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

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

ALSO IN THIS ISSUE:

RESTEK®

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In This Issue

About Restek Corporation

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

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

Restek is *Pure Chromatography.*

www.restek.com

Reflections from the Bench

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

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

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

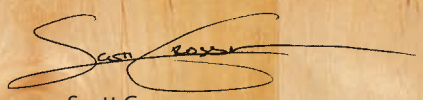
However you look at it, method development comes with a great deal of responsibility. Many of us are developing methods for very important applications in our own industries. So, building an accurate, precise, and robust method that doesn't require a third arm and a lucky rabbit's foot is vital. I appreciate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

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

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

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

Best regards,



Scott Grossman
Quality Control Technical Supervisor

You Have Opinions... and We Want Them

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

‹?› Questions from You

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

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

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

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

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

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

- Chas Simons
Technical Service Manager

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

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

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

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

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

- Jaap de Zeeuw
International GC Specialist

Wrestling with a question of your own?

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

Hot Topics

Click to Quickly Translate Methods and Calculate Flows

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

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

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

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

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

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

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

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

www.restek.com/gc-multiresidue

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

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

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

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

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

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

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

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

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

Restek Signs On with Aegis to Benefit Veterans

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

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

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

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

Photo courtesy of Kelsey Morris, Aegis Sciences Corporation

ChromaBLOGraphy

Topical and Timely Insights

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

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

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

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

Meet with Us Face-to-Face

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

2015 Events Calendar

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

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

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

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

Did you know?

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

Raptor™ LC Columns

Selectivity Accelerated

The Effects of LC Particle Choice on Column Performance:

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

By Sharon Lupo, Ty Kahler, and Paul Connolly

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

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

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

Why Switch from FPP to SPP LC Columns?

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

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

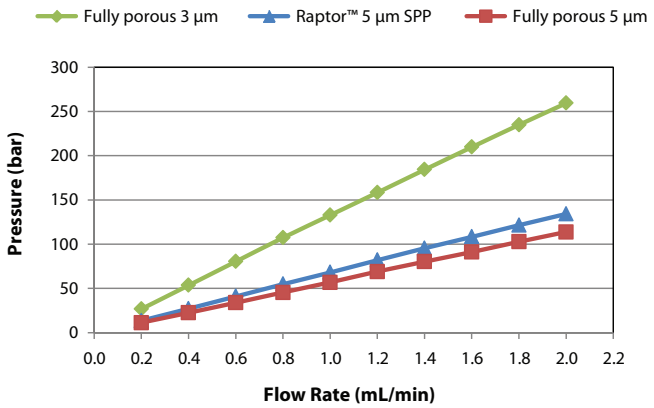
How to Choose between Raptor™ 2.7 vs. 5 µm SPP LC Columns

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

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

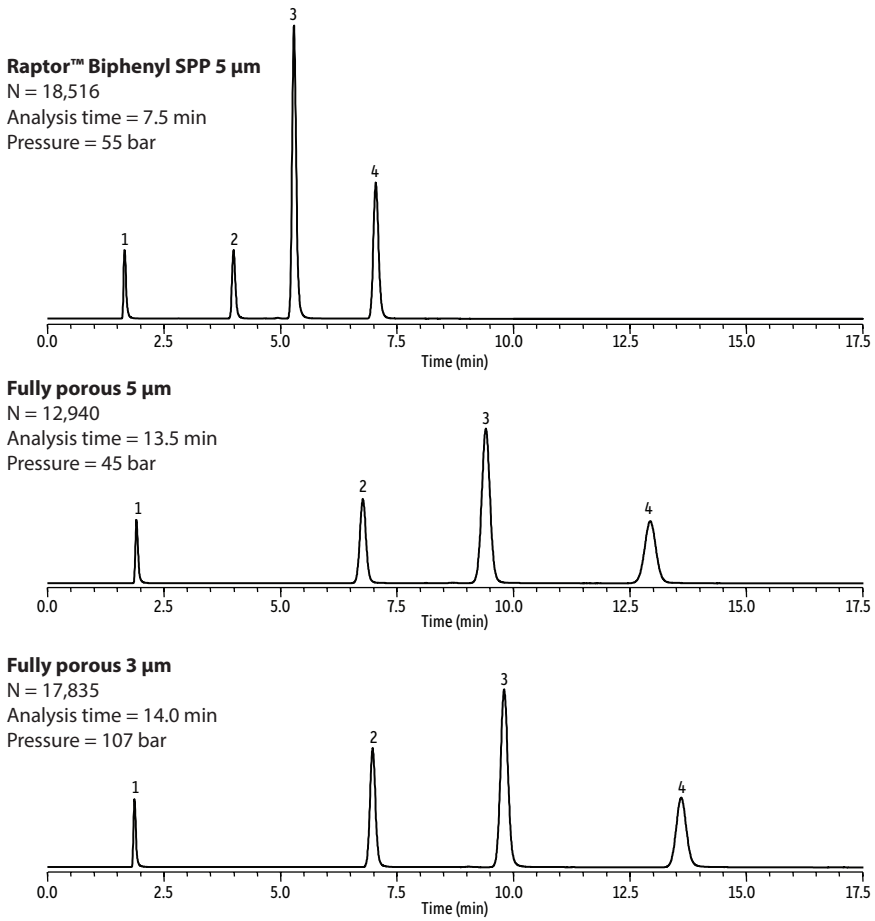
Raptor™ 2.7 µm diameter particle columns exhibit greater efficiency and sensitivity than the 5 µm, but the operating pressures are somewhat higher. Since extra-column peak broadening is most pronounced with short, small-diameter columns packed with small particles, 2.7 µm columns are best suited for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

