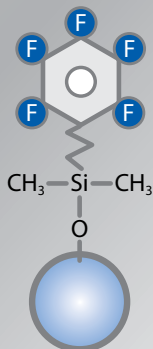
For step-by-step instructions on choosing your USLC™ column and fine tuning your mobile phase, see page 3.



# Stationary Phase: PFP Propyl

Due to its polarity, a cyano stationary phase is often regarded as the most orthogonal to a C18. It is a great choice for the retention and selectivity of bases and amine-containing compounds. Unlike a conventional cyano column, however, the Restek PFP Propyl is much more amenable to LC-MS because it is more reliable and efficient with acidic mobile phases. This versatile column is also compatible with highly aqueous mobile phases and HILIC separations.

## Column Description:



### Stationary Phase Category:

Proprietary end-capped pentafluorophenyl propyl (L43)

### Ligand Type:

Fluorophenyl

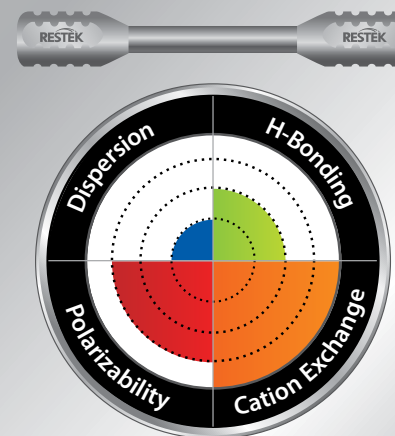
### Properties:

- Increased retention for charged bases and electronegative compounds.
- Capable of reversed phase and HILIC separations.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

### Switch to a PFP Propyl when:

- Limited retention and selectivity are observed on a C18 for basic compounds.
- You need increased retention of hydrophilic compounds.

## Column Interaction Profile:



### Defining Solute Interaction:

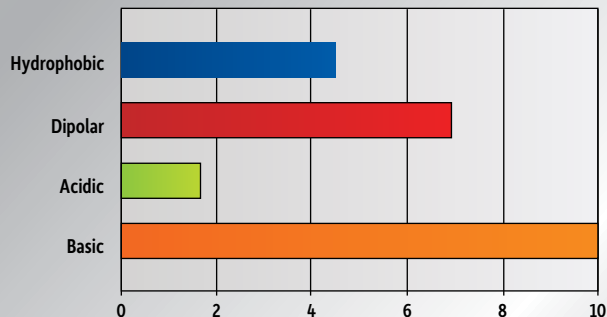
- Cation exchange

### Complementary Solute Interactions:

- Dispersion
- Polarizability



## Solute Retention Profile:



### Target Analyte Structures:

- Nitrogen and Halogenated Species

### Target Analyte Functionalities:

- Protonated amines
- Quaternary ammonium compounds
- Positively charged moieties
- Lewis bases



NOTE: May offer inconsistent results with sulfur-containing compounds.  
For column recommendations, contact Restek Technical Service.

## Fine Tuning Your Mobile Phase:

### If your target analyte is... Consider...

A Basic Moiety	<ul style="list-style-type: none"> <li>• Decreasing acid strength and/or concentration to increase retention relative to neutrals and acids.</li> </ul>
Conjugated or Aromatic	<ul style="list-style-type: none"> <li>• Altering or mixing protic and aprotic solvents to adjust retention and selectivity.</li> </ul>
Ionic or Charged	<ul style="list-style-type: none"> <li>• Increasing buffer strength to decrease retention.</li> </ul>



For step-by-step instructions on choosing your USLC™ column and fine tuning your mobile phase, see page 3.



## There's a USLC™ Column for Nearly Every Instrument Platform, Scale, and Application

Column Line*	Particle Diameter	Use
Pinnacle DB	1.9 µm	UHPLC
Ultra	3 and 5 µm	HPLC

\* In addition to USLC™ stationary phases, Restek also offers additional particle diameters on these column lines as well as additional phases.

Column Class	Column ID
Capillary	<1.0 mm
Microbore	1.0 mm
Narrow bore	2.1–3.0 mm
Standard bore	3.2–4.6 mm
Semi-prep	10–21.2 mm
Prep	30–50 mm

For information on choosing column dimensions and setting instrument parameters, please contact **Restek Technical Service** ([support@restek.com](mailto:support@restek.com)).

## Protect Your LC Columns and Your Separations

Filters and guard cartridges are invaluable for protecting your LC columns and extending their life. Without them, sample impurities, mobile phase contaminants, and even materials from the injector or autosampler can cause particles to collect on the column inlet frit. This buildup can cause an increase in backpressure, split peaks, peak tailing, over-pressure shut-downs, and, ultimately, irreversible column damage.

Adding a filter or guard cartridge as a preventative measure can spare you the significant cost and hassle of frequently replacing your columns. And Restek has a complete line of easy-to-install solutions—even unique combination models with separately replaceable cartridges and filters to make maintenance easier and more cost effective.

Order yours today at  
[www.restek.com/LCguard](http://www.restek.com/LCguard)



## Looking for Additional Help on Using Your USLC™ Column Set?

### Application Note

The information in this selection guide is just the beginning. For a detailed analysis of USLC™ column selectivity data,

visit [www.restek.com/USLCarticle](http://www.restek.com/USLCarticle)

### Technical Service

We're here to help! If you have any questions about choosing the right USLC™ column, adjusting your mobile phases, selecting the right LC accessories, or anything else, call Restek Technical Service at 800-356-1688, ext. 4 or email them at [support@restek.com](mailto:support@restek.com)



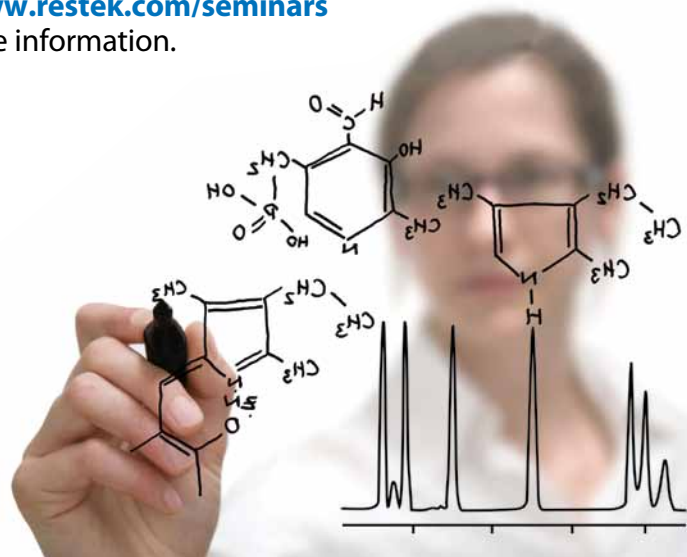
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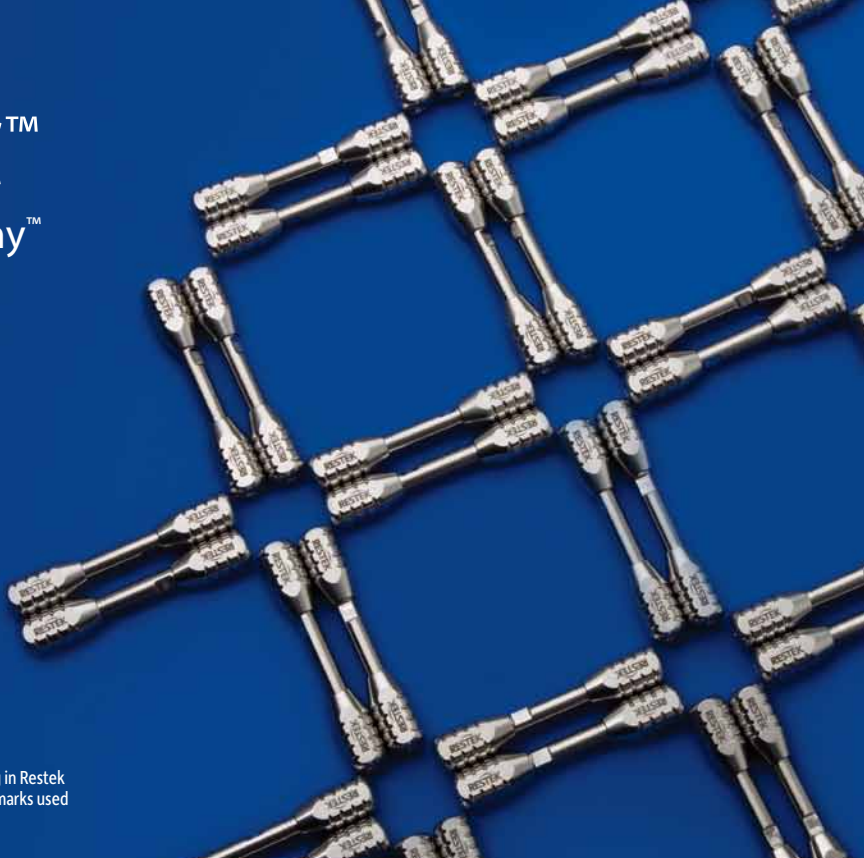




# RESTEK USLC™

Ultra Selective Liquid Chromatography™

*Choose Columns Fast. Develop Methods Faster.*



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## Two Options for Analyzing Potential Genotoxic Impurities in Active Pharmaceutical Ingredients

By Amanda Rigdon, Pharmaceutical Innovations Chemist, Rick Lake, Pharmaceutical Market Development Manager, Claire Heechoon\*, Research Chemist, Roy Helmy\*, Ph.D., Research Fellow, Christopher Strulson\*, Research Assistant, and Margaret Figus\*, Research Chemist

\*Merck & Co., Inc.



*Laboratory needs for analyzing PGIs in API vary. Here we developed both a fast analysis of sulfonate esters on the Rxi®-5Sil MS column, and a comprehensive method for sulfonate esters and alkyl halides on the Rtx®-200 column.*

Compounds that are used in the synthesis of active pharmaceutical ingredients (API), or reaction byproducts that form during synthesis, have the potential to remain as impurities. Some of these compounds are potentially genotoxic impurities (PGI) and may raise concern about cancer and/or birth defects.

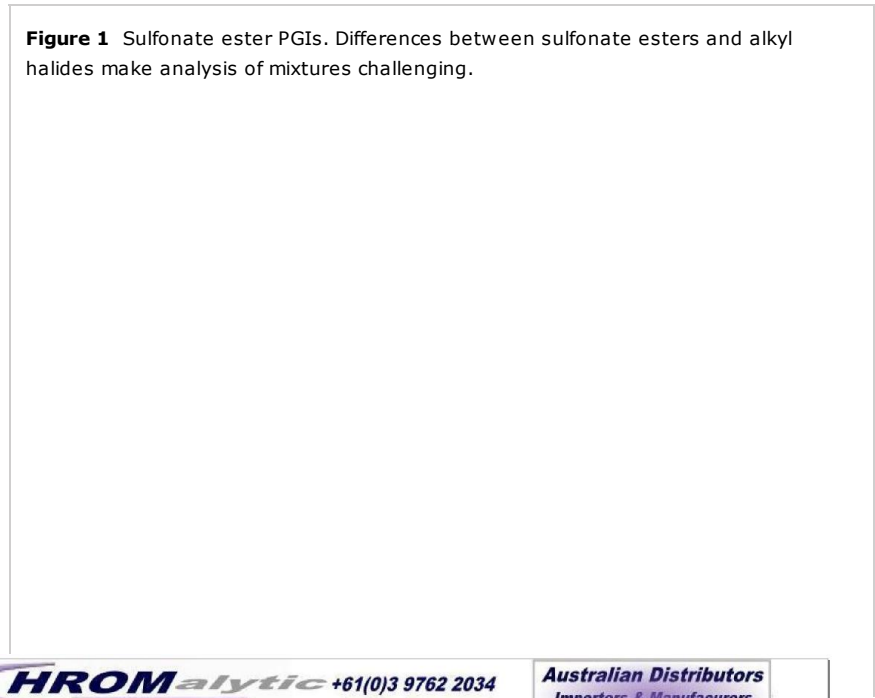
**Scientists from Merck, in collaboration with Restek, have developed a fast method for the analysis of sulfonate esters on the Rxi®-5Sil MS column.**

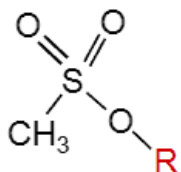
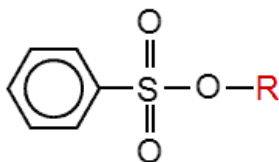
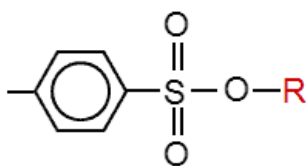
Four structural classes of PGIs are discussed in this article. The first three classes, known collectively as sulfonate esters, include mesylates, besylates, and tosylates (Figure 1). These alkylating sulfonic acid esters may form when sulfonic acid reacts with an alcohol solvent. The first three classes are differentiated by the group that forms an ester with the sulfur: mesylates contain a methyl group, besylates contain a phenyl (benzyl) group, and tosylates contain a toluene group. The fourth class of PGIs tested here, alkyl halides,

consists of short alkyl chains with halogen constituents. Since alkyl halides are polar and very volatile, they are not retained well on thin film stationary phases. This can make analysis of a mixture of sulfonate esters and alkyl halides quite problematic.

Two options for the analysis of PGIs in API have been developed to meet different laboratory needs. The first option is a fast method for the analysis of sulfonate esters on the Rxi®-5Sil MS column. The second option is a comprehensive method for the analysis of both sulfonate esters and alkyl halides on the Rtx®-200 column. Both methods require very little sample preparation, which helps increase laboratory productivity.

**Figure 1** Sulfonate ester PGIs. Differences between sulfonate esters and alkyl halides make analysis of mixtures challenging.

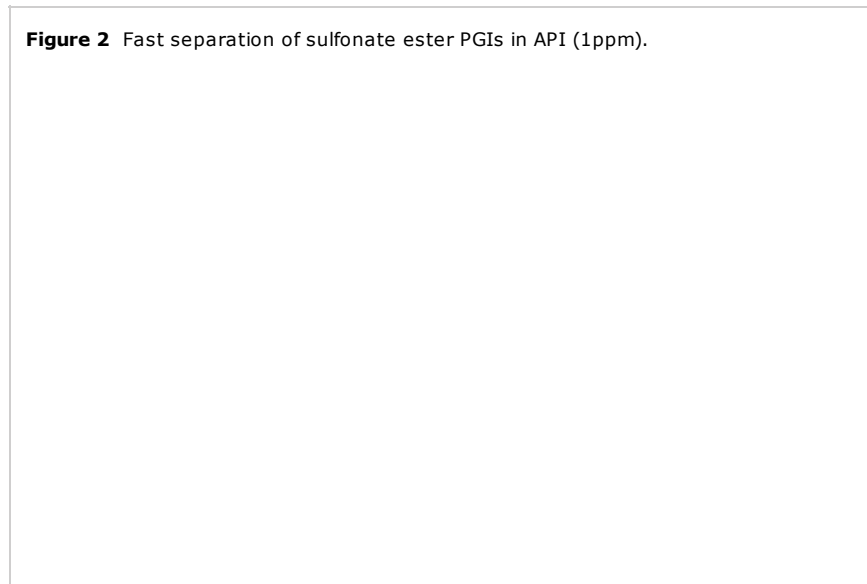


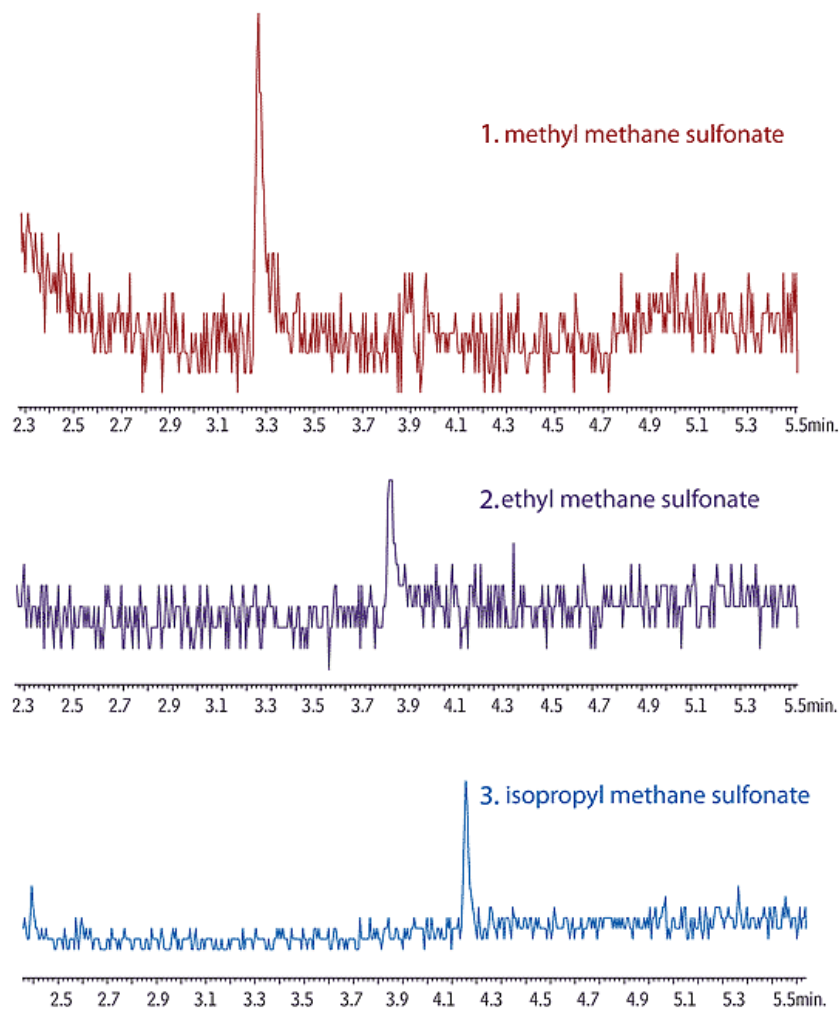
**Mesylate****Besylate****Tosylate****Option 1: Fast Analysis of Sulfonate Esters**

Scientists from Merck, in collaboration with Restek, have developed a fast method for the analysis of sulfonate esters on the Rxi®-5Sil MS column. Since PGIs can differ dramatically from one another, several types of API were spiked and analyzed to ensure the robustness of the method. Figure 2 shows an example of API spiked at 1ppm, which is the threshold for toxicological concern (TTC) for PGIs in API as set by the EMEA. Depending on the dose of API to the patient, it may be necessary to detect levels of impurities as low as 1ppm in order to meet requirements. A linearity study was also performed and shows that this method is linear for sample concentrations from 1ppm to 1,000ppm in API (Figure 3).

The use of a thin film Rxi®-5Sil MS column allows for fast analysis of active PGI compounds. Since the Rxi®-5Sil MS column is very selective toward sulfonate esters, a fast oven program can be used to speed analysis. This method allows for the analysis of selected sulfonate esters in less than 4.5 minutes. However, note that since the Rxi®-5Sil MS column is a mid-polarity stationary phase, the use of polar sample solvents, such as methanol, is not recommended. Due to the polarity of this phase, splitless injections of sample solvents with water may cause peaks to split. The method shown here utilizes a 10:1 split, which allows for the injection of relatively polar sample solvents, such as 90:10 acetonitrile:water.

**Figure 2** Fast separation of sulfonate ester PGIs in API (1ppm).



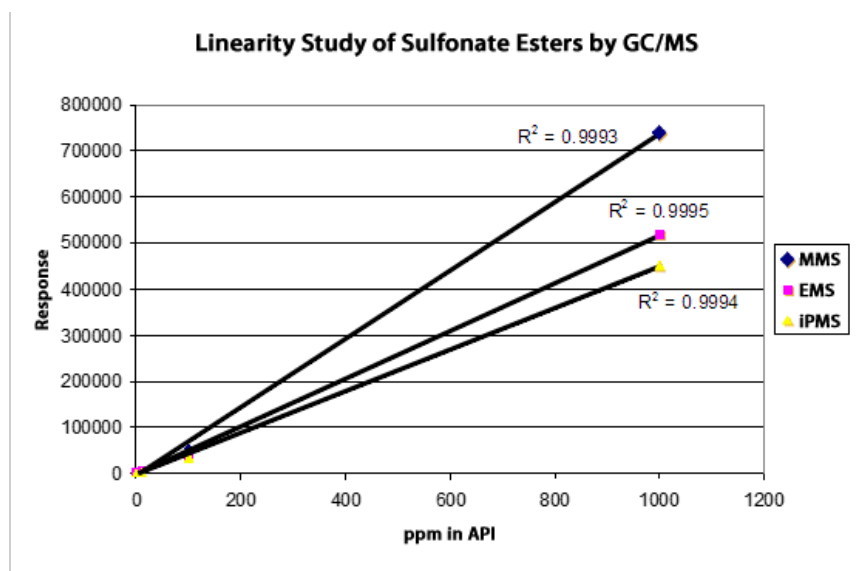


\*Courtesy of Merck & Co., Inc.

GC\_PH01072A

<b>Column</b>	Rxi®-5Sil MS, 20 m, 0.18 mm ID, 0.18 µm (cat.# 43602)
<b>Sample</b>	API
<b>Diluent:</b>	90:10 acetonitrile:water
<b>Conc.:</b>	10 mg/mL
<b>Injection</b>	
Inj. Vol.:	1.0 µL split (split ratio 10:1)
Liner:	Gooseneck Splitless (4mm) w/Wool (cat.# 22405)
Inj. Temp.:	250 °C
<b>Oven</b>	
Oven Temp.:	50 °C (hold 1 min) to 150 °C at 20 °C/min to 250 °C at 50 °C/min
<b>Carrier Gas</b>	He, constant pressure (10.19 psi, 70.3 kPa)
<b>Detector</b>	MS
Mode:	SIM
SIM Program:	80, 109, 123 m/z
Transfer Line Temp.:	280 °C
Ionization Mode:	EI
<b>Acknowledgement</b>	Merck & Co., Inc

**Figure 3** Linearity of fast GC/MS analysis for selected sulfonate esters.



#### Option 2: Comprehensive Method for Analysis of PGIs

Although the thin film Rxi®-5Sil MS column allows for fast analysis of sulfonate esters, the smaller, more polar alkyl halides are not well retained. To take advantage of the halogen constituents on the alkyl halides, a thick film Rtx®-200 column was used to develop this comprehensive method for both volatile alkyl halides and less volatile sulfonate esters. Since the Rtx®-200 column has a fluorinated stationary phase, the alkyl halides are well-retained (Figure 4). Note that all of the alkyl halides elute at a low temperature and some of the more volatile compounds elute prior to the sample solvent (acetonitrile). Because of this, the solvent cut time must be carefully measured. The Rtx®-200 column is also selective for sulfonate esters, providing baseline resolution for 20 out of 22 of the compounds analyzed. Additionally, the increased polarity of the fluorinated Rtx®-200 phase allows for the use of splitless injection of more polar sample solvents, such as methanol.

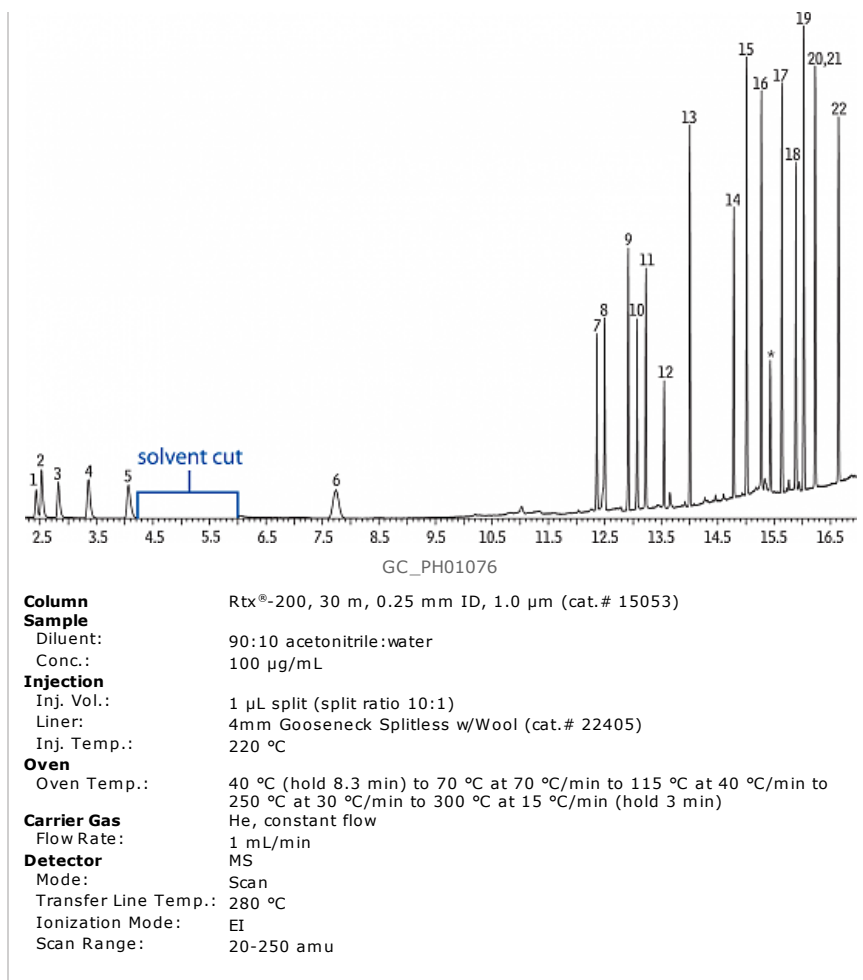
If the analysis of alkyl halides is not a laboratory concern, a thin film Rtx®-200 column may be used for faster analysis of sulfonate esters. A linearity study was conducted to ensure that this method is linear over the expected range of sulfonate esters in API. Samples were prepared at 8 concentrations ranging from 10ng/mL to 10,000ng/mL in 90:10 ACN:H<sub>2</sub>O. This is equivalent to 1-1,000ppm in API for samples prepared at 10mg of API/mL of sample solvent. This method shows acceptable linearity for all 15 sulfonate esters analyzed (Table I).

**Table I** Excellent linearity is also obtained using the comprehensive PGI methodology.

Compound Name	R-squared Value
dimethyl sulfate (DMS)	0.9984
methyl methanesulfonate (MMS)	0.9988
ethyl methanesulfonate (EMS)	0.9988
isopropyl methanesulfonate (iPMS)	0.999
diethyl sulfate (DES)	0.999
diisopropyl sulfate (DPS)	0.9993
di- <i>n</i> -butyl sulfate (DBS)	0.9996
methylbenzene sulfonate (MBS)	0.9991
benzenesulfonic acid ethyl ester (EBS)	0.9995
methyl- <i>p</i> -toluenesulfonate (MTS)	0.9996
<i>p</i> -toluenesulfonic acid ethyl ester (ETS)	0.9996
<i>p</i> -toluenesulfonic acid <i>n</i> -propyl ester (nPTS)	0.9994
benzenesulfonic acid <i>n</i> -butyl ester (nBBS)	0.9995
isopropyl <i>p</i> -toluenesulfonate (iPTS)	0.9996
<i>p</i> -toluenesulfonic acid <i>n</i> -butyl ester (nBTS)	0.9994

8-point standard curves, 1-1,000ng/mL (equivalent to 1-1,000ppm in API matrix)

**Figure 4** Small, polar alkyl halides are well-retained on the fluorinated Rtx®-200 column, as are less volatile sulfonate esters.



### Special Considerations:

#### Sample Preparation

Minimal sample preparation is needed to successfully run these methods. Samples of neat API were simply diluted to a concentration of 10mg/mL and injected into the GC. However, care must be taken with sample solvent selection, since many APIs are in salt forms and cannot be dissolved in 100% organic solvent. To aid dissolution, up to 10% water may be mixed with a water-miscible organic solvent. The sample solvent used in this application was 90:10 acetonitrile:water.

#### System Maintenance

Since samples are prepared at a relatively high concentration, nonvolatile API may build up in the liner and/or on the head of the column after repeated injections. Care should be taken to regularly change liners and seals to avoid problems with chromatography stemming from contaminated parts.

Some PGIs, such as isopropyl benzene sulfonate, break down in both the inlet and column. These breakdown products may persist in the analytical column, causing an elevated baseline that resembles a bleed profile. If isopropyl benzene sulfonate is present in the sample, a longer bake-out time (10-15 min. @ 300°C) is recommended at the end of each analytical run in order to remove any degradation products present on the column.

#### SIM Groups

Care must be taken when choosing SIM groups for PGI compounds during method development. Because these analytes are very similar to one another, many of them share common ions. It is good practice to pick two or three ions to monitor for each compound and to use relatively unique ions for each PGI to aid in peak identification.

#### Conclusion

Since potential genotoxic impurities are of increasing concern for both regulatory bodies and consumers, the importance of effective methods for detection and quantitation of these compounds is growing. As a result of collaboration between Merck and Restek, two easy, sensitive options are now available for the analysis of PGIs in API using inert, selective columns from Restek.

#### RELATED SEARCHES



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# Residual Solvent Analysis

## Complete Solutions for Residual Solvent Testing

- How to successfully implement the USP <467> revision.
- Improve system suitability pass rates with an optimized system.
- Save column evaluation time and expense using a retention time index.



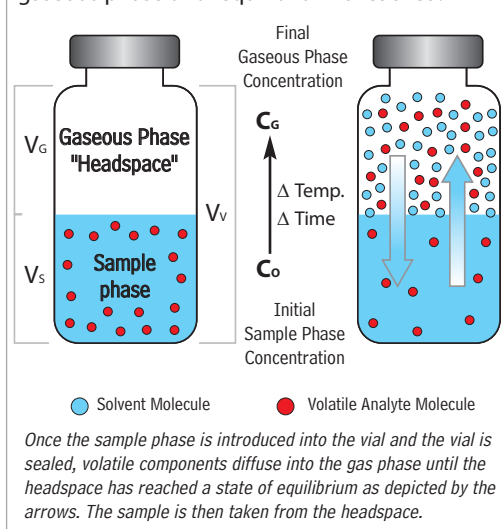
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# The Chemistry of Static Headspace Gas Chromatography

## Improve Method Performance with Fundamentals

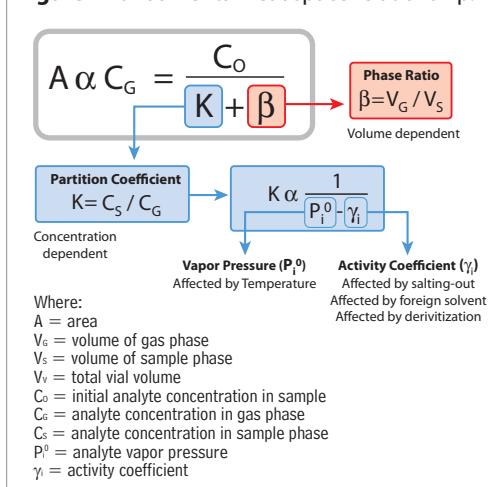
**Figure 1** Volatile components partition into gaseous phase until equilibrium is reached.



Organic volatile impurities (OVIs), commonly referred to as residual solvents, are trace level chemical residues in drug substances and drug products that are byproducts of manufacturing or that form during packaging and storage. Drug manufacturers must ensure that these residues are removed, or are present only in limited concentrations. The International Conference on Harmonization (ICH) Q3C guideline lists the acceptable amounts of solvent residues that can be present. Methodology, both independently developed and compendial, should strive to coincide with this guideline. In this guide, we will take a comprehensive look at residual solvent analysis, in both theory and practice, and illustrate options for the practicing chromatographer.

The analysis of residual solvents is commonly performed using static headspace gas chromatography (HS/GC). The basic premise behind headspace analysis begins with the addition of an exact, known volume or weight of sample into a closed, sealed vial. This creates two distinct phases in the vial—a sample phase and a gaseous phase, or “headspace”. Volatile components inside the sample phase, whether a solid or solution, can be extracted, or partitioned, from the sample phase into the headspace. An aliquot of the headspace can then be taken and delivered into a GC system for separation and detection. If we look at the anatomy of a headspace vial (Figure 1), we can begin to see the relationship of the vial components and how we can control these parameters to create analytical methods.

**Figure 2** Fundamental headspace relationship.



Residual solvent analysis by static HS/GC can be enhanced by careful consideration of two basic concepts—partition coefficient ( $K$ ) and phase ratio ( $\beta$ ). Partition coefficients and phase ratios work together to determine the final concentration of volatile compounds in the headspace of sample vials. Volatile components partition from the sample phase and equilibrate in the vial headspace. Striving for the lowest values for both  $K$  and  $\beta$  when preparing samples will result in higher concentrations of volatile analytes in the gas phase and, therefore, better sensitivity (Figure 2).

### Controlling the Partition Coefficient

The partition coefficient ( $K$ ) is defined as the equilibrium distribution of an analyte between the sample and gas phases. Compounds that have low  $K$  values will tend to partition more readily into the gas phase, and have relatively high responses and low limits of detection.  $K$  can be further described as a relationship between analyte vapor pressure ( $p_i^0$ ) and activity coefficient ( $\gamma_i$ ). In practice,  $K$  can be lowered by increasing the temperature at which the vial is equilibrated (vapor pressure) or by changing the composition of the sample matrix (activity coefficient) by adding an inorganic salt or a solvent of lesser solubility, often referred to as a foreign solvent. High salt concentrations and foreign solvents decrease analyte solubility in the sample phase (decrease activity) and promote transfer into the headspace, thus resulting in lower  $K$  values. The magnitude of this effect on  $K$  is not the same for all analytes. Compounds with inherent low  $K$  values in the matrix will experience little change in partition coefficient in response to the addition of a salt and temperature, while volatile compounds in a matrix of similar polarity will show the largest responses.

### Adjusting the Phase Ratio

The phase ratio ( $\beta$ ) is defined as the volume of the headspace over the volume of the sample in the vial. Lower values for  $\beta$  (i.e., larger sample sizes) will yield higher responses for compounds with inherently low  $K$  values. However, decreasing  $\beta$  will not always yield the increase in response needed to improve sensitivity. When  $\beta$  is decreased by increasing sample size, compounds with high  $K$  values will partition less into the headspace compared to compounds with low  $K$  values and yield correspondingly smaller changes in sensitivity.

## Technical Opportunities

Expand your knowledge and improve your results with Restek.

- Request our free Technical Guide for Static Headspace Analysis. cat.# 59895A
- Review our technical poster on dual column analysis of residual solvents.

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• 2 •

# Achieving USP<467> Compliance

## Your Guide to Successfully Implementing the Revised Method

The USP general chapter <467> Residual Solvents is a widely used compendial method for identifying and quantifying residual solvents when no information is available on what solvents are likely to be present. In an attempt to harmonize with the ICH guidelines, the USP has proposed a more comprehensive method in the current USP 30/NF 25. This revision significantly increases the number of residual solvents to be routinely tested and includes three distinct procedures.<sup>1</sup>

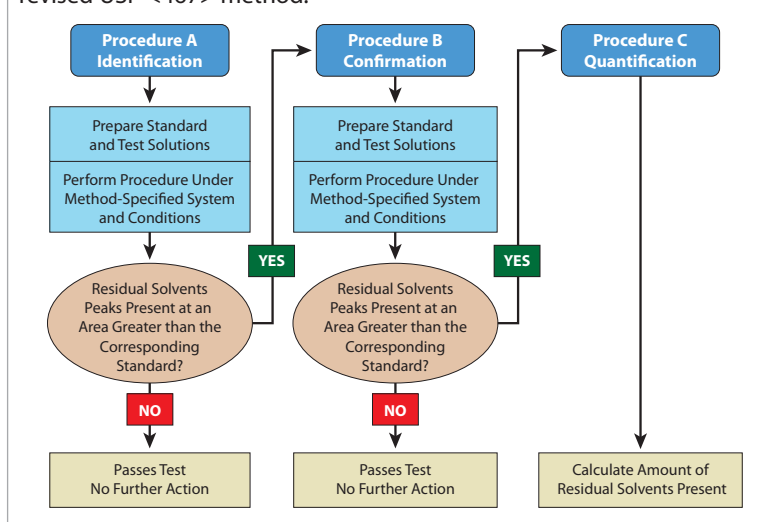
Initially set to become effective July 1, 2007, the implementation of the current version of USP <467> has been delayed until July 1, 2008. Until that time, the Other Analytical Procedures section of the previous version will be retained. However, in preparation for the implementation of the revised method, this application will comply with the procedure and criteria set forth in the USP30/NF25, second supplement (effective December 1, 2007) and the interim revision announcement.

### Overview of Method

The revised USP <467> method consists of a static headspace extraction coupled with a gas chromatographic separation and flame ionization detection. In this guide we demonstrate the USP <467> application using two different types of headspace autosamplers. Procedure A was performed using a pressured loop autosampler and transfer line. Procedure B was performed using a heated syringe injection. Either system can be used to meet method requirements.

USP <467> is divided into two separate sections based upon sample solubility: water-soluble and water-insoluble articles. The methodology for both types of articles is similar, but the diluent used in both standard and sample preparations differs based upon the solubility of the test article. The test method consists of three procedures (A, B, and C), that are designed to identify, confirm, and then quantify residual solvents in drug substances and products (Figure 3).

**Figure 3** Analytical flow chart for residual solvent testing under the revised USP <467> method.



<sup>1</sup> This number of analytes to be tested represents the sum of Class 1 and 2 residual solvents that can be effectively assayed using HS/GC. The actual number of analytes may be more if xylenes, ethyl benzene and *cis/trans* 1,2 dichloroethylene are differentiated, or if circumstances require the quantification of specific Class 3 residual solvents.

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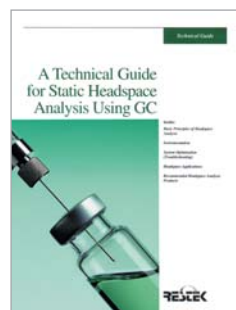
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#### Compatibility concerns?

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lit. cat.# 59895A

## USP-equivalent standards

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### Residual Solvents - Class 1

benzene	10mg/mL	1,1-dichloroethene	40
carbon tetrachloride	20	1,1,1-trichloroethane	50
1,2-dichloroethane	25		

In dimethyl sulfoxide, 1mL/ampul  
cat. # 36279 (ea.)

Quantity discounts not available.

### Residual Solvents Class 2 - Mix A (15 components)

acetonitrile	2.05mg/mL	methylcyclohexane	5.90
chlorobenzene	1.80	methylene chloride	3.00
cyclohexane	19.40	tetrahydrofuran	3.45
cis-1,2-dichloroethene	4.70	toluene	4.45
trans-1,2-dichloroethene	4.70	m-xylene	6.51
1,4-dioxane	1.90	o-xylene	0.98
ethylbenzene	1.84	p-xylene	1.52
methanol	15.00		

In dimethyl sulfoxide, 1mL/ampul  
cat. # 36271 (ea.)

### Residual Solvents Class 2 - Mix B (8 components)

chloroform	60µg/mL	nitromethane	50
1,2-dimethoxyethane	100	pyridine	200
n-hexane (C6)	290	tetralin	100
2-hexanone	50	trichloroethene	80

In dimethyl sulfoxide, 1mL/ampul  
cat. # 36280 (ea.)

Quantity discounts not available.

### Residual Solvents Class 2 - Mix C (8 components)

2-ethoxyethanol	800µg/mL	2-methoxyethanol (methyl Cellosolve®)	250
ethylene glycol	3,100	N-methylpyrrolidone	2,650
formamide	1,100	sulfolane	800
N,N-dimethylacetamide	5,450		
N,N-dimethylformamide	4,400		

In dimethyl sulfoxide, 1mL/ampul  
cat. # 36273 (ea.)

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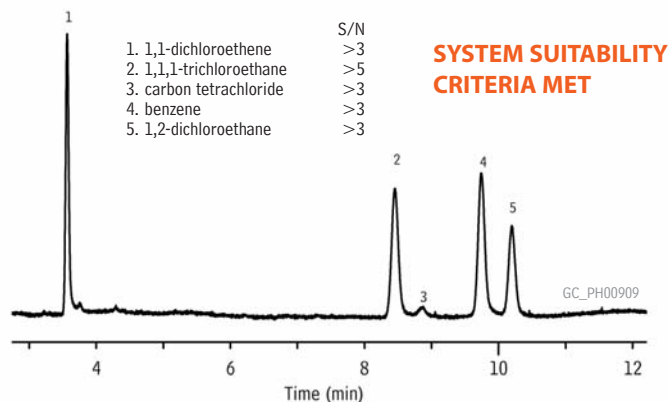
### Analytical Reference Materials

The ICH guideline classifies residual solvents by class according to toxicity. Class 1 compounds are carcinogenic and pose a risk to both the consumer and the environment. The use of these solvents must be avoided or tightly controlled. Class 2 compounds are nongenotoxic animal carcinogens and their concentration should be limited. Both Class 1 and 2 compounds require chromatographic determination and are separated into 3 test mixes: Class 1 Mixture, Class 2 Mixture A, and Class 2 Mixture B. Class 3 compounds have low toxic potential. Concentration levels of up to 0.5% are acceptable and, therefore, they can be assayed by nonspecific techniques, such as weight loss on drying. Class 2 Mixture C is not used in the second supplement of USP 30/NF 25, but contains solvents that are not readily detectable by headspace analysis. These solvents should be assayed by other appropriately validated procedures.

### Procedure A - Identification

Procedure A is the first step in the identification process and is performed on a G43 column to determine if any residual solvents are present in the sample at detectable levels. First, Class 1 standard and system suitability solutions and Class 2 Mix A standard solutions are assayed under the method-specified operating conditions to establish system suitability. All peaks in the Class 1 system suitability solution must have a signal-to-noise ratio not less than 3, the Class 1 standard solution must have a 1,1,1-trichloroethane response greater than 5, and the resolution of acetonitrile and dichloromethane must be not less than 1 in the Class 2 Mixture A solution. When system suitability has been achieved, the test solutions are assayed along with the Class 1 and Class 2 Mixtures A and B standard solutions. If a peak is determined in the sample that matches a retention time and has a greater response than that of a corresponding reference material, then Procedure B is performed for verification of the analyte. In the second supplement of USP 30/NF 25, an exemption is made for 1,1,1-trichloroethane, where a response greater than 150 times the peak response denotes an amount above the percent daily exposure limit. Figures 4 through 6 illustrate the analysis of Class 1, Class 2 Mixture A, and Class 2 Mixture B residual solvent mixes by Procedure A. The resolution between acetonitrile and dichloromethane was easily achieved using an Rtx®-1301 column.

**Figure 4** USP residual solvent Class 1 standard solution on an Rtx®-1301 column (G43).

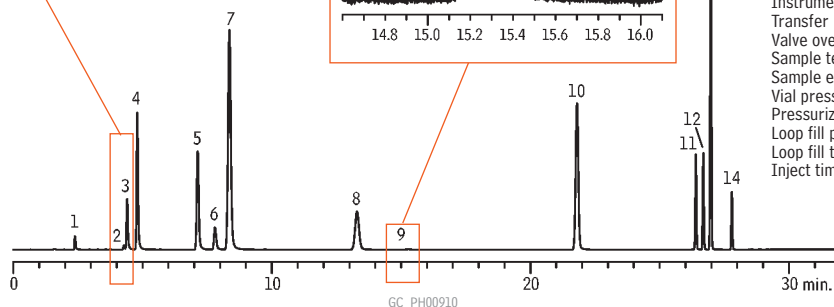
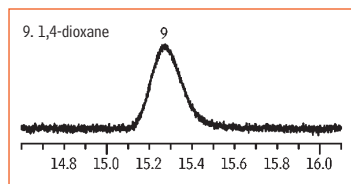
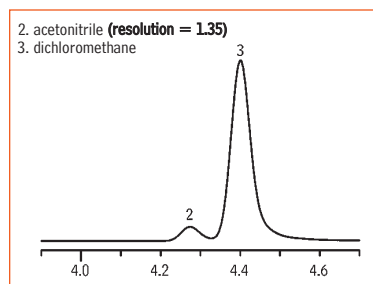


Column: Rtx®-1301, 30m, 0.32mm ID, 1.8µm (cat.# 16092)  
Sample: USP <467> Class 1 standard solution (cat.# 36279) in 20mL headspace vial  
Inj.: headspace injection (split ratio 1:5), 1mm split liner, Siltek® deactivated (cat.# 20972-214.1)  
Inj. temp.: 140°C  
Carrier gas: helium, constant flow  
Flow rate: 2.16mL/min., 35.3cm/sec.  
Oven temp.: 40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)  
Det.: FID @ 240°C

Headspace Conditions  
Instrument: Tekmar HT3  
Transfer line temp.: 105°C  
Valve oven temp.: 105°C  
Sample temp.: 80°C  
Sample equil. time: 45 min.  
Vial pressure: 10psi  
Pressurize time: 0.5 min.  
Loop fill pressure: 5psi  
Loop fill time: 2.00 min.  
Inject time: 1.00 min.

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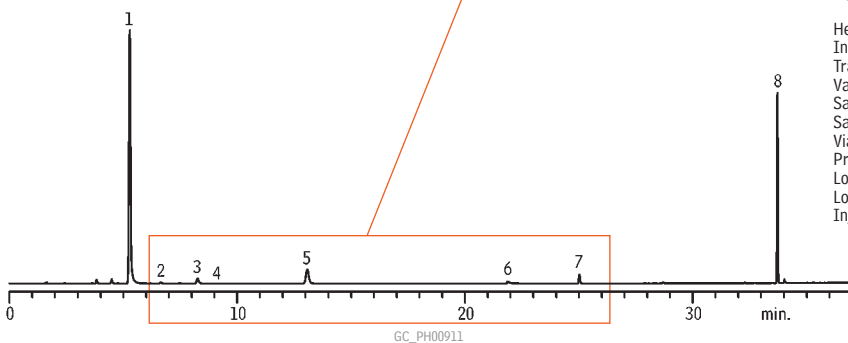
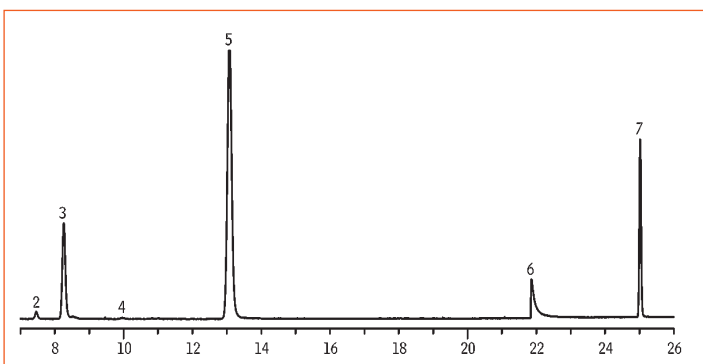
• 4 •

**Figure 5** USP residual solvent Class 2 Mixture A standard solution on an Rtx®-1301 column (G43).**SYSTEM SUITABILITY CRITERIA MET**

1. methanol
2. acetonitrile (resolution=1.35)
3. dichloromethane
4. *trans*-1,2-dichloroethene
5. *cis*-1,2-dichloroethene
6. tetrahydrofuran
7. cyclohexane
8. methylcyclohexane
9. 1,4-dioxane
10. toluene
11. chlorobenzene
12. ethyl benzene
13. *m*-xylene/*p*-xylene
14. *o*-xylene

Column: Rtx®-1301, 30m, 0.32mm ID, 1.8 $\mu$ m (cat.# 16092)  
 Sample: USP <467> Class 2 Mixture B standard solution (cat.# 36280) in 20mL headspace vial  
 Inj.: headspace injection (split ratio 1:5), 1mm split liner Siltek® deactivated (cat.# 20972-214.1)  
 Inj. temp.: 140°C  
 Carrier gas: helium, constant flow  
 Flow rate: 2.16mL/min., 35.3cm/sec.  
 Oven temp.: 40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)  
 Det.: FID @ 240°C

Headspace Conditions  
 Instrument: Tekmar HT3  
 Transfer line temp.: 105°C  
 Valve oven temp.: 105°C  
 Sample temp.: 80°C  
 Sample equil. time: 45 min.  
 Vial pressure: 10psi  
 Pressurize time: 0.5 min.  
 Loop fill pressure: 5psi  
 Loop fill time: 2.00 min.  
 Inject time: 1.00 min.

**Figure 6** USP residual solvent Class 2 Mixture B standard solution on an Rtx®-1301 column (G43).

1. hexane
2. nitromethane
3. chloroform
4. 1,2-dimethoxyethane
5. trichloroethylene
6. pyridine
7. 2-hexanone
8. tetralin

Column: Rtx®-1301, 30m, 0.32mm ID, 1.8 $\mu$ m (cat.# 16092)  
 Sample: USP <467> Class 2 Mixture B standard solution (cat.# 36280) in 20mL headspace vial  
 Inj.: headspace injection (split ratio 1:5), 1mm split liner Siltek® deactivated (cat.# 20972-214.1)  
 Inj. temp.: 140°C  
 Carrier gas: helium, constant flow  
 Flow rate: 2.16mL/min., 35.3cm/sec.  
 Oven temp.: 40°C for 20 min. to 240°C @ 10°C/min. (hold for 20 min.)  
 Det.: FID @ 240°C

Headspace Conditions  
 Instrument: Tekmar HT3  
 Transfer line temp.: 105°C  
 Valve oven temp.: 105°C  
 Sample temp.: 80°C  
 Sample equil. time: 45 min.  
 Vial pressure: 10psi  
 Pressurize time: 0.5 min.  
 Loop fill pressure: 5psi  
 Loop fill time: 2.00 min.  
 Inject time: 1.00 min.

# Achieving USP<467> Compliance (continued from page 5)

## Capillary Column—Procedure A

### Rtx®-1301 (G43) Columns (fused silica)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.80	-20 to 240°C	30-Meter	16092
0.53mm	3.00	-20 to 240°C	30-Meter	16085

## Capillary Column—Procedure B

0.32mm	0.25	40 to 250°C	30-Meter	10624
0.53mm	0.25	40 to 250°C	30-Meter	10625

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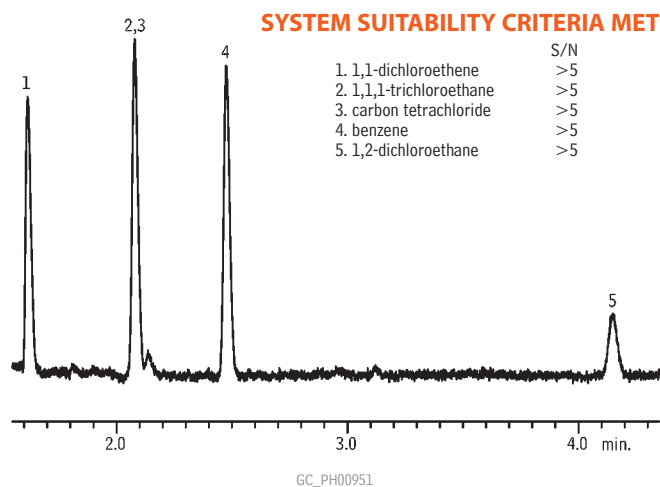
### Procedure B - Confirmation

Once a residual solvent is identified and found to be above the percent daily exposure limit, Procedure B is performed to confirm analyte identity. A G16 capillary column is used here as a confirmation column, because it yields an alternate selectivity compared to a G43 column. The same standard and system suitability preparations are used in Procedures A and B. The system suitability requirements differ here in that the Class 1 standard solution must have a benzene response greater than 5 and the resolution of acetonitrile and *cis*-dichloroethene must not be less than 1 in the Class 2 Mixture A solution, a change from the original version. If the analyte identified in Procedure A again matches the retention time and exceeds the peak response of the reference materials (with the same exception to 1,1,1-trichloroethane), the analyst must quantify the analyte using Procedure C. Figures 7 through 9 illustrate the analysis of Class 1, Class 2 Mixture A, and Class 2 Mixture B residual solvent mixes on a Stabilwax® column. Again, the system suitability requirements were easily met.

### Procedure C - Quantification

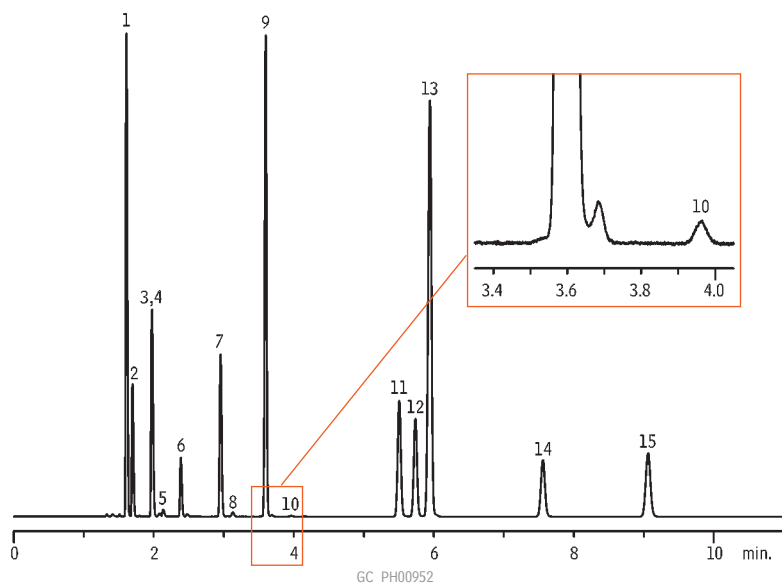
Once a residual solvent has been identified and verified, Procedure C is used to quantify the analyte by analyzing the sample against compound-specific reference materials. Individual standards are prepared by diluting the analyte in solution to a concentration of 1/20 of the concentration limit given under concentration limit Table 1 or 2 of the method. Following the procedure and instrument conditions in either Procedure A or B (whichever provides the most definitive results), a quantifiable result is produced. For water-insoluble articles, the same procedure is followed, except dimethylformamide or dimethylsulfoxide is used as the diluent.

**Figure 7** USP residual solvent Class 1 standard solution on a Stabilwax® column (G16).

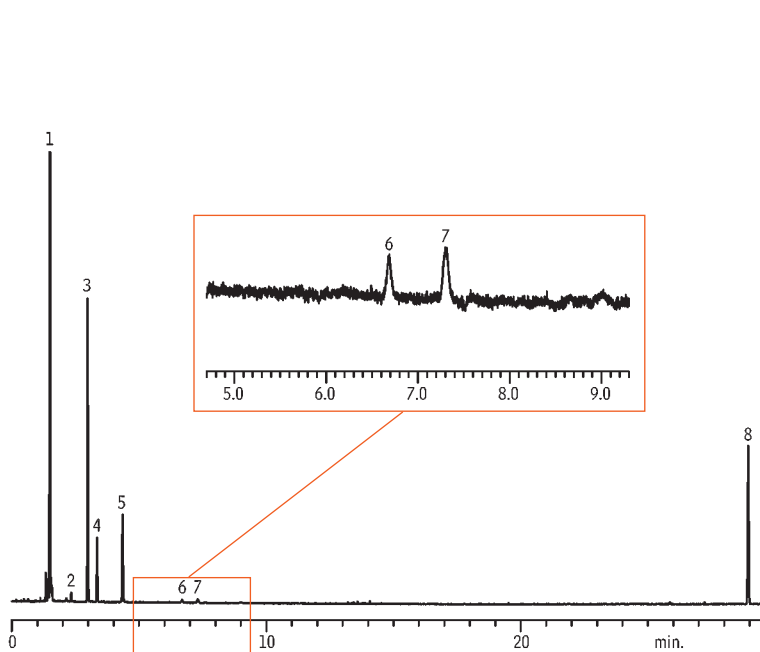


**Figure 8** USP residual solvent Class 2 Mixture A standard solution on a Stabilwax® column (G16).

**SYSTEM SUITABILITY CRITERIA MET—  
RESOLUTION BETWEEN PEAKS 7 & 8 > 1.0**



**Figure 9** USP residual solvent Class 2 Mixture B standard solution on a Stabilwax® column (G16).



1. hexane
2. 1,2-dimethoxyethane
3. trichloroethylene
4. chloroform
5. 2-hexanone
6. nitromethane
7. pyridine
8. tetralin

Column: Stabilwax®, 30m, 0.32mm ID, 0.25µm (cat.# 10624)  
 Sample: USP Stock Standard Residual Solvents Class 2 Mix B (cat.# 36272) in 20mL headspace vial (cat.# 24685), water diluent  
 Inj.: headspace injection (split ratio 1:5), 2mm splitless liner IP deactivated (cat.# 20712)  
 Inj. temp.: 140°C  
 Carrier gas: helium, constant flow  
 Flow rate: 2.15mL/min., 35.2cm/sec.  
 Oven temp.: 50°C for 20 min. to 165°C @ 6°C/min. (hold for 20 min.)  
 Det.: FID @ 250°C

Headspace Conditions  
 Instrument: Overbrook Scientific HT200H  
 Syringe temp.: 100°C  
 Sample temp.: 80°C  
 Sample equil. time.: 45 min.  
 Injection vol.: 1.0mL  
 Injection speed: setting 8  
 Injection dwell: 5 sec.

# Optimize Your Testing Procedure

## Tools, Tips, & Techniques for Improving Method Performance

### Use Smaller Bore Liners for Better Efficiency

#### 1mm Split Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#
1mm Split**		
1.0mm x 6.3mm x 78.5mm	ea.	20972
1.0mm x 6.3mm x 78.5mm	5-pk.	20973

#### 2mm Splitless Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#
2mm Splitless		
2.0mm x 6.5mm x 78.5mm	ea.	20712
2.0mm x 6.5mm x 78.5mm	5-pk.	20713
2.0mm x 6.5mm x 78.5mm	25-pk.	20714

#### Split Liners for Varian 1075/1077 GCs

ID* x OD & Length	qty.	cat.#
1mm Split		
1.0mm x 6.3mm x 72mm	ea.	20970
1.0mm x 6.3mm x 72mm	5-pk.	20971

#### Split Liners for Shimadzu GCs

ID* x OD & Length	qty.	cat.#
1mm Split		
1.0mm x 5.0mm x 95mm	ea.	20976
1.0mm x 5.0mm x 95mm	5-pk.	20977
1.0mm x 5.0mm x 95mm	25-pk.	20978

#### SPME Liners for Shimadzu 17A, 2010, and 2014 GCs

ID* x OD & Length	qty.	cat.#
SPME Liner		
.75mm x 5.0mm x 95mm	ea.	22278
.75mm x 5.0mm x 95mm	5-pk.	22279

#### Zero Dilution Liners for PerkinElmer Auto SYS™ and Clarus GCs

ID* x OD & Length	qty.	cat.#
Zero Dilution Inner Liner		
1.0mm x 2.0mm x 73mm	ea.	22990
1.0mm x 2.0mm x 73mm	5-pk.	22991
Zero Dilution Outer Liner		
2.5mm x 6.2mm x 90mm	ea.	22992
2.5mm x 6.2mm x 90mm	5-pk.	22993

\*Nominal ID at syringe needle expulsion point.

\*\*Use this liner for increased sensitivity.

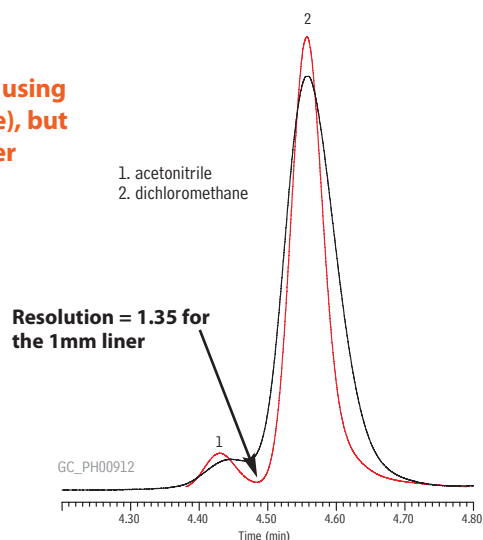
Implementing the revised method for USP<467> can be difficult if the instrument is not optimized correctly. Key issues to address when setting up headspace GC systems include minimizing system dead volume, maintaining inert sample flow paths, and achieving efficient sample transfer. While the second supplement contains a change that allows for modifications to the split ratio, column and liner choices are critical to analytical success.

#### Use Smaller Bore Liners for Better Resolution

The function of an injection port in headspace analysis is very different than in direct liquid injection. In direct injection, the sample is vaporized in the injection port and larger volume liners (e.g., 4mm) are typically used since the liner must be able to accommodate the solvent expansion volume. In contrast, in headspace analysis, the sample is vaporized inside the headspace vial and the resulting gas sample is simply transferred into the injection port via a transfer line or syringe injection. Since solvent vaporization does not occur in the liner, a large volume liner is not needed and, in fact, the use of one can cause deleterious effects such as band broadening and decreased peak efficiency. For headspace applications, a smaller bore liner, preferably 1mm, is recommended. The smaller liner volume reduces band broadening by increasing linear velocity in the liner allowing faster sample transfer and improving resolution (Figure 10).

**Figure 10** Improve system suitability pass rates using smaller bore liners.

**Resolution passes if using a 1 mm liner (red line), but fails with a 4mm liner (black line).**



#### Speed Up Method Development Using a Retention Time Index

ICH guideline Q3C states that residual solvents need only be tested when production or purification processes are known to result in the presence of such solvents. Therefore, in many cases exhaustive testing is not needed and individual validated methods for smaller, specific analyte lists are an option. To simplify column selection and reduce method development time, Restek has created a retention time index for ICH Class 1, 2, and 3 residual solvents on various phases (Table I). To use this index, simply locate the analytes of interest on the list and determine which phase gives the optimal amount of resolution—or difference in retention time—between your target compounds. A critical coelution is indicated by a failure to achieve a retention time difference of greater than 1.5 minutes.