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



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

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



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

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

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

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

LC_GN0556

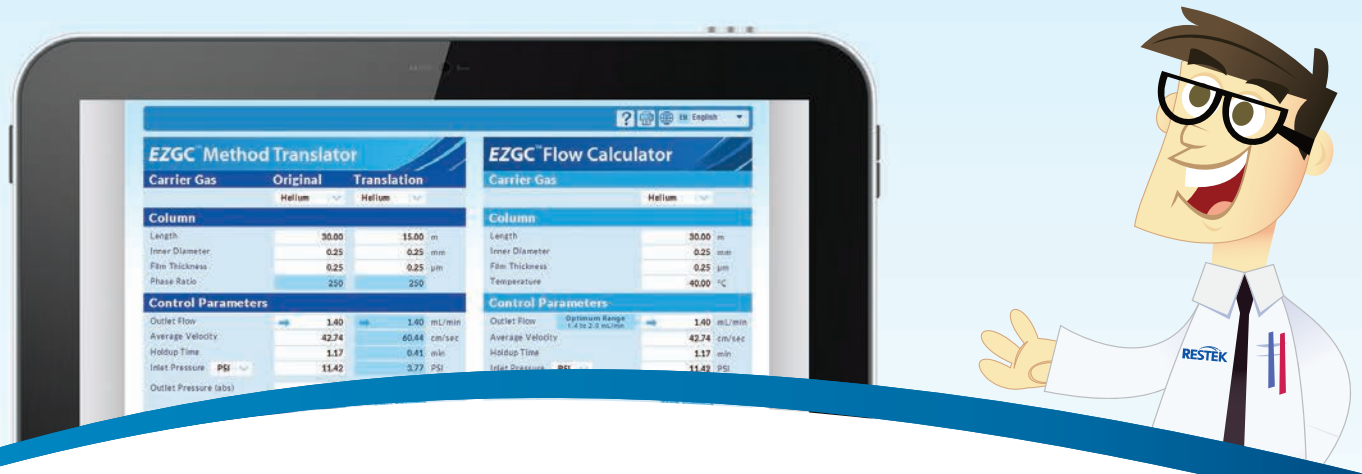
Experience *Selectivity Accelerated*

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

More on SPP and FPP

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

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



Helium to Hydrogen:

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

By Jack Cochran and Jaap de Zeeuw

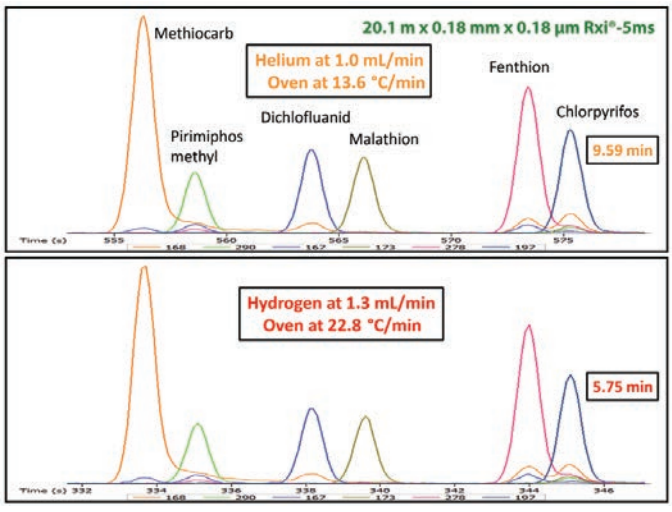
- Improve throughput by translating your GC method from slower helium to faster hydrogen carrier gas.
- Substitute expensive helium GC carrier gas with hydrogen and get the same chromatogram with translation.
- Improve MS detectability by using hydrogen at a lower flow rate without sacrificing separations.