**Table I** Reduce method development time—use a retention time index for column selection.

Retention time data collected using the following conditions:

**G16 Stabilwax®**: 30m, 0.25mm ID, 0.5µm df, Phase ratio: 125, Oven program: 40°C, hold 1 min., to 190°C @ 4°C/min., hold 15 min., Carrier flow: 1.2mL/min., Dead time: 1.38 min. @ 45°C  
**G16 Rtx®-WAX**: 30m, 0.25mm ID, 0.5µm df, Phase ratio: 125, Oven program: 40°C, hold 1 min., to 190°C @ 4°C/min., hold 15 min., Carrier flow: 1.2mL/min., Dead time: 1.40 min. @ 45°C  
**G43 Rtx®-1301**: 30m, 0.25mm ID, 1.0µm df, Phase ratio: 63, Oven program: 40°C, hold 1 min., to 190°C @ 4°C/min., hold 15 min., Carrier flow: 1.2mL/min., Dead time: 1.40 min. @ 45°C  
**G27 Rtx®-5ms**: 30m, 0.25mm ID, 1.0µm df, Phase ratio: 63, Oven program: 40°C, hold 1 min., to 190°C @ 4°C/min., hold 15 min., Carrier flow: 1.1mL/min., Dead time: 1.49 min. @ 45°C  
**G1 Rtx®-1**: 60m, 0.53mm ID, 3.00µm df, Phase ratio: 43, Oven program: 30°C, hold 4 min., to 220°C @ 4°C/min., Carrier flow: 6.3mL/min., Dead time: 2.54 min. @ 35°C  
**Rtx®-200**: 60m, 0.53mm ID, 3.00µm df, Phase ratio: 43, Oven program: 30°C, hold 4 min., to 220°C @ 4°C/min., Carrier flow: 7.8mL/min., Dead time: 2.22 min. @ 35°C

Carrier gas: helium	ICH Class	G16 Stabilwax® Retention Time	G16 Rtx®-WAX Retention Time	G43 Rtx®-1301 Retention Time	G27 Rtx®-5ms Retention Time	G1 Rtx®-1 Retention Time	NA Rtx®-200 Retention Time
1,1,1-trichloroethane	1	3.96	3.49	5.43	5.40	10.82	8.35
1,1,2-trichloroethane	2	15.72	14.28	10.99	9.77	16.75	14.94
1,1-dichloroethane	1	2.23	2.04	2.79	4.41	5.73	4.16
1,2-dichloroethane	1	8.80	7.68	6.15	5.46	10.38	9.74
cis-1,2-dichloroethane	2	6.50	5.65	4.79	2.88	8.71	7.11
trans-1,2-dichloroethane	2	3.63	3.20	3.55	3.54	7.17	5.16
1,2-dimethoxyethane	2	4.80	4.18	6.03	5.54	10.98	10.63
1,4-dioxane	2	8.55	7.49	7.86	7.26	13.54	14.34
1-butanol	3	11.13	10.08	7.18	5.76	11.49	10.13
1-pentanol	3	14.95	13.75	11.19	9.44	16.99	14.95
1-propanol	3	7.69	6.80	4.20	3.37	6.81	6.13
2-butanol	3	7.25	6.44	5.08	4.16	8.51	7.69
2-ethoxyethanol	2	13.99	12.70	8.69	7.36	13.91	13.99
2-methoxyethanol	2	12.42	11.11	6.02	5.14	9.83	10.74
2-methyl-1-propanol	3	9.32	8.40	6.00	4.79	*	*
2-propanol	3	4.81	4.25	3.00	2.55	4.91	4.69
3-methyl-1-butanol	3	13.42	12.25	9.86	8.26	15.28	13.55
acetic acid	3	22.47	20.34	6.52	4.61	8.84	8.96
acetone	3	3.02	2.64	2.89	2.50	4.64	7.68
acetonitrile	2	6.91	5.83	3.28	2.47	4.32	8.89
anisole	3	18.65	17.09	17.12	16.28	25.00	22.84
benzene	1	5.23	4.54	5.98	3.83	11.63	9.17
butyl acetate	3	8.86	7.88	12.12	11.38	19.43	19.63
carbon tetrachloride	1	3.96	3.49	5.61	5.90	11.89	7.42
chlorobenzene	2	13.91	12.54	13.55	13.14	21.56	18.48
chloroform	2	7.31	6.41	5.23	4.64	9.18	6.66
cumene	3	12.36	11.17	16.66	16.69	25.88	20.90
cyclohexane	2	2.16	2.01	5.37	5.89	*	*
dichloromethane	2	5.01	4.33	3.31	3.06	5.87	4.88
dimethylsulfoxide	3	26.47	24.43	16.62	13.01	18.81	30.95
ethanol	3	4.98	4.37	2.52	2.19	4.03	3.80
ethyl acetate	3	4.08	3.56	4.87	4.44	9.04	10.35
ethyl benzene	2	10.72	9.58	13.86	13.81	22.54	18.18
ethyl ether	3	1.72	1.63	2.58	2.67	5.34	3.87
ethyl formate	3	3.16	2.78	3.00	2.78	5.46	6.48
ethylene glycol	2	28.06	26.23	10.77	6.63	12.59	13.86
formamide	2	32.99	30.93	11.85	7.30	12.72	19.93
formic acid	3	24.64	22.09	5.19	2.60	5.59	5.06
heptane	3	1.98	1.86	6.34	6.98	14.18	7.84
hexane	2	1.65	1.58	3.77	4.11	9.06	4.86
isobutyl acetate	3	6.99	6.18	10.39	9.69	17.35	18.02
isopropyl acetate	3	4.26	3.74	6.19	5.71	11.47	12.38
methanol	2	4.23	3.64	1.96	1.80	3.14	2.93
methyl acetate	3	3.19	2.80	3.17	2.93	5.80	7.10
methylbutyl ketone	2	9.10	8.05	11.81	10.50	17.94	20.81
methylcyclohexane	2	2.50	2.30	7.31	7.95	15.49	9.21
methylethyl ketone	3	4.33	3.76	4.90	4.09	7.99	11.55
methylisobutyl ketone	3	6.84	5.97	9.64	8.49	15.35	18.41
m-xylene	2	11.21	10.04	15.46	14.17	23.01	18.78
N,N-dimethylacetamide	2	20.75	19.01	12.95	13.96	21.42	30.00
N,N-dimethylformamide	2	18.04	16.26	13.09	10.23	16.52	26.19
nitromethane	2	11.82	10.31	4.84	3.53	6.30	12.01
N-methylpyrrolidone	2	29.84	27.86	25.09	21.85	29.99	38.08
o-xylene	2	12.79	11.51	15.46	15.26	24.23	20.33
pentane	3	1.49	1.45	2.39	2.62	5.36	3.29
propyl acetate	3	5.98	5.29	8.03	7.44	*	*
p-xylene	2	10.98	9.82	14.29	15.27	22.99	18.69
pyridine	2	12.64	11.24	9.60	8.57	15.40	16.45
sulfolane	2	47.62	43.31	34.02	28.90	36.76	48.67
tert-butylmethyl ether	3	1.94	1.82	3.50	3.59	7.52	5.73
tetrahydrofuran	3	3.63	3.19	5.12	4.90	9.81	9.48
tetralin	2	25.12	23.48	27.49	27.44	37.27	31.72
toluene	2	7.86	6.91	9.80	9.66	17.36	14.00
1,1-diethoxypropane	—	5.42	4.84	11.39	11.38	19.82	15.08
2,2-dimethoxypropane	—	3.11	2.79	5.48	5.55	11.37	8.67
2-chloropropane	—	1.96	1.82	2.67	2.66	5.20	4.61
2-methylpentane	—	1.58	1.52	3.22	3.56	7.72	4.32
acetaldehyde	—	2.05	1.85	1.86	1.84	3.14	3.90
chloroethane	—	1.83	1.71	2.14	2.10	3.97	3.55
chloromethane	—	1.63	1.55	1.70	1.70	3.01	2.73
ethylene oxide	—	2.05	1.86	1.89	2.02	3.59	3.92
formaldehyde	—	2.25	1.57	1.68	1.58	2.66	2.59
isoamyl acetate	—	10.51	9.43	14.84	14.18	22.80	22.62
isooctane	—	1.85	1.75	5.84	6.59	13.66	8.07
isopropyl ether	—	1.86	1.76	4.03	4.23	9.03	5.83
methyl cyclopentane	—	1.91	1.79	4.50	4.93	10.41	5.81
methyl isopropyl ketone	—	4.93	4.29	6.58	5.69	11.04	14.47
methylal	—	2.26	2.06	2.84	2.82	5.65	5.09
trichloroethene	—	6.50	5.70	7.07	7.05	13.58	9.75
water	—	8.24	7.18	1.74	1.68	2.75	2.57

\* Not determined

# Restek Offers An Extensive Selection of Capillary Columns

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#### Rtx®-624 Columns (fused silica)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

G43

ID	df (μm)	temp. limits	30-Meter	60-Meter
0.25mm	1.40	-20 to 240°C	10968	10969
0.32mm	1.80	-20 to 240°C	10970	10972
0.53mm	3.00	-20 to 240°C	10971	10973
ID	df (μm)	temp. limits	20-Meter	40-Meter
0.18mm	1.00	-20 to 240°C	40924	40925

#### Rtx®-1301 Columns (fused silica)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

G43

ID	df (μm)	temp. limits*	30-Meter	60-Meter
0.25mm	0.50	-20 to 270°C	16038	16041
	1.00	-20 to 260°C	16053	16056
	1.40	-20 to 240°C		16016
0.32mm	0.50	-20 to 270°C	16039	16042
	1.00	-20 to 260°C	16054	16057
	1.50	-20 to 250°C	16069	16072
	1.80	-20 to 240°C	16092	16093
0.53mm	0.50	-20 to 270°C	16040	16043
	1.00	-20 to 260°C	16055	16058
	1.50	-20 to 250°C	16070	16073
	3.00	-20 to 240°C	16085	16088

#### Stabilwax® Columns (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

G16

ID	df (μm)	temp. limits	30-Meter	60-Meter
0.25mm	0.25	40 to 250°C	10623	10626
	0.50	40 to 250°C	10638	10641
0.32mm	0.25	40 to 250°C	10624	10627
	0.50	40 to 250°C	10639	10642
	1.00	40 to 240/250°C	10654	10657
0.53mm	1.00	40 to 240/250°C	10655	10658
	1.50	40 to 230/240°C	10669	10672
	2.00	40 to 220/230°C	10670	

### also available

#### Custom Column Lengths:

If you do not see the column dimension you need, call our customer service team, and we will make the column for you.

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

#### Rxi®-5ms Columns (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

G27

ID	df (μm)	temp. limits	30-Meter	60-Meter
0.25mm	0.50	-60 to 330/350°C	13438	13441
	1.00	-60 to 330/350°C	13453	13456
0.32mm	0.50	-60 to 330/350°C	13439	13442
	1.00	-60 to 330/350°C	13454	13457
0.53mm	1.00	-60 to 330/350°C	13455	
	1.50	-60 to 330/350°C	13470	
ID	df (μm)	temp. limits	20-Meter	
0.18mm	0.18	-60 to 330/350°C	13402	
	0.30	-60 to 330/350°C	13409	
	0.36	-60 to 330/350°C	13411	

#### Rtx®-1 Columns (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

G1

ID	df (μm)	temp. limits	30-Meter	60-Meter
0.25mm	0.50	-60 to 330/350°C	10138	10141
	1.00	-60 to 320/340°C	10153	10156
0.32mm	1.00	-60 to 320/340°C	10154	10157
	1.50	-60 to 310/330°C	10169	10172
	3.00	-60 to 280/300°C	10184	10187
	4.00	-60 to 280/300°C	10198	
	5.00	-60 to 260/280°C	10178	10180
0.53mm	1.50	-60 to 310/330°C	10170	10173
	3.00	-60 to 270/290°C	10185	10188
	5.00	-60 to 270/290°C	10179	10183
	7.00	-60 to 240/260°C	10192	10193
ID	df (μm)	temp. limits	20-Meter	40-Meter
0.18mm	0.20	-60 to 330/350°C	40102	40103
	0.40	-60 to 320/340°C	40111	40112

#### Rtx®-200 Columns (fused silica)

(Crossbond® trifluoropropylmethyl polysiloxane)

ID	df (μm)	temp. limits*	30-Meter	60-Meter
0.25mm	0.50	-20 to 310/330°C	15038	15041
	1.00	-20 to 290/310°C	15053	15056
0.32mm	1.00	-20 to 290/310°C	15054	15057
	1.50	-20 to 280/300°C	15069	15072
0.53mm	1.00	-20 to 290/310°C	15055	15058
	1.50	-20 to 280/300°C	15070	15073
	3.00	-20 to 260/280°C	15085	15088
ID	df (μm)	temp. limits	20-Meter	40-Meter
0.18mm	0.20	-20 to 310/330°C	45002	45003
	0.40	-20 to 310/330°C	45011	45012

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### Washerless, leak-tight seals for Agilent GCs

0.8mm ID Dual Vespel Ring Inlet Seal	2-pk./price	10-pk./price
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Siltek Treated	21242	21243
Stainless Steel	21238	21239
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Siltek Treated	21248	21249
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## Dual Vespel® Ring Cross-Disk Inlet Seals for Agilent GCs

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- Washerless, leak-tight seals.

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Gold-Plated	22083	22084
Siltek Treated	22085	22086
Stainless Steel	22087	22088



## Injection Port Weldments for Agilent GCs

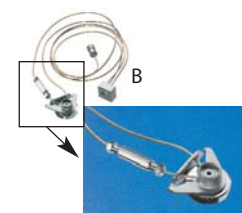
Easily attach your autosampler with pre-installed low dead volume fittings.

### For Agilent GCs with Tekmar Transfer Lines

Description	qty.	cat.#
A) Weldment for Agilent 6890 GCs	ea.	22664
Weldment for Agilent 6890 GCs with optional canister filter	ea.	22668
Weldment for Agilent 5890 GCs	ea.	22666

### For Agilent GCs with OI Purge and Trap Systems

Description	qty.	cat.#
B) Weldment for Agilent 6890 GCs	ea.	22665
Weldment for Agilent 6890 GCs with optional canister filter	ea.	22669
Weldment for Agilent 5890 GCs	ea.	22667



## FID Replacement Jets

### Standard Version

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- Threads specially coated for easy installation and removal.
- Special processing ensures the highest degree of cleanliness.

### High-Performance Version

- Similar to the standard version, but Siltek® treated.
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### Capillary Adaptable FID Replacement Jet for Agilent 5890/6890/6850 GCs

0.011-Inch ID Tip	Similar to Agilent part #	qty.	cat.#	qty.	cat.#
Standard, 0.011-Inch ID Tip	19244-80560	ea.	20670	3-pk.	20671
High-Performance Siltek Treated, 0.011-Inch ID Tip	19244-80560	ea.	20672	3-pk.	20673

### Capillary Dedicated FID Replacement Jet for Agilent 6890/6850/7890 GCs

0.011-Inch ID Tip	Similar to Agilent part #	qty.	cat.#	qty.	cat.#
Standard, 0.011-Inch ID Tip	G1531-80560	ea.	21621	3-pk.	21682
High-Performance Siltek Treated, 0.011-Inch ID Tip	G1531-80560	ea.	21620	3-pk.	21683



did you **know?**

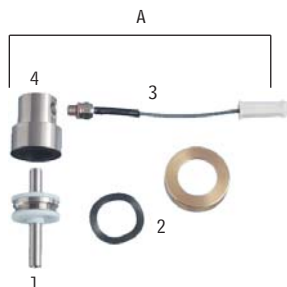
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• 11 •

### Direct Replacement FID Collector Assembly Kit for Agilent 6890/6850/7890 GCs

- Constructed of high-quality stainless steel.
- Meets or exceeds manufacturer's performance.



Description	Similar to Agilent part #	qty.	cat. #
A) FID Collector Assembly Kit (includes insulator)	G1531-60690	kit	21699
FID Collector Assembly Kit w/Siltek Ignitor Castle	—	kit	21132

### Replacement FID Parts for Agilent 6890/6850/7890 GCs

- Meets or exceeds manufacturer's performance.

Description	Similar to Agilent part #	qty.	cat. #
1) FID Collector (includes insulators)	G1531-20690 G1531-20700	ea.	21139
2) FID Collector Nut and Washer	19231-20940 5181-3311	set	21136
3) FID Ignitor*	19231-60680	ea.	21001
4) FID Ignitor Castle	19231-20910	ea.	21137
Siltek FID Ignitor Castle	—	ea.	21135

\*Also fits OI Analytical 4410 detector (similar to OI part # 191833).

### tech tip

Avoid using liquid leak detectors on a capillary system! Liquids can be drawn into the system.

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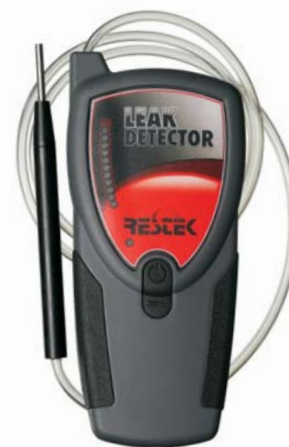
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Description	qty.	cat. #
Leak Detector with Universal Adapter Set	ea	22839



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## Separating NSAIDs through Aromatic Selectivity

### Improve Retention by Using An Allure® Biphenyl HPLC Column

By Rick Lake, Pharmaceutical Innovations Chemist, and Benjamin Smith, Applications Technician

- Optimize retention and selectivity of non-steroidal anti-inflammatory drugs, for better separations.
- Orthogonal separations with simple mobile phase changes.
- Increased retention requires higher organic content, increasing desolvation efficiency in LC/MS.

Non-steroidal anti-inflammatory drugs (NSAIDs), in either prescribed or over-the-counter formulations, are widely used to treat pain, fever, and inflammation. While steroidal anti-inflammatory drugs all share a similar, four-ring chemical structure, NSAIDs have more diverse chemical structures, complicating their analysis. The work we report here is based on three common classes of NSAIDs: arylalkanoic acids, 2-arylpropionic acids (profens), and oxicams.

NSAIDs have a high carbon to heteroatom ratio and, therefore, historically have been separated through reversed phase HPLC on C18 columns. A conventional C18 stationary phase separates compounds based mainly on their overall hydrophobicity. Considering the carbon to heteroatom ratio, this is an effective separation mechanism for NSAIDs. Newer stationary phases are available, however, and we set out to determine if other phases, using other separation mechanisms, such as  $\pi$ - $\pi$  interactions, could be more effective for assaying NSAIDs.

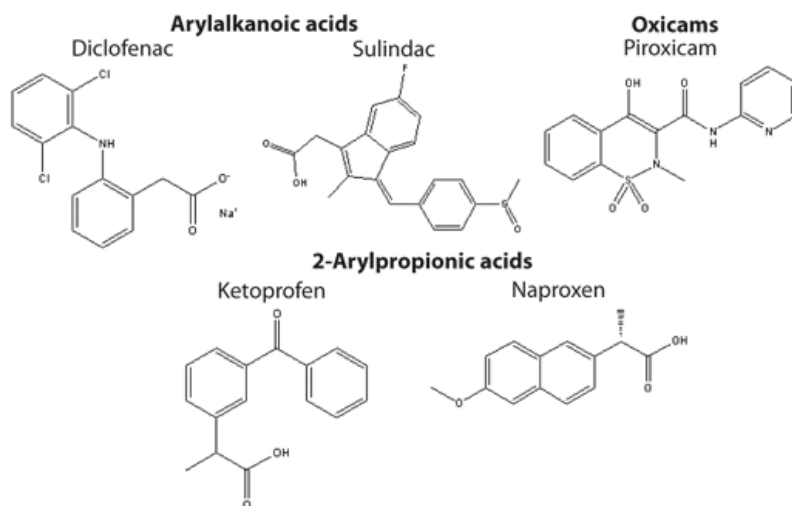
When selecting a stationary phase, it is advantageous to exploit inherent differences in the target analytes' chemical structures. Among these three classes of NSAIDs, there are some common functional groups, like halogens, amines, and carboxylic acids, but no one group is shared across the entire list of analytes (Figure 1). However, all of the target analytes do share one basic structural component — the six-carbon aromatic ring. Aromatic rings are common components of drug molecules, and they can be targeted using a phenyl-based stationary phase.

As a retention mechanism, phenyl stationary phases employ  $\pi$ - $\pi$  interactions between the phenyl groups in the stationary phase and any unsaturated bonds in the analyte. The use of conventional phenyl phases has been somewhat limited due to their moderate retention capacity, relative to that of a C18 phase. Figure 2 illustrates the relative retention capacities of NSAID test probes on an Allure® Biphenyl column, a conventional phenyl column and a C18 column. Note that, in all cases, as commonly seen in practice, the conventional phenyl phase yields only moderate retention compared to that of a C18 column. However, the Allure® Biphenyl phase, which is a stationary phase composed of two phenyl groups bonded end-to-end, easily achieves retention capacities similar to, and even greater than, those of a C18 column when used with a highly organic mobile phase. For this reason, we evaluated the enhanced retention of the Allure® Biphenyl column for assaying NSAIDs through aromatic selectivity.

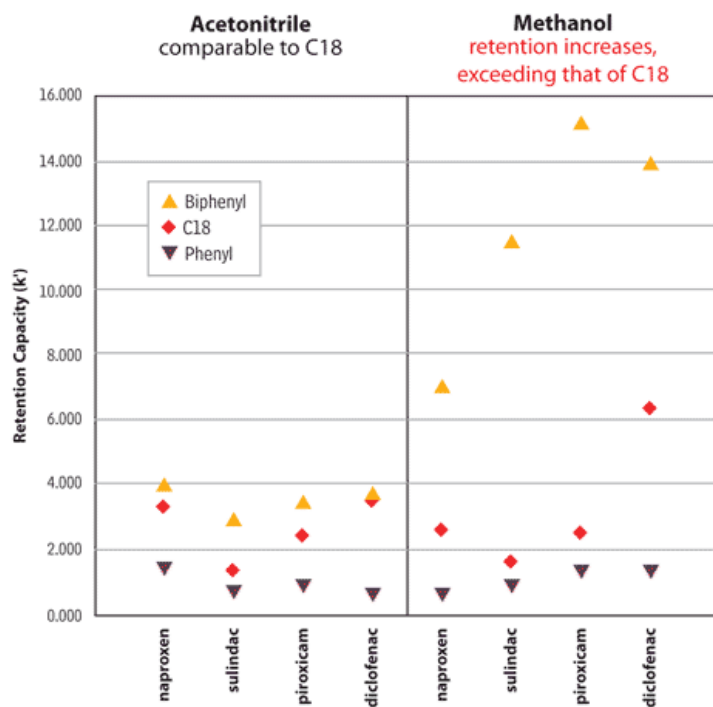
First, we compared the retention characteristics of a conventional C18 column and an Allure® Biphenyl column, using acetonitrile as the organic modifier. As expected, the Allure® Biphenyl column exhibited similar retention under equivalent analytical conditions (Figure 3). But, when we assayed the same analytes using methanol as the organic modifier, we found retention on the Allure® Biphenyl column was *greatly increased*. To maintain the same retention capacities ( $k'$ ) between the columns we had to increase the organic content by 20% (Figure 3). In addition, selectivity between the two columns became dramatically different. Based on these results, we conclude that methanol in the mobile phase enhances  $\pi$ - $\pi$  interactions between aromatic compounds and the biphenyl stationary phase, leading to greater retention and superior selectivity.

An Allure® Biphenyl column, in combination with a methanol-containing mobile phase, significantly improves separations of NSAIDs, or other aromatic drug compounds. Increased retention capacity creates a need for a higher percentage of organic solvent in the mobile phase, to elute the analytes in a timely manner. Increasing the organic content, in turn, increases sensitivity in LC/MS methods, because it optimizes the desolvation efficiency in electrospray interfaces. And this, in turn, makes an Allure® Biphenyl column the best choice for separating aromatics.

**Figure 1** Aromatic rings make NSAIDs candidates for separation through  $\pi$ - $\pi$  interactions.



**Figure 2** The retention capacity of the Allure® Biphenyl phase far exceeds that of conventional phases.



For each analyte all columns were assayed under identical isocratic conditions. The equivalent elutropic strength between acetonitrile and methanol was determined by the relative retention capacities of the C18 phase.

Columns: 5 $\mu$ m, 4.6mm x 150mm

Mobile Phase: 10mM potassium phosphate (pH 2.5): acetonitrile or methanol

Det.: UV @ 254nm

Flow: 1.0 mL/min.

**Figure 3** The versatility of the Allure® Biphenyl phase makes it a great alternative to conventional phase columns, especially in method development.

**Sample:**

Inj.: 5µL

Conc.: ~300µg/mL each component

Sample diluent: mobile phase

1. sulindac
2. piroxicam
3. ketoprofen
4. diclofenac

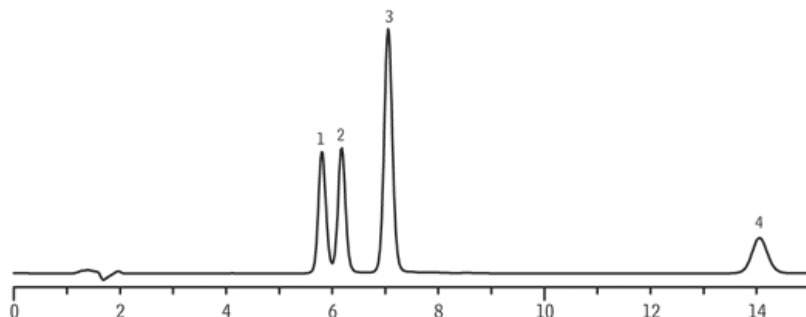
**In acetonitrile, retention of NSAIDs on an Allure® Biphenyl column is comparable to retention on a C18 column and elution order is the same.**

**A) Allure® Biphenyl, 50% acetonitrile****Column:** Allure® Biphenyl (cat.# 9166565)

Dimensions: 150 x 4.6 mm

Particle size: 5µm

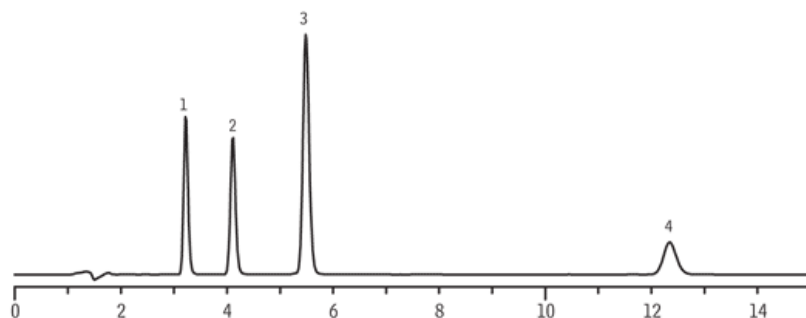
Pore size: 60Å

**B) C18, 50% acetonitrile****Column:** C18

Dimensions: 150 x 4.6 mm

Particle size: 5µm

Pore size: 100Å

**Conditions:**

Mobile phase: 0.5% formic acid in water (pH 2.25):0.1% formic acid in acetonitrile, 50:50 (v/v)

Flow: 1.0mL/min.

Temp.: ambient

Det.: UV @ 254nm

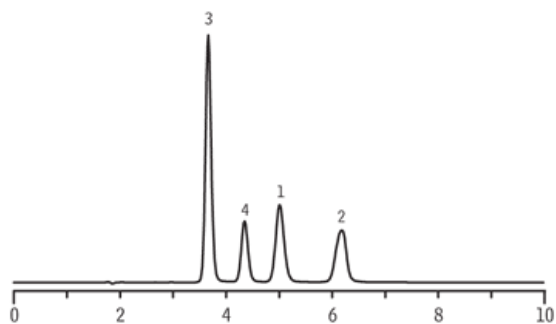
**In methanol, retention capacity & selectivity of NSAIDs are much greater on an Allure® Biphenyl column, compared to a C18 column, and elution order changes.**

**A) Allure® Biphenyl, 90% methanol****Column:** Allure® Biphenyl (cat.# 9166565)

Dimensions: 150 x 4.6 mm

Particle size: 5µm

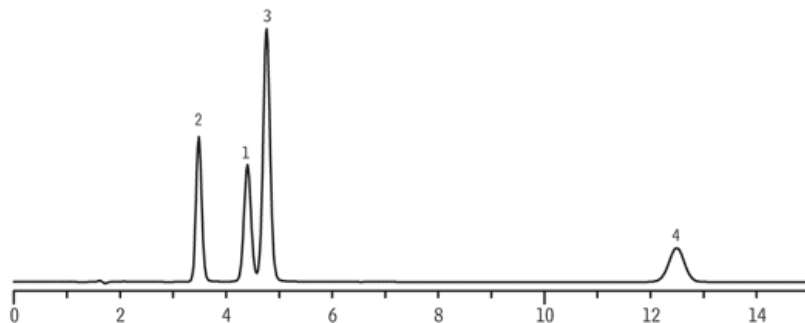
Pore size: 60Å


**B) C18, 70% methanol**
**Column:** C18

Dimensions: 150 x 4.6 mm

Particle size: 5µm

Pore size: 100Å


**Conditions:**

0.5% formic acid in water (pH 2.25):0.1% formic acid in methanol, 30:70 or 10:90

Mobile phase: (v/v)

Flow: 1.0mL/min.

Temp.: ambient

Det.: UV @ 254nm

**RELATED SEARCHES**
[NSAIDs](#), [pi-pi](#)
**RESTEK** Pure Chromatography

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## Rxi<sup>®</sup>-624SiI MS Columns

Exceptionally Inert,  
Low Bleed Columns for  
Volatiles Analysis

- **Optimized selectivity** for volatiles and polar compounds ensures good separations.
- **Highly inert columns** improve accuracy and allow lower detection limits, even for active compounds.
- **Most thermally stable** 624 column available; low bleed, fully MS compatible.

**NEW DIMENSIONS**  
now available!

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

## NEW! Rxi®-624Sil MS Columns:

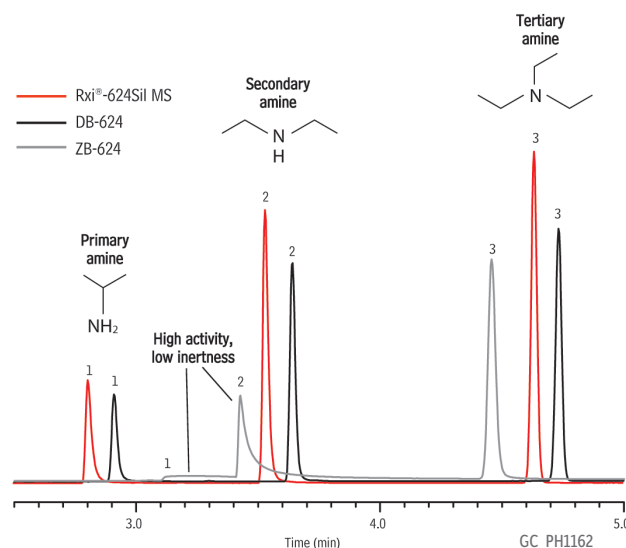
# Increase Confidence in Data Accuracy

While mid polarity 624 columns are widely used for analyzing polar analytes and volatile organic compounds (VOCs), not all columns combine the selectivity needed for critical separations with the high inertness and low bleed that can further improve data quality. Whether you are developing methods for residual solvents, analyzing environmental VOCs, or running other applications for volatile organics, you can improve data quality with Rxi®-624Sil MS columns. These new columns incorporate a new stationary phase chemistry, unique column deactivation, and optimized manufacturing process that is specifically designed to provide the high inertness and thermal stability needed for greater accuracy and lower detection limits. The unique selectivity, inertness, and thermal stability of the Rxi®-624Sil MS column make it ideal for numerous applications, from detecting impurities in pharmaceuticals to monitoring environmental VOCs.

### Exceptional Inertness Provides Better Peak Shape, Higher Sensitivity, and More Accurate Data

Column inertness is difficult to achieve, but critical to improving data quality. The deactivation process used for Rxi®-624Sil MS columns yields a fully passivated surface that is demonstrably more inert than other 624 columns. Comprehensive deactivation results in higher responses, more symmetrical peaks, and easy, accurate integration, even for active compounds at low levels (Figures 1 and 2). Rxi®-624Sil MS columns, with their superior deactivation, provide the inertness needed for improved linearity, greater accuracy, and lower detection limits.

**Figure 1** Highly inert Rxi®-624Sil MS columns provide better peak shape and simplify integration for active compounds at low levels (5 ng on-column).



Highly inert Rxi®-624Sil MS columns give excellent peak symmetry.

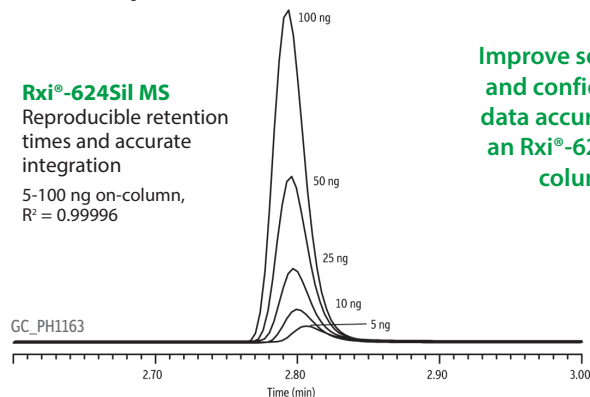
Peaks	Conc. (µg/mL)
1. Isopropylamine	100
2. Diethylamine	100
3. Triethylamine	100

<b>Column</b>	Rxi®-624Sil MS, 30 m, 0.32 mm ID, 1.8 µm (cat.# 13870)
<b>Sample</b>	DMSO
<b>Diluent:</b>	100 µg/mL
<b>Conc.:</b>	
<b>Injection</b>	
Inj. Vol.:	1 µL split (split ratio 20:1)
Liner:	5 mm Single Gooseneck with Wool (cat.# 22973-200.1)
Inj. Temp.:	250 °C
<b>Oven</b>	
Oven Temp:	50 °C (hold 1 min.) to 200 °C at 20 °C/min. (hold 5 min.)
<b>Carrier Gas</b>	He, constant flow
Linear Velocity:	37 cm/sec.
<b>Detector</b>	FID @ 250 °C
<b>Instrument</b>	Agilent/HP6890 GC

**Figure 2** Active compounds like isopropylamine can be more accurately integrated on an Rxi®-624Sil MS column, lowering limits of quantification (LOQs) and increasing data accuracy.

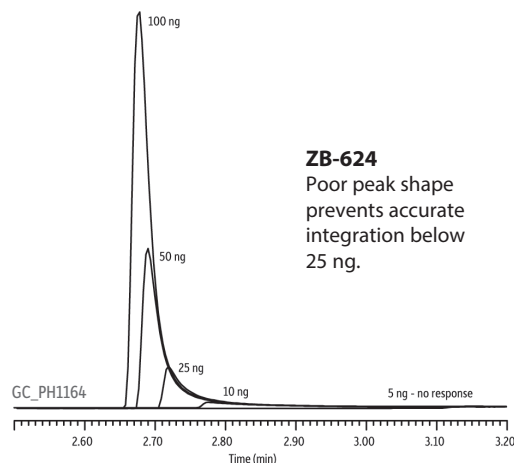
Same conditions as Figure 1.



#### Rxi®-624Sil MS

Reproducible retention times and accurate integration  
5-100 ng on-column,  
 $R^2 = 0.99996$

Improve sensitivity and confidence in data accuracy with an Rxi®-624Sil MS column.



#### ZB-624

Poor peak shape prevents accurate integration below 25 ng.

## Lowest Bleed 624 Available—Assured GC-MS Compatibility

In addition to providing greater inertness and more accurate results for active compounds, the Rxi®-624Sil MS column offers higher temperature stability than any other column in its class (Table I, Figure 3). Even though most 624 columns provide adequate selectivity for polar compounds, poor thermal stability results in stationary phase bleed that can reduce column lifetime, decrease detector sensitivity, and interfere with the quantification of later eluting compounds. The highly effective stationary phase bonding chemistry of the Rxi®-624Sil MS column ensures extremely low bleed up to 320°C. While other 624 columns generate too much bleed to be useful for continuous mass spectrometry work, the Rxi®-624Sil MS column is fully compatible with both quadrupole and ion trap mass spectrometers. In addition to MS compatibility, higher thermal stability results in more stable baselines, longer column lifetimes, and improved method reproducibility.

**Table I** The Rxi®-624Sil MS column has the highest thermal stability of any 624 column.

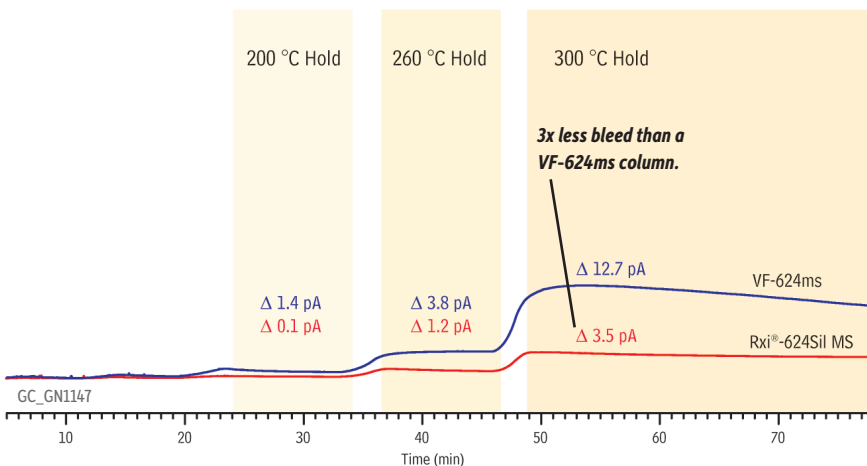
Column	Manufacturer	Maximum Programmable Temperature
Rxi-624Sil MS	Restek	320 °C
VF-624ms	Varian	300 °C
DB-624	Agilent J&W	260 °C
ZB-624	Phenomenex	260 °C

Data obtained from company website or literature for a 30 m x 0.25 mm x 1.4 µm df column.

**Figure 3** The Rxi®-624Sil MS column has the lowest bleed of any column in its class and provides true GC-MS capability.

### High thermal stability Rxi®-624Sil MS columns offer:

- Longer column lifetime.
- GC-MS compatibility.
- Improved method reproducibility.
- Stable baseline.



Columns: 30 m, 0.25 mm ID, 1.4 µm (Columns are of equivalent dimensions and were tested after equivalent conditioning.) Complete analytical conditions for chromatogram GC\_GN1147 are available at [www.restek.com](http://www.restek.com)

## Rxi®-624Sil MS Columns (fused silica)

(midpolarity Crossbond® silarylene phase; similar to 6% cyanopropylphenyl/94% dimethyl polysiloxane)

- Low bleed, high thermal stability column—maximum temperatures up to 320 °C.
- Inert—excellent peak shape for a wide range of compounds.
- Selective—highly selective for residual solvents, great choice for USP<467>.
- Manufactured for column-to-column reproducibility—well-suited for validated methods.

ID	df	temp. limits	20-Meter	30-Meter	60-Meter	75-Meter	105-Meter
0.18mm	1.00µm	-20 to 300/320°C	13865				
0.25mm	1.40µm	-20 to 300/320°C		13868	13869 <b>NEW</b>		
0.32mm	1.80µm	-20 to 300/320°C		13870	13872		
0.53mm	3.00µm	-20 to 280/300°C		13871	13873 <b>NEW</b>	13874 <b>NEW</b>	13875 <b>NEW</b>



## get more

For more information on the new Rxi®-624Sil MS column, visit [www.restek.com](http://www.restek.com) and review our technical literature.

- Volatile Impurities Method Development (flyer PHFL1245)
- Residual Solvent Analysis: Implementing USP<467> (flyer PHFL1018A)
- Optimized Volatiles Analysis Ensures Fast VOC Separations (application note EVAN1271)

## NEW! Rxi®-624Sil MS Columns:

# Assure Reliable Separation of Volatile Impurities in Pharmaceuticals

In the pharmaceutical industry, timing and certainty are everything. Time-to-market is a key driver for new drugs, and efficient batch testing is critical for releasing approved products. Whether developing new methods or conducting routine analysis, increasing productivity depends on choosing the right column for the application. Rxi®-624Sil MS columns provide enhanced retention of polar compounds and volatile analytes, as well as full MS compatibility, making them the best choice for many drug analyses.

### Fast, Effective Method Development

Often, 1 and 5 type columns are used initially for GC-MS method development because of their thermal stability; however, their nonpolar character results in poor retention for polar compounds, which increases method development time. In contrast, effective methods can be developed quickly on mid polarity Rxi®-624Sil MS columns, because they provide greater retention and selectivity for polar compounds as well as good thermal stability. For example, highly volatile, polar alkyl halide genotoxic impurities are difficult to retain on 1s and 5s, but Rxi®-624Sil MS columns provide the retention needed to ensure adequate separation (Figure 4). Increased retention makes GC-MS analysis easier to control and ultimately allows faster method development.

### Improving Results for Routine Analysis

Once a drug is approved, fast, reliable methods are needed for routine batch analysis. Establishing system suitability is an important part of these procedures and a major factor in overall lab productivity. Rxi®-624Sil MS columns provide the optimized selectivity and guaranteed reproducibility needed to increase pass rates. For example, batch throughput can be improved for residual solvent testing under USP <467> by using a column that provides increased resolution for system suitability components (Figure 5). Greater resolution of critical pairs means higher system suitability pass rates, which allows more batches to be analyzed per shift.

Optimized phase chemistry, complete column deactivation, and tightly-controlled manufacturing make Rxi®-624Sil MS columns the best choice for many pharmaceutical applications. With better retention of polar volatiles, lower bleed, and higher inertness, Rxi®-624Sil MS columns can improve lab productivity by allowing new methods to be developed quickly and routine applications to be run more reliably.

## learn more

For more pharmaceutical applications on Rxi®-624Sil MS columns, visit [www.restek.com](http://www.restek.com) and download flyer PHFL1245.

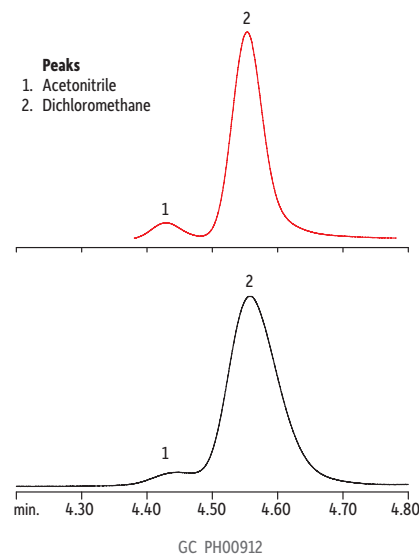
## TECH TIP!



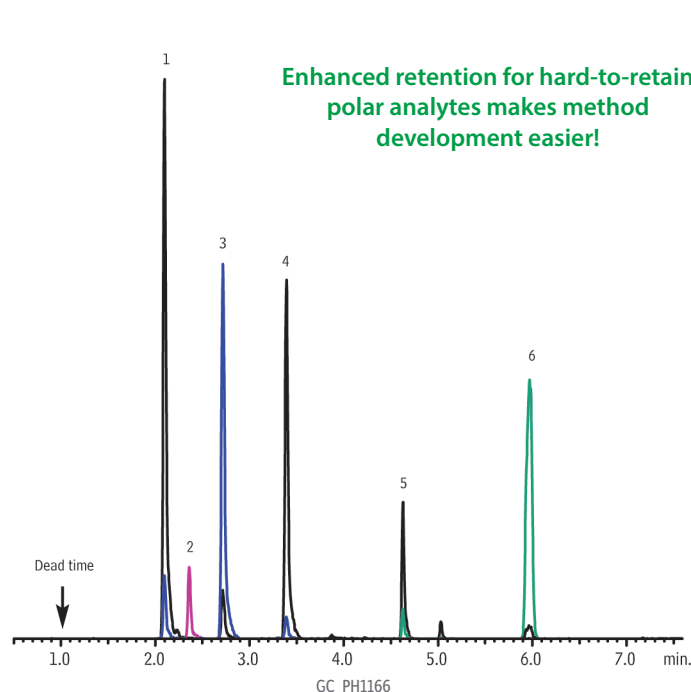
Tim Herring, Technical Service Specialist

When running USP <467> by headspace, using a smaller bore liner (1 mm) can improve system suitability pass rates. Larger bore liners (4 mm) are used with direct liquid injection because the sample is vaporized in the injection port and the liner must be able to accommodate the solvent expansion volume. In contrast, in headspace analysis, the sample is vaporized in a vial instead of the injection port, so a large volume liner is not needed, and in fact it can be deleterious. In headspace methods, using a smaller bore liner reduces band broadening by increasing linear velocity, allowing faster sample transfer and improving resolution.

Resolution passes USP <467> criteria when using a 1 mm liner (red line), but fails if a 4 mm liner is used (black line).



**Figure 4** Polar compounds, such as alkyl halides, are highly retained on mid polarity Rxi®-624Sil MS columns, making method development faster and easier than on a nonpolar 1 or 5 type column.

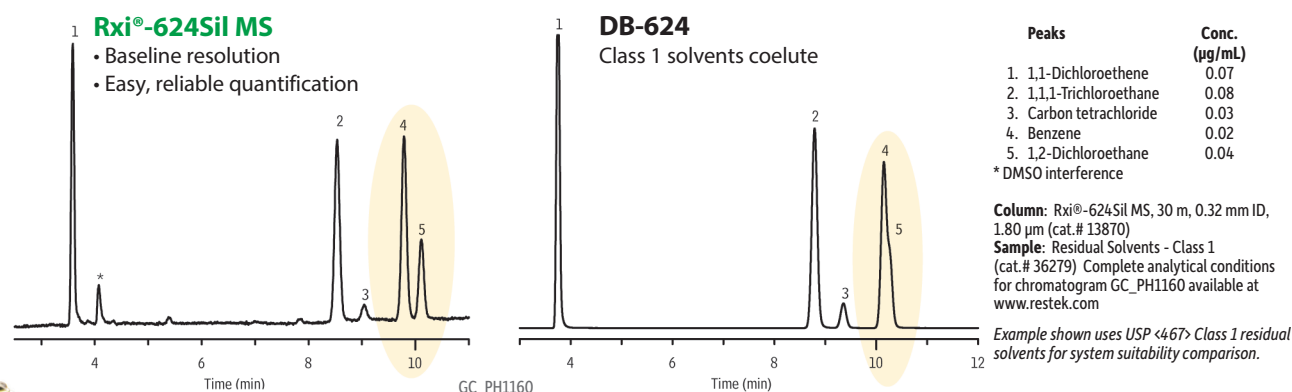


**Peaks**

1. 2-Chloropropane
2. Bromoethane
3. 1-Chloropropane
4. 2-Bromopropane
5. Butyl chloride
6. 1-Bromobutane

**Column** Rxi®-624Sil MS, 20 m, 0.18 mm ID, 1.00 µm (cat.# 13865)  
**Sample**  
**Diluent:** DMSO  
**Conc.:** 1 µg/mL  
**Injection**  
**Inj. Vol.:** 1 µL splitless (hold 0.5 min.)  
**Liner:** 3.5 mm Single Gooseneck Liner with wool placed 3 cm from top (middle) (cat.# 22286)  
**Inj. Temp.:** 220 °C  
**Purge Flow:** 3 mL/min.  
**Oven**  
**Oven Temp:** 40 °C (hold 3 min.) to 200 °C at 20 °C/min.  
**Carrier Gas** He, constant flow  
**Linear Velocity:** 40 cm/sec.  
**Detector** MS  
**Mode:** Scan  
**Transfer Line Temp.:** 280 °C  
**Analyzer Type:** Quadrupole  
**Source Temp.:** 280 °C  
**Solvent Delay Time:** 0.5 min.  
**Ionization Mode:** EI  
**Scan Range:** 30-300 amu  
**Scan Rate:** 5 scans/sec.  
**Instrument** Shimadzu 2010 GC & QP2010+ MS  
**Notes** Ions displayed: 42, 43, 57, 108 m/z

**Figure 5** System suitability pass rates can be improved with Rxi®-624Sil MS columns. The innovative polymer chemistry provides greater resolution of critical pairs that are difficult to separate on other 624 type columns.



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Save time and money with mixes prepared to your specific solvent set and concentrations.  
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## NEW! Rxi®-624Sil MS Columns:

# Increase Sample Throughput for Environmental VOCs

Fast sample throughput is a primary concern for environmental labs interested in improving productivity. Volatiles methods typically are time-consuming, but developing optimized procedures can be challenging because compound lists are extensive and analytes vary significantly in chemical characteristics. The selectivity and inertness of Rxi®-624Sil MS columns make them ideal for optimizing environmental volatiles methods for better resolution and faster analysis time.

Establishing conditions that maximize sample throughput can be difficult, because conditions optimized for speed can result in problematic coelutions, while conditions optimized for resolution can result in long analysis times. The exceptional inertness of Rxi®-624Sil MS columns produces highly symmetrical peaks for active compounds, which improves resolution and allows separations to be maintained even under faster conditions. Here, an optimized method was developed using an Rxi®-624Sil MS column to maintain adequate resolution, while throughput was maximized by synchronizing purge and trap cycles with instrument cycles.

### Improve Productivity and Resolve Critical Pairs

Initially, several critical pairs were chosen for computational modeling using Pro ezGC software. The temperature program first determined by the software provided the best resolution, but also resulted in an analysis time of 19 minutes. Since the purge and trap cycle time was 16.5 minutes, other conditions were evaluated to see if adequate resolution could be maintained using a faster instrument cycle. The final program, shown in Figure 6, reduced instrument downtime by better synchronizing purge and trap cycles with instrument cycles, and also provided excellent resolution. Using these conditions, up to 36 samples can be analyzed following EPA Method 8260 in a typical 12-hour shift.

Labs interested in optimizing both sample throughput and resolution of VOCs can adopt the synchronized conditions established here on Rxi®-624Sil MS columns to maximize productivity and assure accurate, reliable results.

For the complete application, visit [www.restek.com/adv002](http://www.restek.com/adv002)



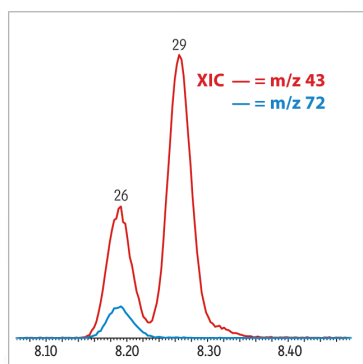
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- Measures volumetric flow for most gases across a range of 0.5-500 mL/min.
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- Data output via USB port.

Go to [www.restek.com/flowmeter](http://www.restek.com/flowmeter) for product features.



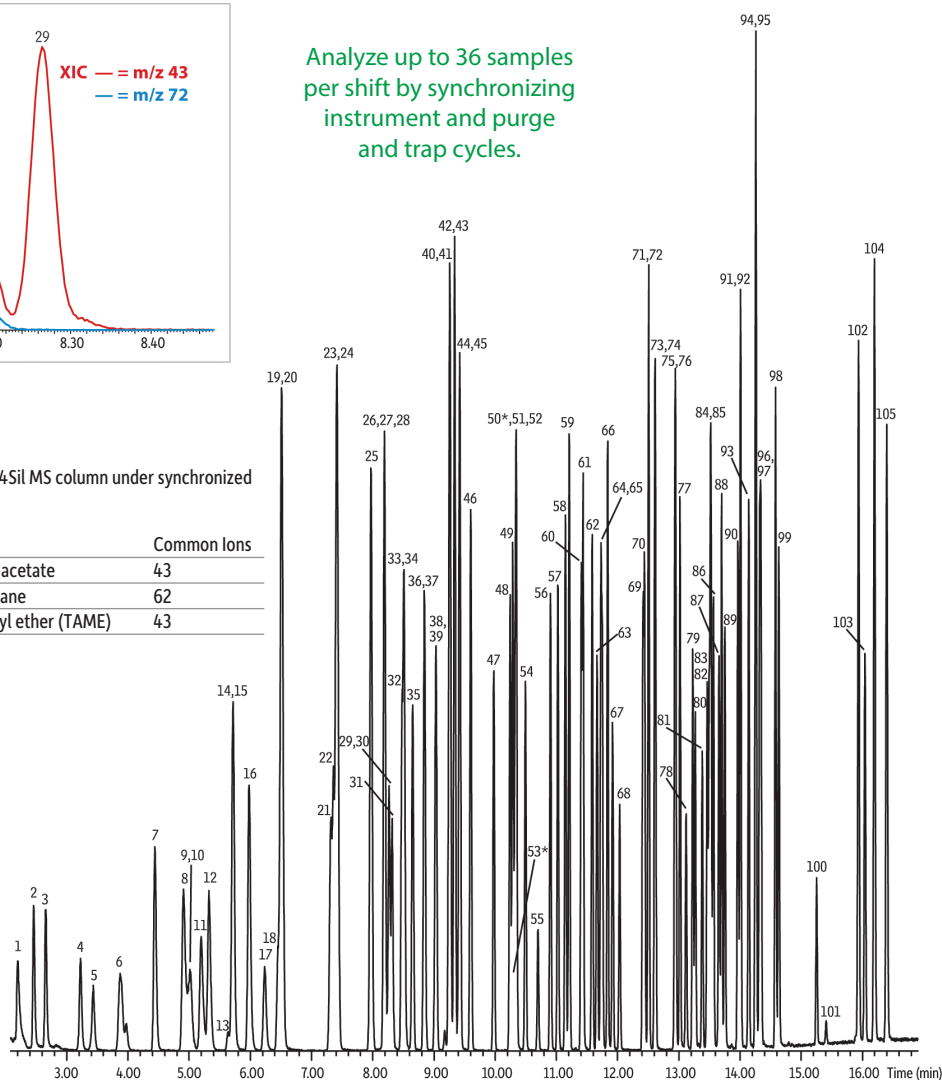
**Figure 6** Using an Rxi®-624Sil MS column under optimized conditions increases productivity by assuring good resolution and minimal downtime when analyzing environmental volatiles.



Analyze up to 36 samples per shift by synchronizing instrument and purge and trap cycles.

Critical pairs resolved using an Rxi®-624Sil MS column under synchronized conditions:

Peak #s	Compounds	Common Ions
26/29	2-butanone (MEK)/ethyl acetate	43
41/42	benzene/1,2-dichloroethane	62
41/45	benzene/ <i>tert</i> -amyl methyl ether (TAME)	43



For peak identifications and conditions visit [www.restek.com](http://www.restek.com) and enter chromatogram GC\_EV1169 in the search function.

## Rxi®-624Sil MS Columns (fused silica)

(midpolarity Crossbond® silarylene phase; similar to 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df	temp. limits	20-Meter	30-Meter	60-Meter	75-Meter	105-Meter
0.18mm	1.00µm	-20 to 300/320°C	13865				
0.25mm	1.40µm	-20 to 300/320°C		13868	13869 (NEW)		
0.32mm	1.80µm	-20 to 300/320°C		13870	13872		
0.53mm	3.00µm	-20 to 280/300°C		13871	13873 (NEW)	13874 (NEW)	13875 (NEW)

63 (of ~193 ) Pharma CT-Update 2015



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## Rxi<sup>®</sup>-624SiI MS

The "**Go To**" GC Column for Fast,  
Effective Volatile Impurities  
Method Development



Visit us at [www.restek.com/pharma](http://www.restek.com/pharma)

# Accelerate Development

## Rxi®-624Sil MS

### The **Go To** GC Column for Fast, Effective Volatile Impurities Method Development

In drug development, time-to-market is everything, but finding the right column can be laborious and time-consuming. Commonly, “624” (6% cyanopropyl phenyl/94% dimethyl polysiloxane) type columns are used for GC/FID impurity analyses to provide the necessary selectivity, but when mass spectrometry is needed, method development often starts with lower bleed “1” (100% dimethyl polysiloxane) and “5” (5% diphenyl/95% dimethyl polysiloxane) type columns. Now, you can get to market faster and more efficiently by using a single column that combines these attributes—the new Rxi®-624Sil MS column. With enhanced retention and selectivity of polar compounds, compatibility with mass spec detectors, and unsurpassed inertness, Rxi®-624Sil MS columns are the most broadly applicable GC columns available to the pharmaceutical industry. Speed up successful method development by making Rxi®-624Sil MS columns your “go to” column of choice for polar impurities.

#### **Go To...**the right column first

For better retention of polar analytes and improved accuracy, peak shape, and response for active compounds.

#### **Go To...**mass spec directly

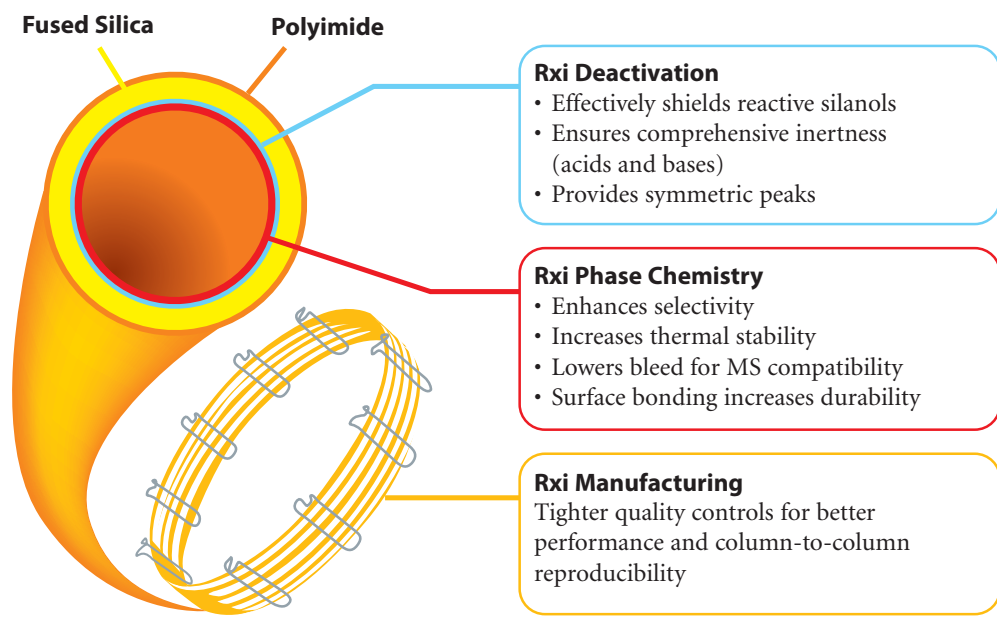
With the lowest bleed 624 column available; stable up to 320 °C, for easy transfer of methods to GC/MS.

#### **Go To...**the next batch faster

With the best-in-class G43 for USP methods.

### How did we create the **Rxi** Column Family?

We've optimized phase chemistry, column deactivation, and our manufacturing process to ensure the comprehensive performance that makes Rxi®-624Sil MS columns the best starting point for method development.

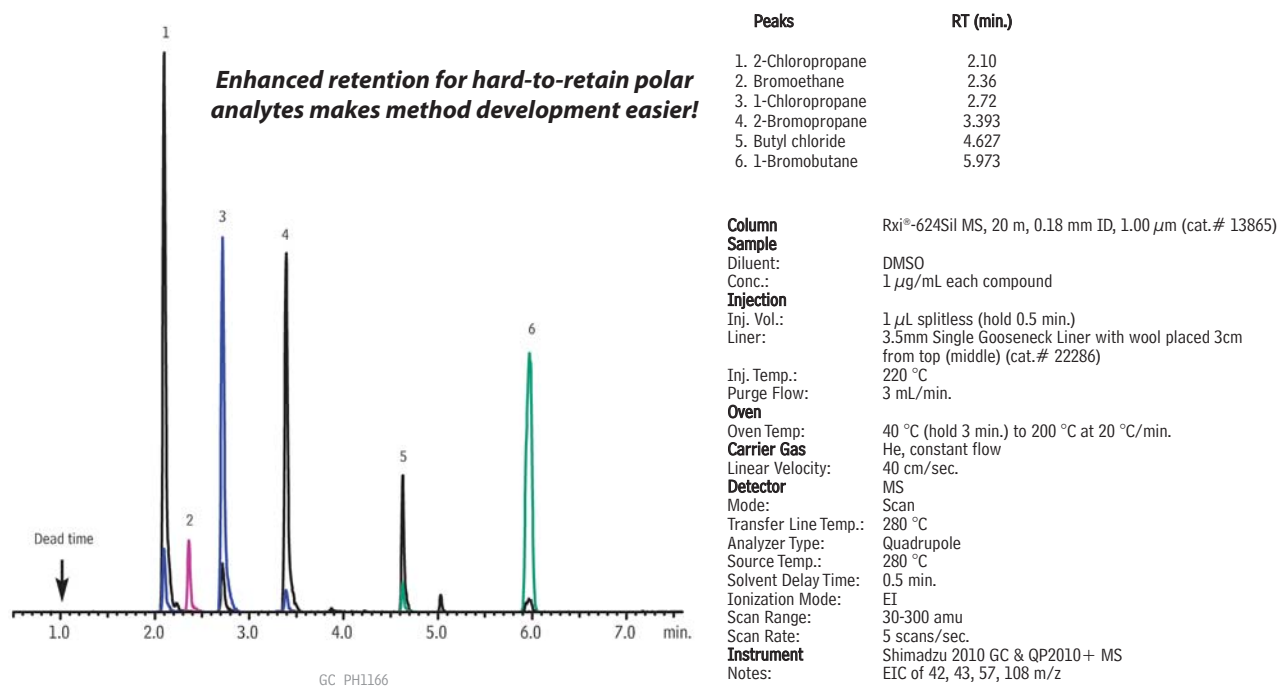


# Go To...the Right Column First

## Balanced Retention Simplifies Method Development For Polar Impurities

While “1s and 5s” are often used initially in GC/MS method development because of their thermal stability, their nonpolar character results in poor retention for polar compounds and costs additional development time. In contrast, midpolarity Rxi®-624Sil MS columns provide improved retention and selectivity for polar compounds and are also more compatible with polar injection solvents. Highly volatile, polar alkyl halide genotoxic impurities, for example, are difficult to retain on 1s and 5s, but the Rxi®-624Sil MS column provides higher retention capacity, making GC/MS analysis easier to control and allowing faster method development (Figure 1).

**Figure 1** Polar compounds, such as alkyl halides, are highly retained on midpolarity Rxi®-624Sil MS columns, making method development faster and easier than on a nonpolar 1 or 5 type column.



Visit [www.restek.com/rxi](http://www.restek.com/rxi) for detailed comparisons and to learn how exceptional Rxi® inertness, bleed, and reproducibility can improve your data.



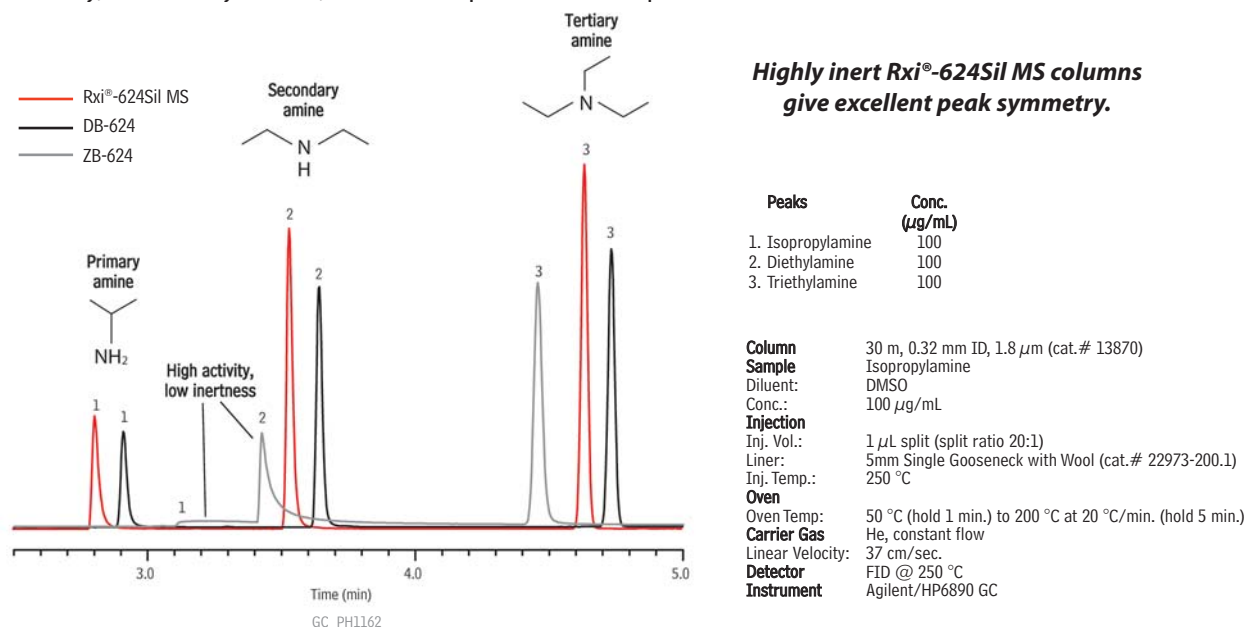
The versatility of an Rxi®-624Sil MS column makes it a perfect fit for Quality By Design.

# Go To...the Right Column First

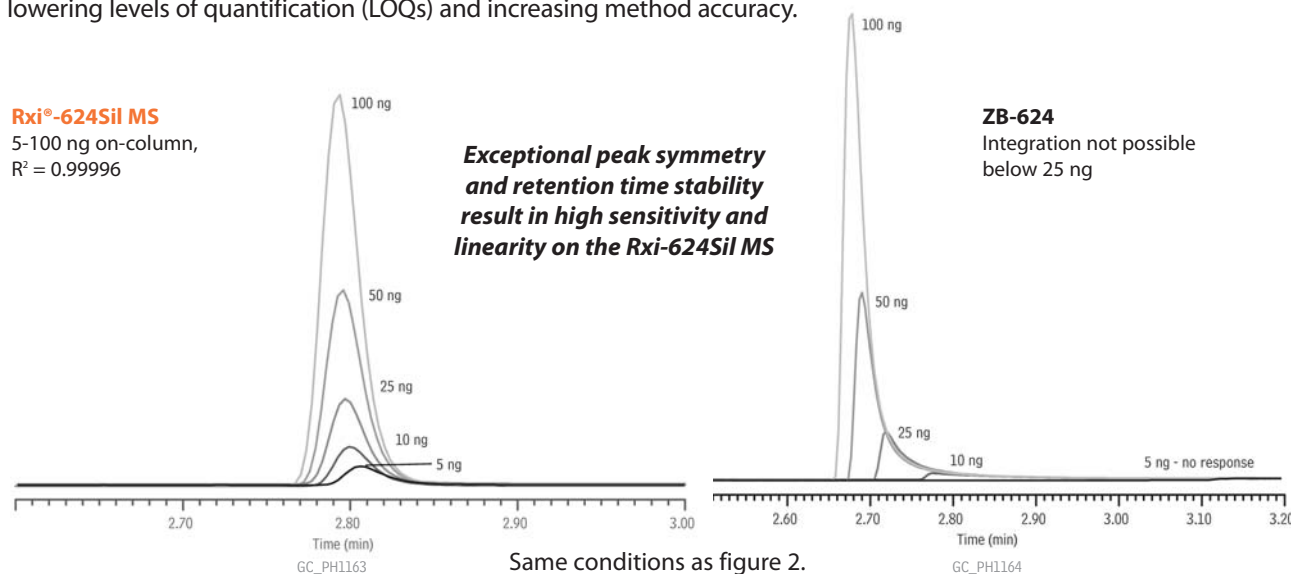
## Balanced Inertness Gives Higher Data Quality— Excellent Peak Symmetry and Reproducibility for Active Compounds

In addition to offering better retention of polar analytes, Rxi®-624Sil MS columns are exceptionally inert, reducing the need to switch columns when developing methods for active compounds, such as amines. Amines are commonly found on pharmaceutical impurities and can interact with surface silanols resulting in a tailing peak. Proper deactivation is the best way to combat this, and Rxi® technology provides the most balanced deactivation, assuring good peak symmetry for both basic and acidic compounds. Columns that are not effectively deactivated for basic compounds produce unacceptable peak tailing (Figure 2). In contrast, the Rxi®-624Sil MS column provides excellent peak shape, which leads to consistent peak integration, improved linearity, and higher method sensitivity (Figure 3).

**Figure 2** Innovative Rxi® deactivation technology provides excellent peak symmetry at 5 ng on-column for primary, secondary, and tertiary amines, which is not possible on competitive columns.



**Figure 3** Primary amines, such as isopropylamine, can be more accurately integrated on an Rxi®-624Sil MS column, lowering levels of quantification (LOQs) and increasing method accuracy.



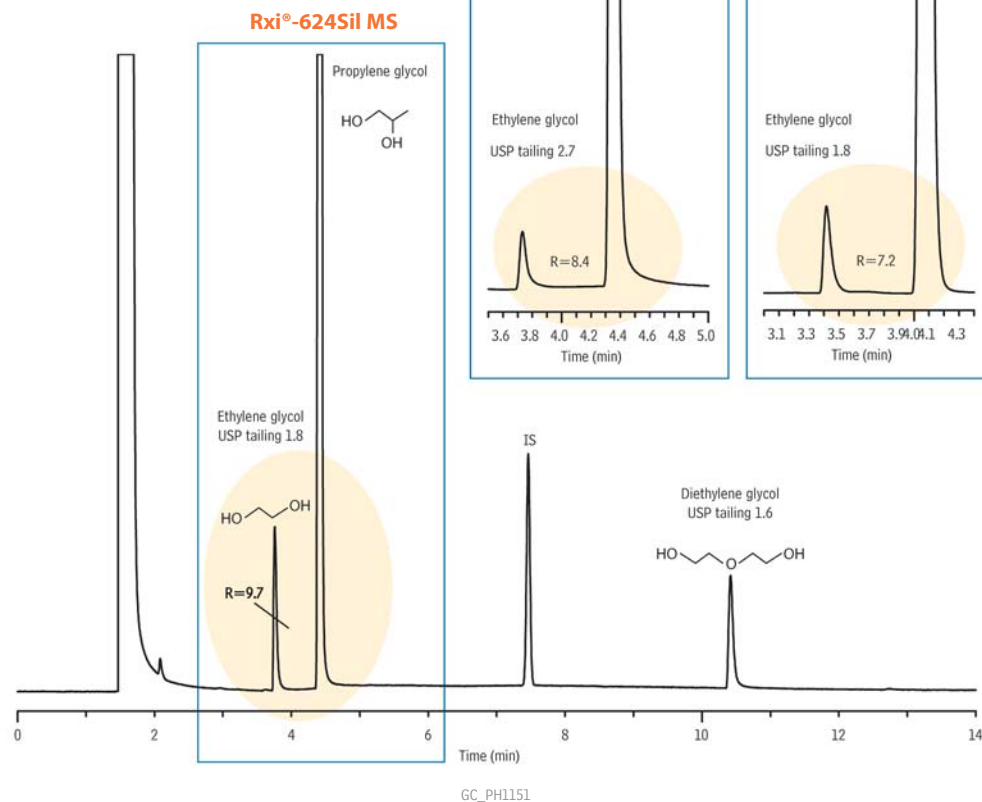
# Go To...the Right Column First

Other active compounds, such as glycols, also exhibit peak tailing as a result of reactivity with the chromatographic system. For example, when analyzing ethylene glycol and diethylene glycol in glycerin according to a new FDA Guidance for Industry, only the Rxi®-624Sil MS column gives the selectivity and peak symmetry needed for these reactive compounds (Figure 4). Satisfy this and other industry guidances quickly, by choosing the right column, the first time.

**Figure 4** Rxi®-624Sil MS columns provide the best overall inertness and selectivity for ethylene glycol and diethylene glycol impurities in glycerin, glycol, or sorbitol solutions.

## Best in Class—Rxi®-624Sil MS

	Rxi®-624Sil MS	ZB-624	DB-624
USP tailing (ethylene glycol)	1.8	2.7	1.8
Resolution (ethylene glycol/propylene glycol)	9.7	8.4	7.2



**Column** 30 m, 0.32 mm ID, 1.80  $\mu$ m (cat.# 13870)  
**Sample** methanol  
**Diluent:**  
**Injection** 1  $\mu$ L split (split ratio 10:1)  
**Inj. Vol.:** 5mm Single Gooseneck with Wool (cat.# 22973-200.1)  
**Liner:** 220 °C  
**Inj. Temp.:**  
**Oven**  
**Oven Temp:** 100 °C (hold 4 min.) to 120 °C at 50 °C/min. (hold 10 min.) to 220 °C at 50 °C/min. (hold 6 min.)  
**Carrier Gas** He, constant flow  
**Linear Velocity:** 40 cm/sec.  
**Detector** FID @ 250 °C  
**Instrument** Agilent/HP6890 GC  
**Notes** Columns tested: Rxi®-624Sil MS, ZB-624, and DB-624

Peaks	RT (min.)	Conc. (mg/mL)
Ethylene glycol	3.757	0.05
Propylene glycol	4.422	2.0
2,2,2-Trichloroethanol (IS)	7.461	0.1
Diethylene glycol	10.416	0.05

## Innovation & Service

"Having a background in LC/MS/MS does not automatically qualify one to run GC/MS. Julie Kowalski spent time with me to help me decide which column would be the best for my application as well as which consumables I would need to do routine maintenance. The time and knowledge she shared with me saved me multiple headaches and will keep me a loyal Restek customer!"

**Richard, Biologist**  
 National Institute of Health

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# Go To...Mass Spec Directly

## High Thermal Stability and Low Bleed for GC/MS Compatibility

While midpolarity 624 type columns offer better retention of polar analytes than 1s and 5s, most 624s have low thermal stability and generate too much column bleed to be useful for mass spec work. However, the Rxi®-624Sil MS column is fully compatible with mass spectrometry, due to stabilizing technology that delivers the highest thermal stability and lowest bleed of any polar capillary column in its class (Table I, Figure 5). Eliminate the need to change columns when mass spec is required—unlike other 624 columns, Rxi®-624Sil MS columns take your method directly to GC/MS. Keep the same 624 retention and selectivity, but leave the bleed behind.

**Table I** The Rxi®-624Sil MS column has the highest thermal stability of any 624 column.

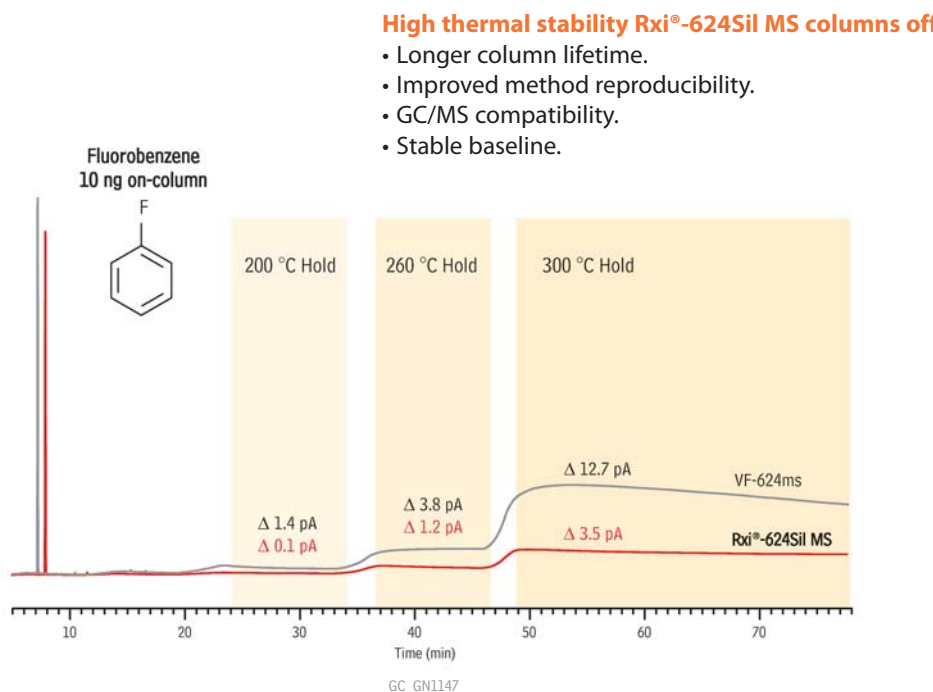
Column	Manufacturer	Highest Temperature Limit (Isothermal)
Rxi®-624Sil MS	Restek	320 °C
VF-624ms	Varian	300 °C
DB-624	Agilent J&W	260 °C
ZB-624	Phenomenex	260 °C



**Ideal for use  
with FID and MS  
detectors.**

Data obtained from company website or literature for a 30 m x 0.25 mm x 1.4 µm df column.

**Figure 5** The Rxi®-624Sil MS column has the lowest bleed of any column in its class, providing true GC/MS capability.



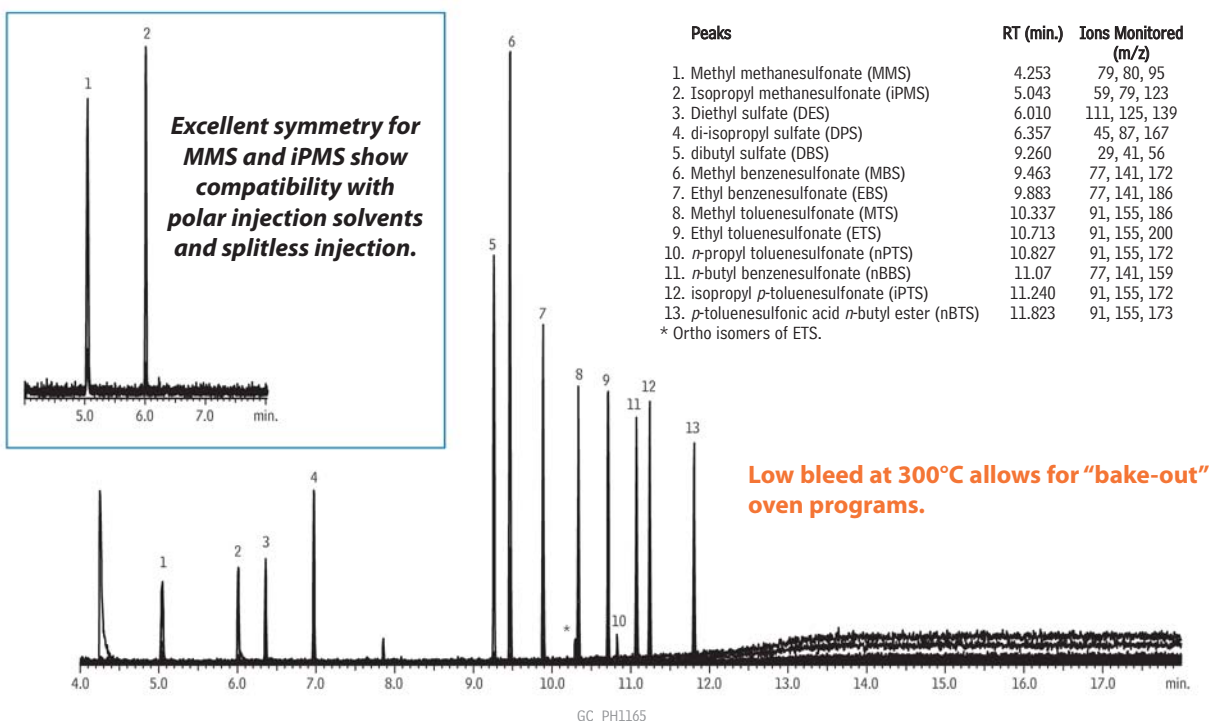
**Column** 30 m, 0.25 mm ID, 1.4 µm (cat.# 13868)  
**Sample** Fluorobenzene (cat.# 30030)  
**Diluent:** methanol  
**Conc.:** 200 µg/mL  
**Injection**  
**Inj. Vol.:** 1 µL split (split ratio 20:1)  
**Liner:** 4mm Split Liner with Wool (cat.# 20781)  
**Inj. Temp.:** 220 °C  
**Oven**  
**Oven Temp:** 40 °C (hold 5 min.) to 60 °C at 20 °C/min. (hold 5 min.) to 120 °C at 20 °C/min. (hold 5 min.) to 200 °C at 20 °C/min. (hold 10 min.) to 260 °C at 20 °C/min. (hold 10 min.) to 300 °C at 20 °C/min. (hold 20 min.)  
**Carrier Gas** He, constant flow  
**Linear Velocity:** 40 cm/sec.  
**Detector** FID @ 250 °C  
**Instrument** Agilent/HP6890 GC  
**Notes** Columns are of equivalent dimensions and were tested after equivalent conditioning.

# Go To...Mass Spec Directly

## Selective, Retentive, and Compatible with Polar Injection Solvents

The Rxi®-624Sil MS column combines the thermal stability of a mass spec friendly column with the selectivity, retention, and injection solvent compatibility needed to analyze polar impurities. For example, when analyzing mesylate, besylate, and tosylate genotoxic impurities by GC/MS, the Rxi®-624Sil MS column provides excellent selectivity, stability, and sensitivity (Figure 6). The innovative stationary phase is compatible with a variety of injection solvents, allowing splitless injection techniques to be used for heightened sensitivity. Bleed-free thermal stability allows oven “bake-out” programs to be used for contaminant removal and longer column life-times.

**Figure 6** The Rxi®-624Sil MS phase is more compatible with polar injection solvents than 1 or 5 type columns, providing higher sensitivity and less time needed for optimizing injection parameters.



**Column** Rxi®-624Sil MS, 20 m, 0.18 mm ID, 1.00  $\mu$ m (cat.# 13865)  
**Sample**  
 Diluent: 90:10 acetonitrile:water  
 Conc.: 500 ng/mL  
**Injection**  
 Inj. Vol.: 1  $\mu$ L splitless (hold 0.5 min.)  
 Inj. Liner: 3.5mm Single Gooseneck Liner with wool placed 3cm from top (middle) (cat.# 22286)  
 Inj. Temp.: 220 °C  
 Purge Flow: 3 mL/min.  
**Oven**  
 Oven Temp: 80 °C (hold 2 min.) to 300 °C at 20 °C/min. (hold 5 min.)  
**Carrier Gas**  
 Carrier Gas: He, constant flow  
 Linear Velocity: 45 cm/sec.

**Detector** MS  
 Mode: SIM  
 Transfer Line Temp.: 280 °C  
 Analyzer Type: Quadrupole  
 Source Temp.: 280 °C  
 Solvent Delay Time: 4 min.  
 Ionization Mode: EI  
**Instrument** Shimadzu 2010 GC & QP2010+ MS  
**Acknowledgement** In collaboration with Merck and Company

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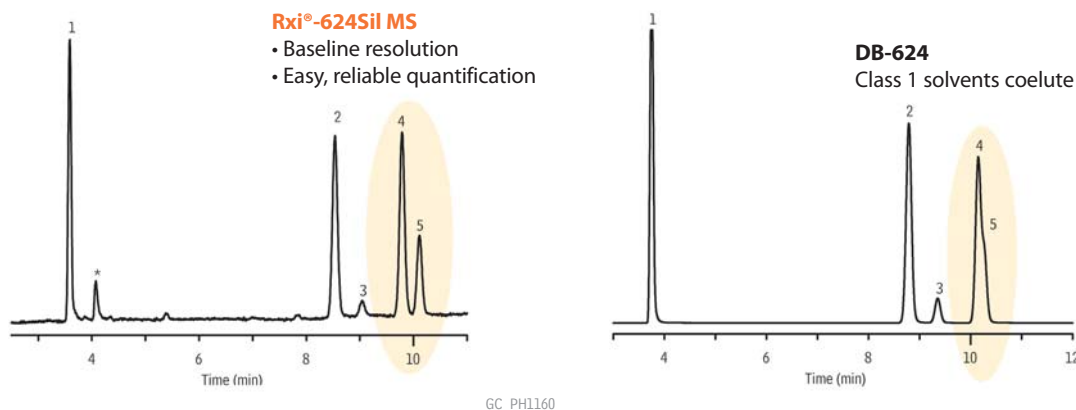
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# Go To...the Next Batch Faster

## Improve Pass Rates with the Best-In-Class G43 for USP <467>

System suitability is a major factor in overall lab productivity, and Rxi®-624Sil MS columns provide the optimized selectivity and guaranteed reproducibility needed to increase pass rates. For example, batch throughput can be improved for USP <467> residual solvents analysis by using a column that provides increased resolution and sensitivity for system suitability components (Figures 7 and 8). Benefits include industry-leading resolution of acetonitrile and dichloromethane, as well as benzene and 1,2-dichloroethane. No other 624 type column performs as well as Rxi®-624Sil MS columns for these critical system suitability requirements.

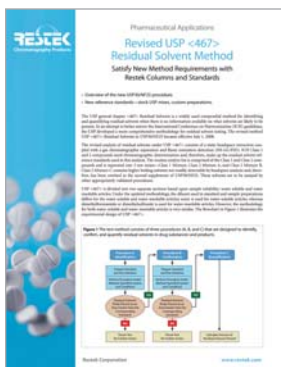
**Figure 7** Improve system suitability pass rates—the innovative polymer chemistry of the Rxi®-624Sil MS column provides greater resolution of critical pairs.



Peaks	RT (min.)	Conc. (µg/mL)	Column	Pressure
1. 1,1-Dichloroethene	3.586	0.07	Rxi®-624Sil MS, 30 m, 0.32 mm ID, 1.80 µm (cat.# 13870)	Equilibration Time: 0.05 min.
2. 1,1,1-Trichloroethane	8.536	0.08	Residual Solvents - Class 1 (cat.# 36279)	Loop Pressure: 5 psi
3. Carbon tetrachloride	9.042	0.03	Diluent: water	Loop Fill Time: 0.1 min.
4. Benzene	9.787	0.02	Injection: headspace-loop split (split ratio 5:1)	Oven
5. 1,2-Dichloroethane	10.112	0.04	Liner: 1mm Split (cat.# 20972)	Oven Temp: 40 °C (hold 20 min.) to 240 °C at 10 °C/min. (hold 20 min.)
* DMSO interference			Headspace-Loop	Carrier Gas
			Inj. Port Temp.: 140 °C	Linear Velocity: 35 cm/sec.
			Instrument: Tekmar HT3	Dead Time: 1.45 min. @ 40 °C
			Inj. Time: 1 min.	Detector
			Transfer Line Temp.: 110 °C	Data Rate: 5 Hz
			Valve Oven Temp.: 110 °C	Instrument
			Sample Temp.: 80 °C	Acknowledgement
			Sample Equil. Time: 60 min.	Agilent/HP6890 GC
			Vial Pressure: 10 psi	Teledyne Tekmar
			Pressurize Time: 0.5 min.	

Columns were tested under identical operating conditions and after identical conditioning.

For the complete application note and technical tips on USP<467>, visit [www.restek.com/usp467](http://www.restek.com/usp467).



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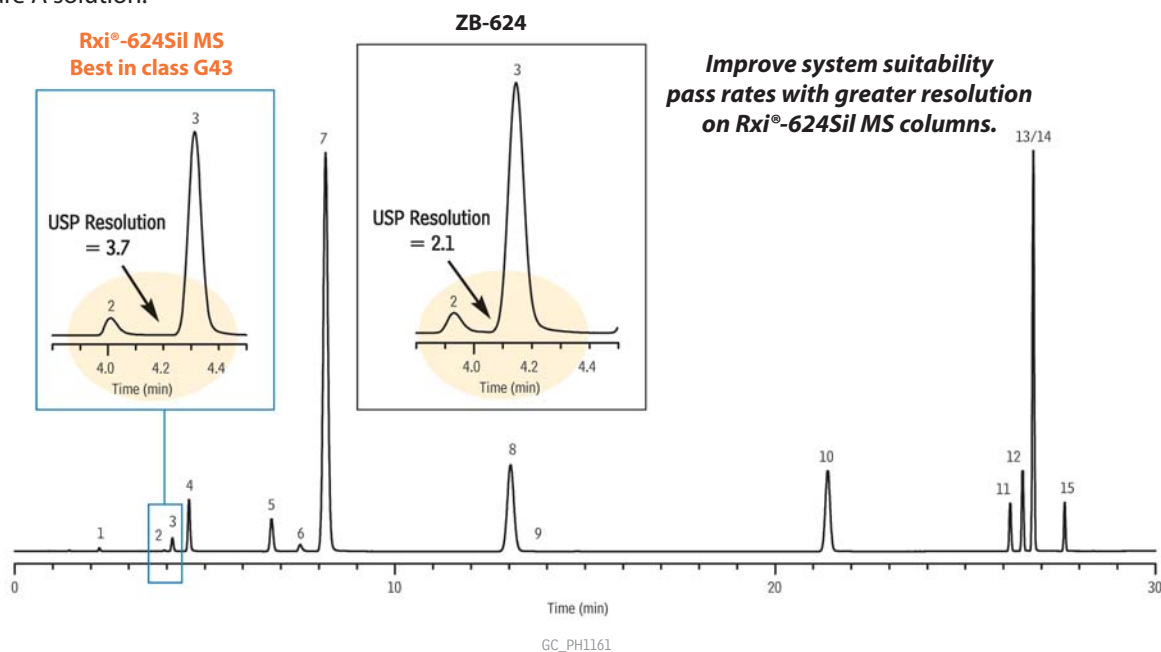
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# Go To...the Next Batch Faster

**Figure 8** The Rxi®-624Sil MS column provides best-in-class system suitability performance for the USP <467> Class 2 Mixture A solution.



Peaks	RT (min.)	Conc. (µg/mL)			
1. Methanol	2.281	25.00	8. Methylcyclohexane	14.099	9.83
2. Acetonitrile	4.009	3.42	9. 1,4-Dioxane	15.054	3.17
3. Dichloromethane	4.313	5.00	10. Toluene	22.018	7.42
4. <i>trans</i> -1,2-Dichloroethene	4.798	7.83	11. Chlorobenzene	26.570	3.00
5. <i>cis</i> -1,2-Dichloroethene	7.028	7.83	12. Ethylbenzene	26.837	3.07
6. Tetrahydrofuran	7.706	5.75	13. <i>m</i> -Xylene	27.147	10.85
7. Cyclohexane	8.708	32.33	14. <i>p</i> -Xylene	27.147	2.53
			15. <i>o</i> -Xylene	27.927	1.63

Columns were tested under identical operating conditions and after identical conditioning. See Figure 7 for conditions.

## Screw-Thread Headspace Vials (18mm)

Description	Volume	Color	Dimensions	100-pk.	1000-pk.
Headspace Vial	10mL	Clear	22 x 45mm	23084	23085
Headspace Vial	10mL	Amber	22 x 45mm	23088	23089
Headspace Vial	20mL	Clear	22 x 75mm	23082	23085
Headspace Vial	20mL	Amber	22 x 75mm	23086	23089

Caps not included.



## Magnetic Screw-Thread Caps (18mm)

Description	Septa Material	100-pk.	1000-pk.
Magnetic Caps and Septa	PTFE/Silicone	23090	23091
Magnetic Caps and Septa	PTFE/Silicone for SPME	23092	23093
Magnetic Caps and Septa	PTFE/Red Chlorobutyl	23094	23095



## Hot Swap Capillary Column Nuts

Quickly change columns for USP <467> Procedures A and B using a Hot Swap Capillary Column Nut

Description	qty.	cat.#
For use with "compact" Agilent-style ferrules.		
Hot Swap Capillary Column Nut	ea.	22348
For use with standard 1/8"-type ferrules.		
Hot Swap Capillary Column Nut	ea.	22347



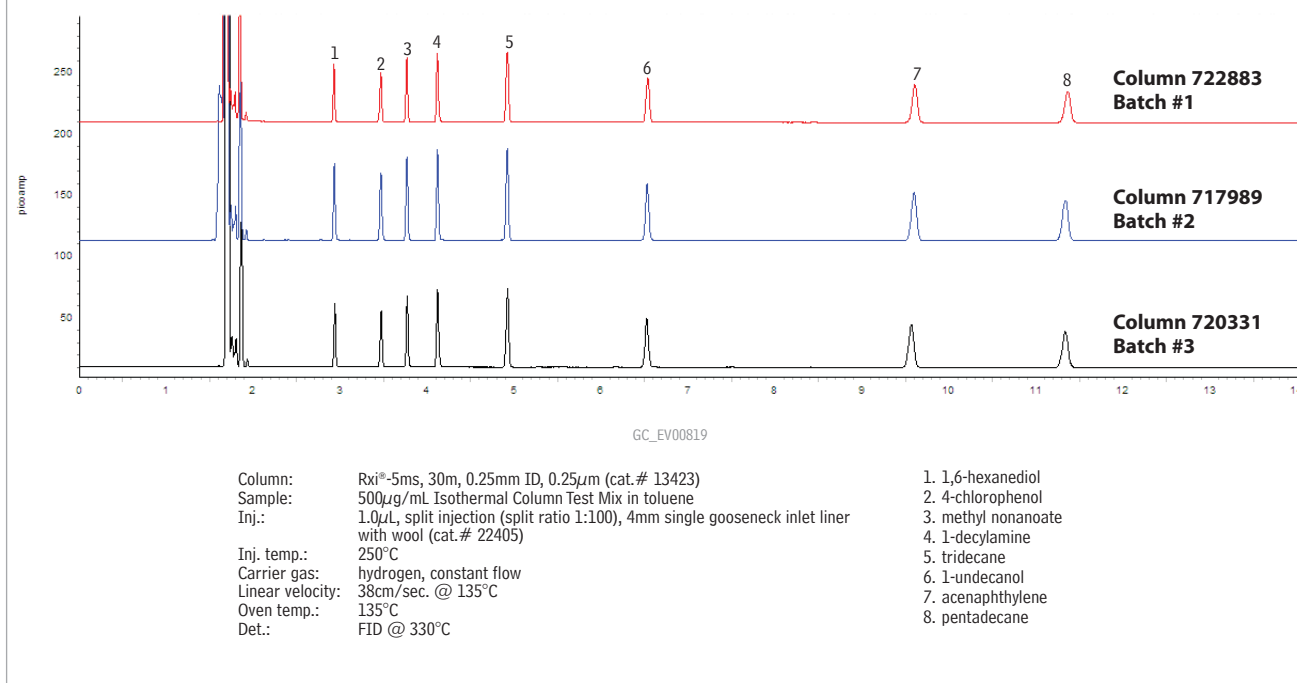
NOTE: For proper operation, oven fan must be kept operational during change out or risk of burn may occur.

# Go To...the Next Batch Faster

## Guaranteed Reliable Column-to-Column Performance

Reliable column-to-column performance also contributes to lab productivity as less column variation means faster setup and more consistent results. Rxi® column technology has enabled Restek to tighten our quality control standards and guarantee consistency. Columns from multiple manufacturing batches show the excellent reproducibility obtained using the new Rxi® manufacturing process (Figure 9). All Rxi® columns are individually tested to assure performance.

**Figure 9** Three manufacturing batches of Rxi® columns show excellent reproducibility.



## Go To...Rxi®-624Sil MS Columns for Faster Method Development

Optimized phase chemistry, column deactivation, and manufacturing make Rxi®-624Sil MS columns the “go to” column for pharmaceutical method development. With better retention of polar compounds than 1 and 5 type columns, lower bleed than any other 624 column, and unsurpassed inertness, Rxi®-624Sil MS columns offer the most comprehensive performance, allowing you to develop successful methods quickly, easily, and reliably. Try one for your next method today.

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"When my research group needed a GC column for a chiral separation, Restek was the only company that offered to provide us with test columns to evaluate. The willingness of Restek to work with us to find a solution to our separation problem is exceptional."

**Joe Dinnocenzo,**  
**Professor of Chemistry**  
 Director, Center for Photoinduced Charge Transfer  
 University of Rochester

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(mid polarity Crossbond® silarylene phase; equivalent to 6% cyanopropylphenyl/94% dimethyl polysiloxane)

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- Inert—excellent peak shape for a wide range of compounds, including acidic and basic compounds.
- Selective—highly selective for residual solvents, great choice for USP<467>.
- Manufactured for column-to-column reproducibility—well-suited for validated methods.

ID	df (μm)	temp. limits	20-Meter	30-Meter	60-Meter
0.18mm	1.00	-20 to 300/320°C	13865		
0.25mm	1.40	-20 to 300/320°C		13868	
0.32mm	1.80	-20 to 300/320°C		13870	13872
0.53mm	3.00	-20 to 280/300°C		13871	



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Description	qty.	cat.#
Leak Detector with Hard-Sided Carrying Case and Universal Charger Set (US, UK, European, Australian)	ea.	22839
Soft-Side Storage Case	ea.	22657
Small Probe Adaptor	ea.	22658

Avoid using liquid leak detectors on a GC! Liquids can be drawn into the system.



### GC/MS Cleaning Kit

Description	qty.	cat.#
Mass Spec Cleaning Kit with Dremel Tool	kit	27194
Mass Spec Cleaning Kit without Dremel Tool	kit	27195
Mass Spec Cleaning Kit Replacement Parts Kit (includes cloths, micro mesh sheets, small and large gloves)	kit	27196



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For Agilent 5971, 5972, GC GC/MS	ea.	23073
For Agilent 5973 & 5975 GC/MS (includes mount for initial installation)*†	ea.	23074
For Agilent 5973 & 5975 GC/MS and LC/MSD (Replacement Multiplier)*†	ea.	23075
For Agilent LC/MSD (includes mount for initial installation)*†	ea.	23076
<b>Electron Multiplier for Applied Biosystems (Sciex)</b>		
For API 300, 3000 & 4000 Applied Biosystems	ea.	23077
<b>Electron Multiplier for Thermo Finnigan GC/MS</b>		
For Thermo TRACE DSQ, DSQII, and Polaris-Q GC/MS	ea.	23081

\*First time installation requires a mount which includes the mechanical housing. After initial installation, only the replacement electron multiplier is required.

†This unit is designed for use in the 5975, 5973 GC and the LC/MSD.





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## Revised USP 467 Residual Solvent Method

### Satisfy New Method Requirements with Restek Columns and Standards

By Rick Lake, Pharmaceutical Innovations Chemist

- Overview of the new USP 30/NF 25 procedure, 2nd supplement & interim revision, effective July 2008.
- New reference standards - stock USP mixes, custom preparations.
- Improve system suitability pass rates by choosing the correct liner size.

#### Background

Organic Volatile Impurities (OVIs), which are commonly referred to as residual solvents, are trace level chemical residues in drug substances and drug products that are byproducts of manufacturing, or that form during packaging and storage. It is the responsibility of the drug manufacturer to ensure that these residues are removed, or are present only in limited concentrations. The International Conference on Harmonization (ICH) publishes a guideline (Q3C) listing the acceptable amounts of solvent residues that can be present. In the ICH guideline, residual solvents are summarized by class, according to their toxicity. Class 1 compounds are carcinogenic compounds that pose a risk to both the consumer and the environment. The use of these solvents is to be avoided, but if they are used, they must be tightly controlled. Class 2 compounds are nongenotoxic animal carcinogens, and concentrations of these compounds should be limited. Chromatographic analysis is needed for both the Class 1 and Class 2 residual solvents. Class 3 compounds, on the other hand, have low toxic potential and may be assayed by nonspecific techniques.

The USP general chapter <467> Residual Solvents is a widely used compendial method used for identifying and quantifying residual solvents when there is no information available on what solvents are likely to be present. In an attempt to better mirror the ICH guidelines, the USP is proposing a more comprehensive methodology in residual solvent testing—the current USP30 NF25. This revised method is more comprehensive, as it increases the number of residual solvents to be routinely tested to 32. This is a much longer analyte list than previously tested and represents the sum of Class 1 and 2 residual solvents. The actual number of analytes may be more if xylenes, ethyl benzene and cis/trans 1,2 dichloroethylene are differentiated or if circumstances require the quantification of specific Class 3 residual solvents. The test method itself is also more extensive and divided into three distinct procedures (A, B and C) for identification, confirmation, and quantification.

Initially set to become effective July 1, 2007, the implementation of the current version of USP <467>: Residual Solvents has been delayed until July 1, 2008. Until that time, the Other Analytical Procedures section of the previous version will be retained. However, looking forward to the implementation of the revised method, this application will comply with the procedure and criteria set forth in the USP30 NF25, second supplement (effective December 1, 2007) and the interim revision announcement.

#### Overview of Method

The revised analysis of residual solvents under USP <467> consists of a static headspace extraction coupled with a gas chromatographic separation and flame ionization detection (HS-GC/FID). ICH Class 1 and 2 compounds need chromatographic determination and therefore make up the residual solvent reference standards used in this analysis. The routine analyte list is comprised of the Class 1 and Class 2 compounds and is separated into 3 test mixes — Class 1 Mixture, Class 2 Mixture A and Class 2 Mixture B. Class 2 Mixture C contains higher boiling solvents not readily detectable by headspace analysis and therefore has been omitted in the second supplement of USP30 NF25 or the interim revision announcement. These solvents are to be assayed by other appropriately validated procedures.

USP <467> is divided into two separate sections based upon sample solubility: water soluble and water insoluble articles. Under the updated methodology, the diluent used in standard and sample preparations differs for the water-soluble and water-insoluble articles; water is used for water-soluble articles, whereas dimethylformamide or dimethylsulfoxide is used for water-insoluble articles. However, the methodology for both water-soluble and water-insoluble articles is very similar. The test method for both sections consists of three procedures (A, B and C) that are designed to identify, confirm and then finally quantify residual solvents found to present in drug substances and products.

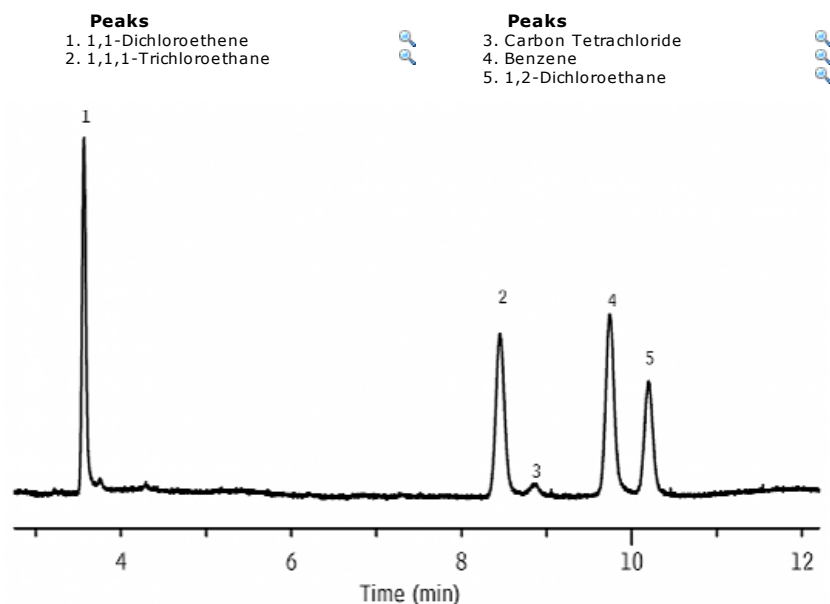
Autosamplers are widely used in headspace analysis and are also included in the USP <467> monograph.

We have purposely performed this application using two different types of headspace autosamplers—a pressurized loop system and a heated syringe. Procedure A was performed using a Tekmar HT3 pressured loop autosampler to demonstrate implementing analyses using a transfer line. Procedure B was performed using an Overbrook Scientific HT200H to demonstrate implementing analyses using a syringe injection.

#### Procedure A - Identification

Procedure A is the first step in the identification process and is performed on a G43 (Rtx®-1301 or 624) column, as a means of determining if any residual solvents are present in the sample at detectable levels. First, Class 1 standard and system suitability solutions and a Class 2 Mix A standard solutions are assayed under the given operating conditions to determine suitability of the chromatographic system. All peaks in the Class 1 system suitability solution must have a signal-to-noise ratio not less than 3, the Class 1 standard solution must have a 1,1,1-trichloroethane response greater than 5, and in the Class 2 Mixture A solution the resolution of acetonitrile and dichloromethane must not be less than 1. When system suitability has been achieved, the test solutions are then assayed along with the Class 1 and Class 2 Mixtures A and B standard solutions. In the event that a peak is determined in the sample that matches a retention time and has a response greater than that of a corresponding reference material, the analyst then proceeds to Procedure B for verification of the analyte. An exemption is made for 1,1,1-trichloroethane, where a response greater than 150 times the peak response denotes an amount above the percent daily exposure limit. Figures 1 through 3 illustrate the analysis of residual solvent mixes Class 1 and Class 2 A and B by Procedure A. The resolution between acetonitrile and dichloromethane is easily achieved using an Rtx®-1301 (or 624) column and a 1mm split liner, coupled to a transfer line injection.

**Figure 1** USP Residual Solvent Class 1 standard solution on an Rtx®-624 (G43) column.

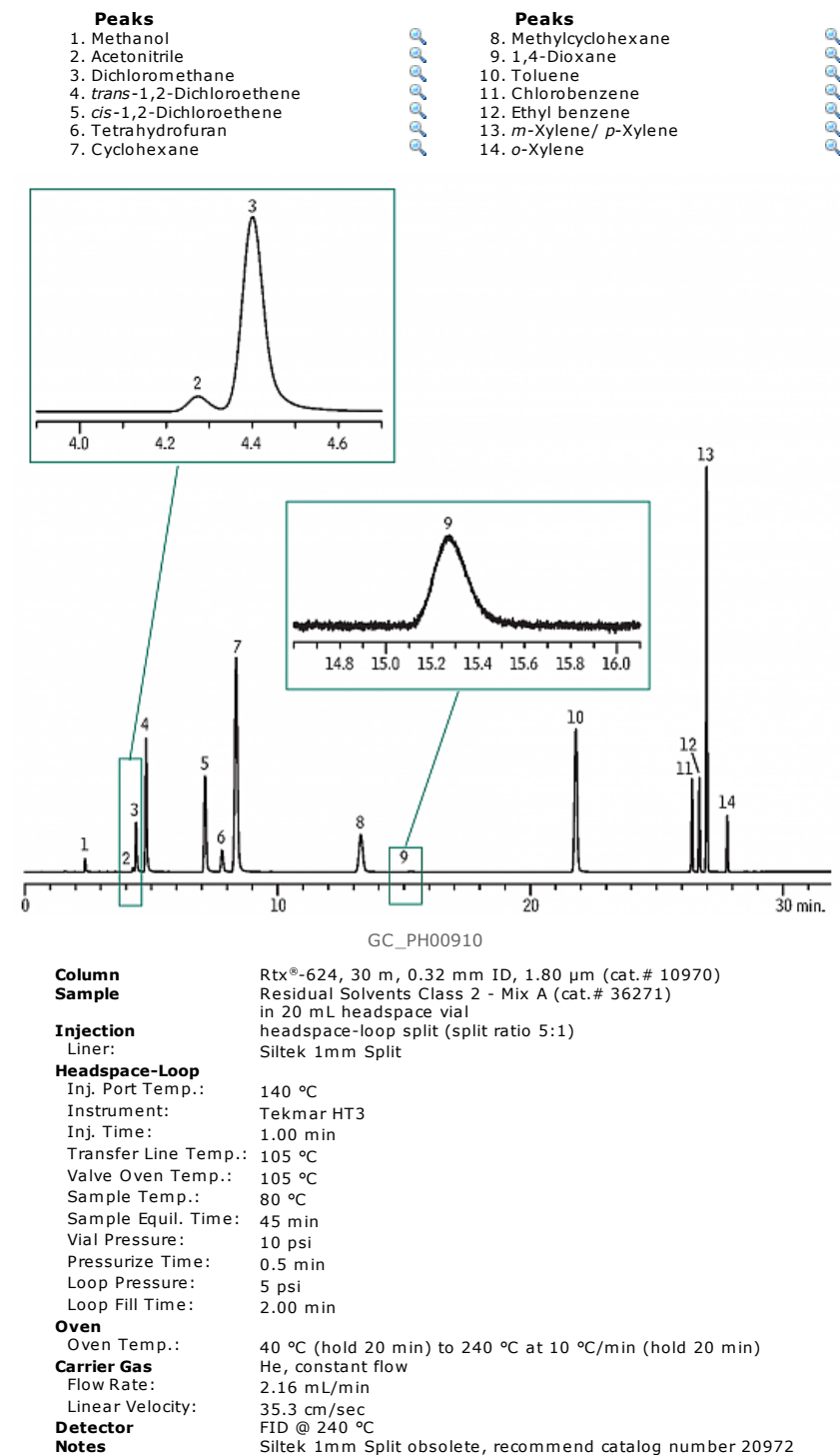


GC\_PH00909

<b>Column</b>	Rtx®-624, 30 m, 0.32 mm ID, 1.80 µm (cat.# 10970)
<b>Sample</b>	Residual Solvents - Class 1 (cat.# 36279)
<b>Injection</b>	headspace-loop split (split ratio 5:1)
<b>Liner:</b>	Siltek® 1mm Split liner
<b>Headspace-Loop</b>	
Inj. Port Temp.:	140 °C
Instrument:	Tekmar HT3
Inj. Time:	1.00 min
Transfer Line Temp.:	105 °C
Valve Oven Temp.:	105 °C
Sample Temp.:	80 °C
Sample Equil. Time:	45 min
Vial Pressure:	10 psi
Pressurize Time:	0.5 min
Loop Pressure:	5 psi
Loop Fill Time:	2.00 min
<b>Oven</b>	
Oven Temp.:	40 °C (hold 20 min) to 240 °C at 10 °C/min (hold 20 min)
<b>Carrier Gas</b>	He, constant flow
Flow Rate:	2.16 mL/min
Linear Velocity:	35.3 cm/sec
<b>Detector</b>	FID @ 240 °C

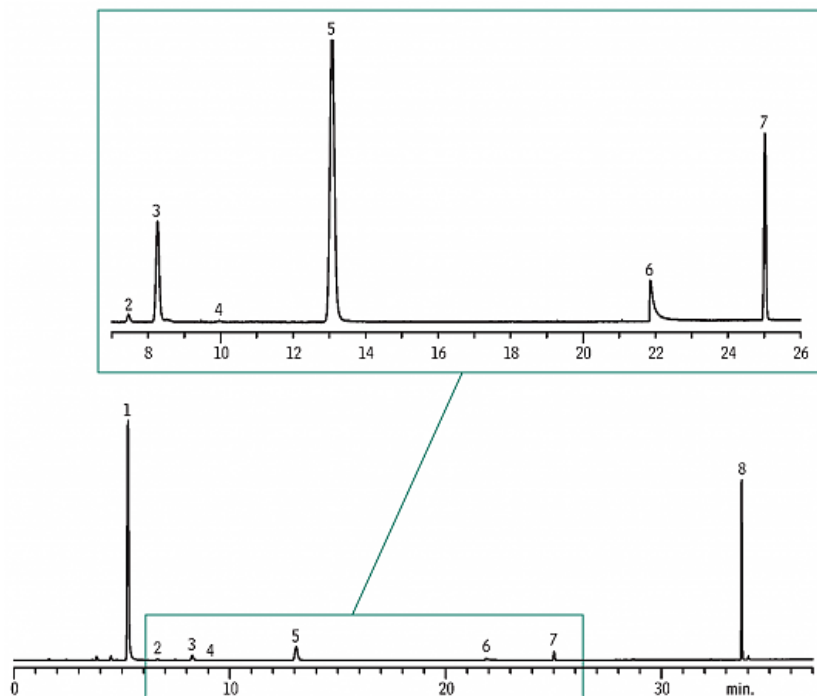
(cat.# 36279) in 20mL headspace vial

Siltek® 1mm Split liner obsolete, recommend catalog number 20972

**Figure 2** USP Residual Solvent Class 2 Mixture A standard solution on an Rtx®-624 (G43) column.**Figure 3** USP Residual Solvent Class 2 Mixture B standard solution on an Rtx®-624 (G43) column.

**Peaks**

1. Hexane
2. Nitromethane
3. Chloroform
4. 1,2-Dimethoxyethane
5. Trichloroethylene
6. Pyridine
7. 2-Hexanone
8. Tetralin



GC\_PH00911

**Column** Rtx®-624, 30 m, 0.32 mm ID, 1.80 µm (cat.# 10970)

**Sample** Residual Solvents Class 2 - Mix B (cat.# 36280)  
in 20 mL headspace vial  
headspace-loop split (split ratio 5:1)

**Injection**  
Liner: Siltek 1mm Split

**Headspace-Loop**  
Inj. Port Temp.: 140 °C  
Instrument: Tekmar HT3  
Inj. Time: 1.00 min  
Transfer Line Temp.: 105 °C  
Valve Oven Temp.: 105 °C  
Sample Temp.: 80 °C  
Sample Equil. Time: 45 min  
Vial Pressure: 10 psi  
Pressurize Time: 0.5 min  
Loop Pressure: 5 psi  
Loop Fill Time: 2.00 min

**Oven**  
Oven Temp.: 40 °C (hold 20 min) to 240 °C at 10 °C/min (hold 20 min)

**Carrier Gas**  
Flow Rate: 2.16 mL/min  
Linear Velocity: 35.3 cm/sec

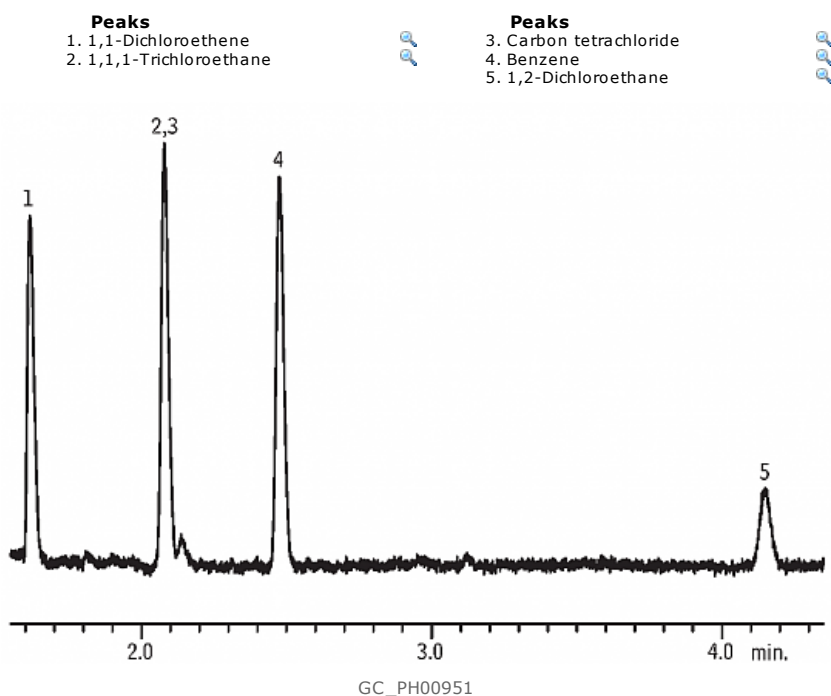
**Detector** FID @ 240 °C

**Notes**  
Sample: USP <467> Class 2 Mixture B standard solution  
(cat.# 36280) in 20mL headspace vial  
  
Siltek 1mm Split obsolete, recommend catalog number 20972

**Procedure B - Confirmation**

Once a residual solvent is identified and found to be above the percent daily exposure limit, Procedure B is then performed to confirm analyte identity. A G16 capillary column (Stabilwax®) is used here as a confirmation column because it yields an alternate selectivity when compared to that of a G43 column. The same residual solvent mixes used in Procedure A, both standard and system suitability preparations, are again analyzed on this column. The system suitability requirements differ here in that the Class 1 standard solution must have a benzene response greater than 5 and the resolution of acetonitrile and cis-dichloroethene must not be less than 1 in the Class 2 Mixture A solution. If the analyte identified in Procedure A again matches the retention time and exceeds the peak response of the reference materials, again with the same exception to 1,1,1-trichloroethane, the analyst must now quantify the analyte using Procedure C. Figures 4 through 6 illustrate the analysis of residual solvent mixes Class 1, Class 2A and B on a Stabilwax® column. Again, the system suitability on a Stabilwax® column, used in conjunction with a 2 mm liner and syringe headspace injection, is easily achieved.

**Figure 4** USP Residual Solvent Class 1 standard solution on a Stabilwax® (G16) column.

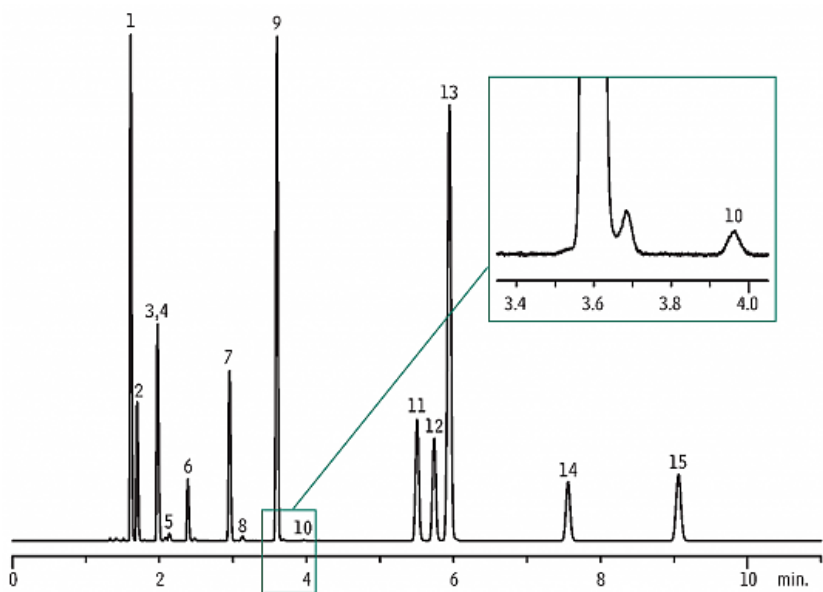


GC\_PH00951

<b>Column</b>	Stabilwax®, 30 m, 0.32 mm ID, 0.25 µm (cat.# 10624)
<b>Sample</b>	Residual solvents - class 1 (cat.# 36279) in 20 mL headspace vial (cat.# 24685)
<b>Diluent:</b>	Water
<b>Injection</b>	
Inj. Vol.:	1.0 µL headspace-syringe split (split ratio 5:1)
Liner:	2 mm splitless (cat.# 20712)
Inj. Temp.:	140 °C
<b>Headspace-Syringe</b>	
Instrument:	Overbrook Scientific HT200H
Syringe Temp.:	100 °C
Sample Temp.:	80 °C
Sample Equil. Time:	45 min
Inj. Speed:	8
Inj. Dwell:	5 sec.
<b>Oven</b>	
Oven Temp.:	50 °C (hold 20 min) to 165 °C at 6 °C/min (hold 20 min)
<b>Carrier Gas</b>	He, constant flow
Flow Rate:	2.15 mL/min
Linear Velocity:	35.2 cm/sec
<b>Detector</b>	FID @ 250 °C

**Figure 5** USP Residual Solvent Class 2 Mixture A standard solution on a Stabilwax® (G16) column.

<b>Peaks</b>		<b>Peaks</b>
1. Cyclohexane		8. Acetonitrile
2. Methylcyclohexane		9. Toluene
3. <i>trans</i> -1,2-Dichloroethene		10. 1,4-Dioxane
4. Tetrahydrofuran		11. Ethyl benzene
5. Methanol		12. <i>p</i> -Xylene
6. Dichloromethane		13. <i>m</i> -Xylene
7. <i>cis</i> -1,2-Dichloroethene		14. <i>o</i> -Xylene
		15. Chlorobenzene



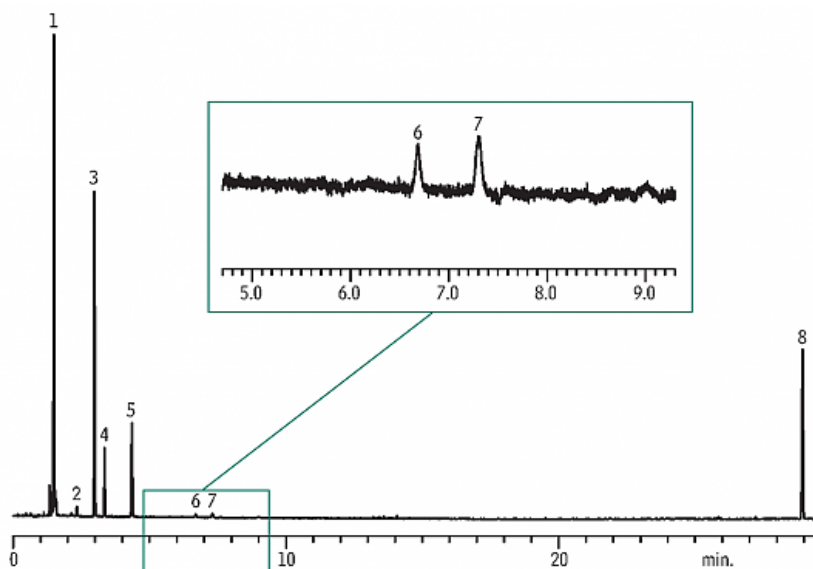
GC\_PH00952

**Column** Stabilwax®, 30 m, 0.32 mm ID, 0.25 µm (cat.# 10624)  
**Sample** Residual solvents class 2 - mix A (cat.# 36271)  
in 20 mL headspace vial (cat.# 24685)  
**Diluent:** Water  
**Injection**  
Inj. Vol.: 1,000 µL headspace-syringe split (split ratio 5:1)  
Liner: 2 mm splitless (cat.# 20712)  
Inj. Temp.: 140 °C  
**Headspace-Syringe**  
Instrument: Overbrook Scientific HT200H  
Syringe Temp.: 100 °C  
Sample Temp.: 80 °C  
Sample Equil. Time: 45 min  
Inj. Speed: 8  
Inj. Dwell: 5 sec.  
**Oven**  
Oven Temp.: 50 °C (hold 20 min) to 165 °C at 6 °C/min (hold 20 min)  
**Carrier Gas**  
Flow Rate: 2.15 mL/min  
Linear Velocity: 35.2 cm/sec  
**Detector** FID @ 250 °C

**Figure 6** USP Residual Solvent Class 2 Mixture B standard solution on a Stabilwax® (G16) column.

- Peaks**
- 1. Hexane
  - 2. 1,2-Dimethoxyethane
  - 3. Trichloroethylene
  - 4. Chloroform
  - 5. 2-Hexanone
  - 6. Nitromethane
  - 7. Pyridine
  - 8. Tetralin





GC\_PH00953

**Column** Stabilwax®, 30 m, 0.32 mm ID, 0.25 µm (cat.# 10624)  
**Sample** Residual solvents class 2 - mix B (cat.# 36272)  
 in 20 mL headspace vial (cat.# 24685)  
 Water

**Diluent:**

**Injection**  
 Inj. Vol.: 1,000 µL headspace-syringe split (split ratio 5:1)  
 Liner: 2 mm splitless (cat.# 20712)  
 Inj. Temp.: 140 °C

**Headspace-Syringe**  
 Instrument: Overbrook Scientific HT200H  
 Syringe Temp.: 100 °C  
 Sample Temp.: 80 °C  
 Sample Equil. Time: 45 min  
 Inj. Speed: 8  
 Inj. Dwell: 5 sec.

**Oven**  
 Oven Temp.: 50 °C (hold 20 min) to 165 °C at 6 °C/min (hold 20 min)

**Carrier Gas**  
 Flow Rate: He, constant flow  
 2.15 mL/min  
 Linear Velocity: 35.2 cm/sec

**Detector** FID @ 250 °C

### Procedure C - Quantification

Lastly, when a residual solvent is identified and verified in both Procedures A and B, the analyst then performs Procedure C to quantify the analyte. This is done by analyzing the sample against the specific, individual reference material for the analyte identified. Individual standards are prepared by diluting the analyte in solution to a concentration of 1/20 of the concentration limit given in the method. Following the procedure given and the instrument conditions in either Procedure A or B, depending upon which procedure provides the most definitive results, a quantifiable result is produced.

### Conclusion

Implementing the revised method for US <467> can be difficult if you do not optimize correctly. Although the second supplement contains a change that allows for modifications to the split ratio, liner and column choices are still important. Both the Rtx®-1301 (624) and the Stabilwax® columns can easily pass system suitability criteria, and the use of smaller bore liners (see Tech Tip) can increase peak efficiency. Restek can supply all your USP <467> materials and can help you optimize your testing within the constraints of the method.

### Thank You!

Instruments provided courtesy of [Overbrook Scientific™](#) and [Teledyne Tekmar](#).



### RELATED SEARCHES

[USP 30 NF 25](#), [ICH](#), [OVI](#), [Q3C](#), [International Conference on Harmonization](#), [solvents](#), [residual solvents](#), [467](#), [USP 467](#), [class 1](#), [class 2](#), [mix a](#), [mixture a](#), [class 2a](#), [mix b](#), [mixture b](#), [class 2b](#), [stabilwax](#), [g16](#), [wax](#), [624](#), [xylene](#)

# Raptor™

LC Columns

## Selectivity Accelerated

- Higher efficiency for drastically faster analysis times.
- Better selectivity for substantially improved resolution.
- Increased sample throughput with existing HPLC instrumentation.
- Long-lasting ruggedness for dependable reproducibility.



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# The Dawn of an Era

Superficially porous particles (commonly referred to as SPP or “core-shell” particles) have been proven to provide fast separations without the need for expensive Ultra High Performance Liquid Chromatography (UHPLC) instruments, thereby increasing sample throughput without capital investment. These particles feature a solid, impermeable core enveloped by a thin, porous layer of silica that decreases the diffusion path and reduces peak dispersion. As a result, they offer significantly higher efficiency than traditional fully porous particles of similar dimensions—often rivaling the efficiency of smaller particles. Core-shell particles changed LC, but they were only the beginning...

## A New Species Has Evolved

Restek is proud to announce that SPP core-shell technology has evolved with the introduction of Raptor™ LC columns. Although column efficiency, which is boosted with superficially porous particles, considerably accelerates analysis time, it has little effect on resolution (i.e., peak separation). Selectivity, on the other hand, has a substantial impact on resolution, but shows minimal improvement in analysis times. New Raptor™ LC columns bond rugged 2.7 and 5 µm superficially porous particles with Restek's unique Ultra Selective Liquid Chromatography™ (USLC®) phases to offer chromatographers the best of both worlds.

By being the first to combine the speed of SPP with the resolution of highly selective USLC® technology, Raptor™ LC columns provide the practicing analyst with the most powerful tools available for fast and efficient method development. And because they are from Restek, Raptor™ LC columns are backed by the manufacturing and quality systems you've come to trust along with the best Plus 1 service in the industry. Choose them for all of your valued assays to experience *Selectivity Accelerated*.

### The History of USLC® Technology

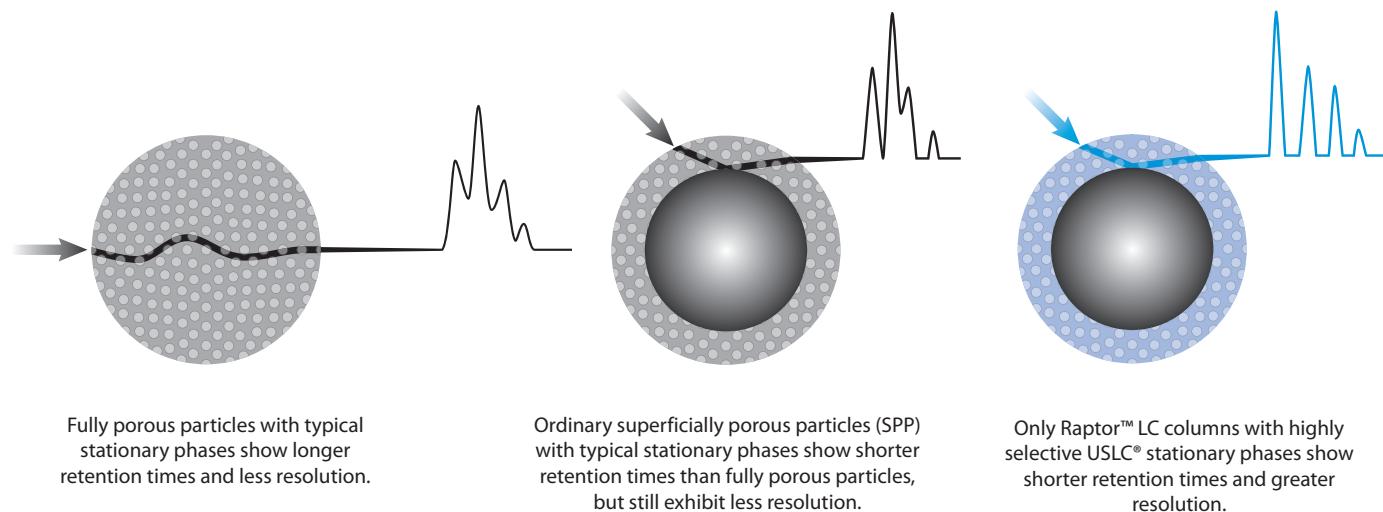
Restek extended the hydrophobic-subtraction model to describe orthogonal selectivity and then applied it to create our unique USLC® stationary phases.

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

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



**Figure 1:** Only Raptor™ LC columns offer the higher efficiency of a superficially porous particle *plus* the improved resolution of USLC® phases.



**Experience *Selectivity Accelerated*. Put Raptor™ LC columns and guards to the test today on your most challenging workflows.**

# Evolutionary Chromatography

It is only possible to fully utilize the efficiency of superficially porous particle technology when it is united with the power of USLC® selectivity. With Raptor™ LC columns, you can speed up method development and enhance sample throughput—without investing in costly UHPLC equipment—to create faster, more reliable, and more sensitive analyses.

- Run faster and avoid lengthy gradient adjustments.
- Separate isobaric and hard-to-resolve compounds with ease.
- Avoid eluting compounds near the void volume and limit ion suppression.
- Skip the complex mobile phases and multiple method modifications.

## Dissecting Raptor™ LC Columns

*A closer look at a new species*

### Adaptive Traits: Raptor™ LC Column

Restek's dedicated R&D group studied every aspect of superficially porous particles (commonly referred to as SPP or "core-shell" particles) to develop the bonding chemistries that are best suited to both the SPP construction and our highly selective USLC® phases. But we didn't stop there. In addition to implementing a new, proprietary column-packing technique, we upgraded our LC column hardware. By looking at not only the particles, but also the packing and hardware, we have made sure that you will get repeatable, rugged performance from each and every Raptor™ LC column.

#### Larger 2 µm Frit

Prevents clogging better than commonly used 0.5 µm frits; boosts column lifetime and helps maintain optimal pressures.

#### Rugged Label

Clearly identifies both flow direction and column; resists solvents and tearing to last as long as your column does.



#### Proprietary Column-Packing Technique

Provides greater pressure stability (600 bar for 2.7 µm; 400 bar for 5 µm); achieves higher linear velocities without sacrificing efficiency or lifetime.



#### Raptor™ SPP Particles

##### Robust 2.7 and 5 µm Particles

Let you run high-speed analyses without UHPLC.

##### Narrow Silica Distribution

Ensures high efficiency and consistent flows.

##### Updated Bonding and QC

Guarantee retention time stability, run to run and column to column.

## Natural Protection: Raptor™ EXP® Guard Column

Regardless of its performance, lifespan, or frit size, we know the LC column is the most expensive consumable used for your chromatographic assay. To help protect your investment and further extend the life of our already-rugged Raptor™ LC columns, we have mated our new superficially porous particles with patent-pending guard column hardware developed by Optimize Technologies. A Raptor™ LC guard column cartridge in an EXP® direct connect holder is the ultimate in column protection.

### Patented Titanium Hybrid Ferrules

Can be installed repeatedly without compromising high-pressure seal.

### Free-Turn® Architecture

Allows you to change cartridges without breaking inlet/outlet fluid connections—and without tools.

### Auto-Adjusting Connection

Provides ZDV (zero dead volume) connection to any 10-32 female port.



### Flexible Design

Replace nut with longer or even tool-free options (below) to best suit your needs.



### Unidirectional Raptor™ Cartridge

#### In-Tandem Development

Made to pair perfectly with Raptor™ LC columns.

#### Superior Packing Technique

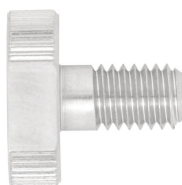
Withstands 600 bar (2.7 µm) / 400 bar (5 µm) operating pressures.

#### Restek® Quality

Backed by the manufacturing and QC systems you trust.

View our full selection of Raptor™ EXP® guard column cartridges at [www.restek.com/raptor](http://www.restek.com/raptor)

## Restek also recommends:



### Hand-Tight Nut (cat.# 25937–25939)

Upgrade the supplied nut to install your Raptor™ EXP® guard column by hand—no tools needed.



### Long Hex-Head Nut (cat.# 25934)

Extend the nut on your Raptor™ EXP® guard column for easier access in tight spaces—no more bumped knuckles.



### EXP® Hand-Tight Coupler (cat.# 25940)

Achieve tool-free 8,700+ psi (600+ bar) seals anywhere in your LC system with EXP® hand-tight couplers and connectors.

Visit [www.restek.com/exp](http://www.restek.com/exp) for more EXP® hex-head fittings, couplers, replacement parts, and more!

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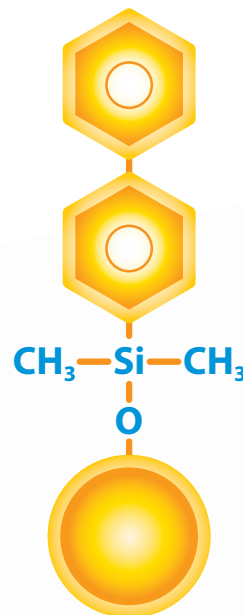
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Stationary Phase: **Biphenyl**

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LC Columns

*Selectivity Accelerated*

Fast, Rugged Raptor<sup>™</sup> Columns  
with Time-Tested Selectivity



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# The Raptor™ Biphenyl Column

With Raptor™ LC columns, Restek chemists became the first to combine the speed of superficially porous particles (also known as SPP or “core-shell” particles) with the resolution of highly selective USLC® technology. This new breed of chromatographic column allows you to more easily achieve peak separation and faster analysis times without expensive UHPLC instrumentation.

Our top priority when developing our new SPP line was to create a version of our innovative Biphenyl. The industry-leading Biphenyl is Restek's most popular LC stationary phase because it is particularly adept at separating compounds that are hard to resolve or that elute early on C18 and other phenyl chemistries. As a result, the rugged Raptor™ Biphenyl column is extremely useful for fast separations in bioanalytical testing applications like drug and metabolite analyses, especially those that require a mass spectrometer (MS). Increasing retention of early-eluting compounds can limit ionization suppression, and the heightened selectivity helps eliminate the need for complex mobile phases that are not well suited for MS detection.

In 2005, Restek was the first to bring you the benefits of the Biphenyl ligand, and we have the experience to maximize the SPP performance of this premier phenyl chemistry for today's challenging workflows.

## Column Description:



### Stationary Phase Category:

Phenyl (L11)

### Ligand Type:

Biphenyl

### Particle:

2.7  $\mu\text{m}$  or 5  $\mu\text{m}$  superficially porous silica (SPP or “core-shell”)

### Pore Size:

90 Å

### Surface Area:

150  $\text{m}^2/\text{g}$  (2.7  $\mu\text{m}$ )  
or 100  $\text{m}^2/\text{g}$  (5  $\mu\text{m}$ )

### Recommended Usage:

pH Range: 1.5–8.0

Maximum Temperature: 80 °C

Maximum Pressure: 600 bar / 8,700 psi (2.7  $\mu\text{m}$ )  
or 400 bar / 5,800 psi (5  $\mu\text{m}$ )

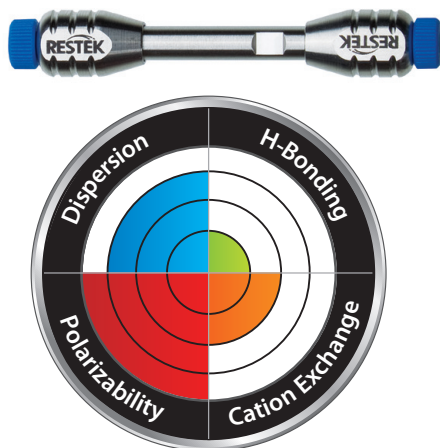
### Properties:

- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

### Switch to a Biphenyl when:

- Limited selectivity is observed on a C18.
- You need to increase retention of hydrophilic aromatics.

## Column Interaction Profile:



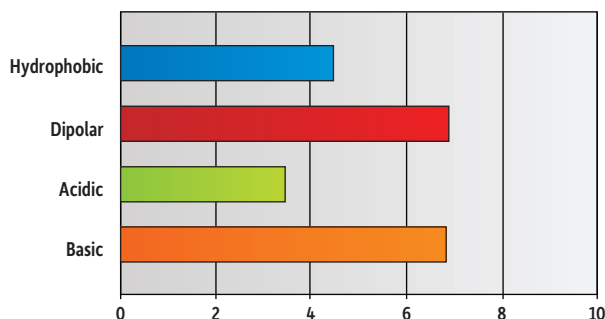
### Defining Solute Interactions:

- Polarizability
- Dispersion

### Complementary Solute Interaction:

- Cation exchange

## Solute Retention Profile:

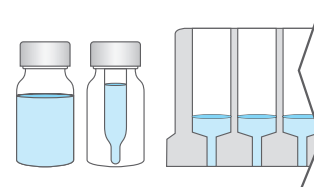


### Target Analyte Structures:

- Aromatic
- Dipolar

### Target Analyte Functionalities:

- Hydrophilic aromatics
- Strong dipoles
- Lewis acids
- Dipolar, unsaturated, or conjugated compounds
- Fused-ring compounds with electron withdrawing groups

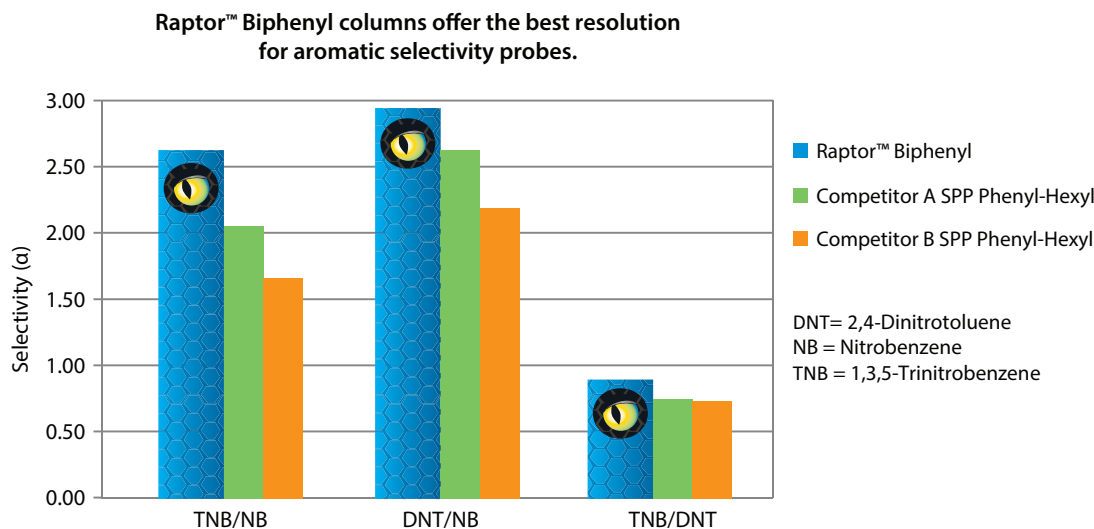


## More Aromatic Selectivity than Ordinary Phenyl-Hexyls

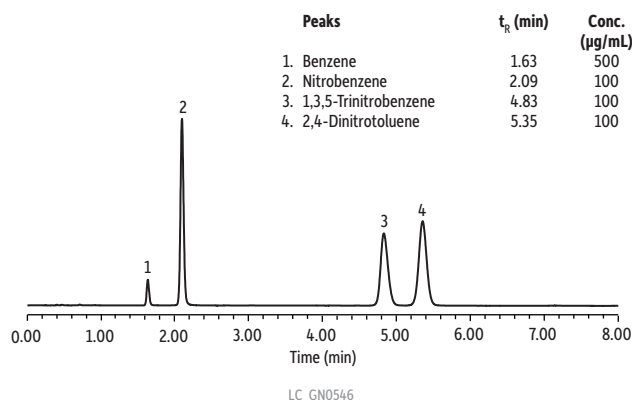
SPP core-shell columns commonly employ traditional phenyl-hexyl stationary phases, but the innovative Biphenyl ligand, developed by Restek's chemists, is the next generation of phenyl column chemistry. It provides greater aromatic selectivity than commercially available phenyl-hexyl columns [1] and a greater degree of dispersion than conventional phenyls. As a result, the Raptor™ Biphenyl allows you to more easily separate bioanalytical compounds like aromatics (Figures 1 and 2), which elute early or are hard to separate on C18 or other phenyl chemistries.

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

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



**Figure 2:** Raptor™ Biphenyl columns show increased retention for compounds containing electron withdrawing groups. Retention and elution order are dramatically different from a traditional C18.



**Column:** Raptor™ Biphenyl (cat.# 9309A55); Dimensions: 50 mm x 4.6 mm ID; Particle Size: 2.7 µm;  
Pore Size: 90 Å; Temp.: 40 °C; **Sample:** Diluent: acetonitrile; Conc.: 100-500 µg/mL; Inj. Vol.: 1 µL  
**Mobile Phase:** water: methanol (50:50); Flow: 1.2 mL/min; **Detector:** Waters Acquity® PDA @ 254 nm;  
**Instrument:** Waters Acquity® UPLC H-Class.

**Part of the USLC® column set!**

**RESTEK®**  **USLC®**

Ultra Selective Liquid Chromatography™

Learn more about USLC® technology, phase profiles, and more at [www.restek.com/uslc](http://www.restek.com/uslc)



## The New Standard for Performance and Durability for SPP Core-Shell Columns

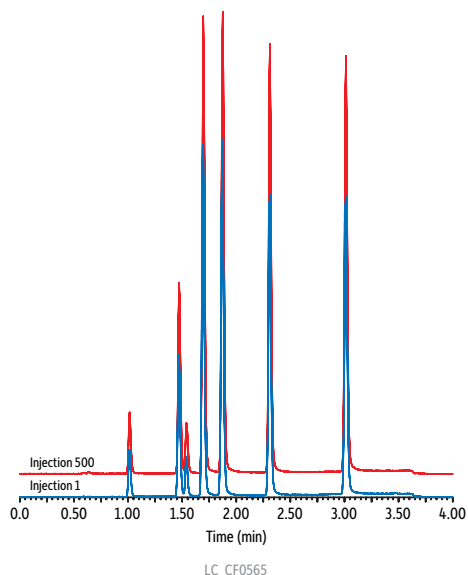
### Pressure Stability:

One of the greatest advantages of an SPP column is the ability to achieve fast, efficient separations by operating at higher linear velocities than are possible with a conventional fully porous particle column. However, these higher velocities can also result in higher back pressures. Raptor™ columns were designed to handle the increased pressures needed to achieve *Selectivity Accelerated*, and handle it far better than other SPP columns on the market (Figure 3).

### Reproducibility:

To help keep your productivity high and your lab budget low, we know that Raptor™ Biphenyl columns must produce exceptional selectivity and fast analysis times not just once, but every time. Ruggedness and repeatability are essential, which is why from the silica and the bonding technique, to the packing process and upgraded hardware, every decision that went into creating this column was made to ensure superlative reproducibility, from injection to injection (Figure 4) and from lot to lot (Figure 5). We also adopted new quality control (QC) specifications to guarantee the retention time stability you need for worry-free MRM analyses.

**Figure 4:** Even after hundreds of injections, a Raptor™ Biphenyl column will provide consistent, reliable data.

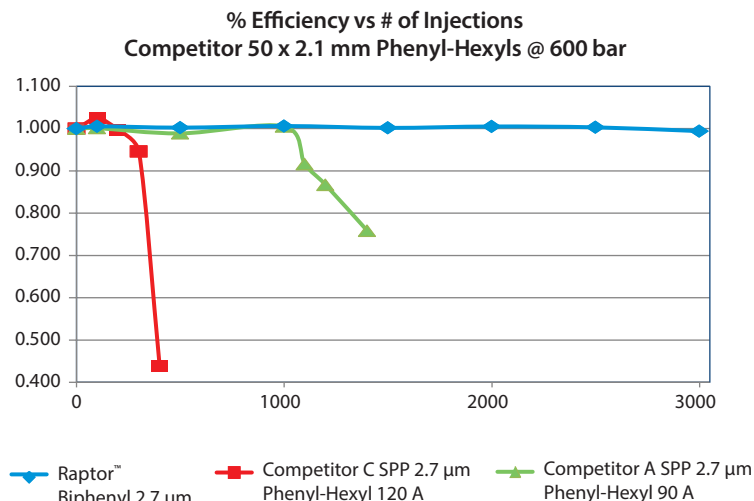


#### Peaks

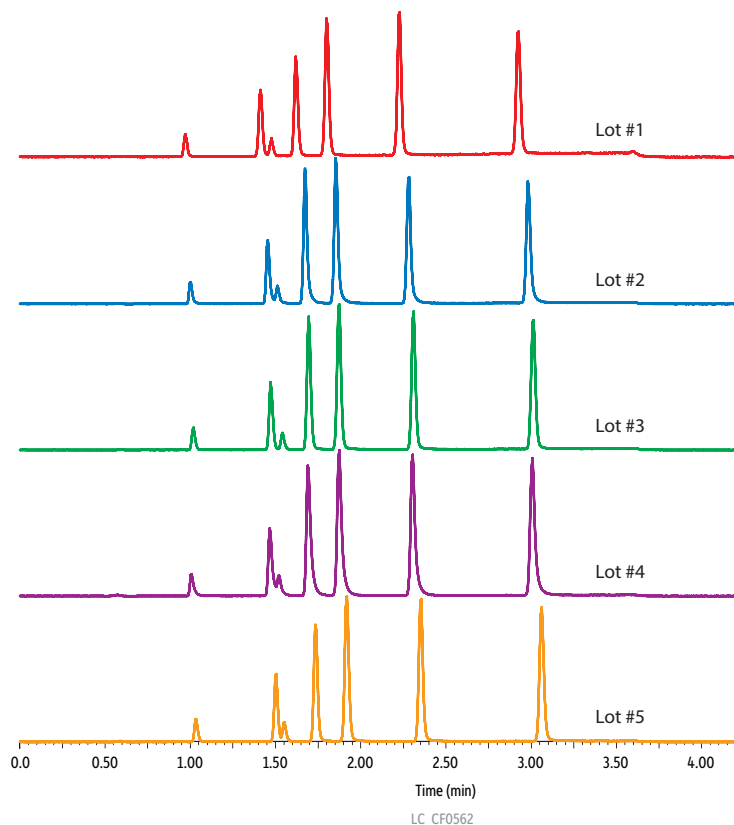
1. Cortisol
2. 11-Deoxycortisol
3. Estradiol
4. Boldenone
5. Testosterone
6. Androstenedione
7. Progesterone

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

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



**Figure 5:** From one lot to the next, every Raptor™ Biphenyl column you purchase will perform the same.



See Figure 4 for compound list and conditions.

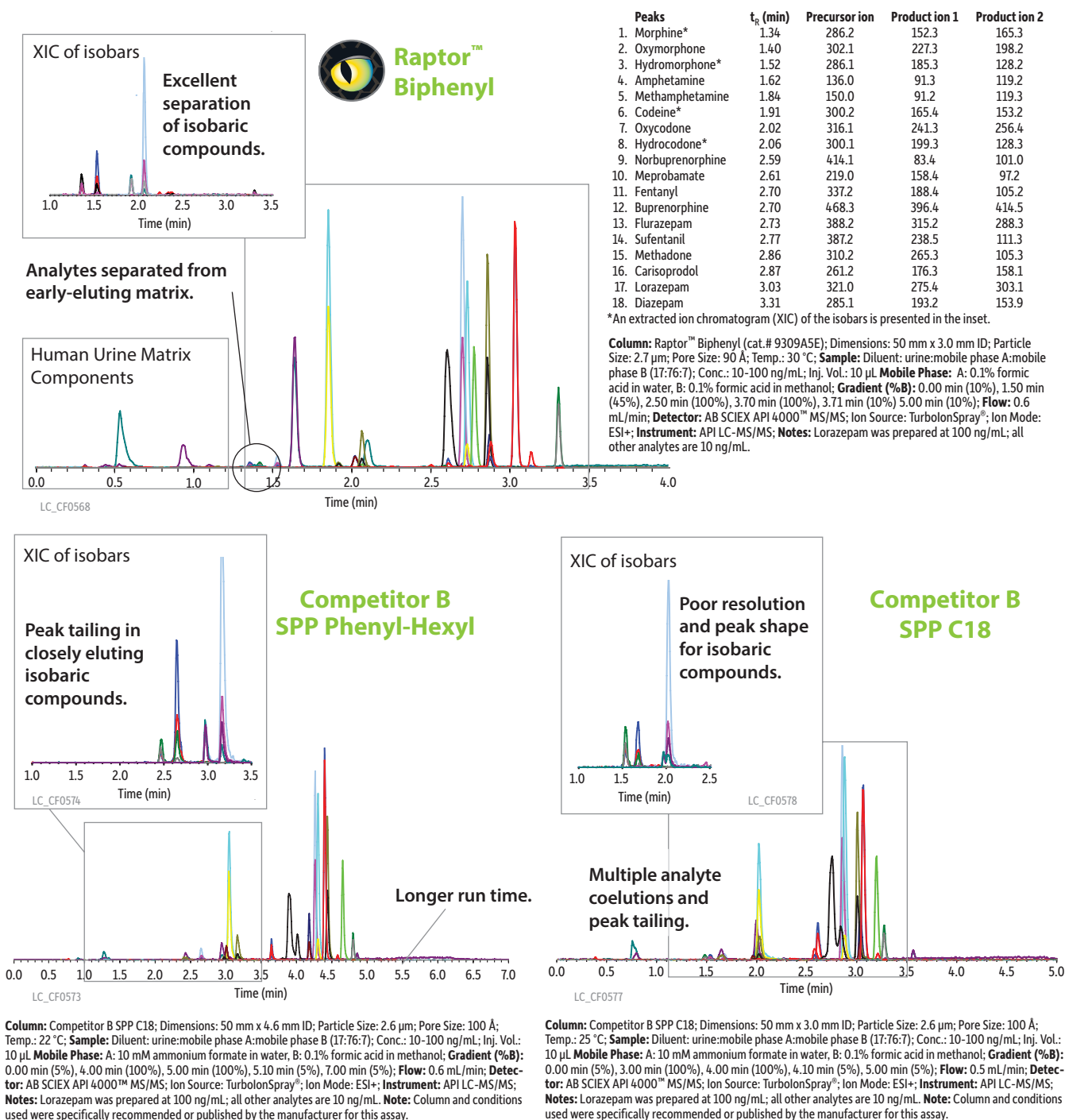
## Clinically Proven to Optimize Your Bioanalytical Workflows

For over a decade, the Restek® Biphenyl has been the column of choice for clinical testing because of its ability to provide highly retentive, selective, and rugged reversed-phase separations of drugs and metabolites. By bringing the speed of SPP to the Biphenyl family, the Raptor™ Biphenyl provides clinical labs with an even faster option for a wide variety of clinical assays.

### Rugged Pain Panels from Urine in Under 3.5 Minutes

Pain panels can be difficult to optimize and reproduce due to the limited selectivity of C18 and phenyl-hexyl phases, but not on the Raptor™ Biphenyl. Complete your pain panel analysis with a 5-minute cycle time and complete isobaric resolution using Raptor™ Biphenyl columns (Figure 6). Popular competitor columns offer tailing peaks, longer run times, and coelutions; the Raptor™ Biphenyl exhibits the selectivity and performance needed for this critical analysis.

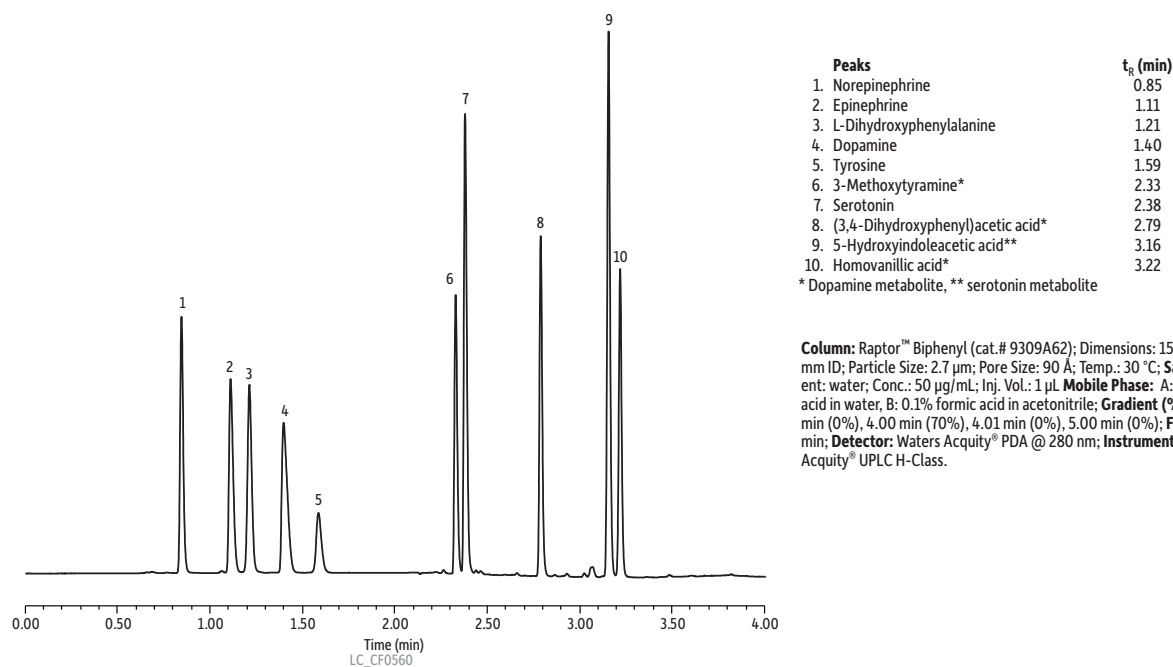
**Figure 6:** Raptor™ Biphenyl columns offer pain panel analyses with complete isobaric resolution in under 5 minutes!



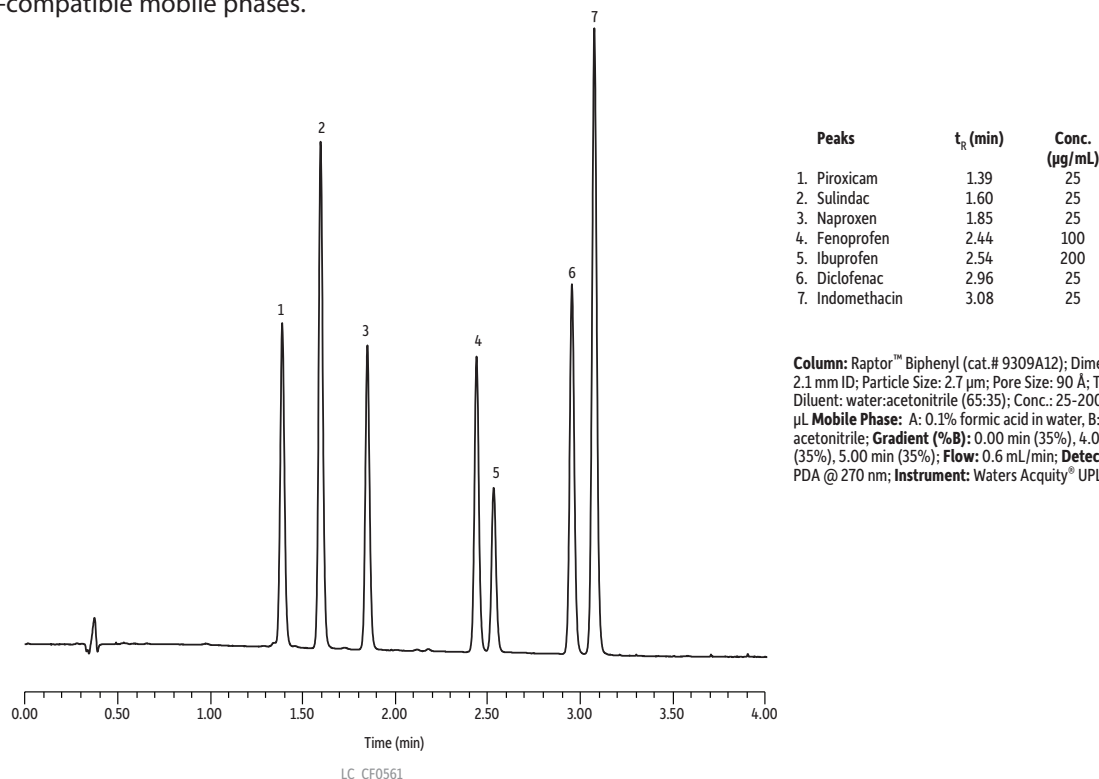
## Catecholamines and NSAIDs Without Ion Pairing, HILIC, or Complex Mobile Phases

Analyzing catecholamine compounds can be problematic by liquid chromatography and often forces chemists to turn to aqueous normal phase / HILIC or ion-pairing reagents that are not well suited for mass spectrometry (MS). Raptor™ Biphenyl columns easily retain and separate these difficult compounds using simple, MS-friendly mobile phases in a time frame that maximizes your productivity (Figure 7). Raptor™ Biphenyl also offers fast, efficient analysis of nonsteroidal anti-inflammatory drugs (NSAIDs) with LC-MS friendly solvents.

**Figure 7:** Separate catecholamine and other neurotransmitter compounds fast without ion pairing or HILIC.



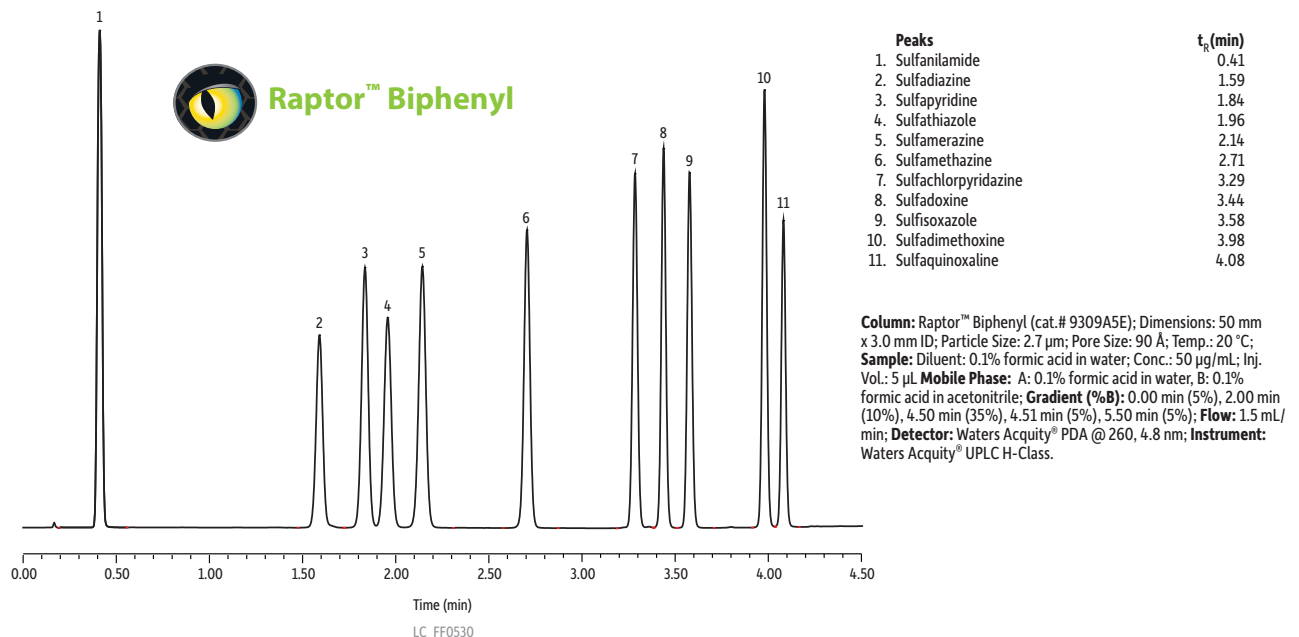
**Figure 8:** Nonsteroidal anti-inflammatory drugs (NSAIDs) are also easily resolved with Raptor™ Biphenyl using UV- and MS-compatible mobile phases.



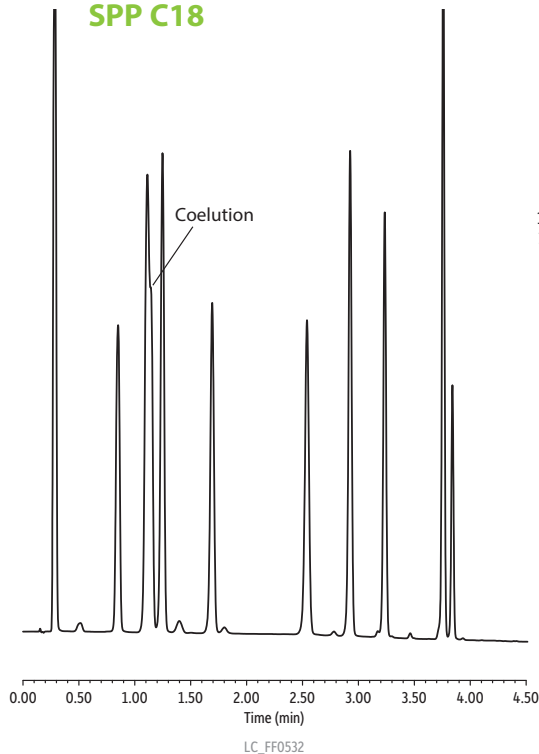
## Fast Analysis of Sulfur Antibiotics Without Coelutions

Even with high-efficiency UHPLC particles, C18 and ordinary phenyl columns fail to achieve baseline separation of sulfonamides. Not only does the Raptor™ Biphenyl have the selectivity to easily and completely separate these difficult compounds (Figure 9), it does so in well under 5 minutes!

**Figure 9:** Sulfonamides pose no problems for analysis, even at high linear velocities. Increased retention of early-eluting sulfanilamide also helps limit ionization suppression.

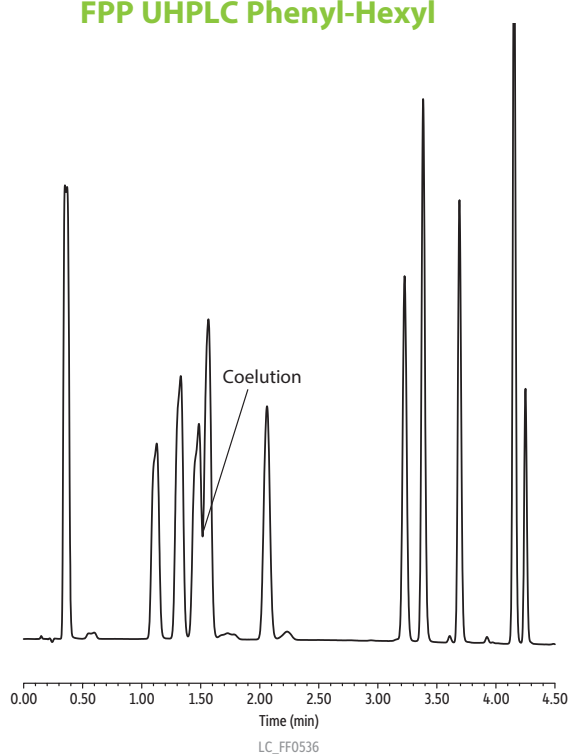


### Competitor B SPP C18



**Column:** Competitor B SPP C18; Dimensions: 50 mm x 3.0 mm ID; Particle Size: 2.6  $\mu$ m; Pore Size: 100 Å; Temp.: 20 °C; **Sample:** Diluent: 0.1% formic acid in water; Conc.: 50  $\mu$ g/mL; Inj. Vol.: 5  $\mu$ L **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (5%), 2.00 min (10%), 4.50 min (35%), 4.51 min (5%), 5.50 min (5%); **Flow:** 1.5 mL/min; **Detector:** Waters Acquity® PDA @ 260, 4.8 nm; **Instrument:** Waters Acquity® UPLC H-Class.

### Competitor D FPP UHPLC Phenyl-Hexyl



**Column:** Competitor D FPP Phenyl-Hexyl; Dimensions: 50 mm x 2.1 mm ID; Particle Size: 1.7  $\mu$ m; Temp.: 20 °C; **Sample:** Diluent: 0.1% formic acid in water; Conc.: 50  $\mu$ g/mL; Inj. Vol.: 5  $\mu$ L **Mobile Phase:** A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile; **Gradient (%B):** 0.00 min (5%), 2.00 min (10%), 4.50 min (35%), 4.51 min (5%), 5.50 min (5%); **Flow:** 0.75 mL/min; **Detector:** Waters Acquity® PDA @ 260, 4.8 nm; **Instrument:** Waters Acquity® UPLC H-Class. **Note:** Flow rate scaled to particle size

# Accelerated Performance and Time-Tested Biphenyl Selectivity for Clinical Diagnostic, Pain, Pharma, and Environmental Labs

## Raptor™ Biphenyl LC Columns

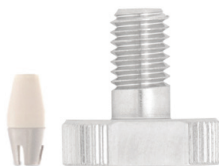


Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
<b>2.7 µm Columns</b>			
30 mm	9309A32	9309A3E	9309A35
50 mm	9309A52	9309A5E	9309A55
100 mm	9309A12	9309A1E	9309A15
150 mm	9309A62	9309A6E	9309A65
<b>5 µm Columns</b>			
30 mm	—	930953E	—
50 mm	9309552	930955E	9309555
100 mm	9309512	930951E	9309515
150 mm	9309562	930956E	9309565
250 mm	—	—	9309575

## EXP® Reusable Fittings for HPLC & UHPLC

for 10-32 fittings and 1/16" tubing

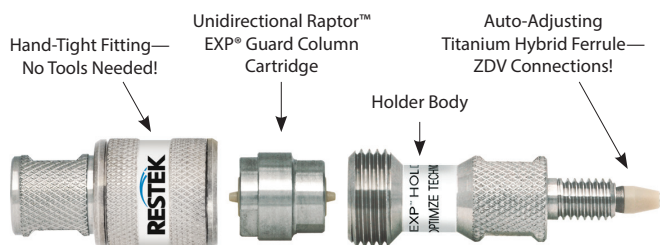
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Description	qty.	cat.#
EXP Hand-Tight Fitting (Nut w/Ferrule)	ea.	25937
EXP Hand-Tight Fitting (Nut w/Ferrule)	10-pk.	25938
EXP Hand-Tight Nut (w/o Ferrule)	ea.	25939

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Protect your investment and extend the life of our already-rugged LC columns and change guard column cartridges by hand without breaking fluid connections—no tools needed!

## EXP® Direct Connect Holder

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	ea.	25808

## Raptor™ EXP® Guard Column Cartridges

Description	Particle Size	qty.	5 x 2.1 mm cat.#	5 x 3.0 mm cat.#	5 x 4.6 mm cat.#
Raptor Biphenyl EXP Guard Cartridge	2.7 µm	3-pk.	9309A0252	9309A0253	9309A0250
Raptor Biphenyl EXP Guard Cartridge	5 µm	3-pk.	930950252	930950253	930950250

Maximum cartridge pressure: 600 bar / 8,700 psi (2.7 µm) or 400 bar / 5,800 psi (5 µm)

Raptor™ SPP LC columns combine the speed of SPP with the resolution of USLC® technology. Learn more at [www.restek.com/raptor](http://www.restek.com/raptor)

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## Organic Volatile Impurities: Retention Time Index

Conditions used to determine retention time data are listed [below](#).

### Retention Time

Compound	ICH Class	G16 Stabil-wax®	G16 Rtx®-WAX	G43 Rtx®-1301	G27 Rxi™-5ms	G1 Rtx®-1	NA Rtx®-200
1,1,1-trichloroethane	1	3.96	3.49	5.43	5.40	10.82	8.35
1,1,2-trichloro- ethene	2	15.72	14.28	10.99	9.77	16.75	14.94
1,1-dichloro- ethene	1	2.23	2.04	2.79	4.41	5.73	4.16
1,2-dichloro- ethane	1	8.80	7.68	6.15	5.46	10.38	9.74
cis-1,2-dichloro- ethene	2	6.50	5.65	4.79	2.88	8.71	7.11
trans-1,2-dichloro- ethene	2	3.63	3.20	3.55	3.54	7.17	5.16
1,2-dimethoxyethane	2	4.80	4.18	6.03	5.54	10.98	10.63
1,4-dioxane	2	8.55	7.49	7.86	7.26	13.54	14.34
1-butanol	3	11.13	10.08	7.18	5.76	11.49	10.13
1-pentanol	3	14.95	13.75	11.19	9.44	16.99	14.95
1-propanol	3	7.69	6.80	4.20	3.37	6.81	6.13
2-butanol	3	7.25	6.44	5.08	4.16	8.51	7.69
2-ethoxyethanol	2	13.99	12.70	8.69	7.36	13.91	13.99
2-methoxyethanol	2	12.42	11.11	6.02	5.14	9.83	10.74
2-methyl-1-propanol	3	9.32	8.40	6.00	4.79	*	*
2-propanol	3	4.81	4.25	3.00	2.55	4.91	4.69
3-methyl-1-butanol	3	13.42	12.25	9.86	8.26	15.28	13.55
acetic acid	3	22.47	20.34	6.52	4.61	8.84	8.96
acetone	3	3.02	2.64	2.89	2.50	4.64	7.68
acetonitrile	2	6.91	5.83	3.28	2.47	4.32	8.89
anisole	3	18.65	17.09	17.12	16.28	25.00	22.84
benzene	1	5.23	4.54	5.98	3.83	11.63	9.17
butyl acetate	3	8.86	7.88	12.12	11.38	19.43	19.63
carbon tetrachloride	1	3.96	3.49	5.61	5.90	11.89	7.42
chlorobenzene	2	13.91	12.54	13.55	13.14	21.56	18.48
chloroform	2	7.31	6.41	5.23	4.64	9.18	6.66
cumene	3	12.36	11.17	16.66	16.69	25.88	20.90
cyclohexane	2	2.16	2.01	5.37	5.89	*	*
dichloromethane	2	5.01	4.33	3.31	3.06	5.87	4.88
dimethylsulfoxide	3	26.47	24.43	16.62	13.01	18.81	30.95
ethanol	3	4.98	4.37	2.52	2.19	4.03	3.80
ethyl acetate	3	4.08	3.56	4.87	4.44	9.04	10.35
ethyl benzene	2	10.72	9.58	13.86	13.81	22.54	18.18
ethyl ether	3	1.72	1.63	2.58	2.67	5.34	3.87
ethyl formate	3	3.16	2.78	3.00	2.78	5.46	6.48
ethylene glycol	2	28.06	26.23	10.77	6.63	12.59	13.86
formamide	2	32.99	30.93	11.85	7.30	12.72	19.93
formic acid	3	24.64	22.09	5.19	2.60	5.59	5.06
heptane	3	1.98	1.86	6.34	6.98	14.18	7.84
hexane	2	1.65	1.58	3.77	4.11	9.06	4.86
isobutyl acetate	3	6.99	6.18	10.39	9.69	17.35	18.02
isopropyl acetate	3	4.26	3.74	6.19	5.71	11.47	12.38
methanol	2	4.23	3.64	1.96	1.80	3.14	2.93
methyl acetate	3	3.19	2.80	3.17	2.93	5.80	7.10
methylbutyl ketone	2	9.10	8.05	11.81	10.50	17.94	20.81
methylcyclohexane	2	2.50	2.30	7.31	7.95	15.49	9.21
methylethyl ketone	3	4.33	3.76	4.90	4.09	7.99	11.55
methylisobutyl ketone	3	6.84	5.97	9.64	8.49	15.35	18.41
m-xylene	2	11.21	10.04	14.29	14.17	23.01	18.78
N,N-dimethyl- acetamide	2	20.75	19.01	12.95	13.96	21.42	30.00

N,N-dimethyl- formamide	2	18.04	16.26	13.09	10.23	16.52	26.19
nitromethane	2	11.82	10.31	4.84	3.53	6.30	12.01
N-methylpyrrolidone	2	29.84	27.86	25.09	21.85	29.99	38.08
<i>o</i> -xylene	2	12.79	11.51	15.46	15.26	24.23	20.33
pentane	3	1.49	1.45	2.39	2.62	5.36	3.29
propyl acetate	3	5.98	5.29	8.03	7.44	*	*
<i>p</i> -xylene	2	10.98	9.82	14.29	14.17	22.99	18.69
pyridine	2	12.64	11.24	9.60	8.57	15.40	16.45
sulfolane	2	47.62	43.31	34.02	28.90	36.76	48.67
<i>tert</i> -butylmethyl ether	3	1.94	1.82	3.50	3.59	7.52	5.73
tetrahydrofuran	3	3.63	3.19	5.12	4.90	9.81	9.48
tetralin	2	25.12	23.48	27.49	27.44	37.27	31.72
toluene	2	7.86	6.91	9.80	9.66	17.36	14.00
1,1-diethoxypropane	—	5.42	4.84	11.39	11.38	19.82	15.08
2,2-dimeth- oxypropane	—	3.11	2.79	5.48	5.55	11.37	8.67
2-chloropropane	—	1.96	1.82	2.67	2.66	5.20	4.61
2-methylpentane	—	1.58	1.52	3.22	3.56	7.72	4.32
acetaldehyde	—	2.05	1.85	1.86	1.84	3.14	3.90
chloroethane	—	1.83	1.71	2.14	2.10	3.97	3.55
chloromethane	—	1.63	1.55	1.70	1.70	3.01	2.73
ethylene oxide	—	2.05	1.86	1.89	2.02	3.59	3.92
formaldehyde	—	2.25	1.57	1.68	1.58	2.66	2.59
isoamyl acetate	—	10.51	9.43	14.84	14.18	22.80	22.62
isooctane	—	1.85	1.75	5.84	6.59	13.66	8.07
isopropyl ether	—	1.86	1.76	4.03	4.23	9.03	5.83
methyl cyclopentane	—	1.91	1.79	4.50	4.93	10.41	5.81
methyl isopropyl ketone	—	4.93	4.29	6.58	5.69	11.04	14.47
methylal	—	2.26	2.06	2.84	2.82	5.65	5.09
trichloroethene	—	6.50	5.70	7.07	7.05	13.58	9.75
water	—	8.24	7.18	1.74	1.68	2.75	2.57
*not determined							

Retention time data collected using the following conditions:

#### Stabilwax® (G16)

Column

dimensions: 30m x 0.25mm x 0.5µm df  
Phase ratio: 125  
Oven program: 40°C, hold 1 min., to 190°C @ 4°C/min., hold 15 min.  
Carrier gas: helium  
Carrier flow  
(mL/min.): 1.2  
Dead time (min.): 1.38 @ 45°C

#### Rtx®-WAX (G16)

Column

dimensions: 30m x 0.25mm x 0.5µm df  
Phase ratio: 125  
Oven program: 40°C, hold 1 min., to 190°C @ 4°C/min., hold 15 min.  
Carrier gas: helium  
Carrier flow  
(mL/min.): 1.2  
Dead time (min.): 1.40 @ 45°C

#### Rtx®-1301 (G43)

Column

dimensions: 30m x 0.25mm x 1.0µm df  
Phase ratio: 63  
Oven program: 40°C, hold 1 min., to 190°C @ 4°C/min., hold 15 min.  
Carrier gas: helium  
Carrier flow  
(mL/min.): 1.2  
Dead time (min.): 1.40 @ 45°C

#### Rxi™-5ms (G27)

Column

dimensions: 30m x 0.25mm x 1.0µm df  
 Phase ratio: 63  
 Oven program: 40°C, hold 1 min., to 190°C @ 4°C/min., hold 15 min.  
 Carrier gas: helium  
 Carrier flow  
 (mL/min.): 1.1  
 Dead time (min.): 1.49 @ 45°C

#### Rtx®-1 (G1)

Column  
 dimensions: 60m x 0.53mm x 3.00µm df  
 Phase ratio: 43  
 Oven program: 30°C, hold 4 min., to 220°C @ 4°C/min.  
 Carrier gas: helium  
 Carrier flow  
 (mL/min.): 6.3  
 Dead time (min.): 2.54 @ 35°C

#### Rtx®-200

Column  
 dimensions: 60m x 0.53mm x 3.00µm df  
 Phase ratio: 43  
 Oven program: 30°C, hold 4 min., to 220°C @ 4°C/min.  
 Carrier gas: helium  
 Carrier flow  
 (mL/min.): 7.8  
 Dead time (min.): 2.22 @ 35°C

#### RELATED SEARCHES

[residual solvents](#), [OVI](#), [ICH](#), [stabilwax](#)



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## Optimized RP-HPLC Method for Hydroxybenzoic Acids

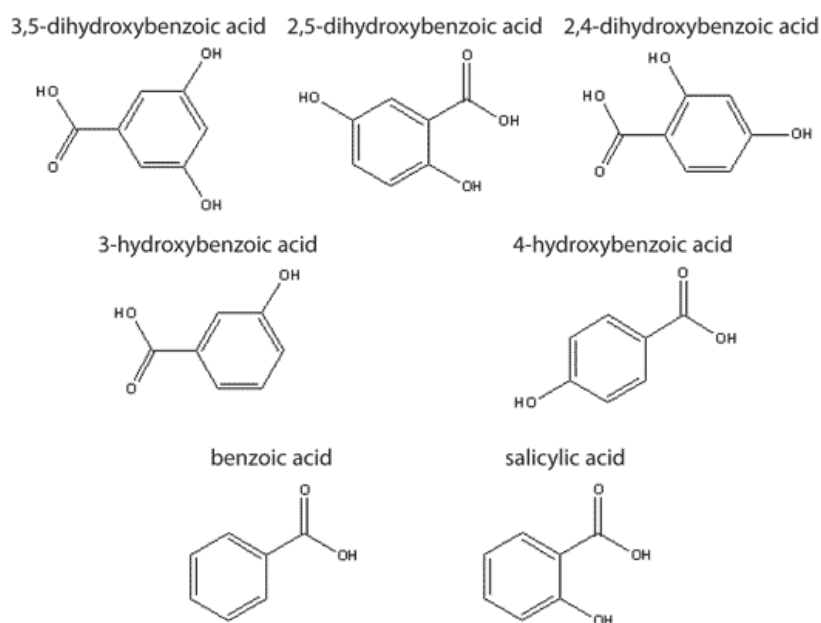
### Balanced Retention for a Range of Polarities, Using an Ultra Aqueous C18 Column

By Rick Lake, Pharmaceutical Innovations Chemist

- Useful retention of more polar and less polar analytes.
- Ultra Aqueous C18 column is compatible with 100% aqueous mobile phases.
- Ideal for samples that encompass a broad range of analyte polarity.

Hydroxybenzoic acids serve as active drug substances (aspirin, for example), as well as preservatives in drug products. In some cases, they represent impurities in drug products. Their analysis sometimes can be difficult, not only because they represent a wide range of applications, but primarily because they encompass a wide range of polarity. Chemically, benzoic acid, the basic structure for these analytes, consists of a benzene ring with a carboxyl group (Figure 1). Hydroxybenzoic acids share the same basic structure, but contain additional hydroxyl groups on the benzene ring (Figure 1). The additional hydroxyl groups' varied positions and numbers create differences among the analytes' overall polarity and solubility. Because these compounds represent such varying chemistry and polarity, finding an alkyl (C18) HPLC column that can effectively assay them all could be very difficult, but such a column could be of value for resolving these compounds from active drugs or from chemically similar impurities.

**Figure 1** Hydroxybenzoic acids share the same basic structure, but have varying polarity.



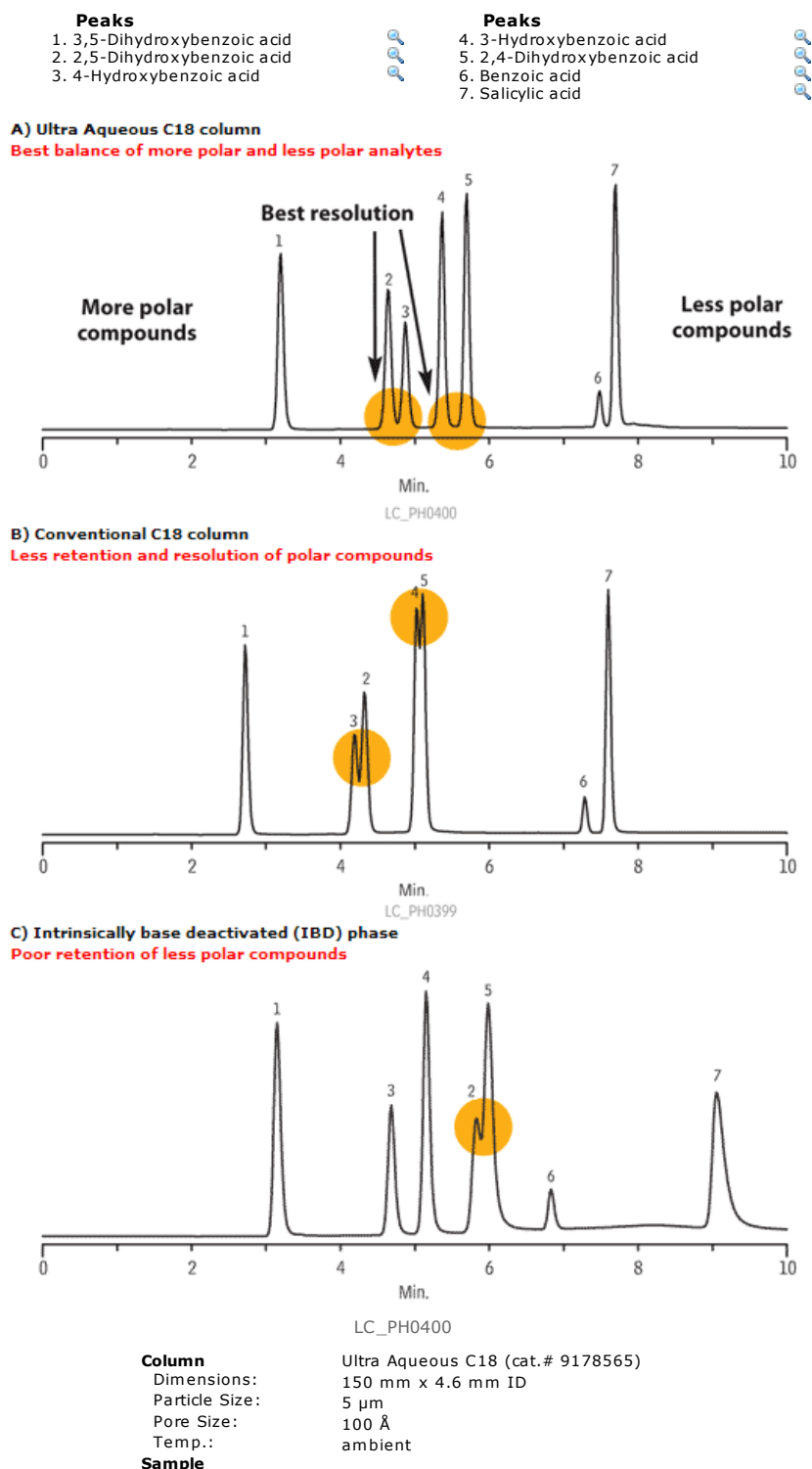
Using identical conditions, we analyzed a group of hydroxybenzoic acids on a conventional C18 stationary phase column, on a C18 column with a polar group within (intrinsic to) the alkyl bonded phase (an IBD phase<sup>2</sup>), and on an Ultra Aqueous C18 column. Our objective was to find the optimum stationary phase for resolving analytes with a varying number of polar functional groups.

Overall, the Ultra Aqueous C18 column provided the best balance of retention for more polar and less polar analytes (Figure 2A), completely resolving our test mix when used with a simple gradient mobile phase. The conventional C18 column exhibited retention very similar to that of the Ultra Aqueous C18 column for the less polar analytes, benzoic acid and salicylic acid, but it showed less retention and resolution for the more polar compounds (Figure 2B). The intrinsically base deactivated column, on the other hand, exhibited opposite characteristics — retention similar to the Ultra Aqueous C18 column for the

more polar compounds, but little retention of the less polar compounds (Figure 2C).

It is well documented that Ultra Aqueous C18 columns are compatible with 100% aqueous mobile phases, because the stationary phase has sufficient polar character to prevent dewetting or hydrophobic collapse.<sup>1</sup> Our current analyses reveal yet another advantage to the slight polar character of this column: by providing the best resolution of analytes exhibiting a wide range of polarity, the Ultra Aqueous C18 column demonstrates that it also can be used to retain, and separate, more polar or less polar compounds — or mixtures of both.

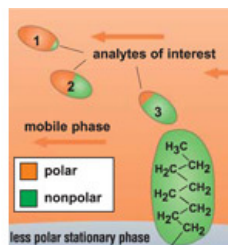
**Figure 2** An Ultra Aqueous C18 column shows suitable retention for polar or nonpolar compounds, providing enhanced selectivity for a broad range of polarity.



Inj. Vol.:	5 µL
<b>Mobile Phase</b>	
A:	20 mM potassium phosphate (pH 2.5)
B:	acetonitrile
	<b>Time (min)%B</b>
	0.00 20
	5.00 50
	10.00 50
Flow:	1.0 mL/min
<b>Detector</b>	UV/Vis @ 210 nm

## Options for Analyzing Polar Compounds

Many types of alkyl phases currently are available to the analyst, making column selection difficult. Although all alkyl phases possess the same basic structure — a specific length of alkyl chain bonded to a silica surface (typically C1-C30, with C18 being the most common) — various attached polar groups create selectivity and retention differences among columns. For example, a conventional C18 phase is comprised of a monomerically bonded straight 18 carbon alkyl chain, meaning every alkyl chain has a single, direct attachment to the silica surface. These phases are excellent for retaining nonpolar compounds, but they show very limited retention for polar compounds. One common bonding technique for increasing retention of polar compounds on an alkyl phase is to attach a polar group within, or intrinsic to, the alkyl phase. These phases, known as intrinsically base deactivated (IBD) phases, show increased retention for polar compounds because the embedded polar groups are capable of interaction with polar portions of analyte molecules. (These phases also have a deactivating effect on basic compounds, by creating an electrostatic barrier.) Polarity also can be added to an alkyl phase by adding polar end caps to active sites on the silica surface, or by adding polar side chains to the alkyl attachment. Interactions with polar compounds also can be increased through the use of a polymeric bonding chemistry.



### Retention by Reversed Phase (RP) HPLC

## References

1. *Ultra Aqueous C18 HPLC Columns: Achieve Stable Retention in 100% Aqueous Mobile Phase* Restek Corporation, 2002 (lit. cat.# 59371).

## Footnotes

- The intrinsically base deactivated (IBD) phase shows increased retention for polar compounds, because the embedded polar groups are capable of interaction with polar portions of analyte molecules.

## RELATED SEARCHES

Ultra, aqueous c18, hydroxybenzoic acids, hydroxyl groups, analgesics



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## General Applications

# Optimize Selectivity & Efficiency in UHPLC Separations

## With More Stationary Phase Choices on 1.9 $\mu\text{m}$ Pinnacle® DB HPLC Columns

By Rick Lake

- Largest variety of stationary phases for UHPLC.
- Faster analyses, uncompromised chromatography.
- 100% Restek manufactured—from base silica to final packed column.

Since the late 1960s, when modern high performance liquid chromatography (HPLC) became a viable tool for practicing chemists, continual advancements have been made in column technology. Over time, the trend has been to pack columns with smaller particle sizes, which necessitates increasing the pressure capabilities of our instrumentation. These trends have brought us to where we are today—Ultra-High Performance Liquid Chromatography (UHPLC). UHPLC is a milestone in the evolution of LC in that columns packed with  $<2\ \mu\text{m}$  particles, used in conjunction with instrumentation capable of handling the resulting high back pressures, make extremely fast and efficient separations possible. UHPLC is a very powerful tool for today's chromatographer, as it can significantly increase the efficiency of a chromatographic separation. In addition, the wider range of usable flow rates makes high-speed separations possible. However, in light of this new technology, it is important that we do not forget the importance of selectivity. In this article, we will look at the principles of resolution and how we can use these concepts, namely selectivity, to maximize the benefits of UHPLC.

**Equation 1:** The resolution equation indicates selectivity has the greatest influence on resolution.

$$R = \frac{1}{4} \sqrt{N} \times \left( \frac{k}{k+1} \right) \times (\alpha - 1)$$

Efficiency    Retention Factor    Selectivity

In past articles we have discussed the physical advantages that are driving interest in small particles, mainly the influence of particle size on usable flow rates and peak efficiency. Although small particles have made faster separations possible, the ultimate goal behind chromatography is still analyte resolution and particle size is only one contributor to this goal. Resolution is the result of three cumulative terms: selectivity, efficiency, and retention. How well we resolve our analytes, and how quickly we do it, depends upon our ability to control these 3 factors. As we can see through the resolution equation (Equation 1), mathematically, the selectivity term has the greatest effect on resolution, indicating that resolution is largely a function of selectivity. Selectivity, in turn, is governed predominantly by analyte interactions with both the stationary and mobile phases. UHPLC, through the use small particle columns, does maximize efficiency (i.e. theoretical plates), but the stationary phase is still the most important consideration when attempting to resolve mixtures of compounds. Ideally, a stationary phase that produces optimum selectivity or allows for the resolution of compounds in a timely manner should be selected.

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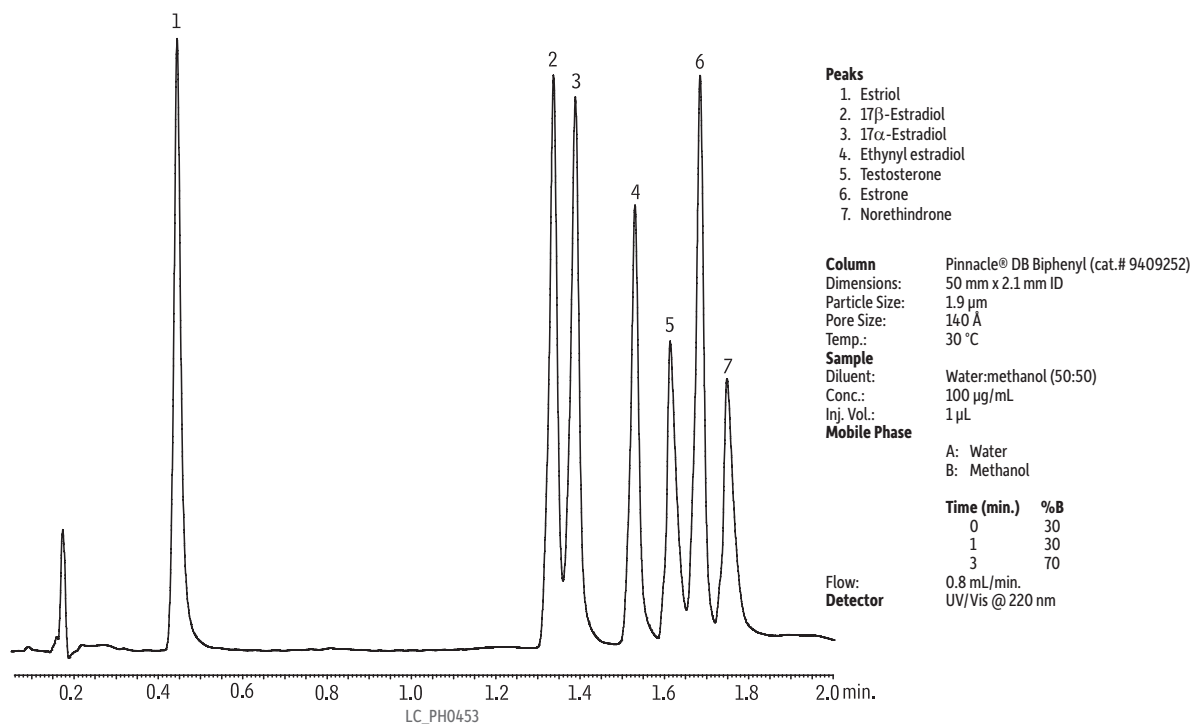
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Previously, some advantages of selectivity in specific separations have been noted. For example, the use of the unique Biphenyl stationary phase has shown excellent selectivity for aromatic or fused ring compounds. When using the Biphenyl stationary phase and combining it with the heightened efficiencies of the Pinnacle® DB 1.9 µm particle size column, we can produce highly selective and fast separations of steroids (Figure 1). A 1.9 µm Pinnacle® DB Biphenyl column can separate a test mix of 7 hormones in under 2 minutes, a feat not possible through C18 selectivity.

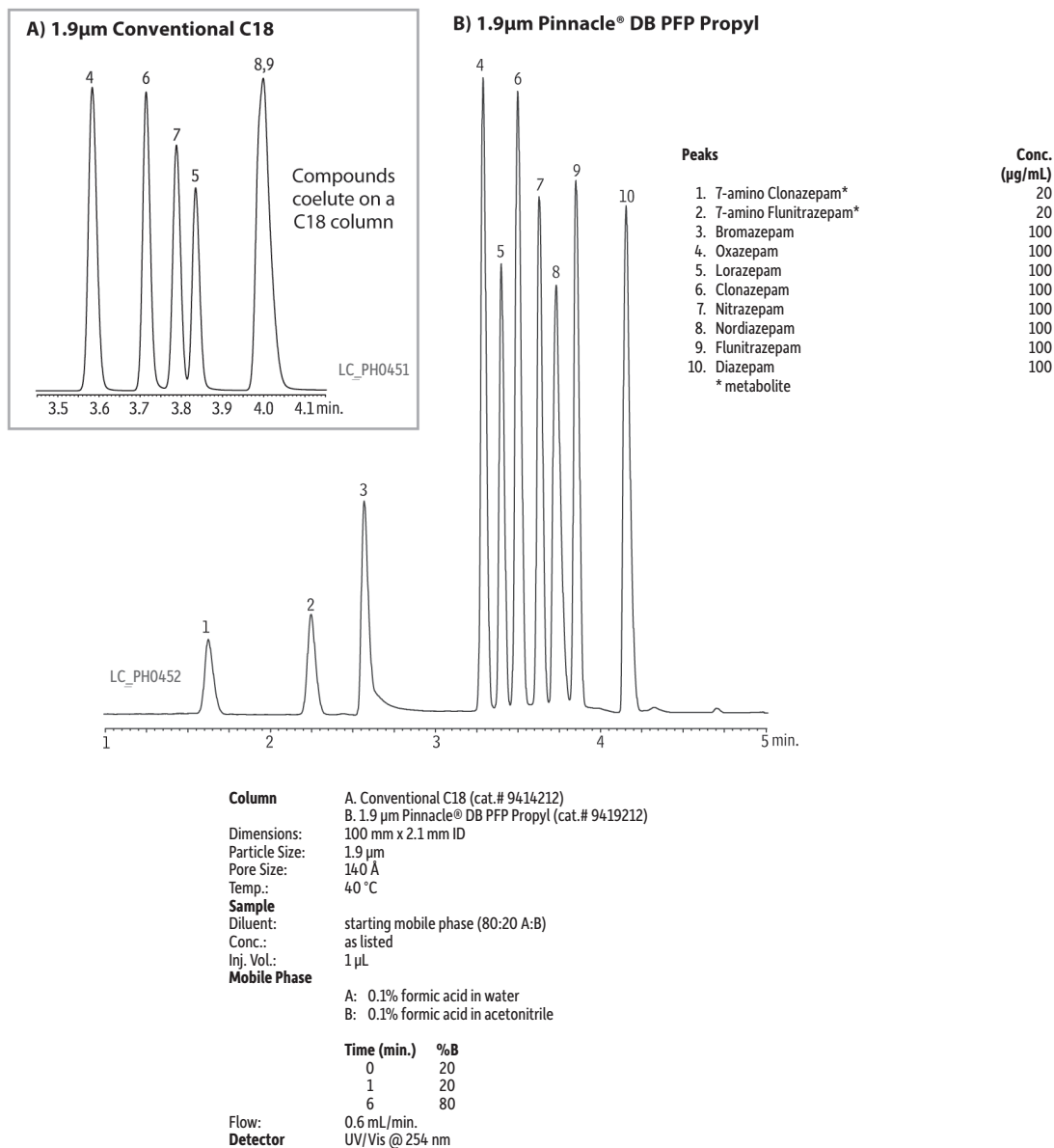
Another example of unique selectivity available on a 1.9 µm particle size column is the PFP Propyl (pentafluorophenyl propyl) stationary phase for halogenated drug compounds. This phase is very selective and retentive for organohalogens or other compounds containing basic or electronegative functionalities. To demonstrate heightened selectivity for halogenated drug compounds, we assayed a test mix of 8 benzodiazepines and 2 metabolites, a mix commonly assayed on a C18 column, in just over 4 minutes with complete resolution (Figure 2). To get the same level of selectivity from a C18 column, a shallower gradient would be needed, prolonging the analysis time. Since the selectivity of the 1.9 µm Pinnacle® DB PFP Propyl column elutes the benzodiazepines in quick succession, a simple gradient still allows for the earlier elution of the more polar metabolites, while maintaining a fast overall run time.

Restek is committed to giving the practicing chromatographer choices, and has therefore sought to deliver the widest selection of stationary phases available with <2 µm particle sizes. The goal of chromatography is always to resolve compounds of interest in the fastest time possible. By combining the benefits of UHPLC with Restek's line of unique stationary phase choices, faster separations become a reality.

**Figure 1:** Restek's 1.9 µm Pinnacle® DB Biphenyl columns are highly selective for steroids, making an extremely fast and selective analysis.



**Figure 2:** Fast, selective analysis of benzodiazepines is made possible by combining the speed of UHPLC with the enhanced selectivity of the 1.9  $\mu\text{m}$  Pinnacle® DB PFP Propyl column.



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## Novel Column Chemistry—High Impact, Low Cost Technology

Content previously published in *Next Generation Pharmaceutical*

*The three areas of development in HPLC that are most significant to the pharmaceutical industry are the implementation of fast LC (UHPLC), the increased applicability of LC mass spectrometry (LC/MS), and the development of novel column chemistries. These three factors have invigorated the HPLC market because they are major contributors to the development of pharmaceuticals and are substantial considerations in laboratory design. Without question, the higher sample throughput of UHPLC and the data quality of LC/MS are very beneficial to an industry striving to improve data quality, while reducing drug development time. However, making LC/MS and UHPLC accessible to the laboratory requires a technology change, a paradigm shift, and a large expenditure in capital equipment. Novel column chemistries, by comparison, are a simple change in an already budgeted consumable that can lead to optimized and more reliable methods—giving a fast return on a minimal investment.*

### Column Chemistry in Today's Laboratory

Within the past decade, HPLC has experienced a renaissance. UHPLC and LC/MS have revitalized the market and made us rethink our technology paradigm. However, in this age of change, the column has been largely overlooked. UHPLC, LC/MS, and novel column chemistry should be viewed as complementary advances that can be dovetailed together, not as separate technology choices. Just as UHPLC and mass spectrometry can be coupled to gain the advantages of both techniques, column chemistry can be added to both UHPLC and LC/MS to make these techniques more reliable and productive. Novel column chemistries are an easy and relatively inexpensive way to help optimize existing analytical resources and build successful technology platforms for drug development.

A day may come when we can use mass spectrometric deconvolution to eliminate the need for chromatographic separation, or use the higher efficiencies of UHPLC to make column selection unnecessary, but we are not there yet. Today, the reality is that technology platforms are still largely comprised of conventional HPLC systems with few open access mass spectrometers available. Even if we had unlimited resources to replace all HPLC systems with UHPLC systems, we still would need the column. UHPLC does increase efficiency, however it has not amassed the number of theoretical plates needed to make column stationary phase obsolete. At this juncture, the column is still an integral part of chromatography, and as long as we intend to resolve mixtures, the chromatography column continues to drive a separation. Choosing advantageous column chemistries is a simple, cost-effective way of making UHPLC and LC/MS work more effectively, thus increasing asset utilization.

### A Novel Phase Explained

Reversed-phase liquid chromatography (RPLC) is arguably the most common analytical tool in pharmaceutical analyses. In a recent study, it was determined that of all RPLC separations 39% employ C18 phases and 12% employ phenyl stationary phases.<sup>1</sup> The main advantage of phenyl phases—and the reason for their recent increase in popularity—is because they can produce orthogonal separations compared to a C18, creating a versatility needed in method development. The selectivity, or peak separation, of a C18 is often called hydrophobic selectivity because it is based upon hydrophobic differences among molecules. Phenyls, on the other hand, can also undergo  $\pi$ - $\pi$  interactions, resulting in aromatic selectivity. In a chromatographic system, these interactions can occur between the  $\pi$  electrons on a stationary phase and the  $\pi$  electrons on a solute, which gives rise to their orthogonal separations.

Phenyl phases offer promising separations for pharmaceutical compounds and are therefore an area of recent phase exploration. One such example of novel column chemistry is the Biphenyl stationary phase. This phase was designed by Restek to make the promising attributes of a phenyl stationary phase more useful for pharmaceutical compounds. The physical arrangement of the Biphenyl stationary phase, two phenyl groups bonded end-to-end, makes this phase distinct from other commercially available phases. Although the structural change is subtle, the practical properties of the column are much more beneficial. A Biphenyl column shows markedly greater aromatic selectivity and hydrophobic retention than other phenyl phases. These attributes maximize versatility and can be used to enhance UHPLC and LC/MS.

### Improve UHPLC Performance with Proper Column Choice

When UHPLC, was introduced into the marketplace in 2004, with the promise of high sample throughput

and decreased drug development time, it was quickly adopted into the pharmaceutical laboratory as a strategic asset. To achieve fast LC, much attention was given to the instrumentation, with relatively little regard for the impact of the analytical column. However, the importance of the column in UHPLC was soon realized. UHPLC in the pharmaceutical laboratory is commonly used to accelerate development of methods which are then scaled to a conventional HPLC-based platform for routine analysis. Because methods need to be scaled from UHPLC to HPLC, UHPLC columns first need to be manufactured under tight specifications and the base silica and phase also need to be available in various common HPLC geometries. Secondly, and more relevant here, the need for selective column phases, is just as great as in HPLC. While UHPLC does produce significant gains in efficiency (theoretical plates) and speed, the gain is not so extreme that column stationary phase is inconsequential. The selectivity of a separation, governed predominantly by analyte interactions with both the stationary and mobile phases, and is arguably the driving force behind separations as it affects resolution to the greatest mathematical degree. Higher quality separations, not just faster separations, are needed by pharmaceutical laboratories. To make the most of UHPLC we need to consider the optimization of both efficiency (UHPLC) and selectivity (novel stationary phases), combining the speed of sub-2 micron particles with the advantageous selectivity of a phase designed for optimal separations. The Biphenyl stationary phase, when used in conjunction with UHPLC, can provide much faster and more effective resolution for drug substances and impurities, which commonly contain aromatic rings or conjugated bonds, and which also commonly differ by levels of unsaturation or electron withdrawing ring substituents.

#### Maximize Use of Your LC/MS Asset

Mass spectrometry is an excellent asset and a powerful tool for improving data quality. However, incorporating high-cost mass spectrometers into laboratory operations can be a considerable investment, making effective resource utilization paramount. To fully utilize the analytical laboratory, a good choice in LC columns today extends the lifetime, performance, and profitability of the mass spectrometer asset tomorrow. In this Biphenyl column example, the benefit is not as much selectivity (the mass spectrometer can provide spectral deconvolution) as it is simple retention. When a stationary phase strongly retains an analyte, the recourse in RPLC is to use higher organic content mobile phases to elute the compounds into the mass spectrometer. This can lead to higher sensitivities as desolvation of the mobile phase becomes more efficient, giving better ionization. Another reason for creating highly retentive phases for mass spectrometry is to eliminate unwanted adduct formation or charge competition from matrix interferences that are less retained by the column. The commonly used C18 is excellent at retaining hydrophobic solutes, but fails when retaining hydrophilic solutes. A Biphenyl phase is capable of retaining both hydrophilic and hydrophobic aromatics better than conventional C18 and phenyl phases, resulting in better mass spectrometer asset utilization.

#### Method Development—Tunable Selectivity from Novel Columns

One of the biggest challenges in method development is finding the optimal stationary phase for a particular separation; this is one of the reasons the industry is moving towards column switching systems that speed up column selection. Whatever the mechanism for column selection, the most important column attribute for method development purposes is versatility. Compared to other phenyl columns, a Biphenyl column offers both heightened aromatic selectivity and a high degree of hydrophobic interaction. Often in HPLC, the mobile phase can be altered to enhance a separation or to get a desired resolution. With a Biphenyl column, this can be more easily achieved. The choice of organic used in the mobile phase can alter the selectivity by switching between these two mechanisms. Using acetonitrile in the mobile phase makes a Biphenyl column more C18-like in its retention and selectivity; methanol, on the other hand, induces aromatic selectivity or  $\pi$ - $\pi$  interactions and makes the Biphenyl column alternately selective. By controlling the desired levels of hydrophobic and  $\pi$ - $\pi$  interactions, or simply mixing methanol and acetonitrile to the appropriate percentages, markedly better selectivity for molecules that differ in degree of unsaturation, position of double bonds, and even electron withdrawing groups within the carbon framework, can be achieved. An added benefit is the increased sensitivity for mass spectrometers due to the higher retention obtained when using mixed methanol and acetonitrile organics. The versatility of a Biphenyl column makes it an excellent tool for the practicing method developer—excellent retention and highly tunable selectivity—an ideal column for column switching systems.

#### Conclusion

Recent advancements in column chemistry offer a simple, cost-effective strategy for strengthening your analytical resources. Although we naturally concentrate on the large capitol expenditures, novel stationary phases can make the chromatographic column a considerable asset. A column specifically designed for optimal chromatography, like Restek's Biphenyl column, can significantly increase the impact of your existing UHPLC and LC/MS resources. Longer instrumentation lifetime, higher sample throughput, more cost-effective analytical procedures, all can be obtained from a minimal investment in novel column chemistry.

[1] R.E. Majors, LC-GC July (2003) 2.

#### RELATED SEARCHES

[reversed phase](#), [biphenyl](#), [RPLC](#), [UHPLC](#)



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## How do small particle size columns increase sample throughput?

### Learning Links

by Rick Lake, Pharmaceutical Innovations Chemist

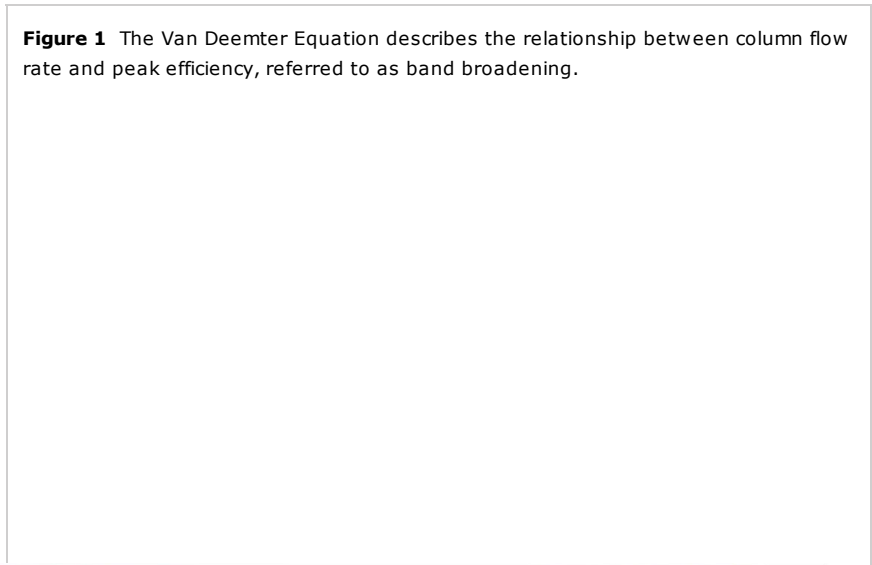
The Van Deemter equation is an empirical formula describing the relationship between plate height ( $H$ , the length needed for one theoretical plate) which is a measure of column efficiency, and linear velocity ( $\mu$ ) (Figure 1). Smaller plate height values corresponds to greater peak efficiencies, as more plates, or analyte partitioning, can occur over a fixed length of column.

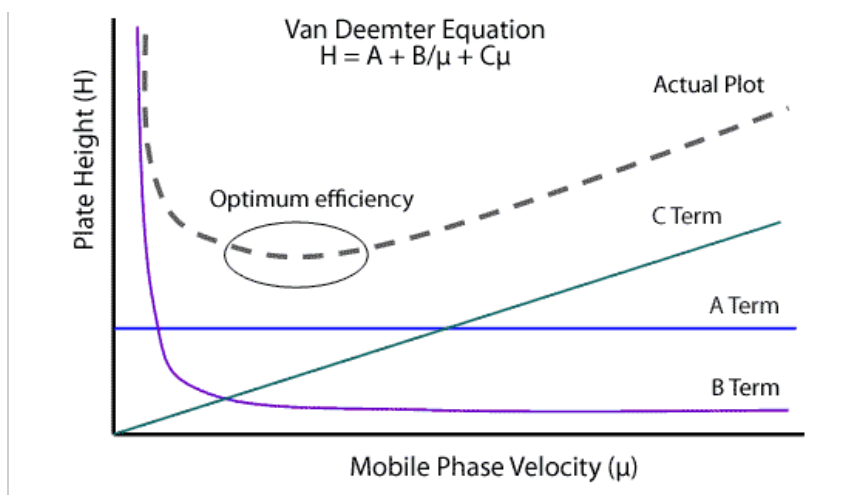
The Van Deemter equation is governed by three cumulative terms: (A) eddy diffusion, (B) longitudinal diffusion, and (C) mass transfer. A loss in peak efficiency can be observed as a wider analyte band, and therefore, these three terms can also be viewed as factors that contribute to band broadening. **Figure 1** illustrates the effect of these terms, both individually and cumulatively. Eddy diffusion, the A term, is caused by a turbulence in the solute flow path and is mainly unaffected by flow rate. Longitudinal diffusion, the B, or difference, term, is the movement of an analyte molecule outward from the center to the edges of its band. Higher column velocities will limit this outward distribution, keeping the band tighter. Mass transfer, the C term, is the movement of analyte, or transfer of its mass, between the mobile and stationary phases. Through this type of diffusion, increased flows have been observed to widen analyte bands, or lower peak efficiencies.

Decreasing particle size has been observed to limit the effect of flow rate on peak efficiency—smaller particles have shorter diffusion path lengths, allowing a solute to travel in and out of the particle faster. Therefore the analyte spends less time inside the particle where peak diffusion can occur. **Figure 2** illustrates the Van Deemter plots for various particle sizes. We notice that as the particle size decreases, the curve becomes flatter, or less affected by higher column flow rates. Smaller particle sizes yield better overall efficiencies, or less peak dispersion, across a much wider range of usable flow rates.

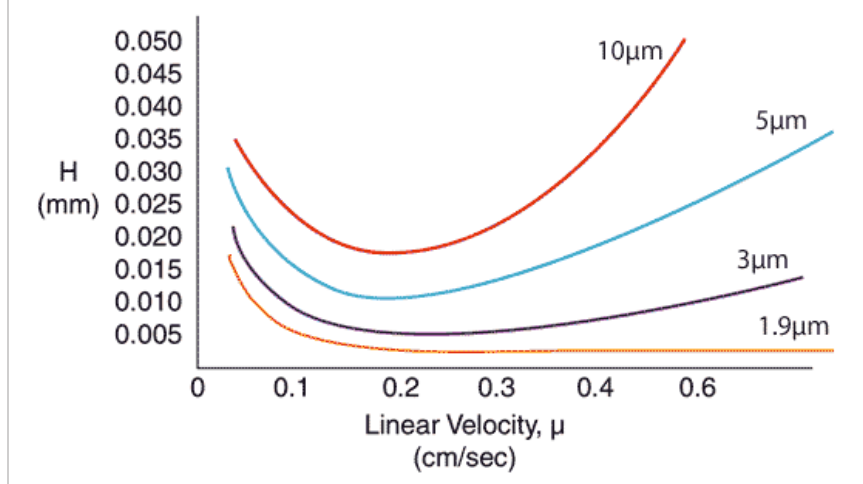
If we look at an empirically determined Van Deemter plot of efficiency versus flow rate, when using a 1.9 $\mu$ m particle size Pinnacle™ DB column (**Figure 3**), the benefit is apparent—column efficiency does not diminish when flow rate increases, as denoted by the relatively flat slope of the curve. Peak efficiency was comparable even when the flow was increased to 1mL/min. This illustrates the most considerable affect that small particles have on chromatographic separations—a much wider range of usable flow rates translates into significantly faster analysis times. This benefit, coupled with a shorter column length needed for similar resolution, allows much higher sample throughput, without the compromising the chromatographic quality of the analytical method.

**Figure 1** The Van Deemter Equation describes the relationship between column flow rate and peak efficiency, referred to as band broadening.

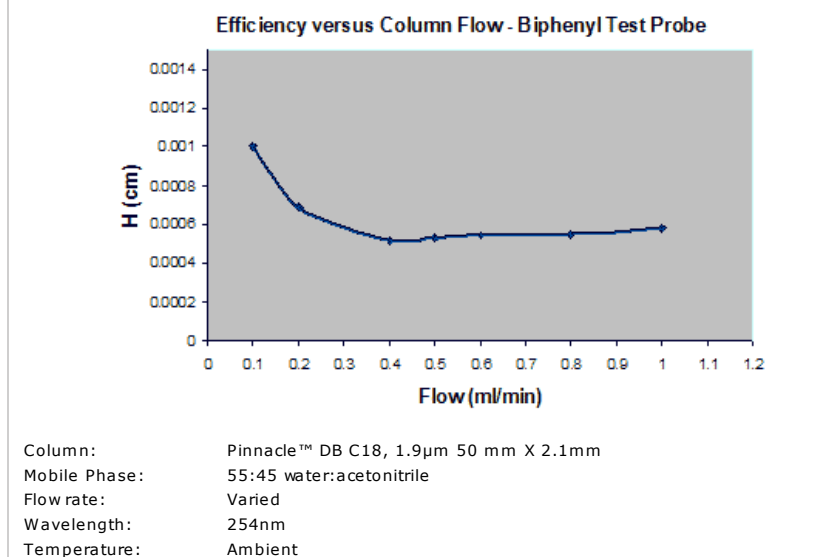




**Figure 2** Smaller particle sizes yield higher overall peak efficiencies and a much wider range of usable flow rates.



**Figure 3** An empirically determined Van Deemter plot shows that column efficiency does not diminish as flow rate increases on a 1.9 $\mu$ m particle size Pinnacle™ DB column—significantly reducing analysis time and increasing sample throughput.



## RELATED SEARCHES

[Van Deemter equation](#), [plate height](#), [eddy diffusion](#), [longitudinal diffusion](#), [mass transfer](#), [efficiency](#), [pinnacle](#)



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## How do intrinsically base-deactivated phases work?

By Rick Lake, Pharmaceutical Innovations Chemist

Analyzing basic compounds can be somewhat troublesome on traditional alkyl stationary phases, namely conventional C18 columns. This is largely due to the interaction of analyte molecules with silanol groups present on the silica surface. To better understand the workings of silanol interactions, it is important to consider the composition of the support material. Silica is the most commonly used support in the production of HPLC columns, mainly because it is well-suited to high-pressure chromatographic separations, giving high efficiencies and good reproducibility. Silica offers bed and pressure stability and is highly porous, which ultimately gives rise to its large surface area, increased bonding capacity and high peak efficiencies. Silica also possesses widely-studied and effective bonding chemistries, making possible diverse analyte selectivities through a wide variety of bonded stationary phases.

If we consider, however, the chemical structure of the silica surface, it is very hydrophilic, acidic, and structurally, is comprised of various forms of silanol groups (Si-OH groups). It is not possible to effectively bond, or attach a stationary phase, to the entire silica surface. Ultimately, it is the surface of the silica particle, or the free silanol groups that causes a majority of the tailing effect of basic compounds. Basic analytes often exhibit tailing on these hydrophobic, reversed phase packings. This is mainly due to the interaction of the protonated form of the base with silanol groups on the silica surface. As this occurs, the molecules undergoing these brief interactions lag behind the main peak band, causing an elongated distribution on the latter half of the peak, or a "tail".

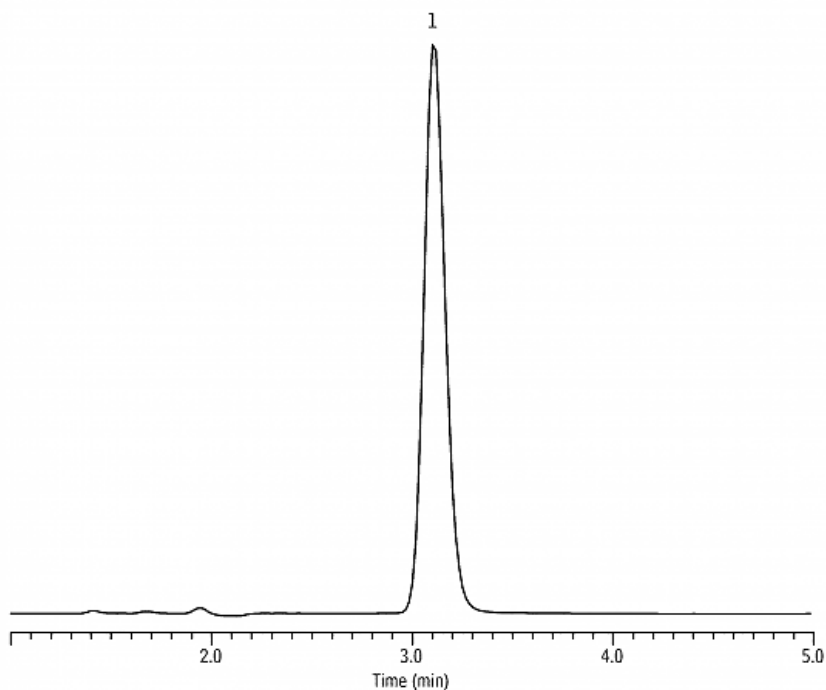
For this reason, column manufacturers have created techniques for limiting the interaction of basic analytes with the surface silica. One very effective means of limiting silanol activity is by bonding a polar group, within, or intrinsic to, the hydrocarbon bonded phase. This bonding chemistry, called intrinsically base deactivated, or IBD, employs either an electrostatic barrier or polar shielding to prevent analytes from interacting with surface silanol groups. The end result is an alkyl stationary phase specifically designed to create optimum peak shape for basic compounds (Figure 1).

**Figure 1** Ultra IBD offers more flexibility in method development giving excellent peak shape for highly basic compounds — even without mobile phase modifiers and regardless of mobile phase pH.

**Mobile phase pH 3 USP tailing 1.10**

**Peaks**  
1. Amitriptyline





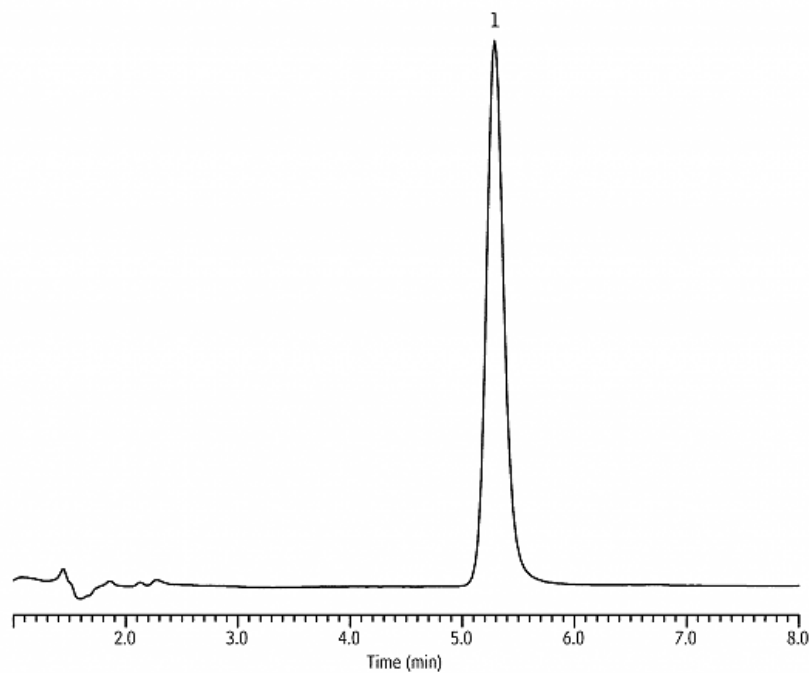
LC\_PH0443

**Column** Ultra IBD (cat.# 9175565)  
 Dimensions: 150 mm x 4.6 mm ID  
 Particle Size: 5  $\mu$ m  
 Pore Size: 100 Å  
 Temp.: ambient  
**Sample**  
 Diluent: mobile phase  
 Conc.: ~100  $\mu$ g/mL  
 Inj. Vol.: 10  $\mu$ L  
**Mobile Phase** 20mM potassium phosphate (pH 3):methanol (10:90)  
 Flow: 1.0 mL/min  
**Detector** UV/Vis @ 254 nm

Mobile phase pH 7 USP tailing 1.13

**Peaks**

1. Amitriptyline



LC\_PH0444

**Column** Ultra IBD (cat.# 9175565)  
 Dimensions: 150 mm x 4.6 mm ID  
 Particle Size: 5  $\mu$ m  
 Pore Size: 100 Å

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Diluent:	mobile phase
Conc.:	~100 µg/mL
Inj. Vol.:	10 µL
<b>Mobile Phase</b>	20mM potassium phosphate (pH 7):methanol (10:90)
Flow:	1.0 mL/min
<b>Detector</b>	UV/Vis @ 254 nm

**RELATED SEARCHES**

[basic](#), [basic compounds](#), [ph3](#), [ph 3](#), [ultra ibd](#), [amitriptyline](#), [ph 7](#), [ph7](#)



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# How Column Inertness Improves the Chromatography of Basic Compounds

*Not all column deactivations are appropriate for analyzing basic compounds. Here we demonstrate the effect of column inertness on peak shape, and discuss its role in improving method accuracy, sensitivity, and development time.*

Basic compounds are often problematic to analyze by gas chromatography. Poor peak symmetry, or tailing, is common and can lead to inconsistent integration and calibrations, as well as losses in sensitivity, due to lower signal-to-noise ratios. In both GC and LC alike, peak tailing of basic compounds can be largely attributed to silanol activity, through an adsorption mechanism where the solutes interact with the exposed silica support in LC or the fused silica capillary in GC. In LC, we have the option of modifying our mobile phase with additives, like triethylamine (TEA), that can act to limit the unwanted silanol interaction. In GC, however, we do not have this option and this puts greater importance on choosing an inert column, such as an Rxi® column, which has a balanced deactivation that is effective toward both basic and acidic compounds. In this article we will discuss the relationship between inertness and system activity, illustrate the effect of improper column deactivation with an example for basic compounds, and discuss options for improving peak symmetry.

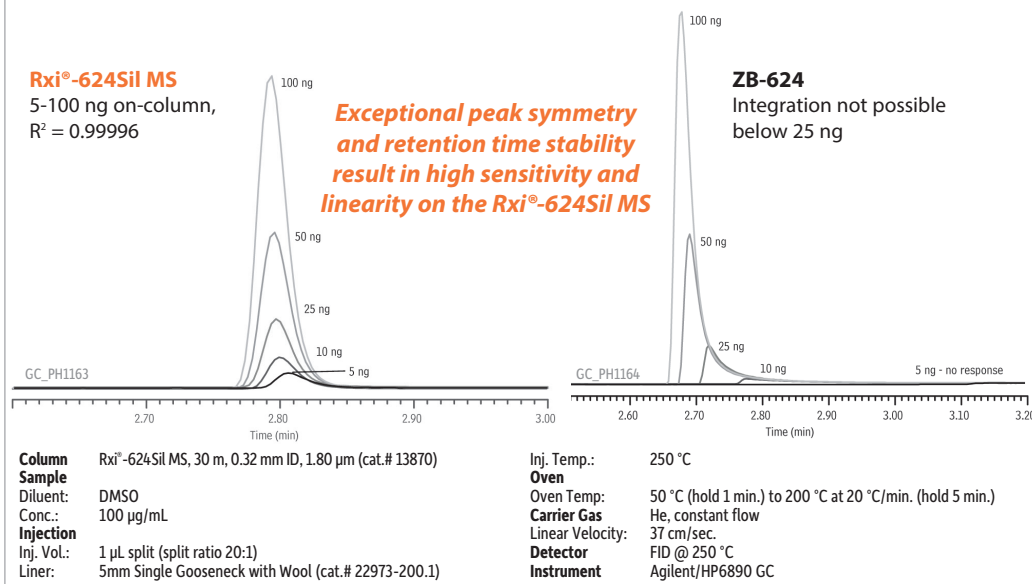
## Understanding Inertness in Gas Chromatography

Glass and metal surfaces are chemically reactive and can undergo solute adsorption, which manifests as chromatographic peak tailing. The entire sample pathway, which includes the liner and the chromatographic column, needs to be inert. Deactivation is simply a means by which column manufacturers treat the sample pathway to eliminate unwanted chemical interaction, which may be more practically viewed as imparting inertness. Capillary column deactivation involves treating the fused silica and creating polymers that limit the residual silanol activity that is the basis for the tailing of basic compounds. Not all deactivation processes are the same, however, and most are tailored to specific solute characteristics. Every manufacturer defines a chemical process, or deactivation technology, for deactivating their capillary columns and liners, and this may vary by column line and manufacturer.

## Diagnosing System Activity

The tailing of basic compounds is certainly familiar to the pharmaceutical researcher as amines are commonly found on active pharmaceutical ingredients and impurities. When troubleshooting our chromatography, improper column deactivation can be easily diagnosed. Peak tailing as a result of solute adsorption is mass dependent. As mass (sample load) increases, tailing becomes less prominent. The ZB-624 column tested in Figure 1 illustrates this point. As the isopropylamine mass on-column drops, tailing

**Figure 1** Rxi®-624Sil MS columns are more inert than ZB-624 columns, resulting in more symmetric peaks that give methods greater accuracy, sensitivity, and reproducibility.



increases and the retention time shifts to the right. If we consider what is occurring here, this chromatography makes complete sense. As mass on-column increases, the amount of solute that is adsorbed is lower in relation to the total solute mass making tailing less apparent. Also, an adsorbed molecule is retained longer, so as the amount of solute adsorbed relative to the total analyte mass increases, i.e. lower concentrations, the retention time shifts to the right.

Evaluating peak symmetry relative to analyte activity can also be used to detect system activity. Since tailing in this case is based upon adsorption, more reactive analytes will show greater tailing. In this example, we analyzed primary, secondary, and tertiary amines—*isopropylamine*, *diethylamine* and *triethylamine*—with analytical conditions set to eliminate the contributions of injection port activity. The ZB-624 peaks in Figure 2 show exactly what we would expect to see from tailing attributed to solute adsorption. The primary amine is the most reactive and therefore exhibits the greatest degree of peak tailing, while the tertiary amine, the weakest in reactivity, remains symmetric throughout these analyses.

#### Options for Reducing Peak Tailing

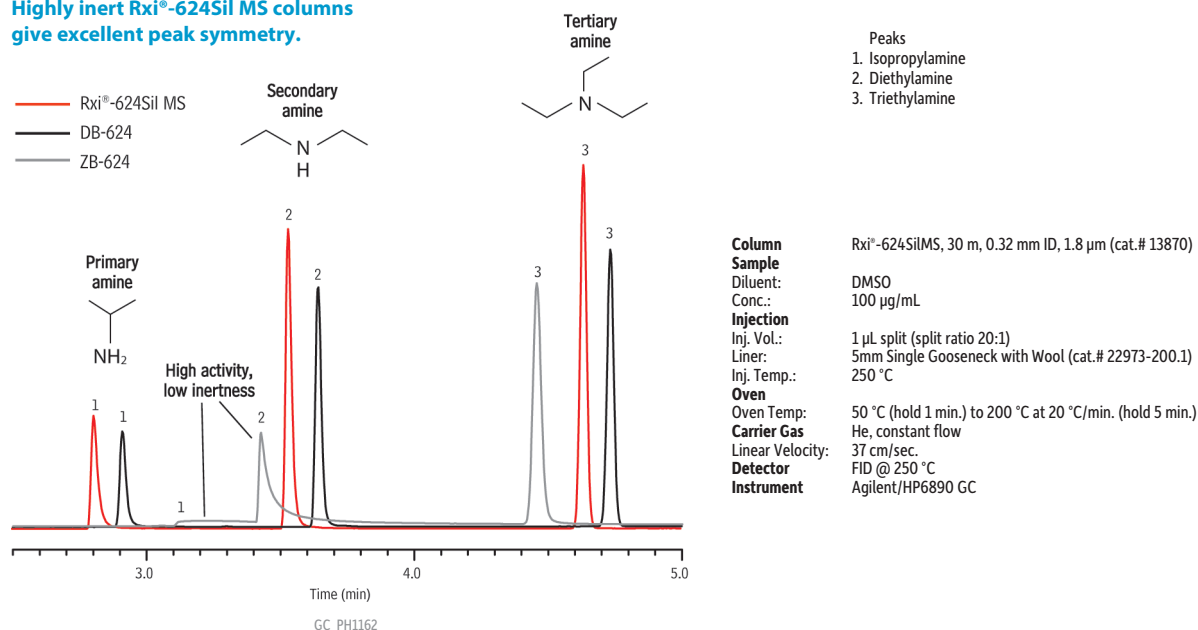
Once we have properly diagnosed system activity, there are certain steps we can take to minimize the deleterious effects on method performance. Since we know that activity is mass dependent, we can simply increase sample load to reduce the effect of improper deactivation. As the mass of solute increases, tailing is less problematic (especially considering that the signal increases while the USP tailing is taken at a consistent 5% peak height). However, increasing sample mass is not always practical or desirable, as it creates a situation where sensitivity and trace analysis is jeopardized. In addition, increased sample loads can contribute to faster column degradation. We can also derivatize compounds into a less reactive state. However, this practice can be lengthy and add uncertainty to methods (derivatization efficiency), so its utility is limited. One simple, easy way to eliminate activity is to consider column inertness. The analytical differences in column inertness can be dramatic. Better sensitivity, linearity, and resolution can all be attributed to column inertness and result in faster, more reliable method development.

#### Balanced Deactivations Make Column Selection Easy

Often, when choosing an analytical capillary column, we focus entirely on the column type and not the column inertness. Specialty deactivations are often very useful when analyzing compounds with similar activity. For example, when analyzing a group of basic compounds, a deactivation specifically targeting basic analytes could be advantageous. We must consider though, this strategy becomes deleterious when we change analyte polarity. Basic deactivation will cause a high degree of peak tailing for acidic compounds, and vice versa. This brings complexity to analytical development when dealing with a wide vari-

**Figure 2** Deactivation processes vary by manufacturer. Rxi® columns provide symmetric peaks for reactive amines, even at trace levels.

Highly inert Rxi®-624Sil MS columns give excellent peak symmetry.

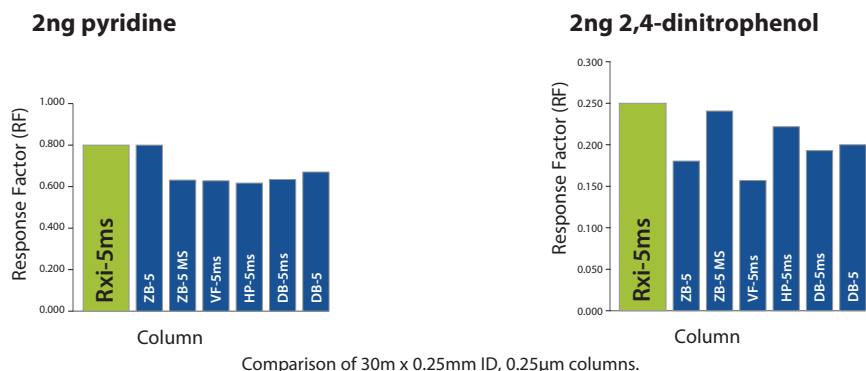


ety of analytes, as is common in pharmaceutical analyses. Rxi® technology was invented to create a practical, comprehensive deactivation that is effective for both acidic and basic compounds (Figure 3). A neutral, or balanced, deactivation as seen in the Rxi® technology, allows for a wider range of compounds—acidic, basic, and polar—to be analyzed with high sensitivity and data quality without the need for changing columns. Rather than spending lab time trying to find the proper column deactivation, researchers can develop methods faster by using columns with a balanced Rxi® deactivation.

### Conclusion

When it comes to peak tailing in GC, we must consider that there are two major areas for solute adsorption, the column and the injection port. Focusing on the column, we can see how choosing a column with an effective deactivation can give us more suitable chromatography—higher sensitivity with decreased sample load, better linearity and accuracy, and increased column lifetimes.

**Figure 3** An Rxi® column gives the best overall performance for both basic and acidic compounds.



### Column Cross-Reference Table

POLARITY	nonpolar	Restek	Phase Composition	Agilent	Varian/Chrompack	SGE	Phenomenex	Machery-Nagel	Supelco
		Rxi-1 ms	100% dimethyl polysiloxane	HP-1ms UI, DB-1ms UI, HP-1, HP-1ms, DB-1 DB-1ms, Ultra-1	VF-1ms CP-Sil 5 CP Sil 5 CB Low Bleed/MS	BP-1	ZB-1 ZB-1ms	Optima-1 Optima-1ms	SPB-1 Equity-1
POLARITY	polar	Rxi-1 HT	100% dimethyl polysiloxane	DB-1HT			ZB-1HT		
		Rxi-5ms	5% diphenyl/ 95% dimethyl polysiloxane	HP-5ms UI, HP-5, HP-5ms, DB-5, Ultra-2	CP-Sil 8 CP Sil 8 CB	BP-5	ZB-5	Optima-5	SPB-5 Equity-5
		Rxi-5Sil MS	5% phenyl, 95% dimethyl arylene siloxane	DB-5ms UI, DB-5ms	VF-5ms CP-Sil 8 CB Low Bleed/MS	BPX-5	ZB-5MS	Optima-5ms	SLB-5
		Rxi-5HT	5% diphenyl/95% dimethyl polysiloxane	DB-5HT	VF-5HT		ZB-5HT		
		Rxi-XLB	arylene/methyl modified polysiloxane	DB-XLB	VF-Xms				
		Rxi-624Sil MS	6% cyanopropylphenyl, 94% dimethyl arylene siloxane	DB-624, HP-624	VF-624ms	BP-624	ZB-624	Optima-624	
		Rxi-35Sil MS	35% phenyl, 65% dimethyl arylene siloxane	DB-35ms	VF-35ms		MR2		
		Rxi-17	50% diphenyl/50% dimethyl polysiloxane	HP-17, DB-17, DB-608	CP-Sil 24 CB		ZB-50		
		Rxi-17Sil MS	50% phenyl, 50% dimethyl arylene siloxane	DB-17ms	VF-17ms	BPX-50			



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## GC Columns

### Rxi®-624Sil MS Columns (fused silica)

(mid polarity Crossbond® silarylene phase; equivalent to 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	1.00	-20 to 300/320°C	20-Meter	13865
0.25mm	1.40	-20 to 300/320°C	30-Meter	13868
0.32mm	1.80	-20 to 300/320°C	30-Meter	13870
0.32mm	1.80	-20 to 300/320°C	60-Meter	13872
0.53mm	3.00	-20 to 280/300°C	30-Meter	13871



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## GC Inlet Liners

### Base-Deactivated Inlet Liners

#### Base Deactivation (BD)

Base deactivation (BD) is ideal for the analysis of basic compounds, such as amines and basic drugs. It prevents analyte adsorption which manifests as either irreproducible results or peak tailing.

For base-deactivated inlet liners, add the corresponding suffix number to the liner catalog number.

qty.	Base-Deactivated Liner		Base-Deactivated Liner w/ Base-Deactivated Wool		Base Deactivated Liner w/CarboFrit	
each	-210.1	addl. cost	-211.1	addl. cost	-229.1	addl. cost
5-pk.	-210.5	addl. cost	-211.5	addl. cost	-229.5	addl. cost
25-pk.	-210.25	addl. cost	-211.25	addl. cost	-229.25	addl. cost



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## Technical Article

# High-Quality Analysis of Pesticides in Cannabis

## 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 cleanup of dirty extracts improves GC inlet and column lifetimes.
- Selective GC columns increase accuracy of pesticide determinations for complex samples.

Over 20 states in the U.S. have legalized the use of recreational or medical cannabis 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 cannabis 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 medical cannabis 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.

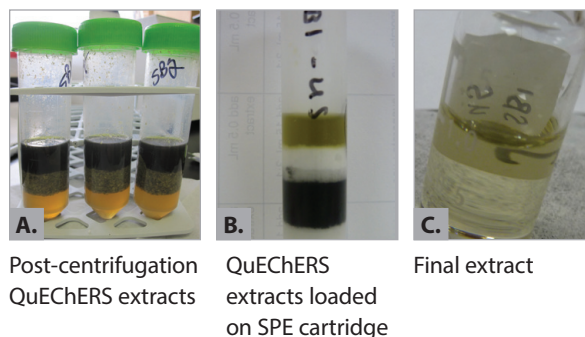
Water was added to finely ground, dry cannabis samples to increase QuEChERS extraction efficiency, especially for more polar pesticides. A vortex mixer was used to shake the solvent

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 Dicolol (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.



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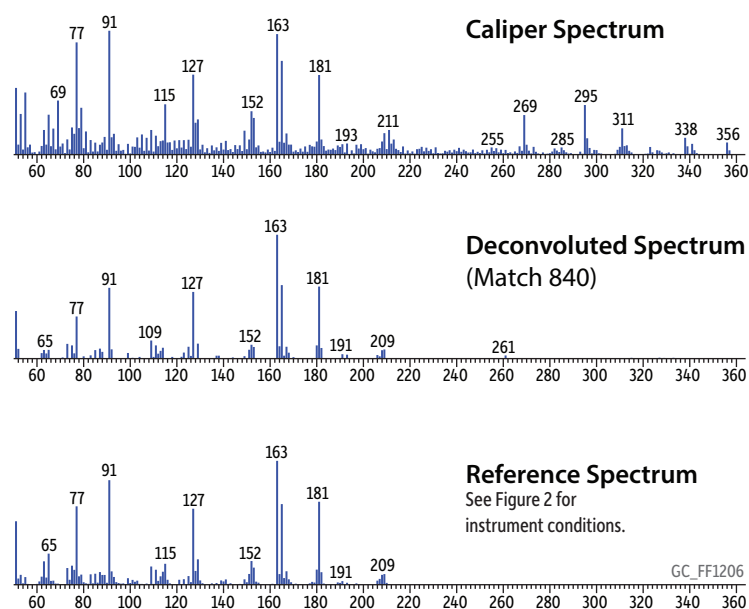
## Orthogonal GC Columns Increase Separation Power for More Accurate Pesticide Results

GCxGC is a powerful multidimensional approach that gives two independent separations in one 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 four 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 cannabis 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.

**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.

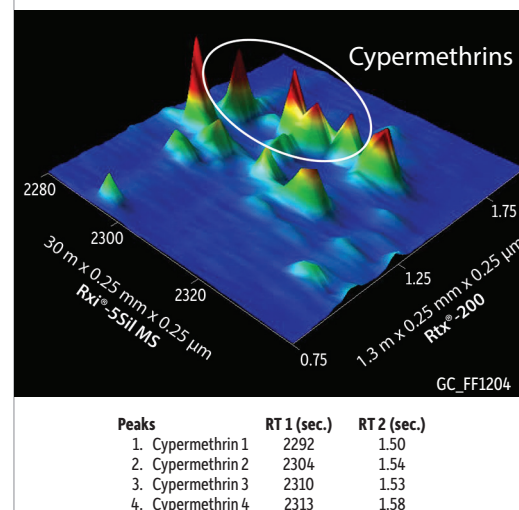
Initially published in *Restek® Advantage*.

**Table I:** Pesticide recoveries for a QuEChERS extract of cannabis 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).



**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](http://www.restek.com) and enter GC\_FF1204 in the search.

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# Fast Screening of Recalled Tylenol® for Tribromoanisole and Related Adulterants

## Using QuEChERS and GC-TOFMS

- Rapid sample preparation with QuEChERS improves turnaround time for emergency response analysis situations.
- Prepackaged QuEChERS extraction salts and snap-and-shoot standards reduce human error and save time.
- Rugged, inert, thermally stable Rxi®-5Sil MS column extends applicability to acids, bases, and higher molecular weight adulterants.

### Introduction

The recent recall of Tylenol® pain reliever and other related products highlights the need for simple, quick sample preparation and a comprehensive analytical method for adulterants in consumer products. The rush to examine a multitude of samples in a short period of time is a common scenario for potential recalls, especially when a contaminant is found in a given product and rapid determinations need to be made to assess how widespread the problem may be.

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample preparation approach, originally developed to prepare fruit and vegetable samples for pesticide residue analysis, is being adopted for other applications and may be useful when rapid screening methods are required. QuEChERS employs a simple solvent shake and centrifugation step, with an optional dispersive solid phase extraction (dSPE) cleanup. In addition to being quick and easy, the use of acetonitrile in QuEChERS allows compounds containing a wide variety of chemical functionalities to be extracted, which is very important when trying to isolate an unknown adulterant. The resulting extract is appropriate for both GC/MS and LC/MS work.

The utility of the QuEChERS method is illustrated here using the aforementioned Tylenol® example, showing the applicability to this problem and, by extension, to others like it. This particular recall was due to the presence of 2,4,6-tribromoanisole (TBA) causing a musty smell in the product and, in some cases, nausea in the consumer [1]. TBA is a known breakdown product of 2,4,6-tribromophenol (TBP), which is a fumigant used on shipping pallets; TBA production occurs through a process actuated by a fungus, *Paecilomyces variotii* [2]. TBA is a common and undesirable odorant in the winemaking industry where it and similar compounds (e.g. trichloroanisole) create a situation known as cork taint [3].

This work demonstrates the potential applicability of QuEChERS sample preparation and GC-TOFMS analysis to screening methods for anisole contaminants. Advantages of methods developed based on QuEChERS and GC-TOFMS may include rapid sample screening and definitive identifications in the presence of significant amounts of matrix.

### Procedure

TBA, TBP, 2,3,4,5-tetrachloroanisole, and pentachloroanisole were spiked into ground up Tylenol® caplets at two different concentrations and extracted using QuEChERS. Several cleanup procedures were performed for comparison and GC analysis was conducted using a sensitive, full mass-range time-of-flight MS.

#### Sample Wetting and Fortification

A bottle of recalled Tylenol® Extra Strength caplets was used for this work, although no odor of TBA was detected. Multiple caplets were ground to a fine powder using a Bamix® Mono Hand Mixer with dry grinder attachment. 1.2 g of powder, equivalent to 2 caplets (500 mg acetaminophen each) was wetted with 9 mL organic-free water for each sample for extraction. After shaking to mix well, wetted powders were fortified as follows; note that spike levels are expressed relative to approximated amount of active ingredient, not formulated product.

- **Unspiked Tylenol®** 100 µL of QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) containing PCBs 18, 28, and 52 (50 µg/mL each); triphenylphosphate (20 µg/mL); tris-(1,3-dichloroisopropyl)phosphate (50 µg/mL); and triphenylmethane (10 µg/mL).
- **~1,000 ng/g spiked Tylenol® (2 samples)** 5 µL of Custom Anisoles Standard #1 (cat.# 564667) containing 2,4,6-tribromoanisole, 2,3,4,5-tetrachloroanisole, and pentachloroanisole at 200 µg/mL each in methanol. 5 µL of Acid Surrogate Mix (cat.# 31025) containing 2,4,6-tribromophenol, 2-fluorophenol, and phenol-d6, diluted to 200 µg/mL in methanol. 100 µL of QuEChERS Internal Standard Mix for GC/MS Analysis.
- **~100 ng/g spiked Tylenol®** 5 µL of Custom Anisoles Standard #1; 5 µL of Acid Surrogate Mix diluted to 20 µg/mL; 100 µL of QuEChERS Internal Standard Mix for GC/MS Analysis.



After fortification, each sample was allowed to soak for 1 hour prior to QuEChERS extraction. Originally the QuEChERS method was developed for high aqueous content fruits and vegetables. Here we used a reduced amount of material and sample wetting in order to increase extraction efficiency for a dry powder.

#### *QuEChERS Extraction*

The EN 15662 QuEChERS method was used for sample extraction [4]. 10 mL of acetonitrile was added to a wet sample. After a 1 minute shake, Q-sep™ Q110 buffering extraction salts (cat.# 26213, 4 g MgSO<sub>4</sub>, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate) were added. Following another 1 minute shake, the sample was centrifuged for 5 minutes at 3,000 U/min. with a Q-sep™ 3000 centrifuge (cat.# 26230).

#### *Extract Cleanup*

Four dispersive solid phase extraction methods (dSPE) were compared. For each, 1 mL portions of QuEChERS extracts were added to tubes containing drying agent and different sorbents such as primary secondary amine (PSA), C18, and graphitized carbon black (GCB) as shown below. The tubes were shaken for 2 minutes and then centrifuged for 5 minutes in the Q-sep™ 3000 centrifuge. The resulting final extracts were then analyzed with GC-TOFMS.

- Q210 (cat.# 26215): 150 mg MgSO<sub>4</sub>, 25 mg PSA
- Q251 (cat.# 26125): 150 mg MgSO<sub>4</sub>, 50 mg PSA, 50 mg C18
- Q252 (cat.# 26219): 150 mg MgSO<sub>4</sub>, 50 mg PSA, 50 mg C18, 50 mg GCB
- Custom dSPE tube: 150 mg MgSO<sub>4</sub>, 50 mg PSA, 50 mg C18, 7.5 mg GCB

#### *GC-TOFMS*

A LECO Pegasus® III GC-TOFMS instrument was used and all data were processed with LECO ChromaTOF® software. Gas chromatography was performed using an Rxi®-5Sil MS column (30 m x 0.25 mm x 0.25 µm, cat.# 13623) with a constant flow of helium at 1.2 mL/min. (40 cm/sec. at 90°C). 1 µL fast autosampler splitless injections were made into a 4mm single gooseneck liner with wool (cat.# 22405) at 250°C. The purge valve time was 60 seconds.

The GC oven program was 90 °C (1 minute), 4 °C/min. to 310 °C (2 minutes). Total run time was 58 minutes.

Electron ionization at 70 eV was used with a source temperature of 225°C. Data acquisition was from 45 to 550 amu at a rate of 5 spectra/sec.

#### *Calibration and Quantification with Matrix-Matched Standards*

Matrix-matched standards were prepared at 100 pg/µL and 10 pg/µL, as these are the expected final concentrations in extracts for Tylenol® spikes (assuming 100% recoveries for the 1,000 and 100 ng/g spikes, respectively). Matrix-matched standards were prepared by adding standard solution to the final extract from a control sample, which had no measurable amounts of the compounds of interest. Actual recoveries were calculated after quantification from one-point calibration in ChromaTOF®. The internal standard method of quantification was employed using PCB 52.

#### **Results**

The concentrations used for spikes in this case were 1,000 and 100 ng/g relative to active ingredient in the starting caplet material (estimated using labeled value). Using QuEChERS combined with GC-TOFMS, modest recoveries of all compounds were realized as can be seen in Table I. In addition, results for duplicate extracts and cleanups for 1,000 ng/g spikes, using either Q210 dSPE tubes or the custom dSPE tubes, were relatively close for each analyte. Although the spiked concentrations are higher than the odor threshold expected for an end product such as Tylenol® (TBA's odor threshold is extremely low, 0.008-0.03 ppt in water and 2-6 ppt in wine [5]), the QuEChERS approach with GC-TOFMS provides a useful technique for screening of contamination at potential levels of health concern, moderate adulteration, and for analyzing source materials such as wood pallets, for contaminants. QuEChERS can produce extracts for up to 24 samples, ready for GC or LC analysis, in less than 60 minutes, a speed conducive to the pressure of responding to a consumer product adulteration issue. In addition, the multi-compound extraction capability of the QuEChERS acetonitrile solvent offers a better chance of isolating potential adulterants from any matrix.

**Table I** Percent recoveries of potential adulterants from QuEChERS extractions: comparison of various dSPE cleanup procedures. (All samples are 1,000 ng/g, unless otherwise noted.)

Compound	RT (sec.)	Q210		Q251		Q252		CustomdSPE	Extract
		Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	1,000 ng/g	100 ng/g
2,4,6-Tribromoanisole	1097.82	82	56	62	68	73	68	59	51
2,4,6-Tribromophenol	1133.62	55	60	40	49	66	53	63	110
2,3,4,5-Tetrachloroanisole	1162.22	71	63	64	64	75	63	67	70
Pentachloroanisole	1256.82	70	67	64	70	71	61	65	60
PCB 52 (IS)	1611.02								

Q210 = 150 mg MgSO<sub>4</sub>, 25 mg PSA

Q251 = 150 mg MgSO<sub>4</sub>, 50 mg PSA, 50 mg C18

Q252 = 150 mg MgSO<sub>4</sub>, 50 mg PSA, 50 mg C18, 50 mg GCB

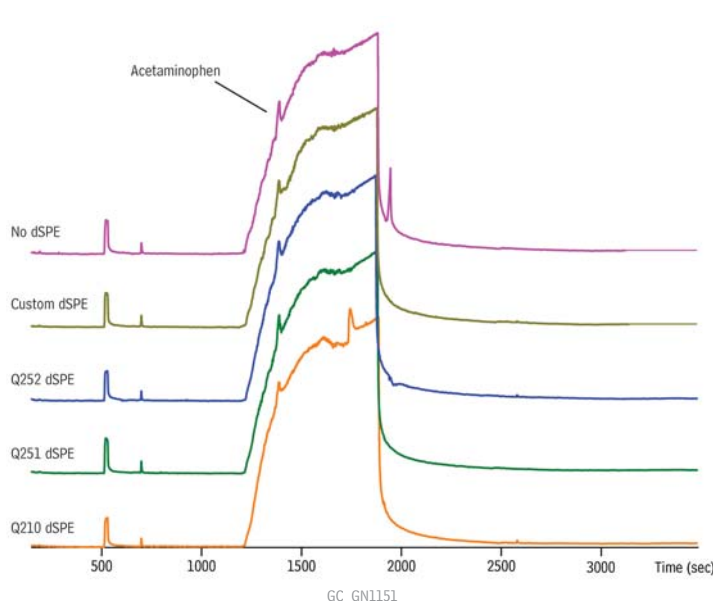
Custom = 150 mg MgSO<sub>4</sub>, 50 mg PSA, 50 mg C18, 7.5 mg GCB

Extract = extraction only, no clean up step was performed

The original QuEChERS approach for fruits and vegetables was developed with a novel dSPE cleanup procedure where an extract is shaken with loose sorbent material (e.g. primary secondary amine, C18, graphitized carbon black) to remove matrix coextractives like fatty acids, lipids, and pigments, that might interfere with targeted residues during instrumental analysis. Although we tried dSPE here, it is less appropriate in this application for two reasons: (1) In a true unknown adulterant situation, sorbents, especially PSA and GCB, might actually remove the adulterant from the extract, in addition to matrix interferences, leaving the adulterant undetected during instrumental analysis. (2) The gross amount of acetaminophen in the extract greatly exceeds the capacity of the dSPE sorbent, which is typically on the order of the 25-50 mg per mL extract.

Due to the overwhelming concentration of acetaminophen in the caplet powder extracts, dSPE cleanup was largely ineffective (Figure 1), but as the acetaminophen was volatile enough to chromatograph, it was not critical to remove it to prevent deposition in the injector and column. Elimination of the dSPE step did not noticeably improve, or degrade, the recovery results for TBA and TBP, or other components (Table I).

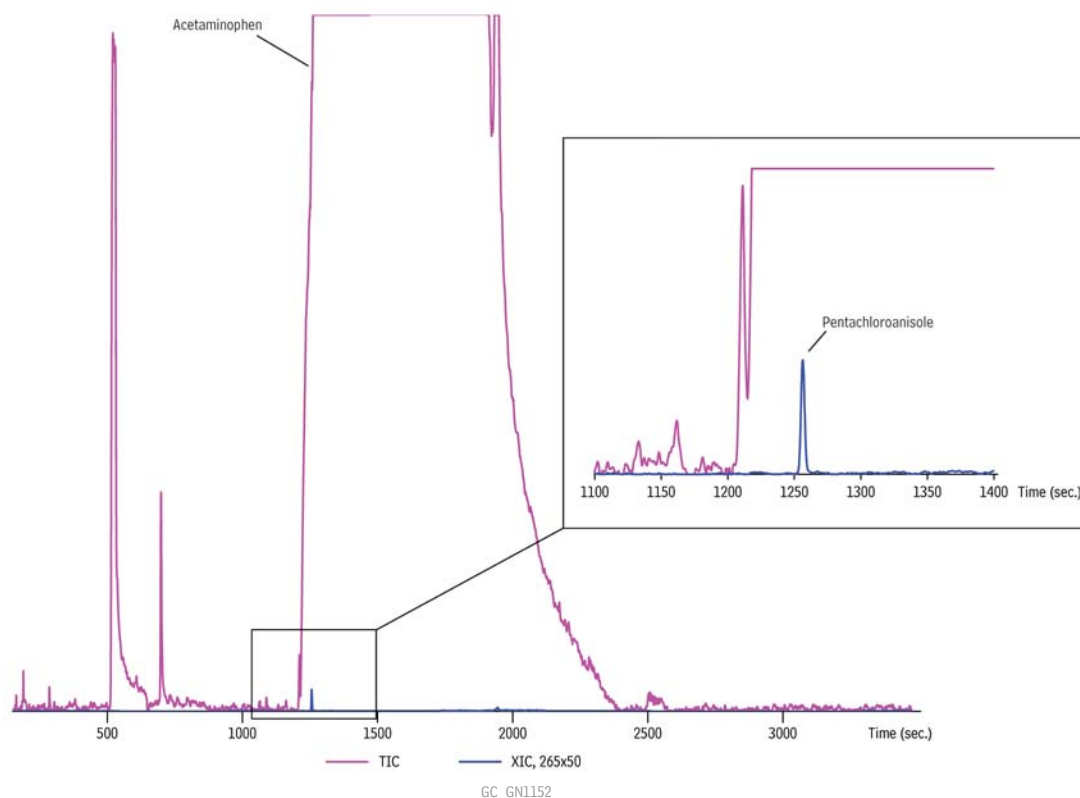
**Figure 1** Chromatograms of QuEChERS extracts of Tylenol® with different dSPE cleanups.



**Column** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)  
**Sample** QuEChERS extracts of Tylenol® spiked with TBA and anisoles  
**Diluent:** Acetonitrile  
**Injection**  
 Inj. Vol.: 1 µL splitless (hold 1 min.)  
 Liner: Gooseneck Splitless (4mm) with Wool (cat.# 22405)  
 Inj. Temp.: 250 °C  
 Purge Flow: 40 mL/min.  
**Oven**  
 Oven Temp: 90 °C (hold 1 min.) to 310 °C at 4 °C/min. (hold 2 min.)  
**Carrier Gas** He, constant flow  
 Flow Rate: 1.2 mL/min.  
 Linear Velocity: 40 cm/sec.  
**Detector** TOFMS  
 Transfer  
 Line Temp.: 290 °C  
 Analyzer Type: TOF  
 Source Temp.: 225 °C  
 Electron Energy: 70 eV  
 Mass Defect: -20 mu/100 u  
 Solvent  
 Delay Time: 2 min.  
 Tune Type: PFTBA  
 Ionization  
 Mode: EI  
 Acquisition  
 Range: 45 to 550 amu  
 Spectral  
 Acquisition Rate: 5 spectra/sec  
**Instrument** LECO Pegasus 4D GCxGC-TOFMS  
**Notes** Q210 = PSA  
 Q251 = PSA and C18  
 Q252 = PSA, C18, and GCB  
 Custom = PSA, C18, and less GCB

One reason to employ dSPE, or another cleanup step, is to remove matrix interferences that can prevent detection of potential adulterants. However, we relied on automated peak find and spectral deconvolution to detect analytes of interest among the overwhelming acetaminophen response. This is particularly evident for pentachloroanisole in the 1,000 ppb spike extract, which eluted well underneath the large acetaminophen peak (Figure 2). The disparity in concentrations is so large that the 265 m/z ion was only visible by magnifying it by 50, yet ChromaTOF® automatically located the peak and produced a deconvoluted spectrum that matched very well with the pentachloroanisole reference spectrum (Figure 3). Although this part of the application was a targeted analysis of TBA, TBP, and other anisoles, to help evaluate QuEChERS extract recoveries for these compounds in a difficult matrix, the peak find and spectral deconvolution algorithms employed here are very useful when looking for unknown contaminants. Pure sample mass spectra lead to better library searching and identification of components.

**Figure 2** Pentachloroanisole was located under the large acetaminophen peak using an automated peak find routine. (1,000 ng/g; QuEChERS extraction only, no dSPE).



**Column** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)  
**Sample** QuEChERS extracts of Tylenol® spiked with TBA and anisoles  
**Diluent:** Acetonitrile  
**Injection**  
 Inj. Vol.: 1 µL splitless (hold 1 min.)  
 Liner: Gooseneck Splitless (4mm) with Wool (cat.# 22405)  
 Inj. Temp.: 250 °C  
 Purge Flow: 40 mL/min.  
**Oven**  
 Oven Temp: 90 °C (hold 1 min.) to 310 °C at 4 °C/min. (hold 2 min.)  
**Carrier Gas** He, constant flow  
 Flow Rate: 1.2 mL/min.  
 Linear Velocity: 40 cm/sec.

**Detector** TOFMS  
 Transfer  
 Line Temp.: 290 °C  
 Analyzer Type: TOF  
 Source Temp.: 225 °C  
 Electron Energy: 70 eV  
 Mass Defect: -20 mu/100 u  
 Solvent  
 Delay Time: 2 min.  
 Tune Type: PFTBA  
 Ionization Mode: EI  
 Acquisition  
 Range: 45 to 550 amu  
 Spectral  
 Acquisition  
 Rate: 5 spectra/sec  
**Instrument** LECO Pegasus 4D GCxGC-TOFMS

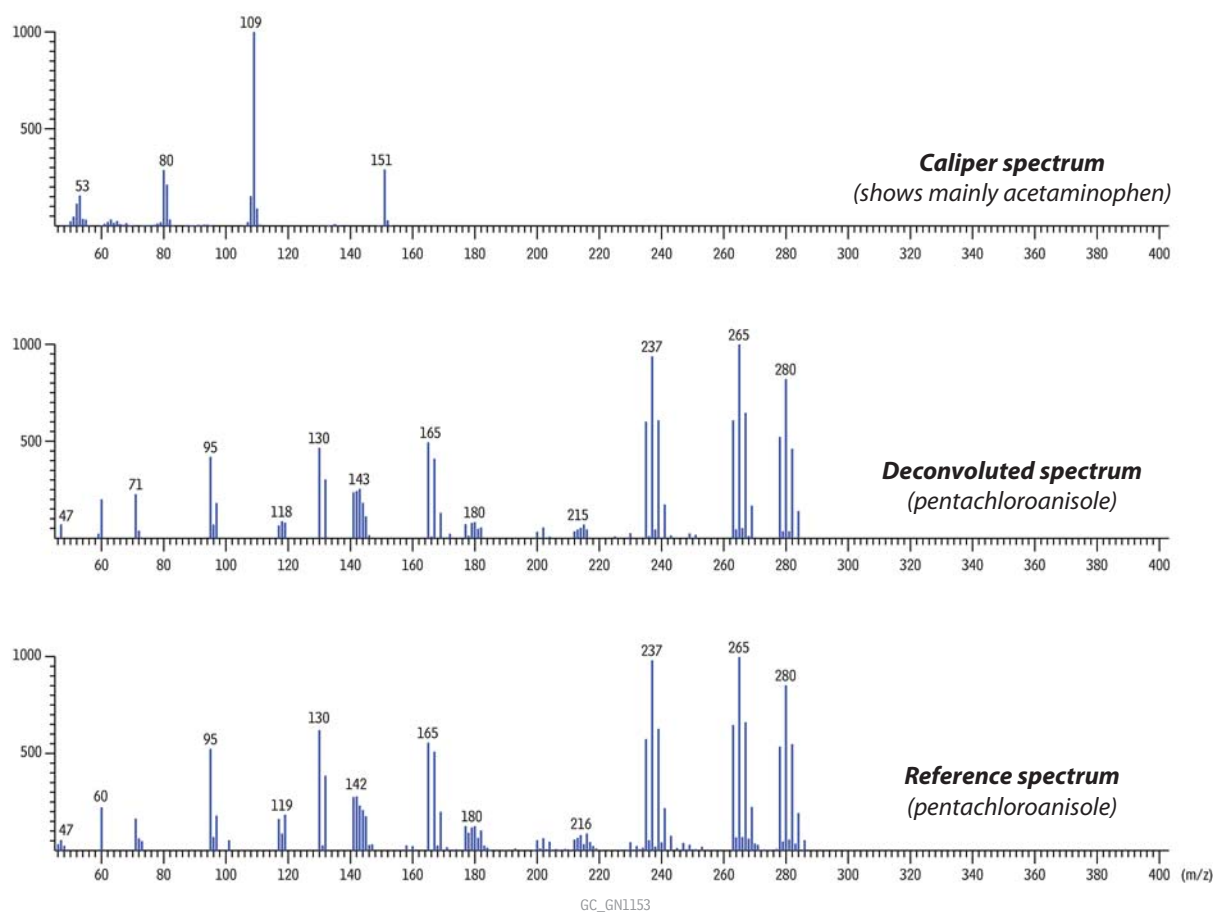
## Conclusions

Shown here is a QuEChERS multi-compound extraction method that rapidly produces samples for GC or LC analysis in consumer product adulteration cases. QuEChERS is simple, efficient, and uses little solvent compared to other extraction methods. QuEChERS and GC with a sensitive, full mass-range TOFMS is a powerful approach to identifying potential adulterants in consumer products.

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**Figure 3** The caliper spectrum taken at the peak apex of pentachloroanisole is representative of the overwhelming acetaminophen peak, but TOFMS allows spectral deconvolution to produce a sample spectrum that matches well with the reference spectrum.



Column: Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25  $\mu$ m (cat.# 13623)  
 Sample: QuEChERS extracts of Tylenol® spiked with TBA and anisoles  
 Diluent: Acetonitrile  
 Injection: 1  $\mu$ L splitless (hold 1 min.)  
 Inj. Vol.: 1  $\mu$ L splitless (hold 1 min.)  
 Liner: Gooseneck Splitless (4mm) with Wool (cat.# 22405)  
 Inj. Temp.: 250 °C  
 Purge Flow: 40 mL/min.  
 Oven: 90 °C (hold 1 min.) to 310 °C at 4 °C/min. (hold 2 min.)  
 Carrier Gas: He, constant flow  
 Flow Rate: 1.2 mL/min.  
 Linear Velocity: 40 cm/sec.

Detector: TOFMS  
 Transfer Line Temp.: 290 °C  
 Analyzer Type: TOF  
 Source Temp.: 225 °C  
 Electron Energy: 70 eV  
 Mass Defect: -20 mu/100 u  
 Solvent Delay Time: 2 min.  
 Tune Type: PFTBA  
 Ionization Mode: EI  
 Acquisition Range: 45 to 550 amu  
 Spectral Acquisition Rate: 5  
 Instrument: LECO Pegasus 4D GCxGC-TOFMS

GC\_GN1153

## Capillary Columns

### Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (μm)	temp. limits	length	qty.	cat. #
0.10mm	0.10μm	-60 to 330/350°C	10m	ea.	43601
0.18mm	0.18μm	-60 to 330/350°C	20m	ea.	43602
0.18mm	0.36μm	-60 to 330/350°C	20m	ea.	43604
0.25mm	0.10μm	-60 to 330/350°C	15m	ea.	13605
0.25mm	0.10μm	-60 to 330/350°C	30m	ea.	13608
0.25mm	0.25μm	-60 to 330/350°C	15m	ea.	13620
0.25mm	0.25μm	-60 to 330/350°C	30m	ea.	13623
0.25mm	0.25μm	-60 to 330/350°C	30m	6-pk.	13623-600
0.25mm	0.25μm	-60 to 330/350°C	60m	ea.	13626
0.25mm	0.50μm	-60 to 330/350°C	15m	ea.	13635
0.25mm	0.50μm	-60 to 330/350°C	30m	ea.	13638
0.25mm	1.00μm	-60 to 325/350°C	15m	ea.	13650
0.25mm	1.00μm	-60 to 325/350°C	30m	ea.	13653
0.25mm	1.00μm	-60 to 330/350°C	60m	ea.	13697
0.32mm	0.25μm	-60 to 330/350°C	15m	ea.	13621
0.32mm	0.25μm	-60 to 330/350°C	30m	ea.	13624
0.32mm	0.50μm	-60 to 330/350°C	30m	ea.	13639
0.32mm	1.00μm	-60 to 325/350°C	30m	ea.	13654
0.53mm	1.50μm	-60 to 310/330°C	30m	ea.	13670



### Make the Switch to Rxi® columns!

Experience what Rxi® did for many others

- Lower detection limits
- Better peak shape
- Accurate results

### Rxi®-5Sil MS with Integra-Guard®

- Extend column lifetime.
- Eliminate leaks with a built-in retention gap.
- Inertness verified by isothermal testing.

Description	qty.	cat.#
15m, 0.25mm ID, 0.25μm Rxi-5Sil MS	ea.	13620-127
30m, 0.25mm ID, 0.25μm Rxi-5Sil MS	ea.	13623-124
30m, 0.25mm ID, 0.25μm Rxi-5Sil MS	ea.	13623-127
15m, 0.25mm ID, 0.50μm Rxi-5Sil MS	ea.	13635-124
30m, 0.25mm ID, 0.50μm Rxi-5Sil MS	ea.	13638-124
30m, 0.25mm ID, 0.50μm Rxi-5Sil MS	ea.	13638-127
30m, 0.32mm ID, 0.50μm Rxi-5Sil MS	ea.	13639-125
30m, 0.32mm ID, 1.00μm Rxi-5Sil MS	ea.	13654-125



## 3-IN-1 TECHNOLOGY

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## Q-sep™ QuEChERS Products

### Q-sep™ 3000 Centrifuge

- Meets requirements of AOAC and European QuEChERS methodology.
- Supports 50 mL, 15 mL, and 2 mL centrifuge tubes.
- Small footprint requires less bench space.
- Safe and reliable—UL, CSA, and CE approved, 1-year warranty.

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

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

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



## Q-sep™ QuEChERS Products (cont.)

### Q-sep™ QuEChERS Tubes

for Extraction and Cleanup of Pesticide Residue Samples from Food Products

- Fast, simple sample extraction and cleanup using dSPE.
- Fourfold increases in sample throughput.
- Fourfold decreases in material cost.
- Convenient, ready to use centrifuge tubes with ultra pure, preweighed adsorbent mixes.

Quick, Easy, Cheap, Effective, Rugged, and Safe, the QuEChERS (“catchers”) method, developed by the USDA Eastern Regional Research Center<sup>1</sup>, has become very popular for extraction and cleanup of pesticide residue samples. Our products are available in three centrifuge tube sizes to meet the needs of both extraction and cleanup of a wide variety of sample matrices following various methods.

The researchers developed a simple two-step procedure. First, the homogenized samples are extracted and partitioned, using an organic solvent and salt solution. Then, the supernatant is further extracted and cleaned, using a dispersive SPE technique. Multiple adsorbents are placed in a centrifuge tube, along with the 1 mL of organic solvent and the extracted residues partitioned from step 1. The contents are thoroughly mixed, then centrifuged, producing a clean extract ready for a variety of GC or HPLC analytical techniques.<sup>2</sup> Validation and proficiency data for the QuEChERS method are available for a wide variety of pesticides in several common food matrices at [www.quechers.com](http://www.quechers.com)



Description	Material	Methods	qty.	cat#
Q110 Kit	4g MgSO <sub>4</sub> , 1g NaCl, 1g TSCD, 0.5g DHS with 50mL Centrifuge Tube	European EN 15662	50 packets & 50 tubes	26235
Q110 Packets	4g MgSO <sub>4</sub> , 1g NaCl, 1g TSCD, 0.5g DHS	European EN 15662	50 packets	26236
Q150 Kit	6g MgSO <sub>4</sub> , 1.5g NaOAc with 50mL Centrifuge Tube	AOAC 2007.01	50 packets & 50 tubes	26237
Q150 Packets	6g MgSO <sub>4</sub> , 1.5g NaOAc	AOAC 2007.01	50 packets	26238
Empty 50mL Centrifuge Tube			50-pk.	26239

#### 2mL Micro-Centrifuge Tubes for dSPE (clean-up of 1mL extract)

Q210	150mg MgSO <sub>4</sub> , 25mg PSA	European EN 15662	100-pk.	26215
Q211	150mg MgSO <sub>4</sub> , 25mg PSA, 25mg C18		100-pk.	26216
Q212	150mg MgSO <sub>4</sub> , 25mg PSA, 2.5mg GCB	European EN 15662	100-pk.	26217
Q213	150mg MgSO <sub>4</sub> , 25mg PSA, 7.5mg GCB	European EN 15662	100-pk.	26218
Q250	150mg MgSO <sub>4</sub> , 50mg PSA	AOAC 2007.1	100-pk.	26124
Q251	150mg MgSO <sub>4</sub> , 50mg PSA, 50mg C18	AOAC 2007.1	100-pk.	26125
Q253	150mg MgSO <sub>4</sub> , 50mg PSA, 50mg GCB		100-pk.	26123
Q252	150mg MgSO <sub>4</sub> , 50mg PSA, 50mg C18, 50mg GCB	AOAC 2007.1	100-pk.	26219

#### 15mL Centrifuge Tubes for dSPE (clean-up of 6mL extract)

Q350	1200mg MgSO <sub>4</sub> , 400mg PSA	AOAC 2007.1	50-pk.	26220
Q351	1200mg MgSO <sub>4</sub> , 400mg PSA, 400mg C18	AOAC 2007.1	50-pk.	26221
Q352	1200mg MgSO <sub>4</sub> , 400mg PSA, 400mg C18, 400mg GCB	AOAC 2007.1	50-pk.	26222
Q370	900mg MgSO <sub>4</sub> , 150mg PSA	European EN 15662	50-pk.	26223
Q371	900mg MgSO <sub>4</sub> , 150mg PSA, 15mg GCB	European EN 15662	50-pk.	26224
Q372	900mg MgSO <sub>4</sub> , 150mg PSA, 45mg GCB	European EN 15662	50-pk.	26225
Q373	900mg MgSO <sub>4</sub> , 150mg PSA, 150mg C18		50-pk.	26226
Q374	900mg MgSO <sub>4</sub> , 300mg PSA, 150mg GCB		50-pk.	26126

PSA—primary and secondary amine exchange material  
GCB—graphitized carbon black

References (not available from Restek)

1. Anastassiades, M., S.J. Lehotay, D. Stajnbaher, F.J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and “Dispersive Solid-Phase Extraction” for the Determination of Pesticide Residues in Produce, J AOAC International, 2003, vol 86 no 22, pp 412-431.
2. Schenck, F.J., SPE Cleanup and the Analysis of PPB Levels of Pesticides in Fruits and Vegetables. Florida Pesticide Residue Workshop, 2002.

### Sorbent Guide

Sorbent	Removes
PSA*	sugars, fatty acids, organic acids, anthocyanine pigments
C18	lipids, nonpolar interferences
GCB**	pigments, sterols, nonpolar interferences
*PSA—primary and secondary amine exchange material	
**GCB—graphitized carbon black	



Free sample packs of SPE cartridges are available by adding -248 to the catalog number when you call to place your order.

## Reference Standards

### QuEChERS Standards

- Ready to use for QuEChERS extractions—no dilutions necessary.
- Support for GC and HPLC with MS, MS/MS, and selective detectors.

Pesticide analysis is fast and simple using QuEChERS methods. Use these cost-effective QuEChERS standards for even greater lab efficiency. Standards are compatible with all major methods, including mini-multiresidue, AOAC, and European procedures. Save time with convenient mixes or make your own blend using our full line of single component solutions.

### QuEChERS Quality Control Standards for GC/MS Analysis

Cat. # 33268:  
PCB 138  
PCB 153

Cat. # 33264:  
anthracene

50 µg/mL each in acetonitrile, 5mL/ampul  
cat. # 33268 (ea.)

100 µg/mL in acetonitrile, 5mL/ampul  
cat. # 33264 (ea.)

### QuEChERS Internal Standard Mix for GC/MS Analysis (6 components)

PCB 18	50 µg/mL
PCB 28	50
PCB 52	50
triphenyl phosphate	20
tris-(1,3-dichloroisopropyl)phosphate	50
triphenylmethane	10

In acetonitrile, 5mL/ampul  
cat. # 33267 (ea.)

### Acid Surrogate Mix (4/89 SOW) (3 components)

- Highest concentrations commercially available.
- Convenient 1mL, 5mL, and 10mL package sizes.
- Reduces laboratory cost per sample extract.

2-fluorophenol  
phenol-d6  
2,4,6-tribromophenol

Each	15-pk.	25-pk.
2,000 µg/mL each in methanol, 1mL/ampul 31025	31025.15	31025.25
10,000 µg/mL each in methanol, 1mL/ampul 31063	31063.15	31063.25
10,000 µg/mL each in methanol, 5mL/ampul 31087	31087.15	31087.25
10,000 µg/mL each in methanol, 10mL/ampul 33029	33029.15	33029.25

### QuEChERS Single-Component Reference Standards

Concentration is µg/mL. ACN=acetonitrile

Compound	Solvent	Conc.	cat. # (ea.)
PCB 18 (5mL)	ACN	50	33255
PCB 28 (5mL)	ACN	50	33256
PCB 52 (5mL)	ACN	50	33257
PCB 138 (5mL)	ACN	50	33262
PCB 153 (5mL)	ACN	50	33263
triphenylmethane (5mL)	ACN	10	33260
triphenylphosphate (5mL)	ACN	20	33258
tris(1,3-dichloroisopropyl)phosphate (5mL)	ACN	50	33259

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Lit. Cat.# GNAN1263

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128 (of ~193 ) Pharma CT-Update 2015

## Explaining the Small Particle Advantage

### Faster Sample Throughput on a 1.9µm Pinnacle™ DB Column

by Rick Lake, Pharmaceutical Innovations Chemist

- Faster analyses, uncompromised chromatography using a 1.9µm Pinnacle™ DB small particle HPLC column.
- Narrow particle size distribution ensures consistent, high efficiencies and longer column lifetimes.
- 100% Restek manufactured—from base silica to final packed column—assures quality and reliability.

*Restek is pleased to introduce an exciting new addition to our family of HPLC columns—the 1.9µm Pinnacle™ DB small particle column. Intended for use in ultra-high pressure liquid separations, the 1.9µm Pinnacle™ DB column combines the benefits of a novel technique with the unmatched quality you expect from Restek. From the manufacturing of the base silica through the packing of the column, Restek performs and tightly controls every step in the manufacturing process, guaranteeing ruggedness and reliability. Here we discuss how and why small particle HPLC columns work, and demonstrate the high efficiency, excellent peak symmetry, and fast analysis times that can be achieved on the 1.9µm Pinnacle™ DB column.*

In HPLC column terminology, particle size refers to the mean diameter of the silica spheres used as the support material to which the stationary phase is bonded. Until recently, the practical particle size limit was around 3µm; smaller particle sizes resulted in backpressures above the limit of conventional LC systems. The advent of LC systems capable of handling higher backpressures (>10000psi) now allows chromatographers to realize the benefits of sub-2µm particle size columns. Smaller particles give rise to greater column efficiencies and a wider range of usable flow rates, resulting in better resolution and higher sensitivity with a significantly faster overall analysis time. **Figure 1** and **Table 1** illustrate the excellent peak shape and higher efficiency commonly seen when using a 1.9µm Pinnacle™ DB C18 column, compared to competitive columns.

To demonstrate the substantial gain in sample throughput that is possible on a small particle column, we assayed a series of parabens under identical conditions on both a C18 column with conventional dimensions and on a 1.9µm Pinnacle™ DB C18 column (**Figure 2**). When comparing the two columns, similar resolution was achieved in a much shorter analysis time on the 1.9µm Pinnacle™ DB C18 column. Next, we doubled the flow rate and assayed the same mix. The resolution and peak efficiencies again were comparable, but the analysis time was cut in half. This illustrates the considerable effect that small particles can have on chromatographic separations; a much wider range of usable flow rates translates into significantly faster analysis times—in this case 10-fold faster, with no loss in chromatographic quality.

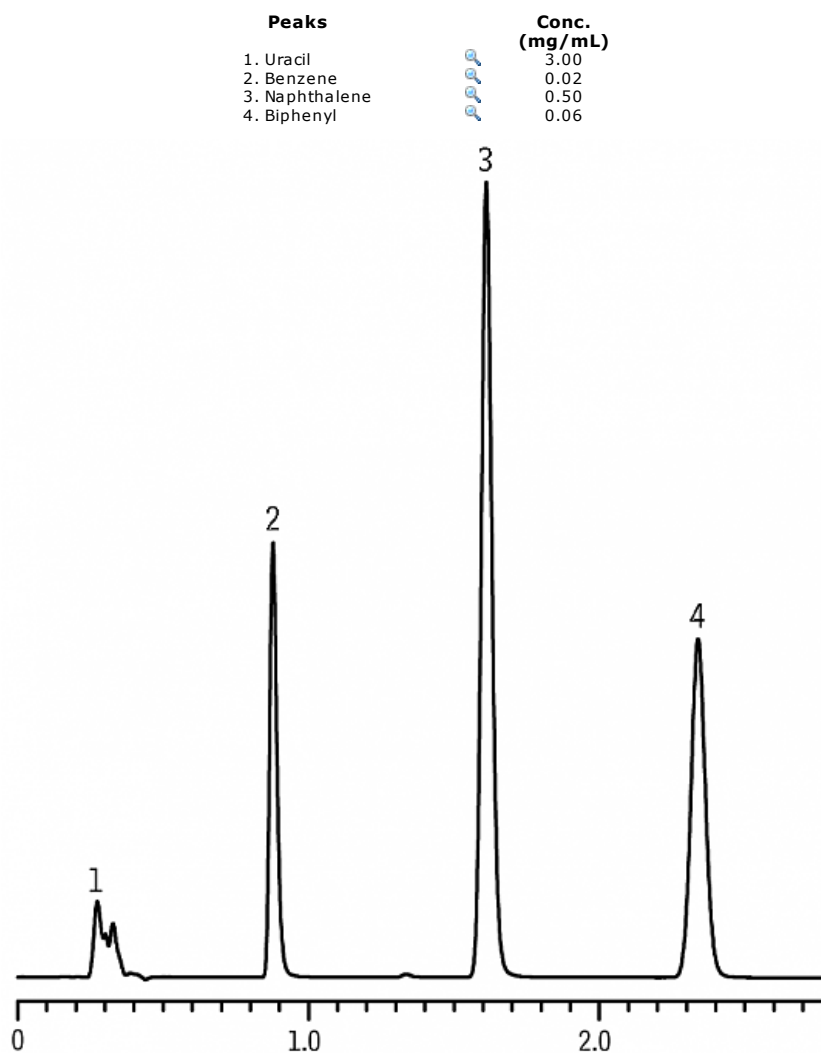
The particle size of an HPLC column is actually the mean of the distribution of all particles used in the manufacturing of the column. In practice, the smaller the particle size distribution, the more uniformly packed the column will be, resulting in higher efficiencies. This distribution is even more critical when manufacturing columns with particle sizes less than 2µm. If the distribution contains many larger particles and is not tightly controlled, the efficiency of the column will suffer and column-to-column reproducibility will vary. More importantly, if the column contains particles less than 1µm (termed “fines”), clogging of the column frit and unwanted column backpressure can result. Restek’s 1.9µm Pinnacle™ DB columns have narrow, symmetric particle size distributions, containing no particles less than 1µm in diameter. **Figure 3** illustrates this exceptional distribution, which is tighter and more accurate than competitive sub-2µm columns.

Restek’s 1.9µm Pinnacle™ DB columns offer practical advantages for today’s chemist across a wide range of analytes from acidic to basic. For higher sample throughput, matched with the reliability and ruggedness of a column made entirely by chromatographers for chromatographers, reach for Restek small particle HPLC columns.

**Table I** Restek’s 1.9µm Pinnacle™ DB offers the highest efficiency of all columns tested (data from the biphenyl peak of a reversed phase test mix).

Column	Efficiency (n/m)	Pressure (psi)	Asymmetry
1.9µm Pinnacle™ DB	217,619	4,500	1.10
Competitor A	177,999	4,400	1.13

**Figure 1** Excellent peak symmetry and efficiency on a 1.9µm Pinnacle™ DB C18 column, using a reversed phase test mix.



LC\_EX0427

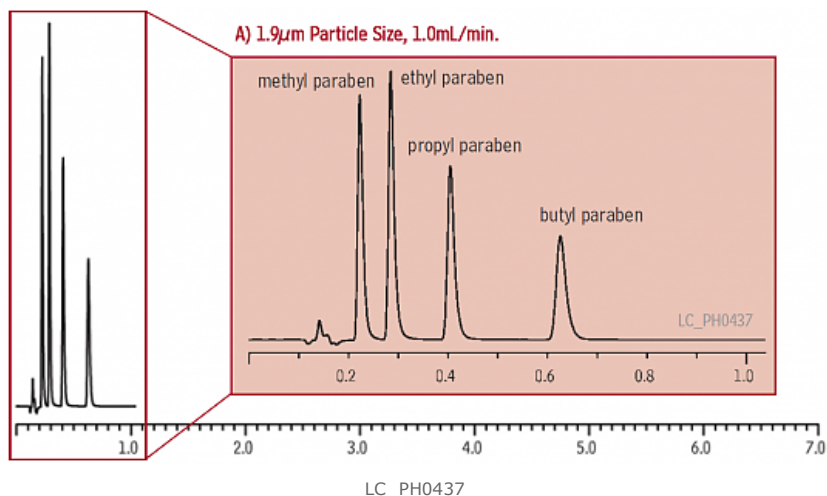
**Column** Pinnacle® DB C18 (cat.# 9414252)  
 Dimensions: 50 mm x 2.1 mm ID  
 Particle Size: 1.9 µm  
 Pore Size: 140 Å  
 Temp.: 25 °C  
**Sample** HPLC Reversed Phase Test Mix #1 (cat.# 35005)  
 Diluent: water:methanol (25:75)  
 Conc.:  
 Inj. Vol.: 2 µL  
**Mobile Phase** water:acetonitrile (45:55)  
 Flow: 0.4 mL/min  
**Detector** UV/Vis @ 254 nm  
**Notes** Performance: (calculations for biphenyl)  
 Efficiency: 217,619 n/m  
 Asymmetry: 1.10  
 Pressure: 4,500 psi

**Figure 2** Restek's 1.9µm Pinnacle™ DB columns can dramatically increase sample throughput—with no loss in resolution.

**Peaks**  
 1. Methylnaraben



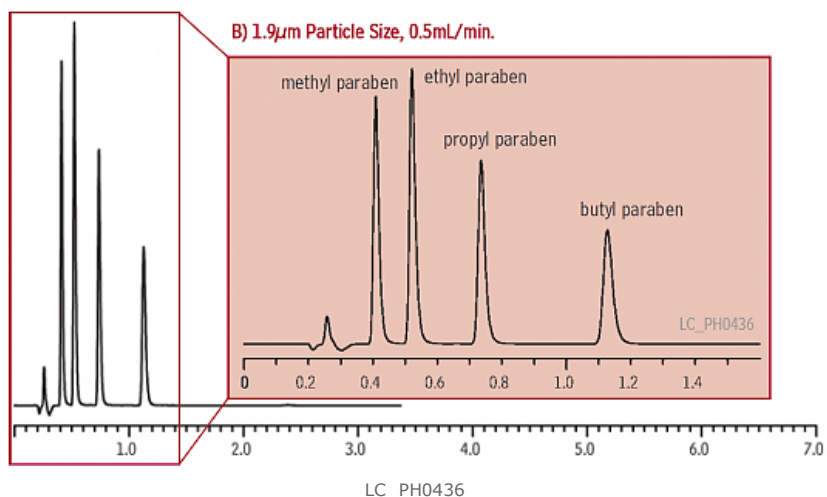
3. Propylparaben  
4. Butylparaben



**Column** Pinnacle® DB C18 (cat. # 9414252)  
**Dimensions:** 50 mm x 2.1 mm ID  
**Particle Size:** 1.9  $\mu$ m  
**Pore Size:** 140 Å  
**Temp.:** ambient  
**Sample**  
**Diluent:** mobile phase  
**Conc.:** ~100  $\mu$ g/mL each component  
**Inj. Vol.:** 2  $\mu$ L  
**Mobile Phase** 0.1% acetic acid in water:acetonitrile (50:50)  
**Flow:** 1.0 mL/min  
**Detector** UV/Vis @ 254 nm

#### Peaks

1. Methylparaben
2. Ethylparaben
3. Propylparaben
4. Butylparaben

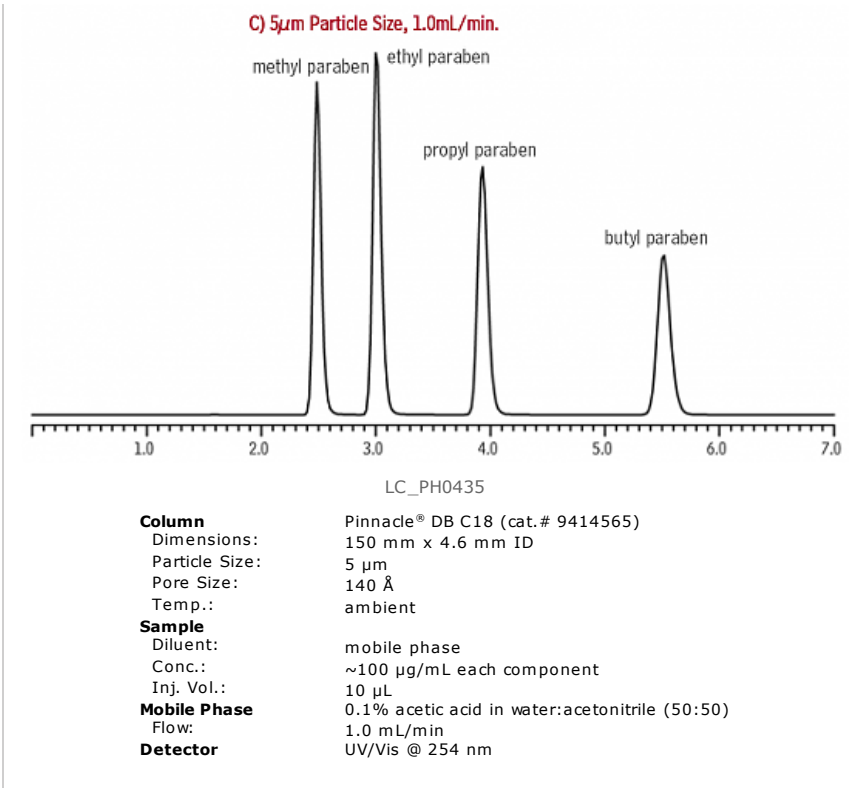


**Column** Pinnacle® DB C18 (cat. # 9414252)  
**Dimensions:** 50 mm x 2.1 mm ID  
**Particle Size:** 1.9  $\mu$ m  
**Pore Size:** 140 Å  
**Temp.:** ambient  
**Sample**  
**Diluent:** mobile phase  
**Conc.:** ~100  $\mu$ g/mL each component  
**Inj. Vol.:** 2  $\mu$ L  
**Mobile Phase** 0.1% acetic acid in water:acetonitrile (50:50)  
**Flow:** 0.5 mL/min  
**Detector** UV/Vis @ 254 nm

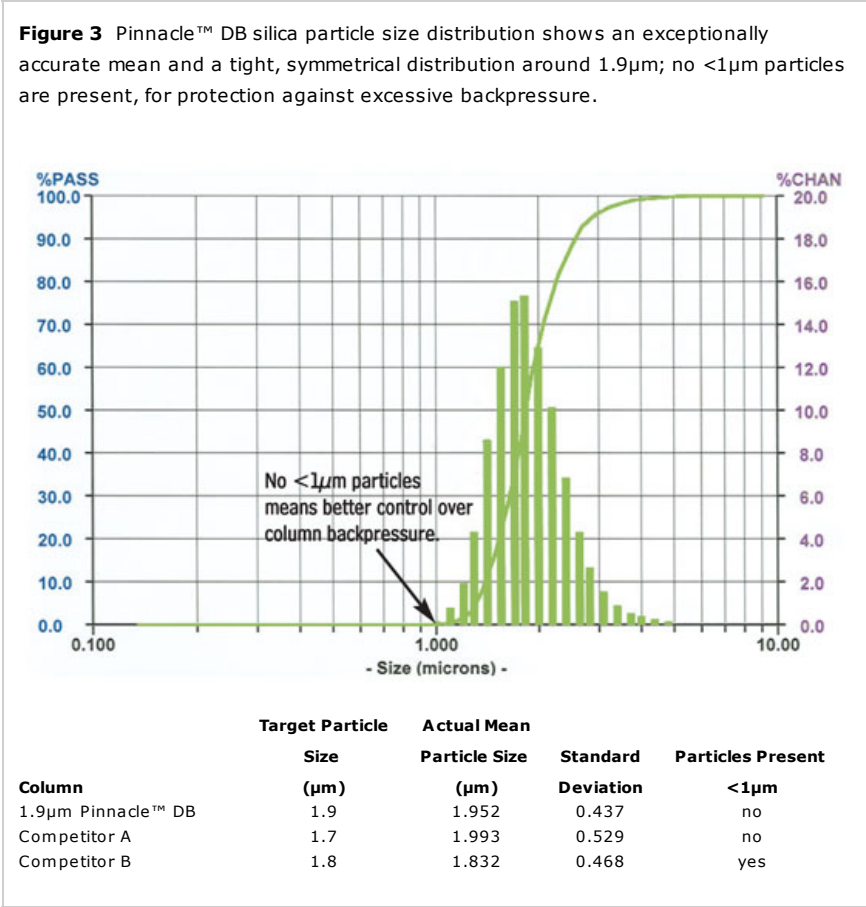
#### Peaks

1. Methylparaben
2. Ethylparaben
3. Propylparaben
4. Butylparaben



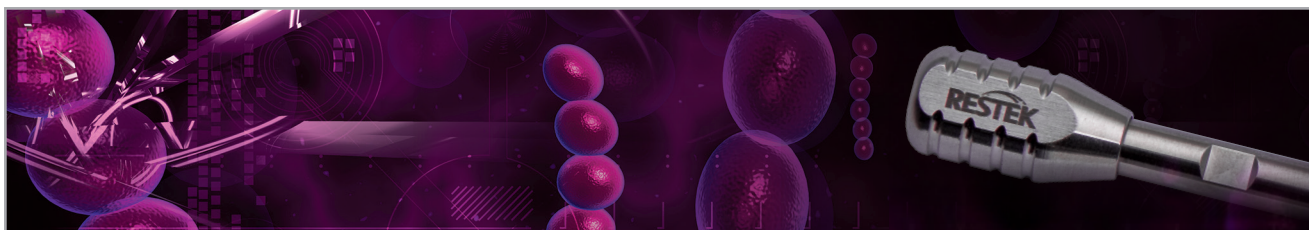


**Figure 3** Pinnacle™ DB silica particle size distribution shows an exceptionally accurate mean and a tight, symmetrical distribution around 1.9µm; no <1µm particles are present, for protection against excessive backpressure.



RELATED SEARCHES

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## Pharmaceutical Applications

# Excellent LC-MS Separation of Penicillins and Cephalosporins Using Ultra IBD Columns

### Introduction

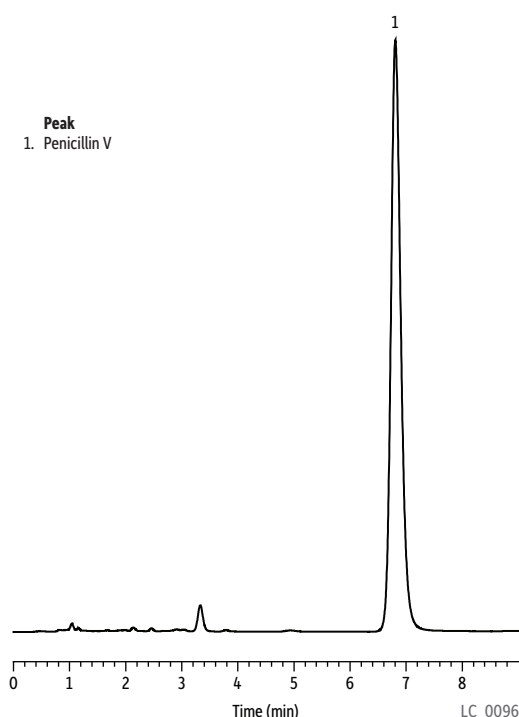
Antibiotics are the most widely used medications in the world. Whether by prescription, addition to animal feed stocks, or use of cleaning agents, everyone in the civilized world is either directly or indirectly exposed to antibiotics in daily life. The overuse of antibiotics, however, has allowed resistant bacteria to thrive. The death of 12,500 people in Guatemala from an episode of Shigella fever can be traced to a simple mutation of the bacterial strain. Research indicated that the bacterium incorporated a single plasmid into its RNA sequence and resultantly became resistant to four different antibiotics. This illustrates the danger of resistance caused by adaptation. To combat resistant bacteria, new antibiotic derivatives must be created to overcome the bacteria's new defense mechanisms. Typically, HPLC columns can be used to analyze penicillins and their structurally related cephalosporins. However, the similarity of many derivatives may require additional interactions to effectively separate related compounds. Restek's Ultra IBD column is better able to resolve these compounds using polar and hydrophobic interactions.

### Background

Penicillins and cephalosporins represent nearly sixty percent of antibiotics worldwide. These antibiotics possess a sulfur atom within a five- or six-membered ring, attached to a fourmember  $\beta$ -lactam ring. They are produced by fermentation processes using either selected fungi or species of *Streptomyces* bacteria. Derivatives are produced in two fashions:

1. Biosynthetic process—The fungus or bacteria are genetically engineered to produce a new derivative, or the starting materials are altered to produce biosynthetic variants during fermentation.
2. Semi-synthetic processes—The materials from a biosynthetic process are converted to chemical derivatives. Penicillin derivatives are created from penicillin G or V, while cephalosporin derivatives are created from cephalosporin C or cephamycin C.

**Figure 1:** Ultra IBD column separates penicillin V from fermentation impurities.



<b>Column</b>	Ultra IBD (cat.# 9175565)
<b>Dimensions:</b>	150 mm x 4.6 mm ID
<b>Particle Size:</b>	5 $\mu$ m
<b>Pore Size:</b>	100 Å
<b>Temp.:</b>	30 °C
<b>Sample</b>	
<b>Diluent:</b>	acetonitrile:water (10:90, v/v)
<b>Conc.:</b>	1.2 mg/mL
<b>Inj. Vol.:</b>	2.5 $\mu$ L
<b>Mobile Phase</b>	10 mM ammonium formate, pH 2.5:acetonitrile (95:5, v/v)
<b>Flow:</b>	1.2 mL/min
<b>Detector</b>	UV/Vis @ 270 nm

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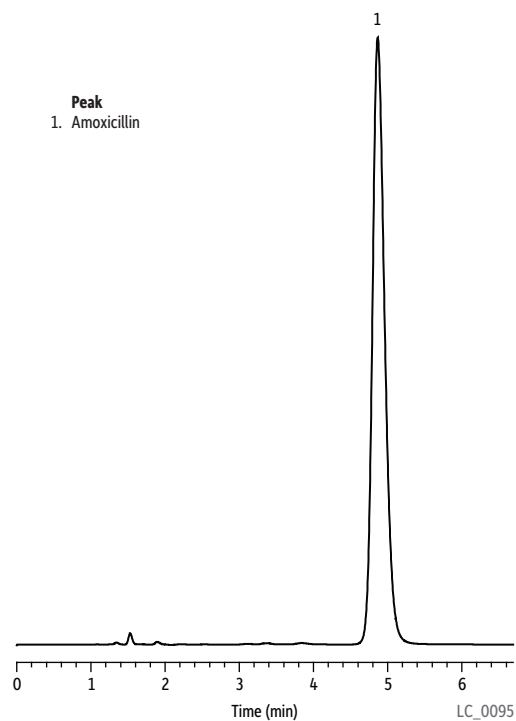
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But biosynthetic fermentation does not produce a “pure” antibiotic. Even after cleanup of the fermentation mash, some side reaction products will remain. Many of these side products are closely related to the primary analyte (Figure 1). Desired products, however, are created in the semi-synthetic process. Penicillin V is converted to amoxicillin through chemical intermediates and varies only slightly in structure (Figure 2). Similar reactions also occur during production of cephalosporin derivatives. The loss of a hydride ion to create a phenyl ring is the only structural difference between cephradine and its side product cephalexin (Figure 3). Semi-synthetic processes are used to create derivatives like cephaloridine.

Unfortunately, many penicillins and cephalosporins are acid labile so that liquid chromatographic (LC) analysis of these molecules only should be performed if the sample is dissolved in a neutral media. Furthermore, if analysis time on the column is prolonged, breakdown of the analytes may occur *in situ* with a mobile phase that is not at a neutral pH. When measuring trace quantities of the analytes, especially by LC–mass spectrometry (MS), maintaining pH near 7.4 may become important for stability and accurate quantitation.

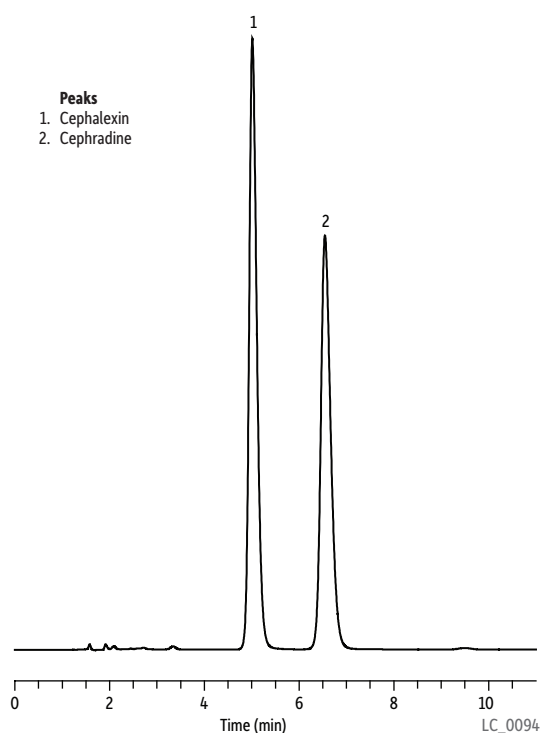
**Figure 2:** Ultra IBD column shows excellent peak shape for amoxicillin.



**Peak**  
1. Amoxicillin

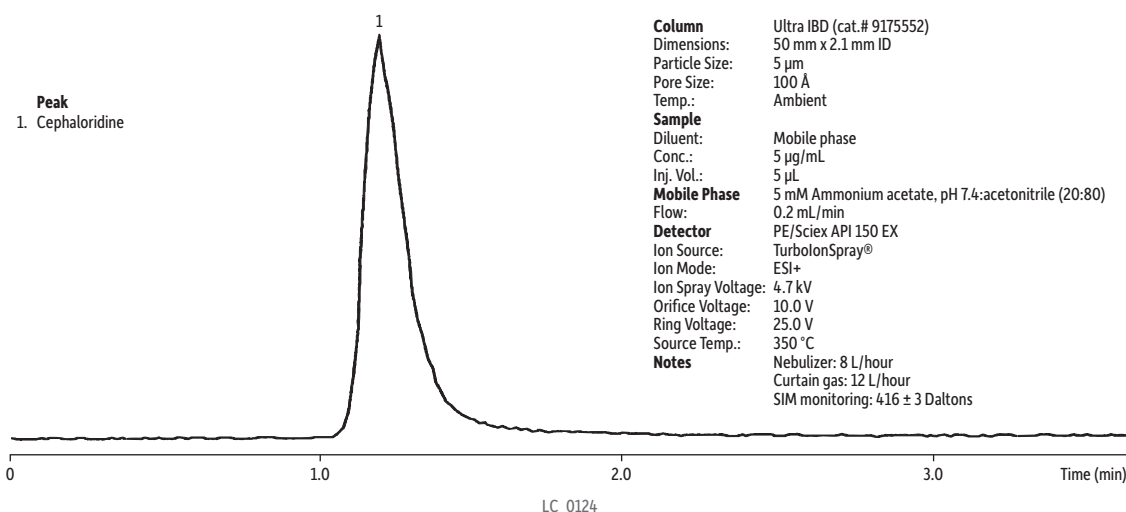
**Column** Ultra IBD (cat.# 9175565)  
**Dimensions:** 150 mm x 4.6 mm ID  
**Particle Size:** 5 µm  
**Pore Size:** 100 Å  
**Temp.:** 30 °C  
**Sample**  
**Diluent:** acetonitrile:water (10:90, v/v)  
**Conc.:** 1.5 mg/mL  
**Inj. Vol.:** 5 µL  
**Mobile Phase**  
**Flow:** 10 mM ammonium formate, pH 2.5:acetonitrile (95:5)  
**Flow:** 1.2 mL/min  
**Detector** UV/Vis @ 270 nm

**Figure 3:** Ultra IBD column shows great separation between cephalexin and cephradine, which differ only by ring structure.



**Peaks**  
1. Cephalexin  
2. Cephradine

**Column** Ultra IBD (cat.# 9175565)  
**Dimensions:** 150 mm x 4.6 mm ID  
**Particle Size:** 5 µm  
**Pore Size:** 100 Å  
**Temp.:** 30 °C  
**Sample**  
**Diluent:** acetonitrile:water (10:90, v/v)  
**Conc.:** 500 µg/mL  
**Inj. Vol.:** 10 µL  
**Mobile Phase**  
**Flow:** 10 mM ammonium formate, pH 2.5:acetonitrile (90:10)  
**Flow:** 1.2 mL/min  
**Detector** UV/Vis @ 270 nm

**Figure 4:** Ultra IBD column allows increased LC-MS sensitivity of cephaloridine in HILIC mode.

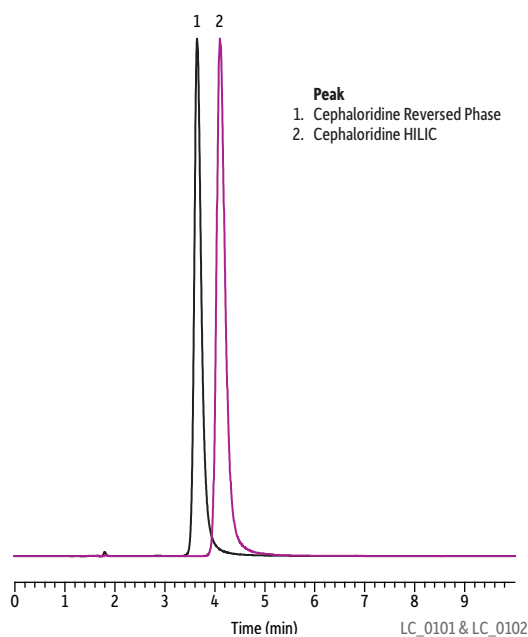
### Discussion of Analysis

The Restek® Ultra IBD phase provides greater versatility for the LC-MS analysis of penicillins and cephalosporins compared to a C18 column. The Ultra IBD column is capable of providing retention for cephaloridine in reversed-phase mode with up to 45% organic solvent in the mobile phase. A conventional C18 column loses all retention near 35% organic solvent. Unlike a C18 column, the IBD is capable of polar interactions in a HILIC mode with analytes that possess charged functional groups. The ability to retain a compound such as cephaloridine in HILIC mode using levels of organic solvents above 50% in the mobile phase will allow increased sensitivity by LC-MS (Figure 4).

The IBD column also provides other chromatographic benefits. The excellent peak shape for cephaloridine in both the reversed- and HILIC modes (Figure 5) increases sensitivity and improves quantitation. Furthermore, the retention of cephalosporin and cephaloridine is essentially unaffected by the pH. This allows full control in the pH range of 2.5 to 8 for optimum stabilization of the cephalosporins and penicillins during analysis, provided hydrolysis is not an issue. The IBD column has a unique blend of hydrophobic and polar character for better resolution of closely related compounds.

### Conclusion

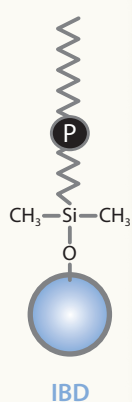
Closely related compounds such as penicillins and cephalosporins may require more than one type of interaction for optimum resolution of closely related components. The Restek® IBD phase provides those interactions using only simple mobile phases. The excellent peak shape, resolution enhancement, and wide pH make it the ideal choice for the analysis of penicillin- and cephalosporin-based antibiotics by HPLC or LC-MS.

**Figure 5:** Ultra IBD column shows excellent peak shape for cephaloridine in both HILIC and reversed-phase modes.

**Column** Ultra IBD (cat.# 9175565)  
**Dimensions:** 150 mm x 4.6 mm ID  
**Particle Size:** 5 µm  
**Pore Size:** 100 Å  
**Temp.:** 27 °C  
**Sample**  
**Diluent:** acetonitrile:water (50:50, v/v)  
**Conc.:** 1 mg/mL  
**Inj. Vol.:** 5 µL  
**Mobile Phase**  
**Flow:** 1.2 mL/min  
**Detector** UV/Vis @ 254 nm

acetonitrile:20mM ammonium phosphate, pH 4.0 (20:80, v/v reversed phase; 80:20, v/v HILIC)

## Product Listings

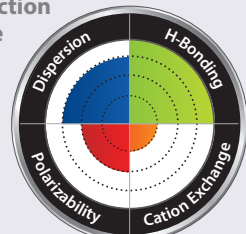


**IBD**

**Column Characteristics:**

particle size:	3 µm or 5 µm, spherical
pore size:	100 Å
carbon load:	12%
end-cap:	no
pH range:	2.5 to 8
temperature limit:	80 °C
USP phase code:	L68
phase category:	polar-embedded alkyl
ligand type:	proprietary polar functional embedded alkyl

**USLC® Column Interaction Profile**



### Ultra IBD Columns (USP L68)

#### Chromatographic Properties

The Restek® IBD is a polar-embedded column that acts as a strong hydrogen bonder and may be the most versatile column available today. With a unique polar group, this column is very retentive and selective for acids. It also provides symmetrical peak shape for strong bases. Restek's IBD is compatible with 100% aqueous mobile phases and can be used under reversed-phase or HILIC conditions to retain very polar, ionic compounds in highly organic mobile phases.

Length	1.0 mm ID cat. #	2.1 mm ID cat. #	3.0 mm ID cat. #	4.6 mm ID cat. #
<b>3 µm Columns</b>				
30 mm	9175331	9175332	917533E	9175335
50 mm	9175351	9175352	917535E	9175355
100 mm	9175311	9175312	917531E	9175315
150 mm	9175361	9175362	917536E	9175365
<b>5 µm Columns</b>				
30 mm	9175531	9175532	917553E	9175535
50 mm	9175551	9175552	917555E	9175555
100 mm	9175511	9175512	917551E	9175515
150 mm	9175561	9175562	917556E	9175565
200 mm	9175521	9175522	917552E	9175525
250 mm	9175571	9175572	917557E	9175575

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### Ultra IBD Guard Cartridges

Guard Cartridges	3-pk. (10 x 2.1 mm)	3-pk. (10 x 4.0 mm)
Ultra IBD Guard Cartridge	917550212	917550210

Pure Chromatography

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## Easy Transfer of HPLC Methods to UHPLC

### Using Fully Scalable Pinnacle® DB Columns

By Rick Lake, Pharmaceutical Innovations Chemist

- Methods on Pinnacle® DB columns are easily transferred from 3 and 5µm to 1.9µm, allowing faster analysis without losing separation quality.
- Pinnacle® DB columns are 100% Restek manufactured—from base silica to final packed column.
- Restek offers the widest selection of stationary phases for UHPLC—more choices mean better selectivity for your analytes.

Ultra High Pressure Liquid Chromatography (UHPLC) is a rapidly growing technique that greatly increases sample throughput. UHPLC takes advantage of HPLC columns packed with <2µm particles, often used at elevated pressures, to significantly reduce analytical run times. By decreasing the particle size of the packing material, the analyst can maximize the number of theoretical plates, making shorter column lengths possible and increasing the range of usable flow rates. Ultimately, the analytical method is shortened without losing separation quality.

UHPLC is commonly used in two ways. First, to increase sample throughput, a conventional HPLC analysis can be scaled-down to UHPLC. Alternatively, to lessen time in method development, a preliminary separation can be developed in UHPLC and then scaled-up to conventional HPLC for routine analysis. In both cases, when transferring an analysis from conventional HPLC to UHPLC, or from UHPLC to HPLC, comparable method parameters must be used to maintain equivalent separations. First and foremost, to maintain selectivity while scaling an analysis, some column properties and operating conditions should remain consistent, while other parameters are optimized. For the analytical column, the pore size, carbon load, and support material must remain the same. Differences in selectivity can be seen when not using equivalent columns. However, when decreasing particle size and column dimensions, it is equally important that certain operating conditions be adjusted properly. To provide guidance, we will identify important method parameters that need to be adjusted and illustrate with an example of method transfer.

In this example, we will perform a scale-down method transfer for sulfonamides from a conventional HPLC analysis to a UHPLC analysis. The initial analysis was conducted on a standard analytical scale 150mm x 4.6mm ID x 5µm HPLC column using gradient elution. To speed the analysis, we decreased the internal diameter of the column to 2.1mm and reduced the particle size to 1.9µm.

The importance of selectivity in a chromatographic separation has been discussed in a previous article, [Optimize Selectivity & Efficiency in UHPLC Separations](#). To ensure that we used the optimum stationary phase for this application, we first compared the selectivity of a Pinnacle® DB Biphenyl column to a C18 column. Sulfonamides are commonly assayed on alkyl phases; however, their fused ring structure (Figure 1) may show enhanced selectivity with a biphenyl phase that is capable of pi-pi interactions. When comparing C18 and biphenyl columns of identical dimensions, under identical conditions, the Pinnacle® DB Biphenyl showed better selectivity towards the early eluting sulfonamides (Figure 2). Therefore, for better selectivity and faster analysis times, we used a Pinnacle® DB Biphenyl stationary phase for this application.

When performing a scale-down procedure, a few simple calculations can be used to determine equivalent run conditions. Let's go through them sequentially.

#### Adjusting Column Size

The first calculation determines the appropriate column length. Keeping the same column length while decreasing the particle size will increase the number of theoretical plates in that given column length. Therefore, column length can be shortened without losing resolution. By adjusting the column length properly, using Equation 1, we can maintain the same separation.

#### Adjusting Injection Volume

Once we have determined the proper column length, we can determine the appropriate injection volume. Decreasing the column internal diameter and length, decreases the overall column volume and sample capacity. Therefore, we must alter the injection volume as described in Equation 2. Please note that since overall column volume has decreased, it is important to match the sample solvent to the starting mobile phase composition. Mismatched sample solvents can cause irreproducible retention times, efficiencies, and

even changes in selectivity.

### Adjusting Flow Rate

Next, flow rate must be adjusted to maintain comparable linear velocity through a column with smaller internal diameter. Linear velocity is defined as the distance mobile phase travels over time, whereas flow rate is the volume of mobile phase that travels over time. To maintain the same linear velocity, which is important in maintaining efficiencies, flow rates must be decreased as column internal diameter decreases. Also, since smaller particle sizes give rise to higher optimal linear velocities, isocratic flow rates should be calculated with particle size taken into account. In this example a gradient elution was used and therefore particle size was not included in the equation. Equation 3 can be used to simply and quickly estimate the adjusted flow rate needed for equivalent chromatography. It is also important to note that  $<2\mu\text{m}$  particle sizes are less affected by higher flow rates, and therefore faster flow rates can be used in isocratic systems without detrimental effects on peak efficiency.

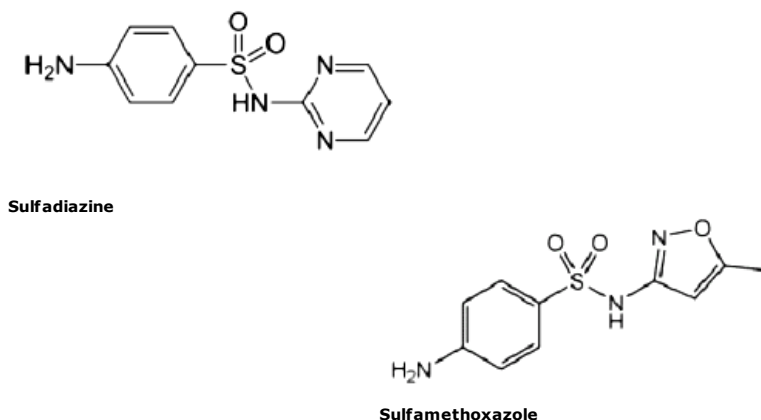
### Adjusting Time Program

Lastly, after we have determined the proper column length, injection volume, and flow rate, we can find the equivalent time needed for gradient or step elutions. As an analytical method is scaled down, the time program needs to also be scaled down to keep the phase interactions the same. Time can be adjusted using Equation 4.

### Conclusion

After determining the equivalent conditions for scaling-down the analysis of sulfonamides, we can see that the separations are equivalent, while the analysis time was greatly reduced (Figure 3). Under conventional HPLC the last compound eluted at 7.2 minutes and under UHPLC the last compound eluted at 2.6 minutes. By following the procedure described here to ensure that the columns are equivalent, scaling analytical procedures from HPLC to UHPLC, or vice versa, easily can be accomplished using Pinnacle® DB columns.

**Figure 1** Chemical structures for example sulfonamides.



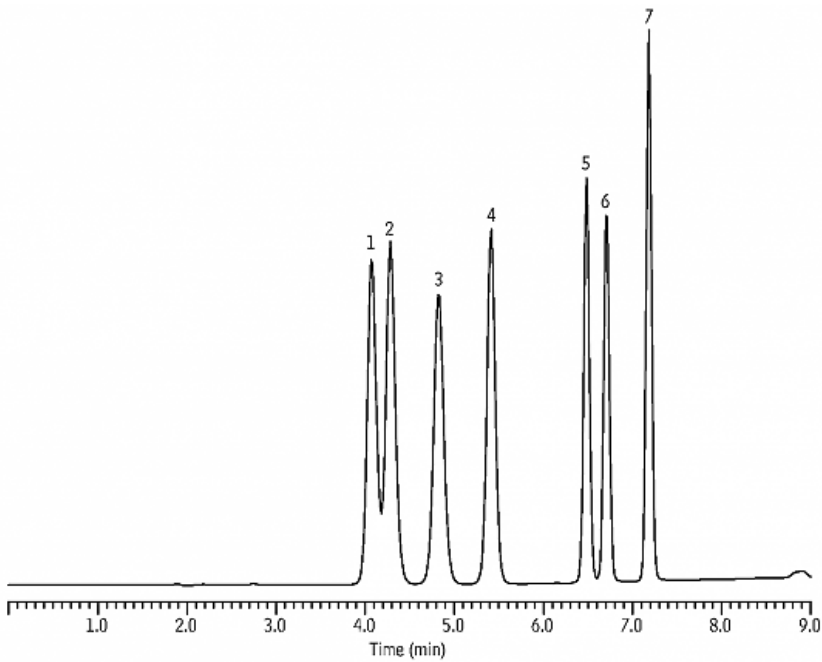
**Figure 2** A  $1.9\mu\text{m}$  Pinnacle® DB Biphenyl column is more selective for sulfonamides than a conventional C18 column.

#### A. Biphenyl Selectivity B. C18 Selectivity

##### Peaks

1. Sulfadiazine
2. Sulfathiazole
3. Sulfamerazine
4. Sulfamethazine
5. Sulfachlorpyridazine
6. Sulfamethoxazole
7. Sulfamethoxine





LC\_PH0461

**Column** Pinnacle® DB Biphenyl (cat.# 9409565)  
**Dimensions:** 150 mm x 4.6 mm ID  
**Particle Size:** 5 µm  
**Pore Size:** 140 Å  
**Temp.:** 30 °C

**Sample**  
**Diluent:** Starting mobile phase (80:20 A:B)  
**Conc.:** 100 µg/mL  
**Inj. Vol.:** 10 µL

**Mobile Phase**  
**A:** 0.1% Formic acid in water  
**B:** 0.1% Formic acid in acetonitrile

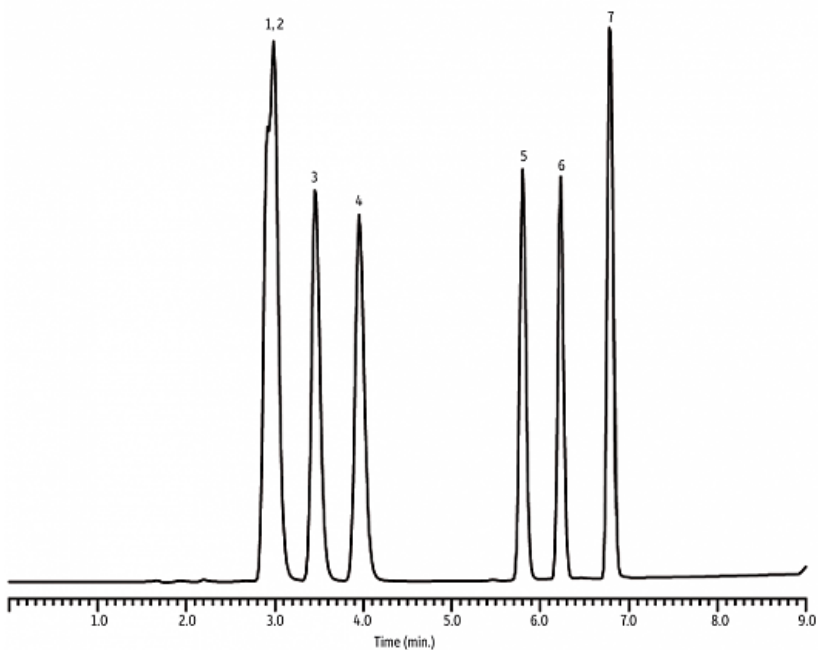
Time (min)	%B
0.00	20
1.0	20
6.0	80
8.0	80

**Flow:** 1.0 mL/min  
**Detector** UV/Vis @ 254 nm  
**Instrument** Shimadzu Prominence

**Peaks**

- 1. Sulfadiazine
- 2. Sulfathiazole
- 3. Sulfamerazine
- 4. Sulfamethazine
- 5. Sulfachlorpyridazine
- 6. Sulfamethoxazole
- 7. Sulfamethoxine





LC\_PH0460

**Column** Conventional C18  
 Dimensions: 150 mm x 4.6 mm ID  
 Particle Size: 5 µm  
 Pore Size: 140 Å  
**Sample** Sulfonamides  
 Diluent: Starting mobile phase  
 Conc.: 100 µg/mL  
 Inj. Vol.: 10 µL  
**Mobile Phase**  
 A: 0.1% Formic acid in water  
 B: 0.1% Formic acid in acetonitrile

**Time (min)%B**

0.0	20
1.0	20
6.0	80
8.0	80

Flow: 1.0 mL/min  
**Detector** UV/Vis @ 254 nm  
 Cell Temp: 30 °C  
**Instrument** Shimadzu Prominence

**Equation 1** Adjusted column length can easily be calculated when scaling from HPLC to UHPLC.

$$L_{C2} = \frac{L_{C1} \cdot dp_2}{dp_1}$$

**Example:**

$$L_{C2} = \frac{150\text{mm} \cdot 1.9\mu\text{m}}{5\mu\text{m}}$$

$$L_{C2} = 57\text{mm}$$

$L_C$  = Column Length  
 $dp$  = Particle Size

**Equation 2** Changing column dimensions requires an adjusted injection volume.

$$V_{I2} = V_{I1} \cdot \left( \frac{d_{C2}^2 \cdot L_{C2}}{d_{C1}^2 \cdot L_{C1}} \right)$$

**Example:**

$$V_{I2} = 10\mu\text{l} \cdot \left( \frac{2.1\text{mm}^2 \cdot 50\text{mm}}{4.6\text{mm}^2 \cdot 150\text{mm}} \right)$$

$$V_{I2} = 0.69\mu\text{l}$$

$V_I$  = Injection Volume  
 $L_C$  = Column Length  
 $d_C$  = Column Diameter

**Equation 3** Changing column internal diameter requires using an adjusted flow rate.

$$F_{C2} = \left( \frac{d_{C2}}{d_{C1}} \right)^2 \cdot F_{C1}$$

**Example:**

$$F_{C2} = \left( \frac{2.1\text{mm}}{4.6\text{mm}} \right)^2 \cdot 1.0 \text{ ml/min}$$

$$F_{C2} = 0.208 \text{ ml/min}$$

$F_C$  = Column Flow  
 $d_C$  = Column Diameter

**Equation 4** When scaling down a gradient method, the time program needs to be adjusted.

$$t_{g2} = t_{g1} \cdot \left( \frac{F_{C1}}{F_{C2}} \right) \cdot \left( \frac{d_{C2}^2}{d_{C1}^2} \right) \cdot \left( \frac{L_{C2}}{L_{C1}} \right)$$

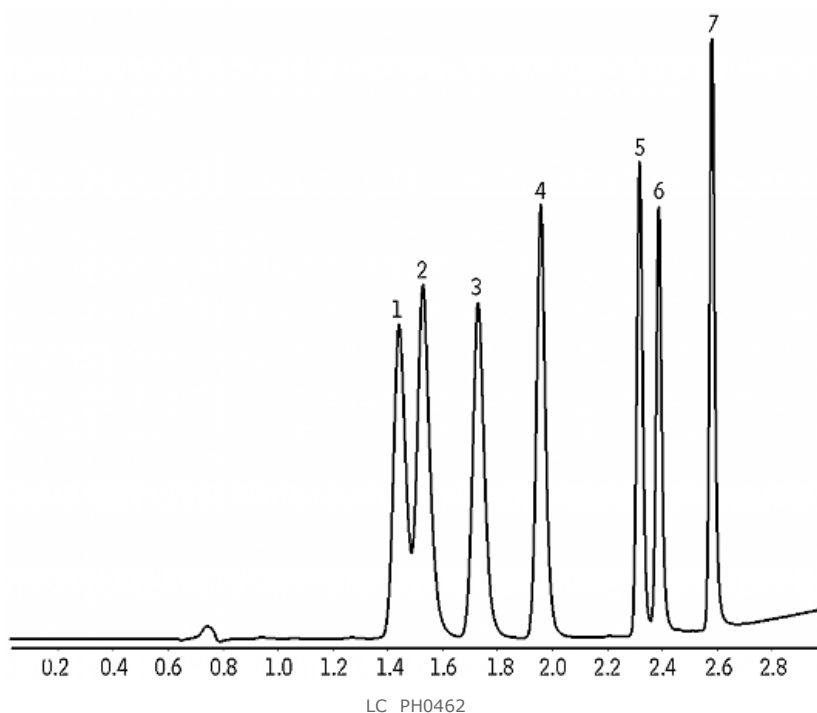
**Example:**

$$t_{g2} = 5\text{min.} \cdot \left( \frac{1.0\text{ml/min.}}{0.2\text{ml/min.}} \right) \cdot \left( \frac{2.1\text{mm}^2}{4.6\text{mm}^2} \right) \cdot \left( \frac{50\text{mm}}{150\text{mm}} \right)$$

$$t_{g2} = 1.7 \text{ min}$$

$t_g$  = Gradient Time  
 $F$  = Column Flow  
 $L_C$  = Column Length  
 $d_C$  = Column Diameter

UHPLC and vice versa.



#### RELATED SEARCHES

[UHPLC](#), [scaling](#), [sulfonamides](#), [pinnacle db](#)

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## Technical Article

# Don't Overestimate Cannabidiol During Medical Cannabis Potency Testing by Gas Chromatography

By Jack Cochran

Accurate potency testing of medical cannabis with gas chromatography (GC) depends principally on choosing a column with the right selectivity; otherwise, coelutions between cannabinoids of interest may cause error in potency measurements. Cannabidiol is one of the chief cannabinoids with pharmacological value and provides relief against nausea, anxiety, and inflammation. Potency testing for medical marijuana is often done using "5-type" GC columns since they are commonly available in most labs. However, on 5-type columns cannabidiol can coelute with cannabichromene, a compound that likely also has medical value and is increasingly becoming part of potency testing. To identify and report both of these compounds accurately, a GC column with a different stationary phase is needed.

### Proper Column Choice Results in More Accurate Potency Data

As shown in Figure 1, cannabinoids are aromatic compounds, meaning they will likely be better separated on a column that contains aromatics in the stationary phase because these stationary phases are more selective for aromatic-containing analytes. A fully non-aromatic stationary phase, like a "1-type" (100% dimethyl polysiloxane) column is not appropriate for this analysis since cannabichromene (CBC) and cannabidiol (CBD) will coelute completely. While 5-type columns (5% phenyl) contain some aromatic component, they generally also produce coelutions for cannabichromene and cannabidiol, depending on the conditions used. At best, CBC and CBD can be only partially resolved on 15 m 5% phenyl columns. Much better separations are obtained on higher phenyl-content phases, such as Rxi®-35Sil MS (35% phenyl type) and Rxi®-17Sil MS (50% phenyl type) columns, as they offer excellent selectivity for aromatic cannabinoids. Not only do both columns resolve cannabichromene and cannabidiol, the chromatograms in Figures 2 and 3 demonstrate that they also separate delta-8-tetrahydrocannabinol (d8-THC), delta-9-tetrahydrocannabinol (d9-THC), cannabigerol (CBG), and cannabinol (CBN). Although both columns perform well, the Rxi®-35Sil MS column is recommended because of the slightly faster analysis time and greater space overall between the peaks of interest.

While stationary phase selectivity is the most important factor in choosing a GC column for cannabinoid analysis, there are some additional aspects of this work that will benefit labs doing medical marijuana potency testing. First, cost savings were achieved by using a 15 m column. When a column with the proper selectivity is used, a 15 m column easily provides the separating power needed for this analysis at about half the cost of a 30 m column. Also, the 0.25 mm x 0.25 µm format has good sample loading capacity and is robust, especially when a proper split injection is used with a Sky® Precision® split liner with wool. Finally, hydrogen carrier gas was used here instead of helium. Using hydrogen provides a faster analysis, increasing sample throughput. Hydrogen carrier gas is a convenient way to speed up run times, increase productivity, and reduce the cost and availability concerns associated with using helium carrier gas.

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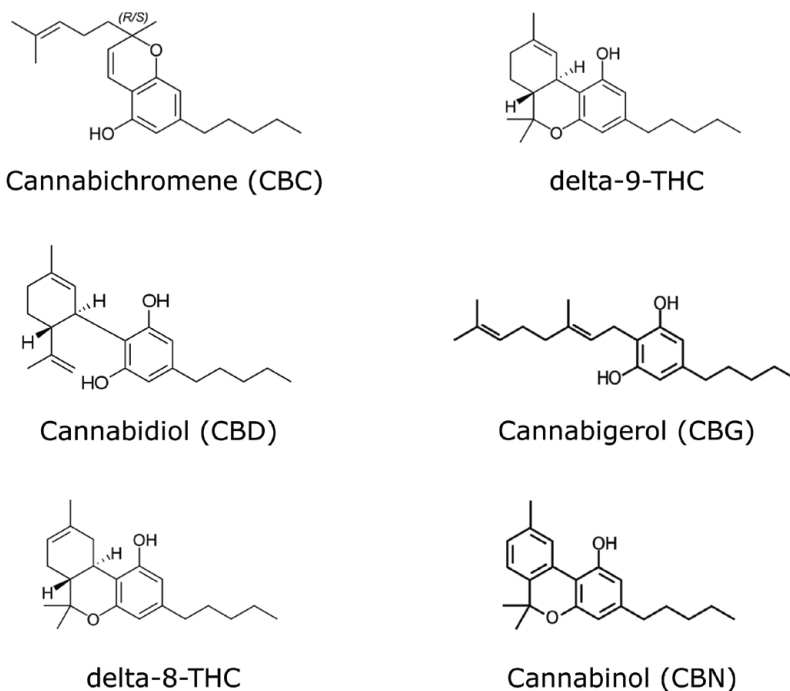
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**Figure 1:** Since cannabinoids are aromatic compounds, a GC column that contains aromatics in the stationary phase will provide much better separations than a column with a non-aromatic phase.

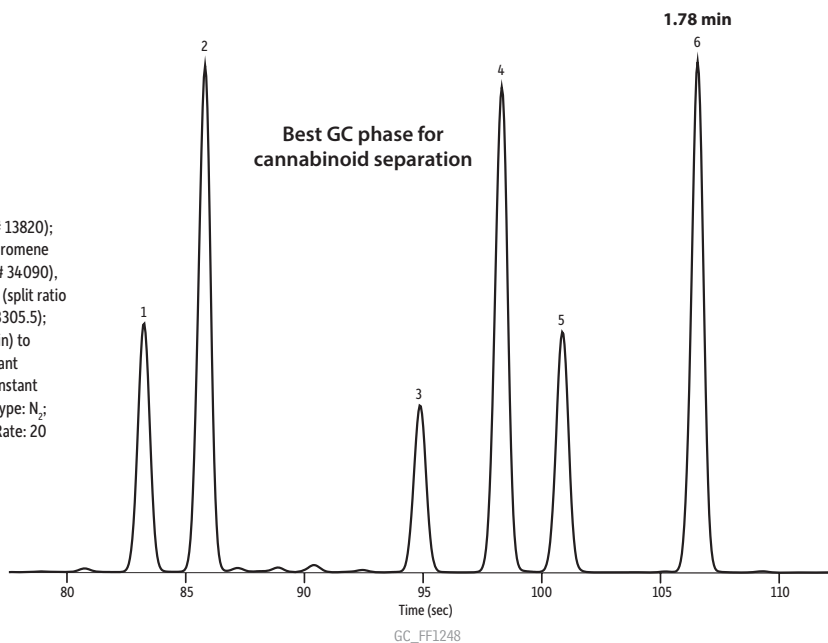


**Figure 2:** The Rxi®-35Sil MS column provides both the best separation and the fastest analysis time, making it the ideal GC column choice for medical cannabis potency testing.

**Peaks**

1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

**Column:** Rxi®-35Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13820);  
**Sample:** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); **Liner:** Sky® 4 mm Precision® liner w/wool (cat.# 23305.5);  
**Inj. Temp.:** 250 °C; **Oven:** Oven Temp.: 225 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.9 min); **Carrier Gas:** H<sub>2</sub>, constant flow; **Flow Rate:** 2.5 mL/min; **Detector:** FID @ 350 °C; **Constant Column + Constant Make-up:** 50 mL/min; **Make-up Gas Type:** N<sub>2</sub>; **Hydrogen flow:** 40 mL/min; **Air flow:** 450 mL/min; **Data Rate:** 20 Hz; **Instrument:** Agilent/HP6890 GC

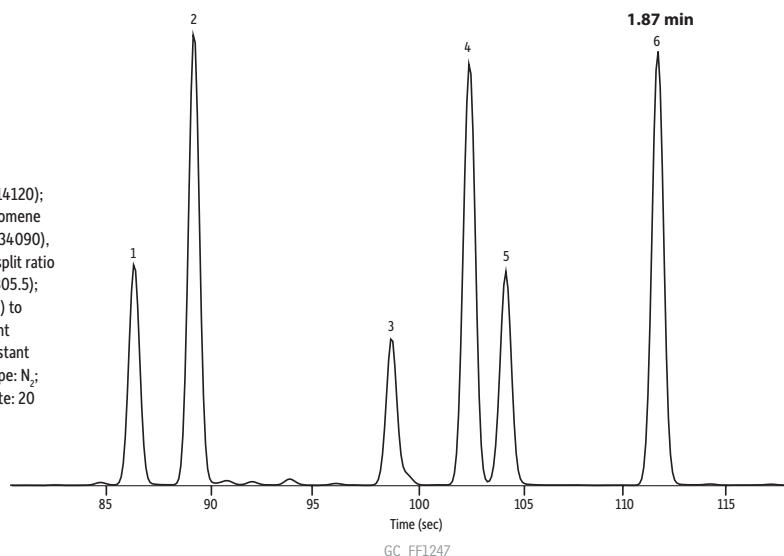


**Figure 3:** Cannabinoids can be effectively separated on an Rxi® 17Sil MS column, but with slightly less resolution than that obtained with the optimal selectivity of the Rxi®-35Sil MS column.

#### Peaks

1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

**Column:** Rxi®-17Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 14120);  
**Sample:** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); **Liner:** Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); **Inj. Temp.:** 250 °C; **Oven:** Oven Temp.: 225 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.9 min); **Carrier Gas:** H<sub>2</sub>, constant flow; **Flow Rate:** 2.5 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N<sub>2</sub>; **Hydrogen flow:** 40 mL/min; **Air flow:** 450 mL/min; **Data Rate:** 20 Hz; **Instrument:** Agilent/HP6890 GC



### Adjusting Conditions for 5-Type Columns

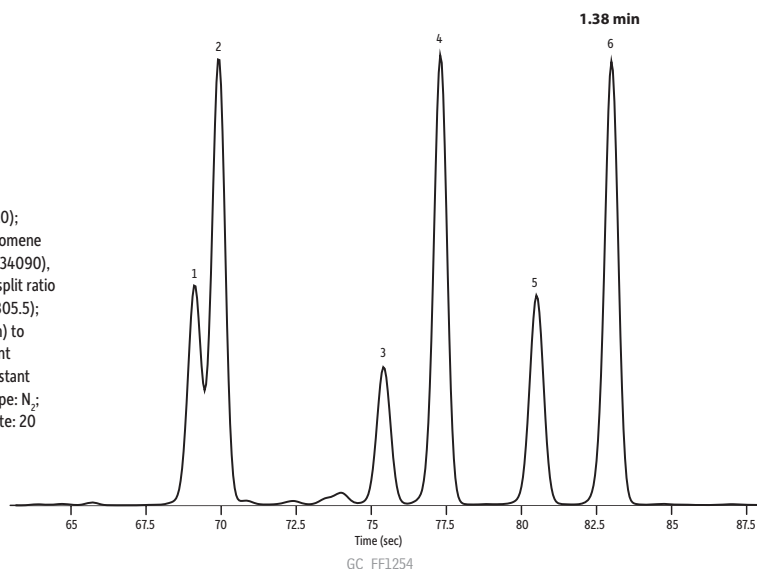
While using an Rxi®-35Sil MS column provides the best selectivity and speed for cannabinoid analysis, cannabidiol potency can be determined in medical cannabis using a 5-type column under certain conditions. If you already have a 5-type column for this work, you can vary the GC conditions, especially carrier flow and oven temperature program, and still separate cannabichromene and cannabidiol, just not as quickly or easily as with the Rxi®-35Sil MS column. Figures 4 and 5 show this analysis on Rxi®-5ms and Rxi®-5Sil MS columns, respectively. Again, the 0.25 mm x 0.25 µm format was used here because it offers better efficiency than wider bore columns (e.g., 0.32 mm and 0.53 mm IDs), which may not separate cannabichromene and cannabidiol under any operational conditions.

**Figure 4:** The selectivity of a 5-type column is not sufficient to fully separate cannabichromene and cannabidiol, resulting in less accurate medical marijuana potency testing.

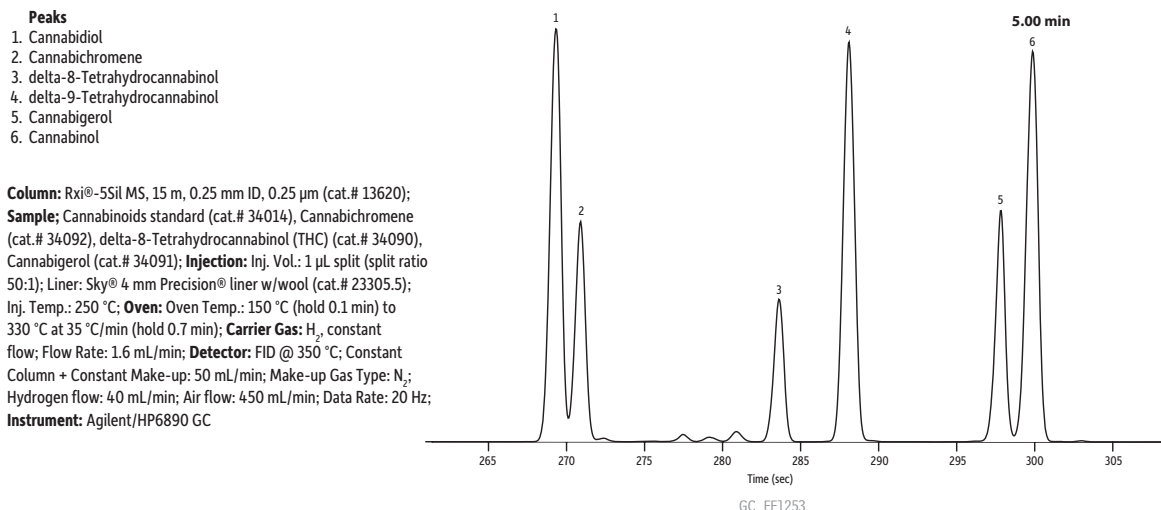
#### Peaks

1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

**Column:** Rxi®-5ms, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13420);  
**Sample:** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); **Liner:** Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); **Inj. Temp.:** 250 °C; **Oven:** Oven Temp.: 250 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.6 min); **Carrier Gas:** H<sub>2</sub>, constant flow; **Flow Rate:** 1.6 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N<sub>2</sub>; **Hydrogen flow:** 40 mL/min; **Air flow:** 450 mL/min; **Data Rate:** 20 Hz; **Instrument:** Agilent/HP6890 GC



**Figure 5:** Rxi®-5Sil MS columns offer better resolution of key cannabinoids than standard 5-type columns, but the incomplete separation and longer analysis time mean further optimization is needed for accurate reporting.



Note that even though these are both 5-type columns, the elution order of cannabichromene and cannabidiol changed. This is due to two things. The first is that Rxi®-5ms and Rxi®-5Sil MS columns differ slightly in selectivity for certain compounds; even though they are both considered 5-type columns, they contain different stationary phases that retain some compounds differently. The second reason is that the GC oven programs are different for the columns, which means that the compounds are eluting at different temperatures. You may be able to further optimize the separation of cannabichromene and cannabidiol on a 5-type column, but the selectivity and faster analysis that can be obtained using a high-phenyl content Rxi®-35Sil MS column make it ideal for potency determinations in medical cannabis.

To sum things up, proper column choice is essential for accurate and robust cannabis potency testing. Using the right column not only gives you more confidence in your potency values, but it also saves you time and money. Switching to hydrogen carrier gas can reduce your costs even further, while increasing sample throughput.

Visit [www.restek.com/medical-cannabis](http://www.restek.com/medical-cannabis) for Restek® GC and LC columns, accessories, reference standards, and other products and resources for medical marijuana analysis.

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**146 (of ~193 ) Pharma CT-Update 2015**

# Developing New Methods for Pesticides in Dietary Supplements

## Advantages of the QuEChERS Approach

*New requirements for dietary supplements to be manufactured under cGMP regulations have created a need for methods to detect pesticides in these complex, largely botanical products. QuEChERS offers a simple, cost-effective approach that can reduce matrix interferences as well as variation among technicians. Here we demonstrate a procedure that incorporates a QuEChERS extraction, cSPE cleanup and GC-TOFMS, resulting in good recoveries for a wide range of pesticide chemistries in dandelion root powder.*

### Introduction

Recently the FDA announced that makers of dietary supplements (e.g. vitamins, herbal and botanical pills, etc.) will have to adhere to current Good Manufacturing Practices (cGMPs), marking a major shift in regulatory oversight and testing for the industry. Previously, compliance was voluntary, but in 2003, due to public and industry concern, the FDA proposed requiring dietary supplement manufacturers to adhere to cGMP standards. The final rule was issued in June 2007 and is in full effect June 2010 [1]. Basic GMPs require implementing comprehensive procedures to ensure product quality and safety. Since many dietary supplements are largely derived from botanical sources, they must be tested for pesticide contaminants in order to meet cGMP regulations. As a result of this requirement, labs are working to develop and validate methods, an endeavor which is complicated by the wide range of pesticides and matrices to be tested.

Labs can begin method development with the FDA Pesticide Analytical Manual (PAM), which includes procedures for plant materials. While PAM Method 303 is an appropriate starting point, it has several disadvantages, including high solvent consumption, manual procedures that contribute to analytical variation, and the inability to extract polar pesticides. As an alternative, we developed a QuEChERS-based method for analyzing pesticides in dietary supplements that has several advantages over PAM 303 (Table I). QuEChERS is an approach that was developed by the USDA Eastern Regional Research Center as a simple, rapid, effective, yet inexpensive way to extract pesticide residues from fruits and vegetables, followed by a novel dispersive solid phase extraction (dSPE) cleanup of the extract. Because of these benefits, the approach has become popular and has been expanded to include numerous other matrices. We chose QuEChERS as an alternative to PAM 303 because of its speed, simplicity, and low solvent use, as well as its ability to produce good extraction efficiencies for relatively polar pesticides [2].

Based on preliminary studies, we knew that while the extraction part of QuEChERS would be successful, the dSPE cleanup step probably did not have the capacity to handle the matrix complexity of most dietary supplements. Thus, we compared dSPE to a cartridge solid phase extraction (cSPE) cleanup and established a procedure that uses a QuEChERS extraction, cSPE cleanup, and GC-TOFMS for accurate determinations of 46 pesticides in dandelion root powder. This approach saves time and can reduce analyst variation by minimizing manual preparation with prepackaged extraction salts and snap-and-shoot standards. As shown in Figure 1, it also uses much less solvent, salt, and sorbent, making it a greener, more cost-effective method than PAM 303.

**Table I** Decrease costs and increase reproducibility with a GMP-friendly QuEChERS approach to analyzing pesticides in dietary supplements.

	PAM 303 Method	QuEChERS + cSPE	Benefits of QuEChERS + cSPE
Solvent used (mL)	1,850	92	20x less solvent; cleaner, greener, & cost-effective
# of Solvents	4	3	
Salt and sorbent used (g)	35	6.6	5x less salt/sorbent
Glassware/lab equipment	<ul style="list-style-type: none"> <li>• Separatory funnel (1L capacity)</li> <li>• Filter apparatus</li> <li>• Florisil column</li> </ul>	<ul style="list-style-type: none"> <li>• Centrifuge</li> <li>• SPE manifold</li> </ul>	Fast, easy batch processing
Manual preparation	<ul style="list-style-type: none"> <li>• Salt solution</li> <li>• Standards</li> <li>• Florisil column</li> </ul>	None—prepackaged salts and cSPE cartridge are ready to use	Highly reproducible; less manual prep means less human error

## Procedure

### Sample Wetting and Fortification

Fully processed dandelion root powder obtained from a dietary supplement manufacturer was used for this work. The powder was wetted and then fortified with 46 pesticides representing different chemical classes that have been previously reported in dietary supplements [3]. Typically, QuEChERS methods use 10-15 grams of material with high water content (>80%). Therefore, to prepare for a QuEChERS extraction with a dry commodity, it is critical to use a reduced amount of material and wet it with water prior to extraction. In this work, 1 g of dietary supplement powder was combined with 9 mL of water. After shaking to mix well, the wetted supplement was fortified with 200 µL of a 2 ng/µL pesticides spiking solution resulting in a 400 ng/g spike level, relative to the original commodity. Also, 100 µL of QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) was added. The sample was then allowed to soak for 2 hours prior to extraction.

**Figure 1** QuEChERS extraction and cSPE cleanup simplifies sample prep for pesticides in dietary supplements.

#### QuEChERS Extraction

1. **Wet** 1 g of matrix powder with 9 mL of water. Fortify as necessary, then soak 2 hours.
2. **Add** 10 mL acetonitrile.
3. **Shake** 1 min.
4. **Add** Q-sep™ Q110 extraction salts.
5. **Shake** 1 min.
6. **Centrifuge** 5 min. at 3,000 U/min.



#### cSPE Cleanup

1. **Prepare** 6mL Resprep® Combo SPE Cartridges as follows. Add magnesium sulfate to a level approximately half the height of either the GCB or PSA bed. Rinse cartridge with 20 mL of 3:1 acetonitrile:toluene.
2. **Load** 1 mL of extract on cartridge and elute with 50 mL 3:1 acetonitrile:toluene.
3. **Evaporate** to approximately 0.5-1 mL using dry nitrogen gas and a 35-40°C water bath.
4. **Add** 3 mL toluene and evaporate to just under 0.5 mL.
5. **Rinse** evaporation vessel with toluene and adjust final volume to 0.5 mL.

**Solvent Usage: 92 mL, 3 solvents**

#### PAM Extraction

1. **Weigh** 20-25 g and fortify as necessary.
2. **Add** 350 mL 65:35 acetonitrile:water.
3. **Blend** 5 min. and filter.
4. **Transfer** to a 1 L separatory funnel and add 100 mL petroleum ether (hexanes).
5. **Shake** 1-2 min.
6. **Add** 10 mL saturated sodium chloride and 600 mL water.
7. **Shake** 45 seconds and allow layers to separate.
8. **Wash** organic layer with 100 mL water and transfer to a graduated cylinder.
9. **Wash** organic layer again with another 100 mL water and transfer to cylinder.
10. **Add** 15 g sodium sulfate to organic fraction.
11. **Shake** vigorously, then evaporate to ~100 mL.



#### PAM Cleanup

1. **Prepare** a Florisil® cleanup column as follows. Add Florisil® to a 22 mm x 300 mm column to a height of 4 inches, then top with ½ inch sodium sulfate.
2. **Transfer** extract to column for cleanup.
3. **Elute** in 3 separate fractions as follows:
  - a. 200 mL 6% diethyl ether in petroleum ether.
  - b. 200 mL 15% diethyl ether in petroleum ether.
  - c. 200 mL 50% diethyl ether in petroleum ether.
4. For each fraction: **evaporate** solvent, **adjust** final volume, and **add** internal standards as necessary for GC injection.

**Solvent Usage: 1,850 mL, 4 solvents**

### QuEChERS Extraction

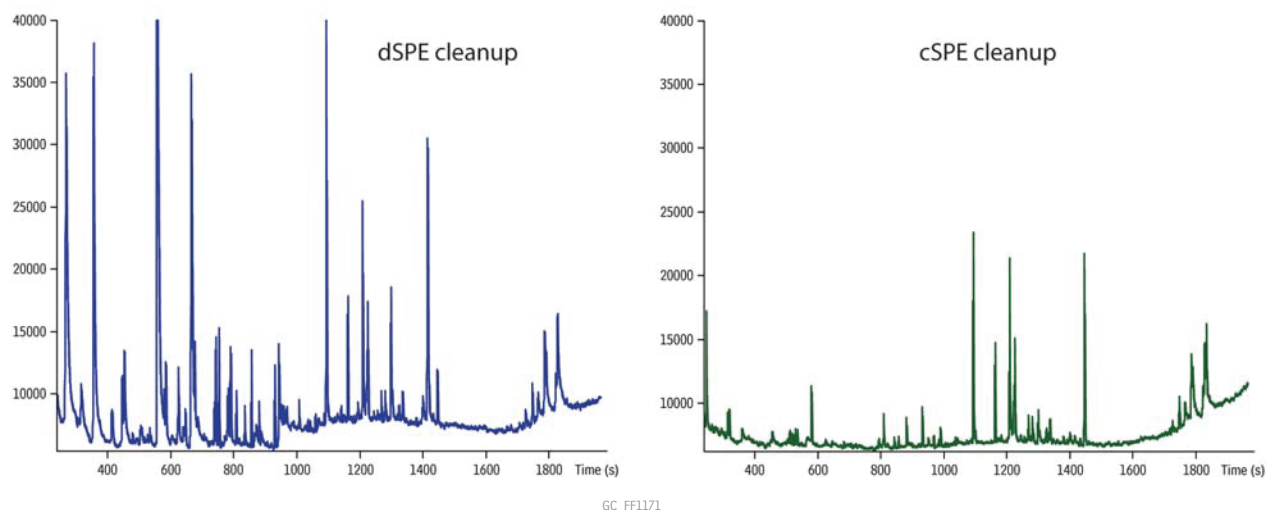
The EN 15662 QuEChERS method was used for sample extraction [4]. 10 mL of acetonitrile was added to the wetted sample. After a 1 minute shake, Q-sep™ Q110 buffering extraction salts (cat.# 26213, 4 g MgSO<sub>4</sub>, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate) were added. Following another 1 minute shake, the sample was centrifuged for 5 minutes at 3,000 U/min. with a Q-sep™ 3000 centrifuge (cat.# 26230). Lastly, 5 µL of quality control standard anthracene (cat.# 33264) was added to a 1 mL aliquot of extract to indicate fatal losses of planar compounds to CarboPrep® 90 during cleanup.

### Extract Cleanup

Two approaches were explored for extract cleanup: dSPE and cSPE. For dSPE, 1 mL of extract was added to a Q210 dSPE tube containing 150 mg MgSO<sub>4</sub> and 25 mg PSA (cat.# 26215), shaken for 2 minutes, and then centrifuged for 5 minutes. The resulting final extract was then analyzed by GC-TOFMS.

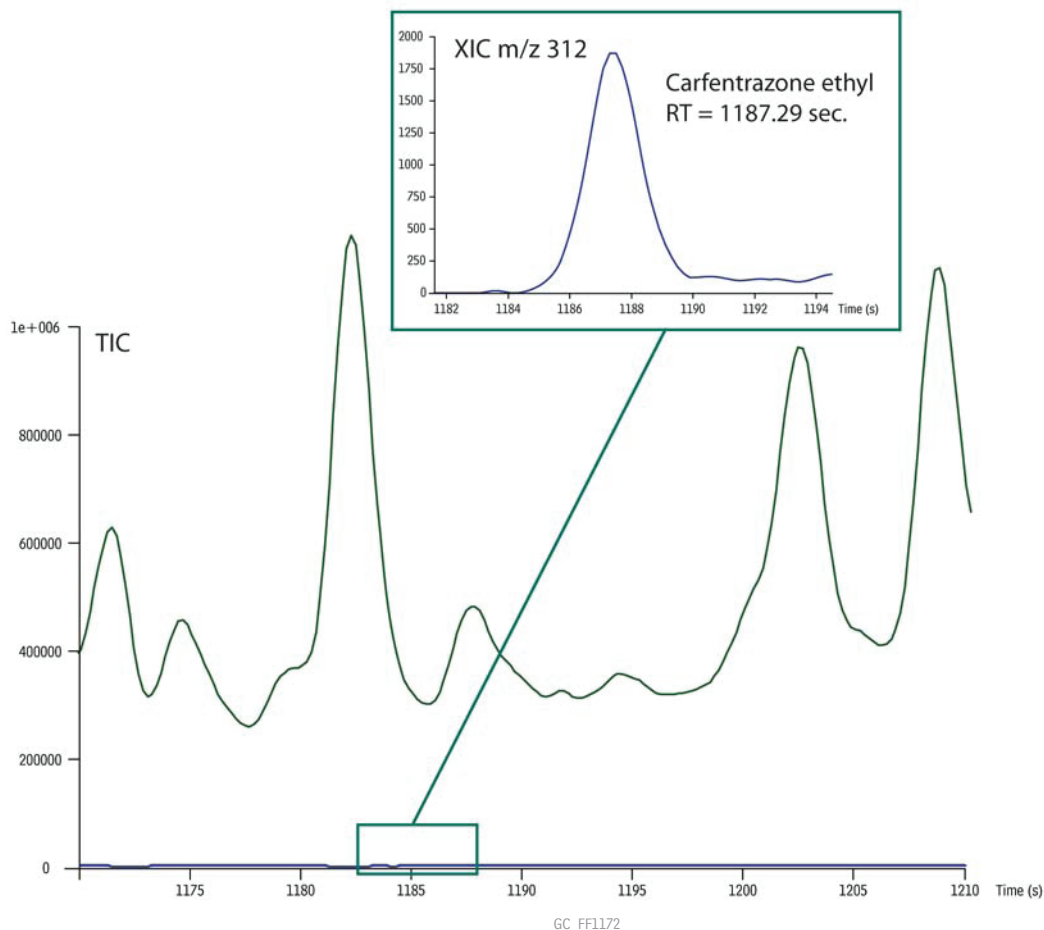
For cSPE cleanup [5], 1 mL of extract was processed with a 6 mL Resprep® Combo SPE Cartridge (cat.# 26194), which is designed for pesticide residue cleanup and contains 500 mg CarboPrep® 90 and 500 mg primary secondary amine (PSA). To prepare the SPE cartridge, magnesium sulfate was first added to a level approximately one-quarter height of the total bed; then the cartridge was rinsed with 20 mL of 3:1 acetonitrile: toluene, which was discarded. For cleanup, 1 mL of extract was loaded onto the prepared cartridge and then eluted with 50 mL 3:1 acetonitrile: toluene. The eluent was then evaporated and solvent exchanged using dry

**Figure 2** QuEChERS extracts of pesticides in dietary supplements benefit from cSPE cleanup, which minimizes matrix interferences by removing more sugars and fatty acids than dSPE.



<b>Column</b>	Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)
<b>Sample</b>	Dandelion Root powder spiked with 46 pesticides QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) anthracene for quality control (cat.# 33264) 80 pg/µL
<b>Conc.:</b>	80 pg/µL
<b>Injection</b>	
Inj. Vol.:	1 µL splitless (hold 1.5 min.)
Liner:	5mm Splitless with wool (cat.# 22975-200.1)
Inj. Temp.:	250 °C
<b>Oven</b>	
Oven Temp:	90 °C (hold 1.5 min.) to 340 °C at 8 °C/min.
<b>Carrier Gas</b>	He, constant flow
Flow Rate:	1.5 mL/min.
<b>Detector</b>	MS
<b>Mode:</b>	
Analyzer Type:	TOF
Source Temp.:	225 °C
Electron Energy:	70 eV
Ionization Mode:	EI
Acquisition Range:	45-550 amu
Acquisition Rate:	5 spectra/sec.
<b>Instrument</b>	LECO Pegasus 4D GCxGC-TOFMS
<b>Notes</b>	Sample Preparation: Wetting: 1 g of Dandelion Root powder combined with 9 mL of water, shaken well, fortified with pesticides and QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267), soak for 2 hours.  Extraction: 10 mL acetonitrile added then addition of Q-sep™ Q110 (cat.# 26213), centrifuge with Q-sep™ 3000 Centrifuge (cat.# 26230).  dSPE: cleanup procedure according to EN 15662 method, add control standard anthracene (cat.# 33264) to 1 mL extract, add this to Q-sep™ Q110 dSPE tube (cat.# 26213), shake, centrifuge.  cSPE: add control standard anthracene (cat.# 33264) to 1 mL extract, add magnesium sulfate to cartridge, use 6mL Combo SPE Cartridge containing 500mg CarboPrep® 90/500mg PSA (cat.# 26194), concentrate via evaporation.

**Figure 3** Using TOFMS allows definitive identification and quantification, even when matrix components coelute with target analytes. (Inset: carfentrazone ethyl S/N = 105; extracted ion chromatogram, m/z 312.)



**Column** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25  $\mu$ m (cat.# 13623)  
**Sample** Dandelion root powder spiked with 46 pesticides  
 QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267)  
 anthracene for quality control (cat.# 33264)

**Solvent:** toluene  
**Conc.:** 80  $\mu$ g/ $\mu$ L

**Injection**  
**Inj. Vol.:** 1  $\mu$ L splitless (purge valve time 1.5 min.)  
**Liner:** 5mm Splitless with wool (cat.# 22975-200.1)  
**Inj. Temp.:** 250 °C

**Oven**  
**Oven Temp:** 90 °C (hold 1.5 min.) to 340 °C at 8 °C/min.  
**Carrier Gas** He, constant flow  
**Flow Rate:** 1.5 mL/min.

**Detector** MS  
**Mode:** Full mass range  
**Analyzer Type:** TOF  
**Source Temp.:** 225 °C  
**Electron Energy:** 70 eV  
**Ionization Mode:** EI

**Acquisition**  
**Range:** 45-550 amu  
**Acquisition Rate:** 5 spectra/sec.  
**Instrument** LECO Pegasus 4D GCxGC-TOFMS

**Notes**  
**Sample Preparation:**  
 Wetting: 1 g of dandelion root powder combined with 9 mL of water, shaken well, fortified with pesticides and QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267), soak for 2 hours.  
 Extraction: 10 mL acetonitrile added, then Q-sep™ Q110 (cat.# 26213), centrifuge with Q-sep™ 3000 Centrifuge (cat.# 26230).  
 cSPE: add control standard anthracene (cat.# 33264) to 1 mL extract, add magnesium sulfate to cartridge, use 6 mL Combo SPE Cartridge containing 500 mg CarboPrep® 90/500 mg PSA (cat.# 26194), concentrate via evaporation

**Table II** This QuEChERS-based method provides good recoveries for a variety of pesticides found in dietary supplements.

Compound	RT (sec.)	Recovery (%)	Class	Type
1,2,3,5-Tetrachlorobenzene	418.0	46	Organochlorine	Chemical intermediate
Pentachlorobenzene	587.0	51	Organochlorine	Metabolite
Tetrachloronitrobenzene	648.8	72	Organochlorine	Fungicide
2,3,5,6-Tetrachloroaniline	678.0	64	Organochlorine	Fungicide
alpha-BHC	739.4	69	Organochlorine	Insecticide
Hexachlorobenzene	744.4	56	Organochlorine	Impurity
Pentachloroanisole	754.6	62	Organochlorine	Metabolite
beta-BHC	780.5	88	Organochlorine	Insecticide
Pentachloronitrobenzene	784.2	62	Organochlorine	Fungicide
Pentachlorobenzonitrile	790.0	70	Organochlorine	Impurity
gamma-BHC	791.2	85	Organochlorine	Insecticide
Diazinon	816.6	71	Organophosphorus	Insecticide
Chlorothalonil	819.2	100	Organochlorine	Fungicide
delta-BHC	836.4	85	Organochlorine	Insecticide
Pentachloroaniline	857.6	75	Organochlorine	Metabolite
Pentachlorothioanisole	931.2	66	Organochlorine	Metabolite
PCB 52	932.0	-	Organochlorine	Internal standard
Chlorpyrifos	952.6	92	Organophosphorus	Insecticide
Dacthal	958.8	83	Organochlorine	Herbicide
Parathion	963.2	91	Organophosphorus	Insecticide
Heptachlor epoxide	1008.4	93	Organochlorine	Metabolite
Procymidone	1027.4	100	Organonitrogen	Fungicide
Endosulfan I	1059.8	70	Organochlorine	Insecticide
4,4'-DDE	1094.6	90	Organochlorine	Metabolite
Dieldrin	1097.8	91	Organochlorine	Insecticide
Myclobutanil	1100.6	100	Organonitrogen	Fungicide
Endosulfan II	1141.6	110	Organochlorine	Insecticide
Oxadixyl	1149.4	100	Organonitrogen	Fungicide
4,4'-DDD	1152.2	98	Organochlorine	Insecticide, Breakdown product
2,4'-DDT	1155.0	94	Organochlorine	Insecticide
Carfentrazone ethyl	1188.0	110	Organonitrogen	Herbicide
Endosulfan sulfate	1194.8	105	Organochlorine	Metabolite
Fenhexamid	1202.4	94	Organonitrogen	Fungicide
4,4'-DDT	1203.8	96	Organochlorine	Insecticide
Piperonyl butoxide	1237.6	93	Other	Insecticide synergist
Iprodione	1261.0	110	Organochlorine	Fungicide
Cypermethrin 1	1466.8	130	Pyrethroid	Insecticide
Cypermethrin 2	1474.8	86	Pyrethroid	Insecticide
Cypermethrin 3	1478.6	75	Pyrethroid	Insecticide
Cypermethrin 4	1481.8	100	Pyrethroid	Insecticide
Pyraclostrobin	1538.0	92	Organonitrogen	Fungicide
Fluvalinate 1	1541.4	100	Pyrethroid	Insecticide
Fluvalinate 2	1546.8	94	Pyrethroid	Insecticide
Difenoconazole 1	1562.0	99	Triazole	Fungicide
Difenoconazole 2	1566.6	81	Triazole	Fungicide
Azoxystrobin	1596.0	93	Organonitrogen	Fungicide

nitrogen gas and a 35–40 °C water bath. Evaporation was allowed to proceed until approximately 0.5–1 mL eluent was left, at which point about 3 mL of toluene was added. The mixture was evaporated to just under 0.5 mL, and then the evaporation vessel was rinsed with toluene to bring the sample to a final volume of 0.5 mL. The resulting final extract was then analyzed by GC-TOFMS.

### Standards

Matrix-matched standards were prepared at 80 pg/μL, as 80 pg/μL is the expected final concentration in extract of the 400 ng/g matrix spikes (assuming 100% recoveries). Matrix-matched standards were prepared by adding standard solution to the final extract (post-cleanup) from a control sample. Actual recoveries were calculated by comparing peak areas for fortified samples that were extracted and cleaned, to areas of a matrix-matched standard, using the internal standard quantification method.

### GC-TOFMS

A LECO Pegasus III GC-TOFMS instrument was used and all data were processed with LECO ChromaTOF™ software. Gas chromatography was performed using an Rxi®-5Sil MS column (30m x 0.25mm x 0.25μm, cat.# 13623). Instrument conditions are shown in Figure 1. Temperature and flow settings yielded an analysis time of 32.75 minutes.

### Results

One aspect of this investigation was to compare the applicability of two sample cleanup methods, dSPE and cSPE for QuEChERS extracts of pesticides in dietary supplements. While dSPE has the advantage of improved speed and less solvent usage, it does not have the sorbent capacity to adequately clean up these samples (Figure 2). Since cSPE uses more sorbent, it is a better choice for dietary supplements (and other complex samples, e.g. spices, essential oils) as it can remove more matrix components, such as fatty acids, sugars, and pigments. QuEChERS methods developed for dietary supplements of botanical origin can benefit from the extra sorbent capacity of cSPE, which reduces GC inlet/column contamination and chromatographic interference from complex botanical matrices.

Even with effective extraction and cleanup techniques, dietary supplements can be challenging to analyze due to their complexity. Coelutions are common and pesticide residues can be overwhelmed by abundant matrix compounds not only qualitatively, but also by interfering with quantification masses. Figure 3 plots the total ion chromatogram (TIC) and extracted ion m/z 312 corresponding to the quantitation mass for carfentrazone ethyl. It is clear that target pesticide signals can be obscured in the TIC. LECO ChromaTOF™ software was able to identify target pesticides by comparison with reference spectra using automatic peak find and spectral deconvolution algorithms, along with calibration and quantification. TOFMS makes this powerful data processing possible with very fast acquisition rates and unbiased mass spectra, and by having pg level sensitivity in full mass range mode, which allows the potential for finding non-target pesticides. An alternate GC/MS approach for targeted pesticides in dietary supplements would be to use selected ion monitoring with a typical quadrupole mass spectrometer.

Overall, the combination of QuEChERS extraction, cSPE cleanup, and GC-TOFMS used in this method produced good recoveries for most compounds tested (Table II). Although early eluting compounds trended toward lower recoveries, most analytes, including more polar compounds, showed excellent recoveries. The potential for good recoveries of polar pesticides is a major advantage to QuEChERS methods; this difference is due to the use polar acetonitrile as the extraction solvent, rather than petroleum ether (hexanes) which is used in PAM 303. The lower recoveries here of early eluting compounds may be due to evaporative loss during concentration steps, due to their higher volatility. Additionally, in the case of planar compounds, reduced recoveries may be due to interaction with the CarboPrep® 90 sorbent used to remove pigments and other matrix compounds, although the planar quality control standard, anthracene, did not show drastic losses during cSPE. Overall, the chromatography and recovery results seen for a broad range of pesticides in dandelion root demonstrate the utility of the QuEChERS approach for dietary supplement testing.

### Conclusion

Demonstrated here is a QuEChERS approach that helps accomplish the pesticide testing now required for dietary supplements. The basic methodology presented here for dandelion root can be modified for other analytes and matrices and illustrates the advantages of the QuEChERS approach for labs developing cGMP methods. Analytical benefits include reduced interferences and good recoveries, even of polar compounds. Other benefits include an overall savings of both materials and prep time compared to the PAM 303 method, and better expected reproducibility due to the straight-forward procedure with fewer manual preparations.

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### Pesticide Residue Cleanup SPE Cartridges

- Convenient, multiple adsorbent beds in a single cartridge.
- For use in multiple-residue pesticide analysis, to remove matrix interferences.

SPE Cartridge	qty.	cat#
6mL Combo SPE Cartridge Packed with 500mg CarboPrep 90/500mg Aminopropyl, Polyethylene Frits	30-pk.	26193
6mL Combo SPE Cartridge Packed with 500mg CarboPrep 90/500mg PSA, Polyethylene Frits	30-pk.	26194
6mL SPE Cartridge Packed with 500mg PSA, Polyethylene Frits	30-pk.	26195
6mL Combo SPE Cartridge Packed with 200mg CarboPrep 200 and 400mg PSA, PTFE Frits	30-pk.	26127
6mL Combo SPE Cartridge Packed with 250mg CarboPrep 200 and 500mg PSA, PTFE Frits	30-pk.	26128
6mL Combo SPE Cartridge Packed with 500mg CarboPrep 200 and 500mg PSA, PTFE Frits	30-pk.	26129



### Q-sep™ QuEChERS Tubes

for Extraction and Clean-Up of Pesticide Residue Samples from Food Products

- Fast, simple sample extraction and cleanup using dSPE.
- Fourfold increases in sample throughput.
- Fourfold decreases in material cost.
- Convenient, ready to use centrifuge tubes with ultra pure, preweighed adsorbent mixes.

Description	Material	Methods	qty.	cat#
<b>50mL Centrifuge Tubes for Sample Extraction</b>				
Q110	4g MgSO <sub>4</sub> , 1g NaCl, 1g trisodium citrate dihydrate, 0.5g disodium hydrogen citrate sesquihydrate	European EN 15662	50-pk.	26213
Q150	6g MgSO <sub>4</sub> , 1.5g NaOAc	AOAC 2007.1	50-pk.	26214
Empty 50mL Centrifuge Tube	—	European EN 15662, AOAC 2007.1	25-pk.	26227
<b>2mL Micro-Centrifuge Tubes for dSPE (clean-up of 1mL extract)</b>				
Q210	150mg MgSO <sub>4</sub> , 25mg PSA	European EN 15662	100-pk.	26215
Q211	150mg MgSO <sub>4</sub> , 25mg PSA, 25mg C18	—	100-pk.	26216
Q212	150mg MgSO <sub>4</sub> , 25mg PSA, 2.5mg GCB	European EN 15662	100-pk.	26217
Q213	150mg MgSO <sub>4</sub> , 25mg PSA, 7.5mg GCB	European EN 15662	100-pk.	26218
Q250	150mg MgSO <sub>4</sub> , 50mg PSA	AOAC 2007.1	100-pk.	26124
Q251	150mg MgSO <sub>4</sub> , 50mg PSA, 50mg C18	AOAC 2007.1	100-pk.	26125
Q253	150mg MgSO <sub>4</sub> , 50mg PSA, 50mg GCB	—	100-pk.	26123
Q252	150mg MgSO <sub>4</sub> , 50mg PSA, 50mg C18, 50mg GCB	AOAC 2007.1	100-pk.	26219
<b>15mL Centrifuge Tubes for dSPE (clean-up of 6mL extract)</b>				
Q350	1200mg MgSO <sub>4</sub> , 400mg PSA	AOAC 2007.1	50-pk.	26220
Q351	1200mg MgSO <sub>4</sub> , 400mg PSA, 400mg C18	AOAC 2007.1	50-pk.	26221
Q352	1200mg MgSO <sub>4</sub> , 400mg PSA, 400mg C18, 400mg GCB	AOAC 2007.1	50-pk.	26222
Q370	900mg MgSO <sub>4</sub> , 150mg PSA	European EN 15662	50-pk.	26223
Q371	900mg MgSO <sub>4</sub> , 150mg PSA, 15mg GCB	European EN 15662	50-pk.	26224
Q372	900mg MgSO <sub>4</sub> , 150mg PSA, 45mg GCB	European EN 15662	50-pk.	26225
Q373	900mg MgSO <sub>4</sub> , 150mg PSA, 150mg C18	—	50-pk.	26226
Q374	900mg MgSO <sub>4</sub> , 300mg PSA, 150mg GCB	—	50-pk.	26126

### Sorbent Guide

Sorbent	Removes
MgSO <sub>4</sub>	excess water
PSA*	sugars, fatty acids, organic acids, anthocyanine pigments
C18	lipids, nonpolar interferences
GCB**	pigments, sterols, nonpolar interferences
*PSA—primary and secondary amine exchange material	
**GCB—graphitized carbon black	



**Q-sep™**  
innovative chromatography solutions

### Q-sep™ 3000 Centrifuge

for QuEChERS

- Meets requirements of AOAC and European QuEChERS methodology.
- Supports 50 mL, 15 mL, and 2 mL centrifuge tubes.
- Small footprint requires less bench space.
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Centrifuge includes 50 mL tube carriers (6), 50 mL conical tube inserts (6), 4-place 15 mL tube carriers (6), and 2 mL tube adaptors (24).

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



### Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623

### QuEChERS Quality Control Standards for GC/MS Analysis

Cat. # 33268:	Cat. # 33264:
PCB 138	anthracene
PCB 153	
50µg/mL each in acetonitrile, 5mL/ampul	
cat. # 33268 (ea.)	
100µg/mL in acetonitrile, 5mL/ampul	
cat. # 33264 (ea.)	

### QuEChERS Internal Standard Mix for GC/MS Analysis (6 components)

PCB 18	50µg/mL
PCB 28	50
PCB 52	50
triphenyl phosphate	20
tris-(1,3-dichloroisopropyl)phosphate	50
triphenylmethane	10
In acetonitrile, 5mL/ampul	
cat. # 33267 (ea.)	

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# Determining Pesticides in Dietary Supplements with QuEChERS Extraction, Cartridge SPE, and GCxGC-TOFMS

*Regulatory requirements are driving the development of new multiresidue pesticide methods for dietary supplements. Minimizing matrix interference is critical for data accuracy. The novel approach employed here combines QuEChERS extraction, cartridge SPE cleanup, and GCxGC-TOFMS analysis, and results in good recoveries across a range of compounds found in these complex matrices.*

## Introduction

Dietary supplement manufacturers must now comply with the current Good Manufacturing Practice (cGMP) regulations that also guide the manufacture of pharmaceuticals. cGMPs require testing that ensures product safety, and, since many dietary supplements are botanically based, pesticide residue methods are among the new analyses being developed. Methods that minimize matrix interference are especially important, as plant-based dietary supplements are extremely complex and data integrity can depend on removing or reducing matrix contributions.

Existing procedures for agricultural commodities are a good starting point for multiresidue pesticide methods. For example, the QuEChERS approach to sample extraction and cleanup was first developed as a fast, easy way to prepare fruit and vegetable samples for pesticide analysis, but it can also be applied to other areas. In recent work [1], we used a QuEChERS extraction method [2] with cartridge solid phase extraction (cSPE) cleanup to prepare dietary supplement samples for pesticide residue analysis by GC/MS. For dandelion root samples, matrix interferences were substantially reduced by using the higher capacity cSPE cleanup, and recoveries for a wide range of pesticides reported in dietary supplements [3] were very good. However, in more complex samples, quantification bias appeared for some pesticides, leading us to consider a relatively new technique, comprehensive two-dimensional gas chromatography (GCxGC) with time-of-flight MS.

GCxGC offers greater potential for accurate pesticide determinations than single dimension GC, because resolution is enhanced by applying two independent separations to a sample in one analysis. GCxGC involves a serial column configuration (differing phases) separated by a thermal modulator. A separation is performed on the first column, and then effluent from the first column is continually (and quickly) focused and injected onto the second column. By keeping the second column short, a series of high speed chromatograms are generated, and the first column separation can be maintained. Separation results are plotted as a retention plane (column 1 time x column 2 time). Use of orthogonal stationary phases optimizes peak resolution.

This work shows the application of QuEChERS, cSPE, and GCxGC-TOFMS with an Rxi®-5Sil MS x Rtx®-200 column combination to quantify pesticides in dietary supplements. The approach used here reduces matrix interferences and improves accuracy relative to one dimensional GC-TOFMS.



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**Tubes & Standards**

**for multi-residue pesticide analysis from food and other matrices**



## Experimental

### Sample Wetting and Fortification

Samples of powdered dandelion root, sage, and finished product (a combination of botanicals) were obtained from a dietary supplement manufacturer and used for this work. Since the QuEChERS method was originally developed for high aqueous content fruits and vegetables, modification is necessary when testing dry samples. For powders, such as those used here, using a reduced amount of sample and then adding water increases extraction efficiency. Therefore, 1 g of powder was wetted with 9 mL organic-free water for each sample. After shaking to mix well, wetted powders were fortified as described below and then allowed to soak for 1 hour prior to QuEChERS extraction.

- *Unspiked Dietary Supplement*

Each control sample was fortified with 100  $\mu$ L of QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) containing PCBs 18, 28, and 52 (50  $\mu$ g/mL each), triphenylphosphate (20  $\mu$ g/mL), tris-(1,3-dichloroisopropyl)phosphate (50  $\mu$ g/mL), and triphenylmethane (10  $\mu$ g/mL).

- *400 ng/g Spiked Dietary Supplement*

Each spike was fortified with 200  $\mu$ L of a 2 ng/ $\mu$ L standard that contained 46 pesticides, representing different chemical classes, previously reported in dietary supplements [3]. 100  $\mu$ L of QuEChERS Internal Standard Mix for GC-MS Analysis was also added.

### QuEChERS Extraction

The EN 15662 QuEChERS method was used for sample extraction [2]. 10 mL of acetonitrile was added to each wet sample. After a 1 minute shake, Q-sep™ Q110 buffering extraction salts (4 g  $\text{MgSO}_4$ , 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydroxide citrate sesquihydrate; cat.# 26235) were added. Following another 1 minute shake, the sample was centrifuged for 5 minutes at 3,000 g with a Q-sep™ 3000 centrifuge (cat.# 26230).

### Extract Cleanup

Dispersive SPE (dSPE) cleanup is typically associated with the QuEChERS approach, but previous work indicated sorbent capacity with the EN dSPE PSA tubes was inadequate [1]; therefore, several different cleanup procedures were compared, including various dSPE cleanups and a cartridge SPE (cSPE) cleanup.

For dSPE, 1 mL portions of QuEChERS extracts were added to Q210 tubes (cat. # 26215) containing 150 mg  $\text{MgSO}_4$  and 25 mg primary secondary amine (PSA). The tubes were shaken for 2 minutes and then centrifuged for 5 minutes in the Q-sep™ 3000 centrifuge. Supernatant extract was removed by Pasteur pipette for analysis. This procedure was also followed for other samples using tubes containing different sorbent materials, such as graphitized carbon black (GCB). Sorbents tested were Q211 (150 mg  $\text{MgSO}_4$ , 25 mg PSA, 25 mg C18; cat.# 26216), Q213 (150 mg  $\text{MgSO}_4$ , 25 mg PSA, 7.5 mg GCB; cat.# 26218), and Q252 (150 mg  $\text{MgSO}_4$ , 50 mg PSA, 50 mg C18, 50 mg GCB; cat.# 26219).

For cSPE, a 6 mL Resprep® Combo SPE Cartridge (cat.# 26194) containing 500 mg CarboPrep® 90 and 500 mg PSA for pesticide residue cleanup was used. Anhydrous  $\text{MgSO}_4$  was added on top to a level approximately one-quarter height of the total bed followed by a cartridge rinse with 20 mL 3:1 acetonitrile:toluene, which was discarded. 1 mL of QuEChERS dietary supplement extract was then loaded onto the cartridge and eluted with 50 mL 3:1 acetonitrile:toluene. The eluent was evaporated and solvent exchanged using dry nitrogen gas and a 35-40 °C water bath. Evaporation proceeded until approximately 0.5-1 mL extract was left, at which point about 3 mL of toluene was added. The extract was evaporated to just under 0.5 mL and the evaporation vessel was rinsed with toluene to bring the sample to a final volume of 0.5 mL.

The resulting final extracts for all matrices, with cleanup by either a dSPE procedure or cSPE, were analyzed by both GC-TOFMS and GCxGC-TOFMS.

### GC-TOFMS

A LECO Pegasus® 4D GCxGC-TOFMS instrument was used and all data were processed with LECO ChromaTOF® software. One-dimensional gas chromatography was performed using a 30 m x 0.25 mm x 0.25  $\mu$ m Rxi®-5Sil MS column (cat.# 13623) with a constant flow of helium at 1.5 mL/min. 1  $\mu$ L fast autosampler splitless injections were made into a 5 mm single gooseneck liner with wool (cat.# 22405) at 250 °C. The purge valve time was 90 seconds. The GC oven program was 90 °C (1.5 min.), 8 °C/min. to 340 °C. Electron ionization at 70 eV was used with a source temperature of 225 °C. Data acquisition was from 45 to 550 u at a rate of 5 spectra/sec.

### GCxGC-TOFMS

The LECO Pegasus® 4D GCxGC-TOFMS was operated in comprehensive two-dimensional gas chromatography mode with a 30 m x 0.25 mm x 0.25  $\mu$ m Rxi®-5Sil MS column (cat.# 13623) connected to a 1.5 m x 0.18 mm x 0.20  $\mu$ m Rtx®-200 column (cut from a 10 m column, cat.# 45001) with a deactivated Universal Press-Tight® Connector (cat.# 20429). These orthogonal phases were chosen to maximize peak separation. Instrument conditions are shown in Figure 1.

### Calibration and Quantification with Matrix-Matched Standards

Matrix-matched standards for each matrix were prepared at 80 pg/ $\mu$ L, representing 100% recovery of pesticides in a final extract, by adding standard solution to the final extract from an unspiked sample. Actual recoveries were calculated after quantification from one-point calibration in ChromaTOF®. The internal standard method of quantification was employed using PCB 52.

## Results

We previously demonstrated that the dispersive SPE cleanup approach of QuEChERS, specifically 25 mg PSA per mL extract, was too weak to remove matrix interferences for complex dietary supplement extracts [1]. We saw similar results here for all matrices, even though we employed higher amounts of PSA and additional sorbents, including GCB, which is typically excellent for removing pigments and other compounds. In contrast, cartridge SPE has much higher capacity for removing matrix interferences and resulted in acceptable quantification for the dandelion root samples. However, even with cSPE cleanup, the sage and finished product extracts still showed quantification bias for some pesticides when using one-dimensional GC/MS, due to the overwhelming complexity of the matrix (Table I).

**Table I** GC-TOFMS and GCxGC-TOFMS recovery comparison for QuEChERS extracts and cartridge SPE cleanups of dietary supplements.

Compound	Dandelion			Sage		Finished Product	
	Quant Mass	GC Rec %	GCxGC Rec %	GC Rec %	GCxGC Rec %	GC Rec %	GCxGC Rec %
1,2,3,5-Tetrachlorobenzene	216	46	56	65	61	52	58
Pentachlorobenzene	250	51	57	75	68	55	60
Tetrachloronitrobenzene	261	72	64	93	85	57	64
2,3,5,6-Tetrachloroaniline	229	64	69	92	83	63	66
alpha-HCH	219	69	70	88	84	69	68
Hexachlorobenzene	284	56	61	74	67	62	61
Pentachloroanisole	265	62	73	77	78	62	64
beta-HCH	219	88	102	95	90	80	81
Pentachloronitrobenzene	237	62	70	97	87	65	68
Pentachlorobenzonitrile	275	70	74	81	81	71	72
gamma-HCH	219	85	76	100	87	83	72
Diazinon	179	71	72	98	103	70	64
delta-HCH	219	85	95	97	91	86	82
Pentachloroaniline	265	75	84	95	85	73	74
Pentachlorothioanisole	246	66	76	82	76	68	68
PCB 52	292	ISTD	ISTD	ISTD	ISTD	ISTD	ISTD
Chlorpyrifos	314	92	86	106	98	75	80
Dacthal	301	83	95	101	94	79	78
Parathion	291	91	94	89	91	90	80
Heptachlor epoxide	353	93	84	109	90	69	76
Procymidone	283	104	107	102	99	97	85
Endosulfan I	195	70	90	84	86	92	89
4,4'-DDE	318	90	100	84	88	102	106
Dieldrin	263	91	99	94	87	89	80
Myclobutanil	179	103	109	102	97	93	92
Endosulfan II	195	109	103	86	91	159	163
Oxadixyl	132	101	109	Int	97	86	91
4,4'-DDD	235	98	101	105	105	89	95
2,4'-DDT	235	94	102	88	90	86	82
Carfentrazone ethyl	312	112	106	102	100	88	93
Endosulfan sulfate	387	105	117	119	94	111	92
Fenhexamid	177	94	75	Int	85	110	86
4,4'-DDT	235	96	110	106	100	102	89
Piperonyl butoxide	176	93	106	123	93	73	91
Iprodione	187	112	125	Int	87	58	83
Cypermethrin	163	98	107	Int	88	Int	72
Pyraclostrobin	132	92	109	90	74	85	88
Fluvalinate	250	99	112	95	88	85	88
Difenoconazole	265	90	102	98	78	85	83
Azoxystrobin	344	93	105	118	80	52	86

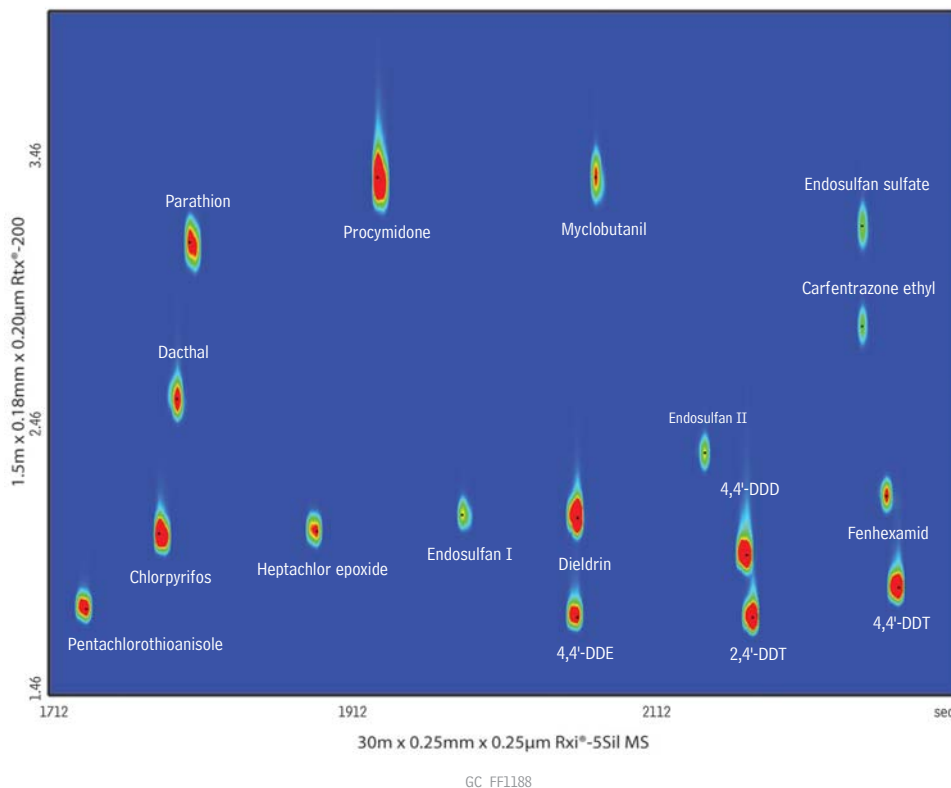
PCB 52 is the Internal Standard.

Cypermethrin, Fluvalinate, and Difenoconazole represent values from summed isomers.

Int = interference that prevented quantification.

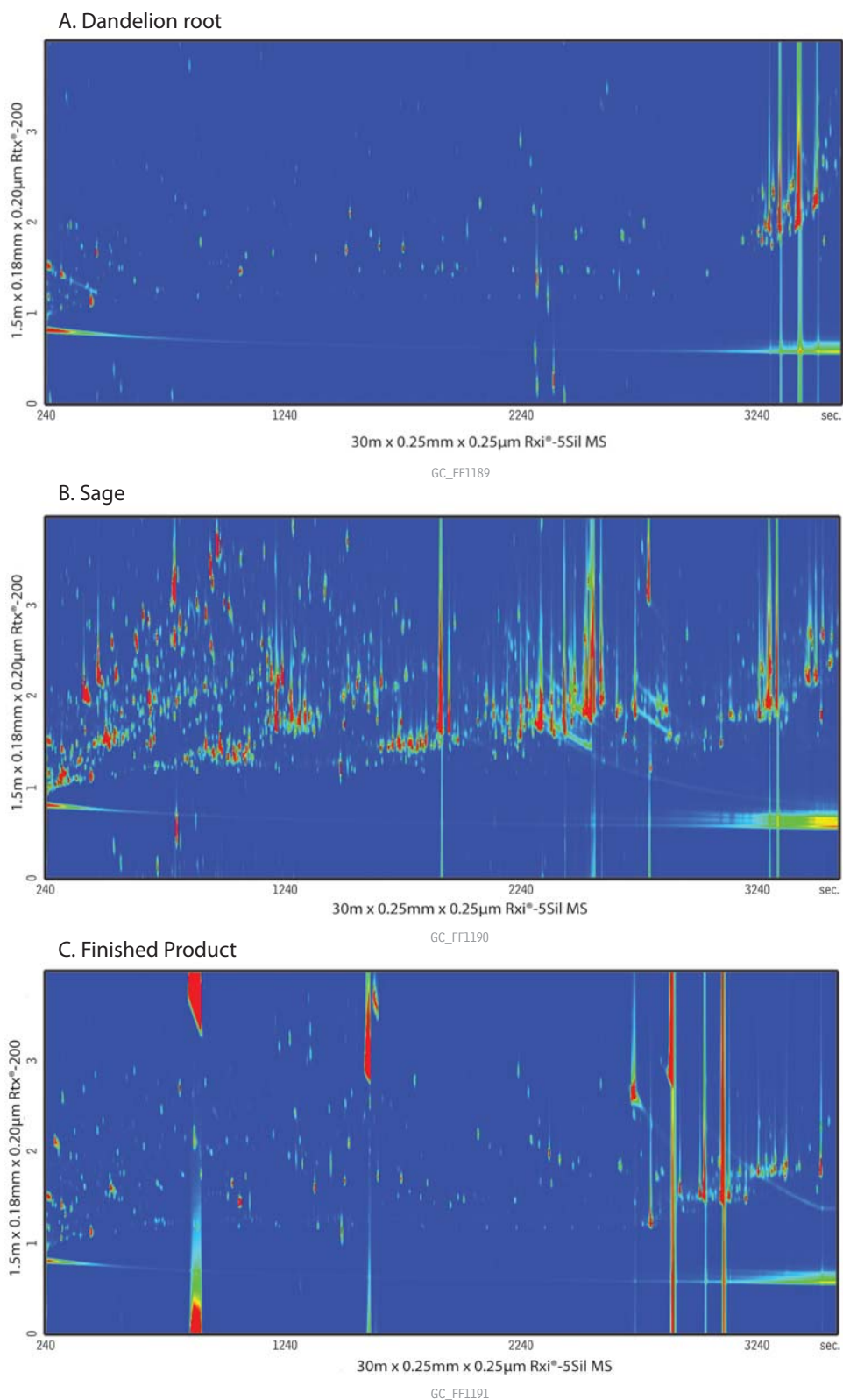
GCxGC allows two independent separations in one analytical run, which not only increases resolution among pesticides (Figure 1), but also spreads out all peaks, increasing the qualitative and quantitative accuracy of trace residue determinations in complex samples (Figure 2). Its specific value in the case of sage and finished product extracts was to allow the unbiased quantification of Oxadixyl, Fenhexamid, Iprodione, and Cypermethrin (Table I). As shown in Figure 3, Fenhexamid in sage was separated just enough when using GCxGC to not only get an accurate recovery value (Table I), but also to yield a mass spectrum that matches well with the reference spectrum (Figure 4).

**Figure 1** GCxGC-TOFMS separation of a dietary supplement pesticide standard. GCxGC-TOFMS allows the separation 2,4'-DDT and 4,4'-DDD along the second dimension (Rtx®-200 column). These compounds coelute in the first dimension (Rxi®-5Sil MS column) and have very similar mass spectra.



**Column:** Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25  $\mu$ m (cat.# 13623); Rtx®-200 1.5 m, 0.18 mm ID, 0.20  $\mu$ m (cat.# 45001); **Sample:** Mixed pesticide standard; Diluent: toluene; Conc.: 2 ng/ $\mu$ L; **Injection:** Inj. Vol.: 1  $\mu$ L splitless (hold 1 min.), Liner: Gooseneck Splitless (4mm) w/Wool (cat.# 22405); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min.; **Oven:** Oven Temp: Rxi®-5Sil MS: 80 °C (hold 1 min.) to 310 °C at 4 °C/min. (hold 1.5 min.), Rtx®-200: 90 °C (hold 1 min.) to 320 °C at 4 °C/min. (hold 1.5 min.); Carrier Gas: He, constant flow; Flow Rate: 1.8 mL/min.; **Modulation:** Modulator Temp. Offset: 25 °C; Second Dimension Separation Time: 4 sec.; Hot Pulse Time: 1.2 sec.; Cool Time between Stages: 0.8 sec.; **Detector:** TOFMS; Transfer Line Temp.: 290 °C; Analyzer Type: TOF; Source Temp.: 225 °C; Electron Energy: 70 eV; Mass Defect: -20 mu/100 u; Solvent Delay Time: 4 min.; Ionization Mode: EI; Acquisition Range: 45 to 550 amu; Spectral Acquisition Rate: 100 spectra/sec; **Instrument:** LECO Pegasus 4D GCxGC-TOFMS

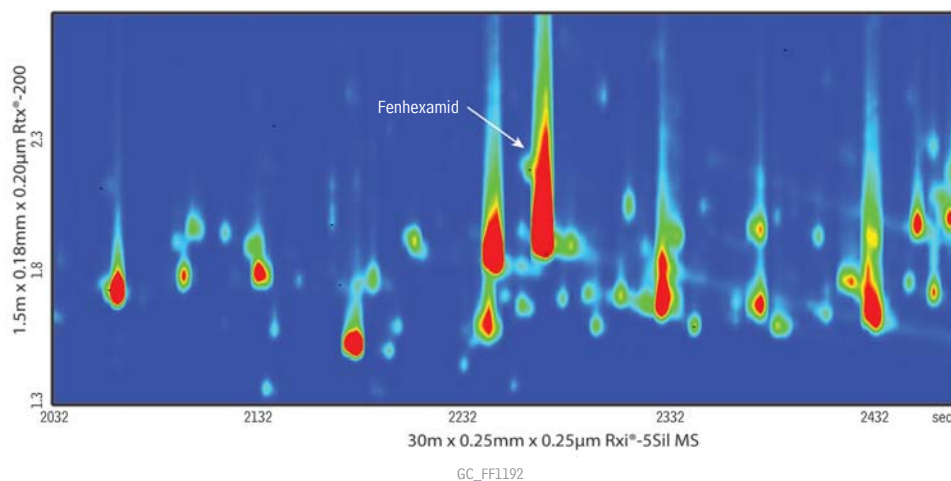
**Figure 2** GCxGC-TOFMS can be used to separate compounds that coelute in complex dietary supplement matrices when analyzed by single dimension GC-TOFMS.



See Figure 1 for conditions.

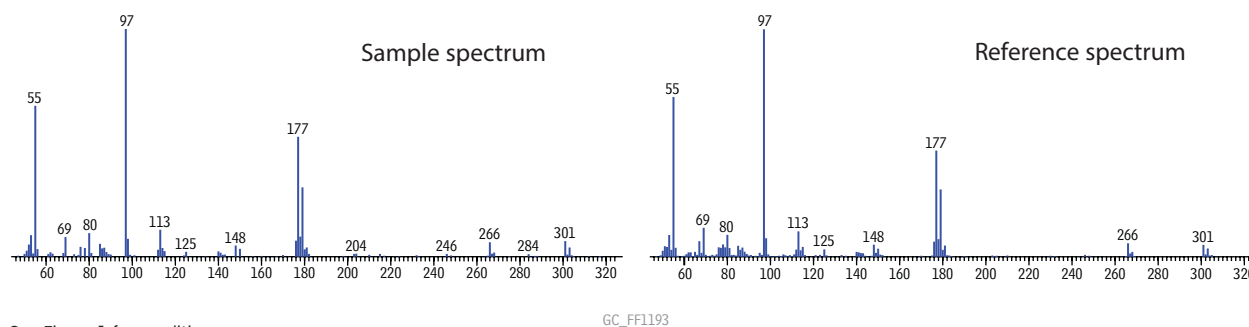
**Figure 3** Fenhexamid coelutes with a major interference in one-dimensional GC-TOFMS, but the slight separation achieved with GCxGC allows quantification.

GCxGC-TOFMS extracted ion contour plot for the quantification mass (177 m/z) of Fenhexamid in sage extract.



See Figure 1 for conditions.

**Figure 4** The excellent match between sample and library Fenhexamid spectra is achieved with the GCxGC separation using Rxi®-5Sil MS and Rtx®-200 columns.



See Figure 1 for conditions.

A more subtle correction on recovery for gamma-hexachlorocyclohexane (Lindane) in sage was achieved when using GCxGC-TOFMS by separating an isobaric interference that coeluted with Lindane in one-dimensional GC-TOFMS. This GCxGC separation is shown in Figure 5 as the peak immediately above gamma-hexachlorocyclohexane. A 100% recovery value was reported in Table I for GC-TOFMS, but a plot of the chlorine isotope m/z ions associated with the 219 ion used for Lindane quantification, indicates a high bias on the 219 ion versus a standard (Figure 6). In addition, the peak apexes do not line up properly for the Lindane in sage, another indication of coelution for one-dimensional GC. The 87% recovery value from GCxGC, although lower, is more accurate.

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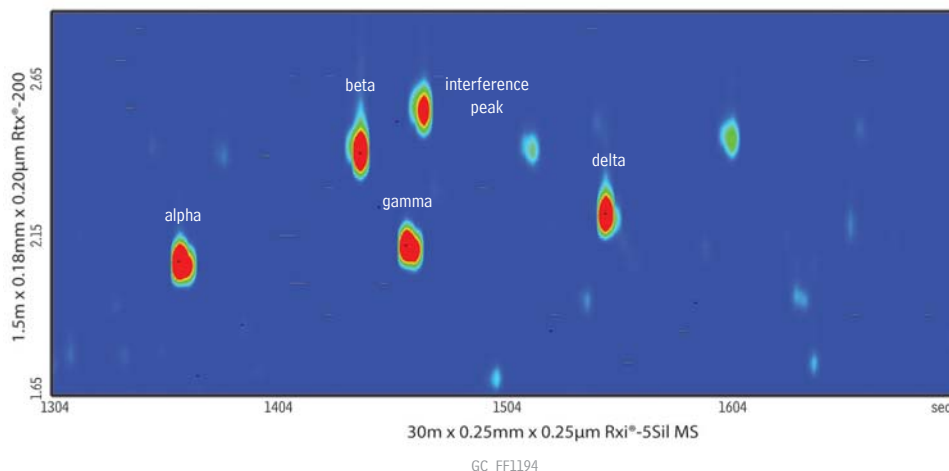


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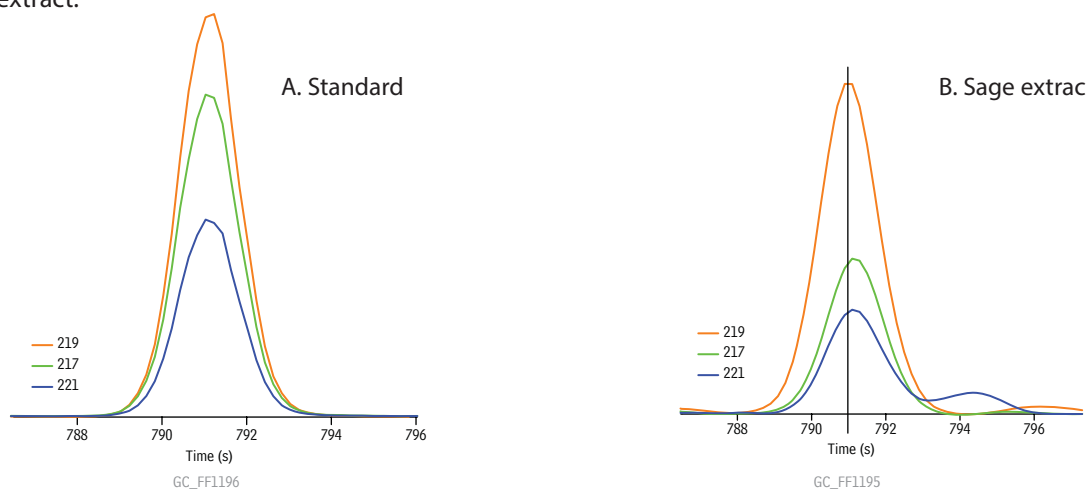
**Figure 5** The interference just above the gamma-HCH peak at  $m/z$  219 causes high quantification bias in one-dimensional GC-TOFMS, but the peaks are fully resolved and can be accurately quantified by GCxGC-TOFMS.

GCxGC-TOFMS extracted ion contour plot for the quantification mass (219  $m/z$ ) of the hexachlorocyclohexanes in sage extract.



See Figure 1 for conditions.

**Figure 6** The correct chlorine isotope pattern for HCH can be seen in the standard, but is inaccurate for the sage extract due to a coeluting compound. In addition, the peak apexes for the ions do not align for the HCH in the sage extract.



**Column:** Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25  $\mu$ m (cat.# 13623); **Sample:** Diluent: Toluene; **Injection:** Inj. Vol.: 1  $\mu$ L splitless (hold 1.5 min.); Liner: Gooseneck Splitless (4mm) w/Wool (cat.# 22405); Inj. Temp.: 250  $^{\circ}$ C; Purge Flow: 40 mL/min.; **Oven:** Oven Temp: 90  $^{\circ}$ C (hold 1.5 min.) to 340  $^{\circ}$ C at 8  $^{\circ}$ C/min.; Carrier Gas: He, constant flow, Flow Rate: 1.5 mL/min.; **Detector:** TOFMS; Transfer Line Temp.: 300  $^{\circ}$ C; Analyzer Type: TOF; Source Temp.: 225  $^{\circ}$ C; Electron Energy: 70 eV; Mass Defect: -20 mu/100 u; Solvent Delay Time: 4 min.; Ionization Mode: EI; Acquisition Range: 45 to 550 amu; Spectral Acquisition Rate: 5 spectra/sec; **Instrument:** LECO Pegasus 4D GCxGC-TOFMS

**Notes:** See application note PHAN1251 for extraction and cleanup details.

## Conclusions

QuEChERS is a fast, solvent-saving approach originally developed for fruits and vegetables that can be extended to other matrices. As shown here, QuEChERS extraction with cartridge SPE cleanup of dietary supplement samples resulted in good recoveries for many pesticides, but a more powerful instrumental method such as GCxGC-TOFMS is sometimes necessary to minimize the impact of matrix interference in these complex samples. The benefits of GCxGC-TOFMS are maximized by using orthogonal stationary phases, such as Rxi®-5Sil MS and the Rtx®-200 columns, which allow optimized GCxGC separations.

## References

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2. Foods of Plant Origin—Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE (QuEChERS-method). (EN 15662 Version 2008).
3. J.W. Wong, M.S. Wirtz, M.K. Hennessy, F.J. Schenck, A.J. Krynitsky, S.G. Capar, Acta Hort. (ISHS) 720 (2006) 113.

# Product Listing

## Q-sep™ QuEChERS Sample Prep Packets & Tubes

- Ready-to-use tubes, no glassware required.
- Preweighed, ultra-pure sorbents.
- Convenient, method-specific standards.

Description	Material	Methods	qty.	cat.#
<b>Extraction Salt Packets and 50mL Centrifuge Tubes</b>				
Q110 kit	4g MgSO <sub>4</sub> , 1g NaCl, 1g TSCD, 0.5g DHS with 50mL Centrifuge Tube	European EN 15662	50 packets & 50 tubes	26235
Q110 packets	4g MgSO <sub>4</sub> , 1g NaCl, 1g TSCD, 0.5g DHS	European EN 15662	50 packets	26236
Empty 50mL Centrifuge Tube			50-pk.	26239
<b>2mL Micro-Centrifuge Tubes for dSPE (clean-up of 1mL extract)</b>				
Q210	150mg MgSO <sub>4</sub> , 25mg PSA	European EN 15662	100-pk.	26215
Q211	150mg MgSO <sub>4</sub> , 25mg PSA, 25mg C18		100-pk.	26216
Q213	150mg MgSO <sub>4</sub> , 25mg PSA, 7.5mg GCB	European EN 15662	100-pk.	26218
Q252	150mg MgSO <sub>4</sub> , 50mg PSA, 50mg C18, 50mg GCB	AOAC 2007.01	100-pk.	26219

## Q-sep™ 3000 Centrifuge for QuEChERS

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

## Pesticide Residue Cleanup SPE Cartridges

- Convenient, multiple adsorbent beds in a single cartridge.
- For use in multiple-residue pesticide analysis, to remove matrix interferences.

SPE Cartridge	qty.	cat.#
6mL Combo SPE Cartridge		
Packed with 500mg CarboPrep 90/500mg PSA, Polyethylene Frits	30-pk.	26194

## Rtx®-200 (fused silica)

(midpolarity phase; Crossbond® trifluoropropylmethyl polysiloxane)

ID	df (μm)	temp. limits*	15-Meter
0.18mm	0.20μm	-20 to 310/330°C	45001

## Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (μm)	temp. limits	30-Meter
0.25mm	0.25μm	-60 to 330/350°C	13623

## 5.0mm ID Straight Inlet Liner w/ Wool

ID* x OD & Length	qty.	cat.#
Straight, Intermediate Polarity (IP), Semivolatiles Wool, 5.0mm x 6.5mm x 78.5mm	ea.	22975-231.1
5.0mm x 6.5mm x 78.5mm	5-pk.	22976-231.5

## 4.0mm ID Single Gooseneck Inlet Liner w/ Wool

ID* x OD & Length	qty.	cat.#
Single Gooseneck, Intermediate Polarity (IP), Deact. Wool, 4.0mm x 6.5mm x 78.5mm	ea.	22405
4.0mm x 6.5mm x 78.5mm	5-pk.	22406
4.0mm x 6.5mm x 78.5mm	25-pk.	22407
Single Gooseneck, Intermediate Polarity (IP), Semivolatiles Wool, 4.0mm x 6.5mm x 78.5mm		
4.0mm x 6.5mm x 78.5mm	ea.	20798-231.1
4.0mm x 6.5mm x 78.5mm	5-pk.	20799-231.5
4.0mm x 6.5mm x 78.5mm	25-pk.	20800-231.25

## Press-Tight® Connectors

- Deactivated Press-Tight® connectors assure better recovery of polar and nonpolar compounds.
- Siltek® treated connectors are ideal for organochlorine pesticides analysis.
- Fit column ODs from 0.33–0.74mm (Restek 0.1mm–0.53mm ID).
- Made from inert fused silica.

Description	5-pk.	25-pk.	100-pk.
Universal Press-Tight Connectors	20400	20401	20402
Universal Press-Tight Connectors, Deactivated	20429	20430	
Universal Press-Tight Connectors, Siltek Treated	20480	20449	

## QuEChERS Quality Control Standards for GC/MS Analysis

Cat.# 33268:	Cat.# 33264:
PCB 138	anthracene
PCB 153	

50μg/mL each in acetonitrile, 5mL/ampul  
cat. # 33268 (ea.)

100μg/mL in acetonitrile, 5mL/ampul  
cat. # 33264 (ea.)

## QuEChERS Internal Standard Mix for GC/MS Analysis

(6 components)

PCB 18	50μg/mL	tris-(1,3-dichloroisopropyl)phosphate	
PCB 28	50		
PCB 52	50		50
triphenyl phosphate	20	triphenylmethane	10
In acetonitrile, 5mL/ampul			
cat. # 33267 (ea.)			

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## Column Choice: A Critical Factor for Successful UHPLC Integration

Content previously published in *Next Generation Pharmaceutical*

*Ultra High Pressure Liquid Chromatography (UHPLC) is an increasingly popular platform for analytical method development in the pharmaceutical laboratory. Higher productivity can be realized with this technique, ultimately offsetting some of the rising costs of drug development. However, the challenge we now face is seamlessly integrating methods developed using UHPLC into pharmaceutical labs, which are still dominated by conventional HPLC systems. Column choice is a critical factor in successfully transferring methods between UHPLC and HPLC—poor choices ultimately cause failure or costly delays in development and transfer. Here, we discuss what attributes are needed from the analytical column, and how these qualities contribute to the successful integration of UHPLC technology into pharmaceutical labs.*

High Pressure Liquid Chromatography (HPLC) is the cornerstone analytical technique of the pharmaceutical laboratory; it is employed in every segment of drug development, from discovery through final product testing. However, HPLC is fundamentally restricted by the pressure limitations of the instrumentation, which effectively limit the particle sizes used in column packings to 3 micron or larger size particles. Recently, the advent of Ultra High Pressure Liquid Chromatography (UHPLC) spurred the next leap in liquid chromatographic techniques, offering higher pressure limits, faster throughput, and the promise of ultimately lowering operating costs. Higher productivity can be achieved by developing methods using <2 micron particle size columns in conjunction with UHPLC instrumentation, however, the UHPLC method generally must be scaled to conventional HPLC for routine analysis.

### The Next Hurdle—Integration

The benefits of UHPLC addressed the need of the pharmaceutical industry for higher productivity, so it has been implemented rather quickly and into diverse segments of the drug development process. Now, the integration and optimization of this novel technique is the challenge. How do we best utilize it in an industry that is still largely dominated by conventional HPLC systems? UHPLC can be integrated in two primary ways. First, to increase sample throughput, a conventional HPLC analysis can be transferred to UHPLC (scale down). Alternatively, to lessen time in method development, a preliminary separation can be developed in UHPLC and then transferred to conventional HPLC for routine analysis (scale up). In both cases, in order to realize the practical and financial benefits of UHPLC, the methodology needs to be easily and routinely transferred between conventional HPLC and UHPLC. Since the common element between these two technologies is not the instrumentation, but rather the separation achieved, the properties of the analytical column are critical in ensuring the success of the method transfer.

### Successful Transfer Starts with Fully Scalable Silica

The basis for method transfer, or scaling, is the silica support material used to produce the column packings. To maintain the separation and ease method transfer while scaling an analysis, the properties of the silica used in the columns must remain the same. The columns used both in the UHPLC and HPLC methods must be built from the same base material and differ only in particle size. Other differences in the base silica can cause variation in chromatographic properties such as peak shape, retention time, and even selectivity. These differences can result in significant additional time spent optimizing the HPLC operating conditions in order to obtain the same results achieved during the UHPLC separation. Fully-scalable columns, such as the Pinnacle® DB line, are designed specifically for scaling and use a consistent base support for both HPLC and UHPLC size columns.

Scaling methods is a mathematical process. We can calculate the HPLC parameters, like column length, flow rate, injection volume and time program, from the corresponding UHPLC parameters. But, this can only be truly mathematical if the physical properties of the columns are consistent. Let's look at particle size for example. The main distinction between UHPLC and HPLC columns is the particle size used in the packing material. UHPLC columns employ a smaller particle, less than 2 micron in diameter. In column terminology, particle size refers to the mean diameter of the silica spheres used as the support material to which the stationary phase is bonded. This does not indicate that all particles inside the column are of that specific diameter, but rather that diameter is actually the mean of the distribution of all particles used in the manufacturing of the column. For scaling purposes, it is important to consider both the accuracy of the listed particle size as well as the width of the particle size distribution inside the column. Only columns with tightly controlled manufacturing specifications perform reliably in scaled method transfers.

Accuracy and precision of the particle size becomes increasingly important as the particle size decreases. In practice, the smaller the particle size distribution, the more consistent the column packing. This distribution is even more critical when manufacturing columns with particle sizes of less than 2 microns. If this distribution contains many larger particles, and is not tightly controlled, the efficiency of the column will suffer and column-to-column reproducibility may vary. More importantly, if the column contains particles less than 1 micron (termed "fines"), clogging of the column frit and unwanted column backpressure can result. Choosing a column with a narrow and tightly controlled particle size distribution is critical for achieving optimum performance and easy scalability.

### Phase Choices—Key to Getting Speed *and* Selectivity

Fundamentally, the overall goal in both UHPLC and HPLC is still chromatographic resolution, whether between analytes or between an analyte and the sample matrix. Although UHPLC has given us the capability to use <2 micron HPLC packing materials and provides us with a significant increase in peak efficiency, as well as a drastic reduction in analysis time, the increase in the number of theoretical plates is not large enough to ignore the importance of the chromatographic stationary phase. If we consider the factors that contribute to resolution, we can better see the significance of the column parameters. How well we resolve our analytes, and how quickly we do it, depends upon our ability to control three factors: selectivity ( $\alpha$ ), retention capacity ( $k'$ ) and efficiency ( $N$ ). The smaller particles used in UHPLC primarily affects the efficiency, or  $N$  term, of the resolution equation. While this can improve and speed up a separation (smaller particles give rise to greater column efficiencies and a wider usable range of flow rates), it is only one contributor towards the goal of resolution, and minor one at that.

Selectivity, which is governed predominantly by analyte interactions with both the stationary and mobile phases, is arguably the driving force behind separations as it affects resolution to the greatest degree. One limitation we currently see in implementing UHPLC is the need for more selective stationary phases—higher quality separations, not just faster separations. One UHPLC column line, the Pinnacle® DB line, includes the widest variety of stationary phase chemistries for UHPLC, including phases commonly used in pharmaceutical analyses, as well as some unique application-specific phases. Resolution, simply, is separation in time, and to optimize resolution using UHPLC we need to optimize both efficiency **and** selectivity. This can only be realized by having a significant variety of different phase chemistry choices available on UHPLC silica.

### The Biphenyl Example

To ease method transfer from UHPLC to HPLC, we need columns that are fully scalable, but it is equally important that the stationary phases we use in UHPLC are not limited to that platform. By selecting a stationary phase that produces optimum selectivity for the specific compounds of interest, we can maximize the benefit of UHPLC. For example, the use of a biphenyl stationary phase can greatly enhance the separation of aromatic drug compounds. A biphenyl stationary phase differs from a C18 phase in selectivity and offers greater retention than a phenyl phase. When a biphenyl phase is bonded to a highly efficient <2 micron particle size column, we can produce fast, highly selective separations. This benefit is fully maximized in a column line, such as the Pinnacle® DB line, which offers a biphenyl phase on a fully scalable silica. The nine other phases also available in this line offer similar examples.

### Conclusion

The UHPLC columns available for method development will ultimately determine what columns are used in subsequent routine HPLC analyses, so having a wide range of available phase chemistries in UHPLC columns is advantageous. The full benefit of UHPLC can only be fully realized if the scalability of the analytical column and selectivity of the phase are carefully considered during method development. Selecting a column line for UHPLC that is designed for scalability, with tight manufacturing controls on consistent silica, that is offered on a wide variety of stationary phases is the best way to ensure successful method transfer between UHPLC and HPLC platforms.

### RELATED SEARCHES

[scaling methods](#), [accuracy](#), [method transfer](#), [HPLC](#), [UHPLC](#)



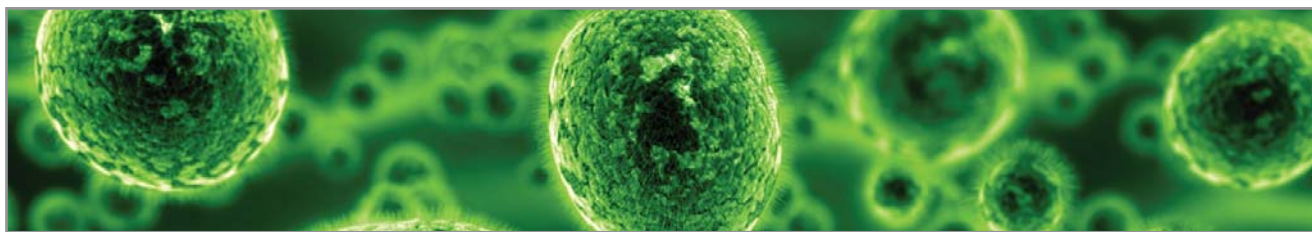
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## Bioanalytical Applications

# Characterizing Cellular Fatty Acid Methyl Ester (FAME) Profiles to Identify Bacteria Using Gas Chromatography

By Radomír Čabala



Dr. Radomír Čabala is the Head of the Toxicology Department at the General University Hospital in Prague, Czech Republic. His current research interests include the characterization of solute-solvent interactions by means of inverse GC; development, optimization, and testing of microextraction techniques; and comprehensive GC. (Radomir.Cabala@vfn.cz)

Practical, accurate methods for bacterial identification are of great interest in fields ranging from clinical diagnostics to food safety. Traditional methods, based on staining or growth, often are not specific enough to identify closely related species, which has led to the development of molecular methods based on DNA sequence or protein expression. Today, ELISA and other immunochemical techniques are commonly used, but gas chromatography can also be an effective approach. When paired with highly inert, low-bleed columns of appropriate selectivity, GC is one of the most efficient analytical separation methods available. The fatty acid methyl ester (FAME) analyses shown here illustrate the potential of GC-TOFMS for distinguishing clinically relevant species and identifying low-level compounds in these complex biological samples.

In the first example, the fatty acid composition of cell membranes from two Gram-negative bacteria, *Bordetella bronchiseptica* and *Bordetella pertussis*, were compared. *B. bronchiseptica* rarely infects humans, but it can cause infectious bronchitis and is resistant to macrolides and cephalosporins. *B. pertussis* is the dangerous agent of pertussis (whooping cough) in humans and produces several toxins. These species have a very close evolutionary relationship, which can be seen in their genetic similarity and cellular fatty acid profiles. To determine if these species could be distinguished based on FAME analysis, samples were first prepared from bacterial cell wall lysate by transesterification with sodium methanolate. Samples were then analyzed on a DANI Master GC-TOFMS equipped with an Rxi®-17Sil MS column. This column was chosen based on its selectivity for the cellular fatty acids. Even without optimizing the method for separation efficiency, good separation of the saturated acid methyl esters and their respective unsaturated counterparts was achieved (Figure 1). This separation also shows that *B. bronchiseptica* produces hexadecanoic and hexadecenoic acids at a different ratio than *B. pertussis*. In addition, 2-hexyl-cyclopropane-octanoic acid is present in the *B. bronchiseptica* sample, but not in the *B. pertussis* sample. Cell membrane lysates of *B. bronchiseptica* were also cultured at 24 °C and 37 °C to investigate the effect of temperature. It is commonly observed that at lower temperatures microorganisms increase the fraction of unsaturated acids in the cell membrane in order to maintain membrane fluidity. This effect is evident for the pairs of hexadecanoic/hexadecenoic and octadecanoic/octadecenoic acids (Figure 2).

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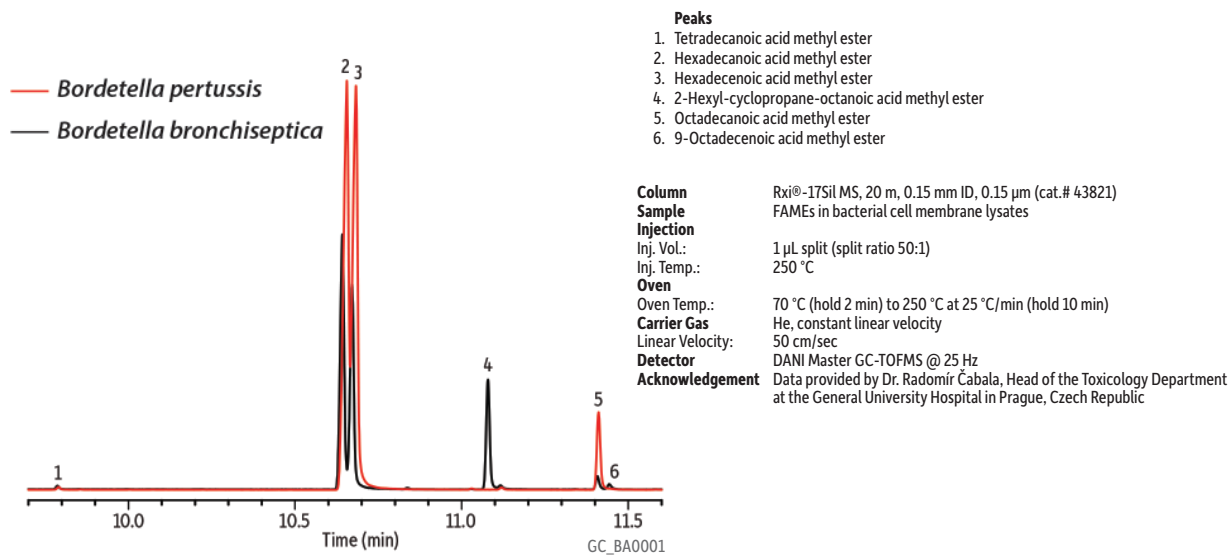
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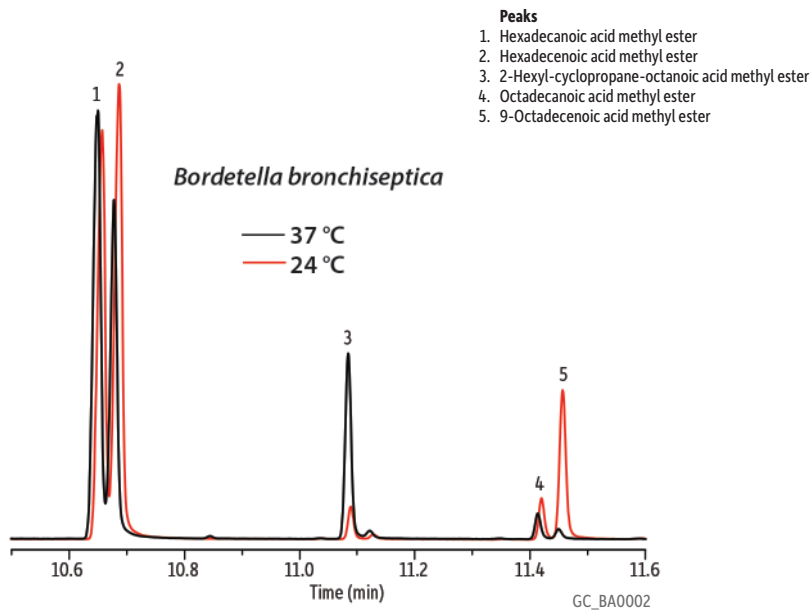
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**Figure 1:** Differences in FAME profiles in cell membrane lysates can be useful for bacterial identification.

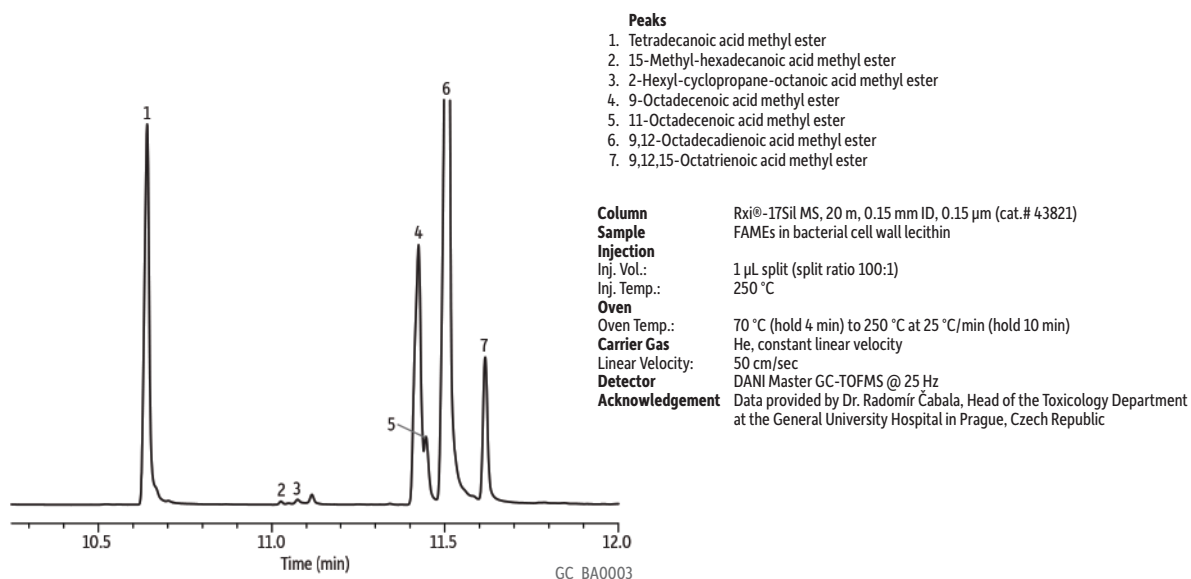


**Figure 2:** As expected, the ratio of unsaturated to saturated fatty acids in the cell membrane changes with temperature.

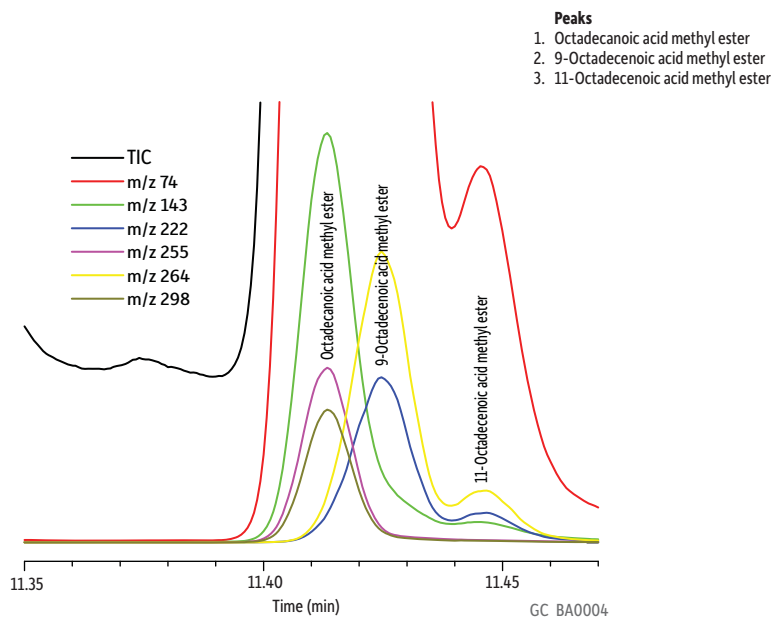


See Figure 1 for instrument conditions.

**Figure 3:** Octadecanoic acid coelutes with the unsaturated octadecenoic acids and cannot be distinguished.



**Figure 4:** TOFMS allows octadecanoic acid to be positively identified (match factor 835) even in the presence of coeluting peaks.



See Figure 3 for instrument conditions.

In the second example, we investigated whether octadecanoic acid could be determined in phosphatidylcholin (lecithin) isolated from the cell membrane of the Gram-positive bacterium *Bacillus subtilis*. This bacterium produces a cyclopeptide surfactin that is one of the most potent natural antibiotics known. The problem arising here is the severe coelution of low levels of octadecanoic acid with the excess of unsaturated octadecenoic acids (Figure 3). Data were collected at 25 Hz, which was determined to be the optimum frequency, but octadecanoic acid was not chromatographically resolved. The signal processing feature of the TOF detector and the deconvolution option of the DANI Master Lab software allowed successful separation of the selected m/z signals and reliable compound identification of octadecanoic acid (match factor 835) based on the NIST 08 spectral database (Figure 4).

The examples presented here illustrate the potential utility of GC-TOFMS and an Rxi®-17Sil MS column for bacterial identification through cellular fatty acid analysis. The efficiency and selectivity of the column and power of the instrument allow FAME profiles to be compared and a low-level coeluting compound (octadecanoic acid) to be distinguished.

### Acknowledgements

The author would like to thank to Restek Corporation for the kind donation of the capillary columns and DANI Instruments for use of the Master GC-TOFMS.

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## Beyond C18—Increase Retention of Hydrophilic Compounds Using Biphenyl Columns

By Amanda Rigdon, Pharmaceutical Innovations Chemist and Rick Lake, Pharmaceutical Market Development Manager



*Searching for a better way to retain hydrophilic aromatic drug compounds?*

*Biphenyl phases, such as the Pinnacle™ DB Biphenyl column, provide greater retention than alkyl phases. Use a Biphenyl column to separate difficult-to-retain polar aromatics from unretained matrix contaminants.*

Many drug classes include compounds with aromatic ring structures, some of which also contain a sulfone or sulfoxide group. Both sulfur groups have dipole moments, adding a hydrophilic character to compounds containing these functional groups. The analysis of hydrophilic compounds on a traditional alkyl column (e.g., C18) can be problematic, since alkyl columns depend on hydrophobic (dispersive) interactions for retention. Since the sulfone and sulfoxide groups contain  $\pi$  bonds, the Biphenyl column's affinity toward compounds containing these bonds makes it a logical choice when increased retention of compounds containing these groups is desired.

**Biphenyl columns are much more effective than alkyl, phenyl, or phenyl hexyl phases when increased retention of hydrophilic aromatics is desired.**

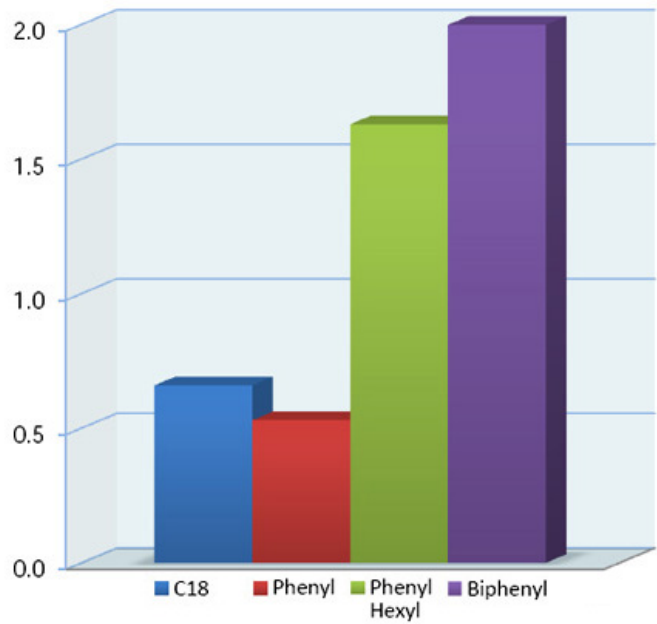
To explore the selectivity of the biphenyl phase towards sulfur-containing aromatic compounds, phenyl sulfone, a simple probe, was analyzed on alkyl (C18), phenyl, phenyl hexyl, and Biphenyl columns to determine the relative retention of each phase, as measured by capacity factor ( $k'$ ). In order to ensure separation of analytes from unretained contaminants, a minimum  $k'$  value of 2 is recommended for most analyses, however in cases where there is little to no matrix interference, a  $k'$  of 1 may be acceptable. The data in Figure 1 show that phenyl sulfone is retained to a much greater degree on the Pinnacle™ DB Biphenyl column, than on the other phases tested ( $k' = 2.08$ ). This is due to the unique retention mechanism of the biphenyl stationary phase, which can interact with both the hydrophobic aromatic ring and the hydrophilic sulfone group through

$\pi$ - $\pi$  interactions. Although the phenyl stationary phase also allows for the use of  $\pi$ - $\pi$  interactions, the biphenyl phase has a larger electron cloud and is significantly more retentive.

To further test the retention of the Biphenyl column, a second set of probes, consisting of compounds in the NSAID family, was analyzed. Tenoxicam, which contains a sulfone group, and sulfinpyrazone, which contains a sulfoxide group, were analyzed along with a void marker (uracil). Although these compounds are more complex than the probe used in the first experiment, the same pattern of retention was observed (Figure 2). The Pinnacle™ DB Biphenyl column exhibited the greatest retention for tenoxicam. With  $k'$  values of 0.33 on the C18 and 0.49 on the phenyl columns, tenoxicam shows almost no retention on these stationary phases. The phenyl hexyl phase performed slightly better with a  $k'$  value of 1.52 for tenoxicam. However, when tenoxicam was analyzed on the Biphenyl column under the same conditions, the  $k'$  value increased to 2.22, a value much more likely to provide adequate resolution from matrix components. Sulfinpyrazone, a less polar compound, also followed the same pattern of retention (Table I).

The improved retention for hydrophilic aromatics shown here is due to the unique  $\pi$ - $\pi$  interaction retention mechanism of the Biphenyl phase. This mechanism is particularly useful for analysis of sulfone- and sulfoxide-containing drug compounds, which are not easily retained on alkyl or phenyl phases. The Biphenyl phase provides greater retention than alkyl and phenyl phases and is ideal for separating difficult-to-retain polar aromatics from unretained matrix contaminants.

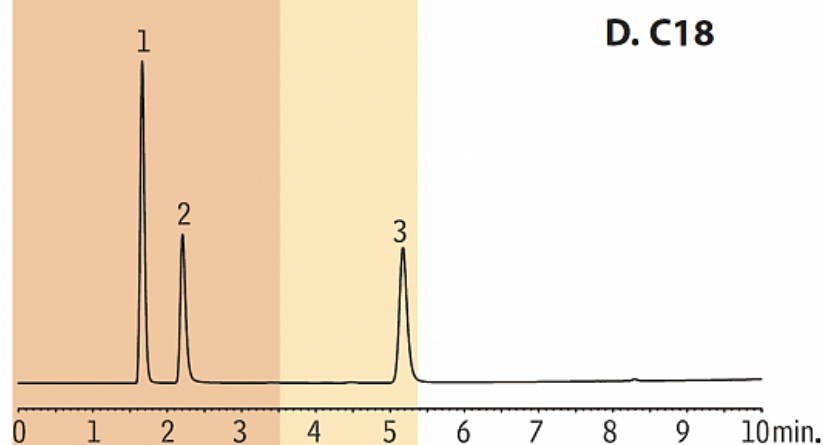
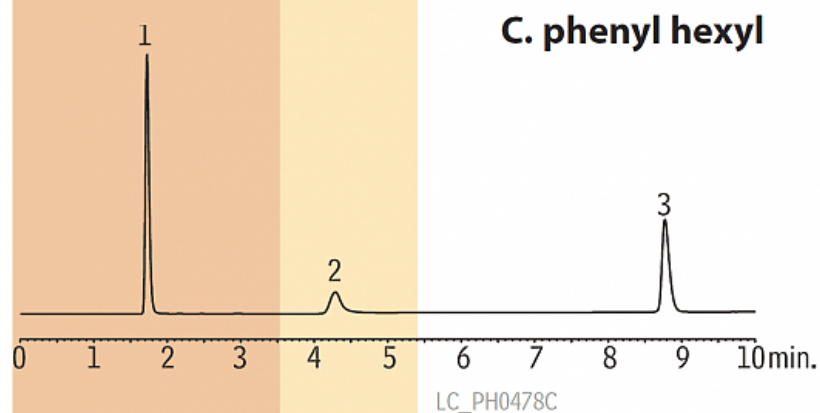
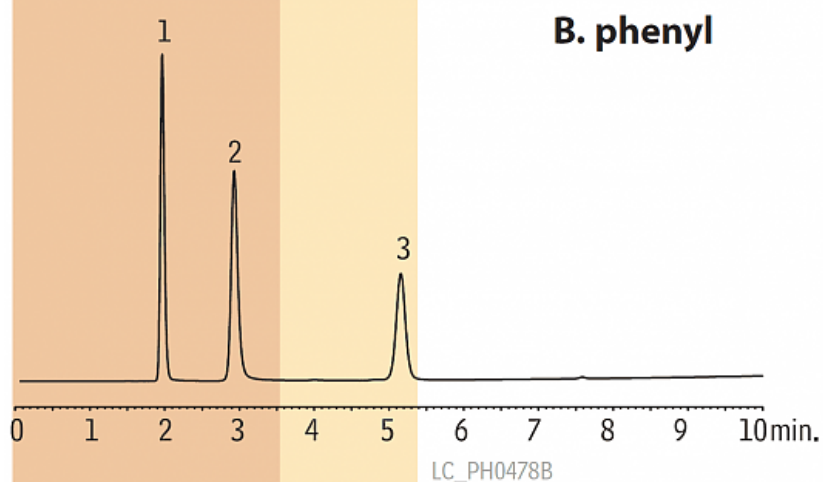
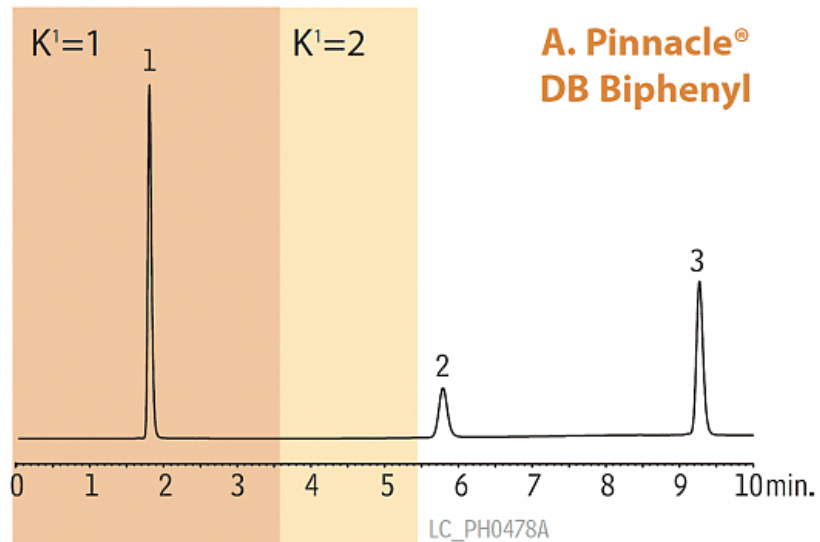
**Figure 1** The Biphenyl phase is more retentive for phenyl sulfone than other alkyl and phenyl phases.



**Figure 2** Only the Biphenyl phase retains both test probes to  $k' > 2$ , the level recommended to ensure separation from unretained matrix contaminants.

- Peaks**
- 1. Uracil (void marker)
  - 2. Tenoxicam
  - 3. Sulfinpyrazone





## LC\_PH0478D

## LC\_PH0478A

**Column** Pinnacle® DB Biphenyl (cat.# 9409565)  
**Dimensions:** 150 mm x 4.6 mm ID  
**Particle Size:** 5 µm  
**Pore Size:** 140 Å  
**Temp.:** 30 °C  
**Sample**  
**Diluent:** 0.1% formic acid in water:methanol (40:60)  
**Conc.:** 100 µg/mL each component (see peak list)  
**Inj. Vol.:** 10 µL

**Mobile Phase**  
**A:** 0.1% formic acid in water  
**B:** methanol

**Time (min)%B**

0.00	60
2.0	60
8.0	90
20.0	90
20.1	60

**Flow:** 1.0 mL/min  
**Detector** UV/Vis @ 254 nm  
**Instrument** Shimadzu Prominence  
**Notes** Column B: phenyl  
 Column C: phenyl hexyl  
 Column D: C18  
 Detector: Shimadzu PDA (SPD-M20A)

*Note: a minimum  $k'$  value of 2 is generally recommended to fully separate target analytes from matrix contaminants.*

**Table I** Biphenyl columns show improved retention of sulfone- and sulfoxide-containing aromatic drugs.

K' Value

	<b>Biphenyl</b>	<b>Phenyl hexyl</b>	<b>Phenyl</b>	<b>C18</b>
Tenoxicam	2.23	1.39	0.637	0.235
Sulfinpyrazone	4.18	3.90	1.88	1.89

**RELATED SEARCHES**

[NSAID](#), [pinnacle](#), [pi-pi](#), [aromatic](#), [tenoxicam](#), [sulfinpyrazone](#), [biphenyl](#), [unsaturated](#), [hydrophilic](#)

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## Assaying Local Anesthetics by GC/FID

### Optimizing System Suitability, Using an Rxi®-5ms Column

By Rick Lake, Pharmaceutical Innovations Chemist

- Rxi®5ms column assures excellent peak shapes for basic compounds.
- Stable, reproducible retention times.
- Easy conformance to stringent system suitability criteria.

Local anesthetics are biologically active compounds that reversibly inhibit the propagation, or broadcasting, of signals along nerve cell pathways. Because of this action, they are widely used as drug compounds to produce temporary analgesia (loss of pain) and paralysis (loss of muscle movement). Anesthetic compounds are formulated into a large number and wide variety of drug products, ranging from over-the-counter topical ointments to clinical injectables, and they often are formulated in combination with other active ingredients. Therefore, many analyses of local anesthetics involve manufacturing assays, like potency and stability assays, which require high throughput and reproducible results. These assays require the fulfillment of system suitability criteria and, for this reason, we investigated assaying local anesthetics by GC/FID, using common system suitability parameters as evaluation criteria.

By GC standards, a local anesthetic is a high molecular weight, weakly basic, active compound. We took these characteristics into account when we chose the column and inlet liner for this application. Considering that these analytes are basic and active, the deactivation of the inlet liner and capillary column is very important. For superior inertness, we chose to use an Rxi®-5ms column.

When analyzing high molecular weight compounds — the normal case in pharmaceutical assays — discrimination and irreproducible injections sometimes occur, primarily due to incomplete vaporization of the analytes. This can be especially problematic for analysts who must meet stringent system suitability criteria. Some liners, like the laminar cup and cup splitter, were designed specifically for samples containing high molecular weight compounds. These liner designs aid in sample vaporization, but at a cost of reduced internal volumes and intricate flow paths that can cause poor reproducibility when such liners are used with a solvent that has a large expansion volume, like methanol. In this application, we used our conventional, intermediate polarity deactivated, split liners packed with intermediate polarity deactivated wool. Wool in the liner provides a large surface area, for rapid vaporization, but the liner still delivers a uniform vapor cloud to the split point.

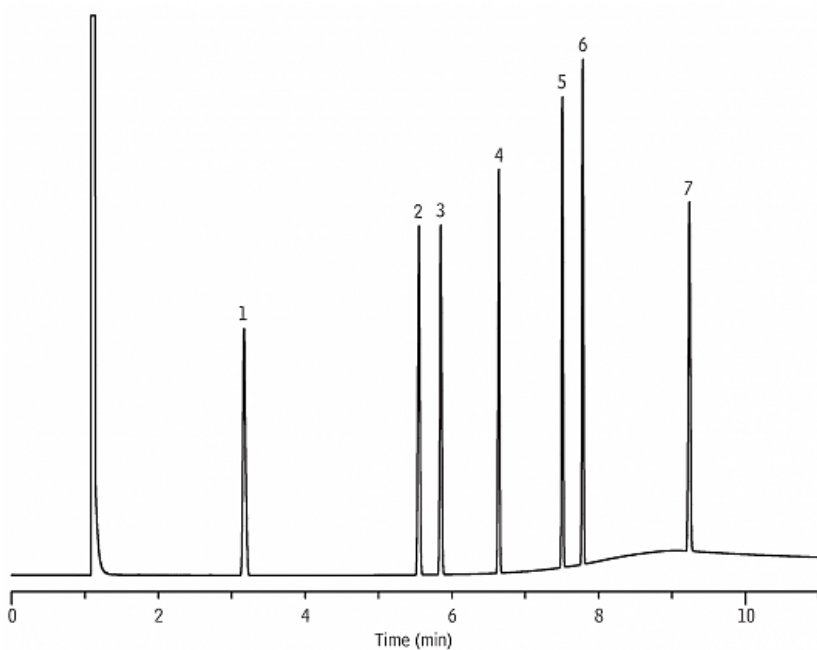
Under these conditions, chromatography from a six-replicate system suitability analysis (Figure 1) was well within normal acceptance criteria (Table 1). USP tailing, approximately 1.00 for all analytes, shows the exceptional inertness of the Rxi®-5ms column. In addition, retention times and area responses were extremely stable. The Rxi®-5ms column, coupled with an appropriate inlet liner, provides the stability and deactivation necessary to afford easier conformance to system suitability criteria.

**Figure 1** An Rxi®-5ms column provides excellent peak shape and stable retention times for basic compounds, for easier conformance to system suitability criteria.

#### Peaks

1. Benzocaine
2. Prilocaine
3. Lidocaine
4. Procaine
5. Tetracaine
6. Bupivacaine
7. Dibucaine





GC\_PH00860

**Column** Rx1®-5ms, 30 m, 0.53 mm ID, 1.00 µm (cat.# 13455)  
**Sample**  
 Diluent: methanol  
 Conc.: 50 µg/mL each component  
**Injection**  
 Inj. Vol.: 1.0 µL split (split ratio 10:1)  
 Liner: 4mm Split w/Wool (cat.# 20781)  
 Inj. Temp.: 250 °C  
**Oven**  
 Oven Temp.: 200 °C (hold 4 min) to 320 °C at 30 °C/min (hold 3 min)  
**Carrier Gas**  
 Flow Rate: 5.0 mL/min  
**Detector** FID @ 300 °C

**Table 1** An Rx1®-5ms column provides exceptionally stable retention times and area responses.

Compound	Peak Area (%RSD)	Retention Time (%RSD)	USP Tailing	Efficiency
benzocaine	0.85	0.03	1.00	55858
prilocaine	1.36	0.02	1.00	(isothermal)
lidocaine	1.01	0.02	1.00	
procaine	1.83	0.03	1.00	
tetracaine	1.78	0.01	1.00	
bupivacaine	1.64	0.02	1.02	
dibucaine	1.17	0.06	1.00	
Mean	1.38	0.03	1.00	

six-replicate system suitability analysis

#### RELATED SEARCHES

[Rx1-5ms, anesthetics, basic compounds](#)

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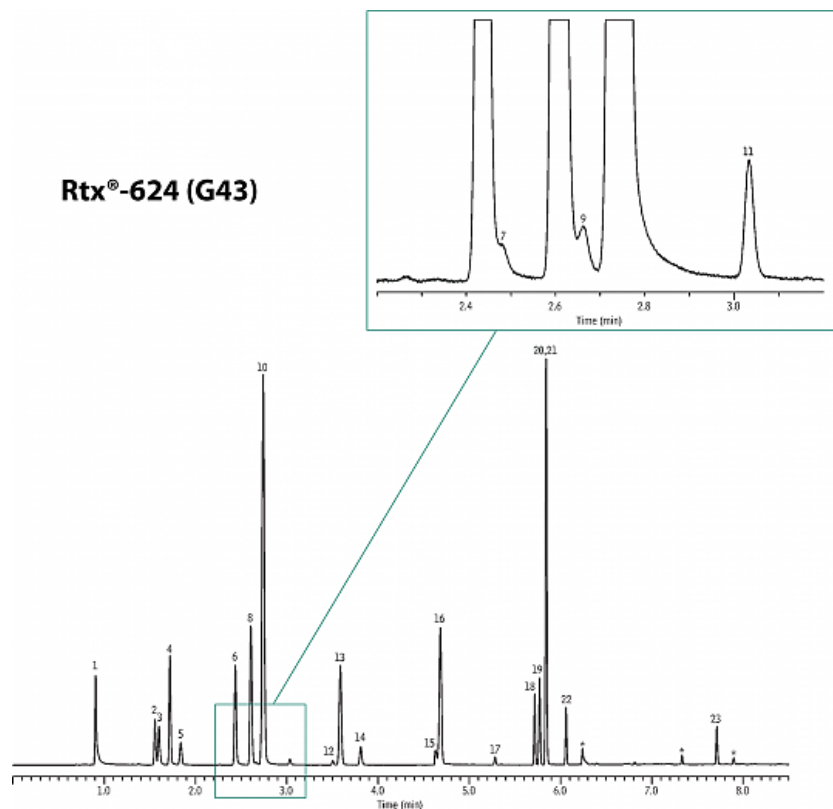
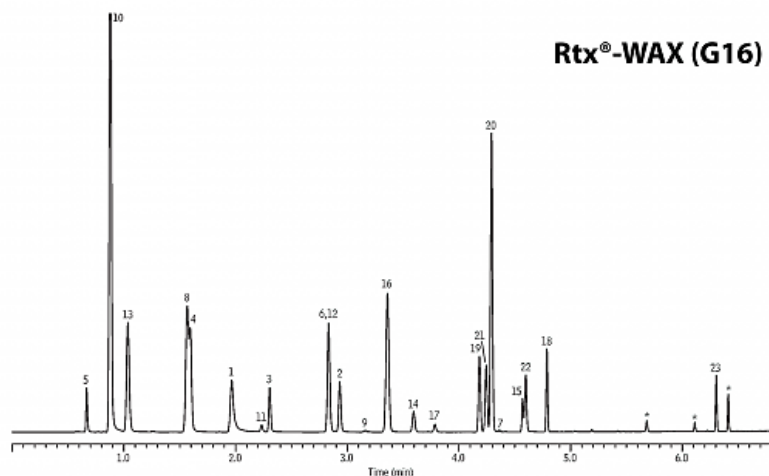


The MACH system allows independent temperature programming of up to four columns, simultaneously.

**Figure 2:** Detect and confirm residual solvents with one injection, using Restek columns in a Gerstel MACH System. ([View Larger](#))

Peaks		Peaks	
1. Methanol	🔍	12. Trichloroethylene	🔍
2. Acetonitrile	🔍	13. Methylcyclohexane	🔍
3. Dichloromethane	🔍	14. 1,4-Dioxane	🔍
4. <i>trans</i> -1,2-Dichloroethylene	🔍	15. Pyridine	🔍
5. Hexane	🔍	16. Toluene	🔍
6. <i>cis</i> -1,2-Dichloroethylene	🔍	17. 2-Hexanone	🔍
7. Nitromethane	🔍	18. Chlorobenzene	🔍
8. Tetrahydrofuran	🔍	19. Ethyl benzene	🔍
9. Chloroform	🔍	20. <i>m</i> -Xylene	🔍
10. Cyclohexane	🔍	21. <i>p</i> -Xylene	🔍
11. 1,2-Dimethoxyethane	🔍	22. <i>o</i> -Xylene	🔍
		23. Tetralin	🔍

\* septum components

**Rtx®-624 (G43)****Rtx®-WAX (G16)**

GC\_PH00845

**Columns**

Rtx®-624 20 m, 0.18 mm ID, 1.00  $\mu$ m (cat.# 40924)  
and Rtx®-WAX (G16) 20m, 0.18mm ID, 0.4 $\mu$ m (custom)  
using IP Deactivated Guard Column 5 m, 0.32 mm ID (cat.# 10044)  
with Universal "Y" Press-Tight Connector (cat.# 20405)

**Sample**

Residual Solvents Class 2 - Mix A (cat.# 36271)  
Residual Solvents Class 2 - Mix B (cat.# 36272)

**Diluent:**

0.5mL DI water/~0.2g sodium sulfate in 20mL headspace vial;  
components at 0.10 - 6.00 $\mu$ g

**Injection****Headspace-Trap**

Instrument: Tekmar HT3  
Inj. Temp.: 220 °C  
Transfer Line Temp.: 220 °C  
Valve Oven Temp.: 220 °C  
Standby flow rate: 50 mL/min  
Trap Standby Temp: 40 °C  
Trap Sweep Temp: 40 °C  
Preheat Mixer: On  
Preheat Mixer Time: 2.0 min  
Preheat Mixing Level: 5  
Preheat Mixer  
Stabilize Time: 0.5 min  
Sample Temp.: 80 °C  
Sweep Flow Rate: 75 mL/min  
Sweep Flow Time: 5.0 min

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Temp:	245 °C
Desorb:	1.0 min @ 250 °C
Trap Bake Temp:	260 °C
Trap Bake Time:	6.0 min
Trap Bake Flow:	450 mL/min
<b>Oven</b>	
Oven Temp.:	250 °C
<b>Carrier Gas</b>	He
<b>Detector</b>	FID @ 250 °C
Make-up Gas Flow	
Rate:	45 mL/min
<b>Instrument</b>	Agilent 6890 with Gerstel Modular Accelerated Column Heater (MACH)
<b>Notes</b>	Sample preheat time: 15.00 min.
	Flow rate: Constant Flow, Column 1: 0.85mL/min.; Column 2: 0.99mL/min.
	Gerstel temps.:
	Column 1: 50°C (2 min.) to 80°C at 20°C/min. (1 min.), to 200°C at 40°C/min. (2 min.)
	Column 2: 35°C (2 min.) to 60°C at 100°C/min. (1 min.), to 200°C at 40°C/min. (2 min.)

## References

1. Restek Advantage 2006 vol. 1, pp.14-15 (2006); request: lit. cat.# 580035.
2. Direct inquiries about the Gerstel MACH System to Gerstel Inc. Phone: 410-247 5885; e-mail: [sales@gerstelus.com](mailto:sales@gerstelus.com)

## RELATED SEARCHES

*Gerstel MACH, Rtx-WAX, Rtx-624, G16, G43, ICH Q3C, regulated solvents, Class 1 compounds, Class 2 compounds, Class 3 compounds, carcinogens, non-genotoxic, headspace, residual solvents, stabilwax, OVI*



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