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

Increase Sample Throughput with Faster Separations

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

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



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

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

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

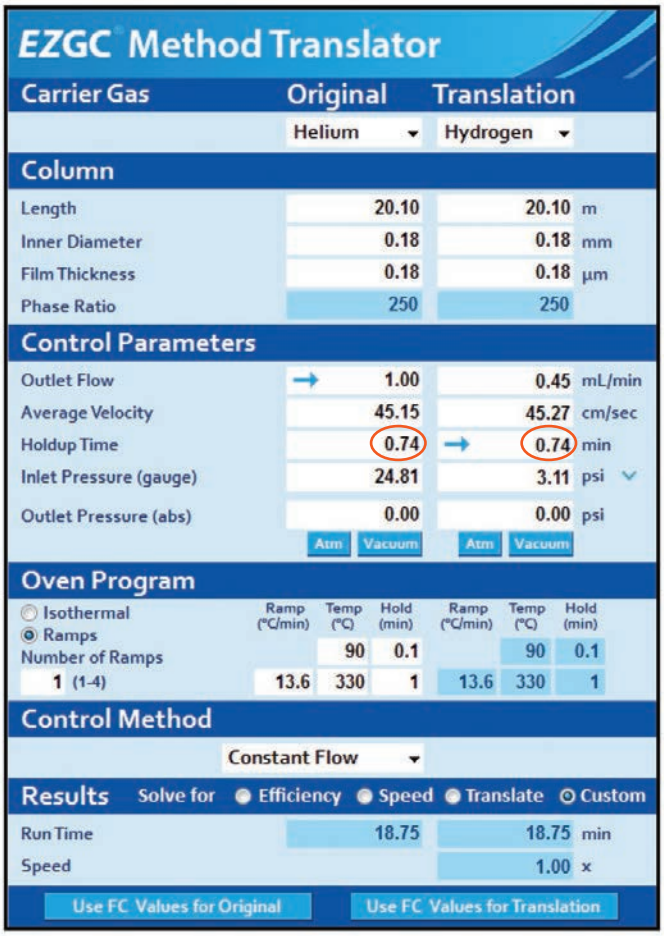
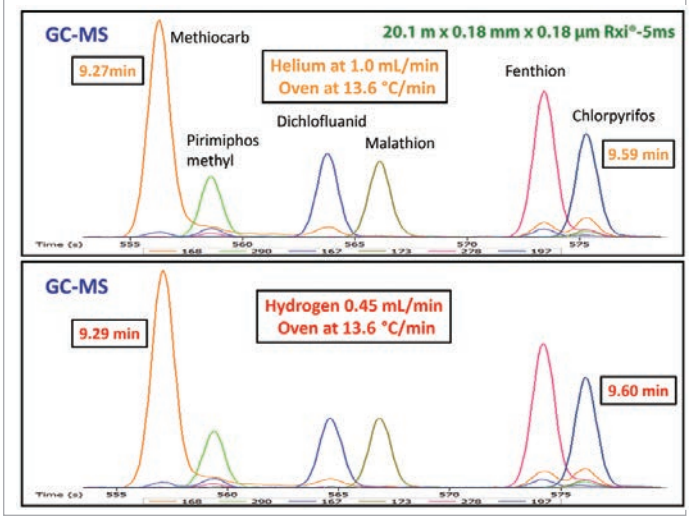


Figure 3: Get the advantage of switching to hydrogen, without having to reset retention time windows. Use the *EZ*GC® method translator/flow calculator to establish conditions that give the same retention times as your original method.



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

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

By Scott Adams

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

Split Injections

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

Sky® Precision® liner with wool for Agilent® GCs

Sky® Cyclo liner for Agilent® GCs

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

Splitless Injections

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

Sky® single taper with wool for Agilent® GCs

Sky® single taper without wool for Agilent® GCs

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

Programmable Temperature Vaporization (PTV) Injections

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

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

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

tech **tip**

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

Optimizing an Agilent-Style Splitless Inlet

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

By Chris Rattray and Jack Cochran

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

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

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

Setting up for CSR-LVSI Success

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

Example Application: Lower Detection Limits for Volatile Drinking Water Contaminants

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

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

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

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

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

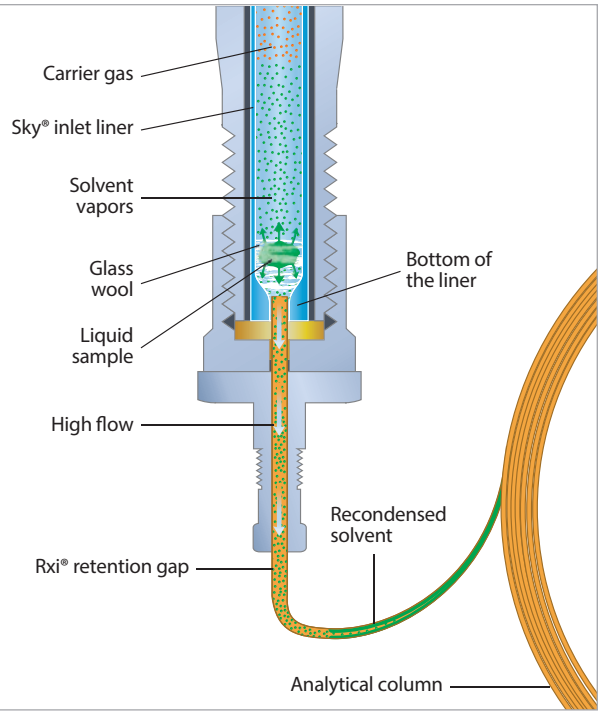


Table I: Starting points for CSR-LVSI method optimization.

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

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

References

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

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

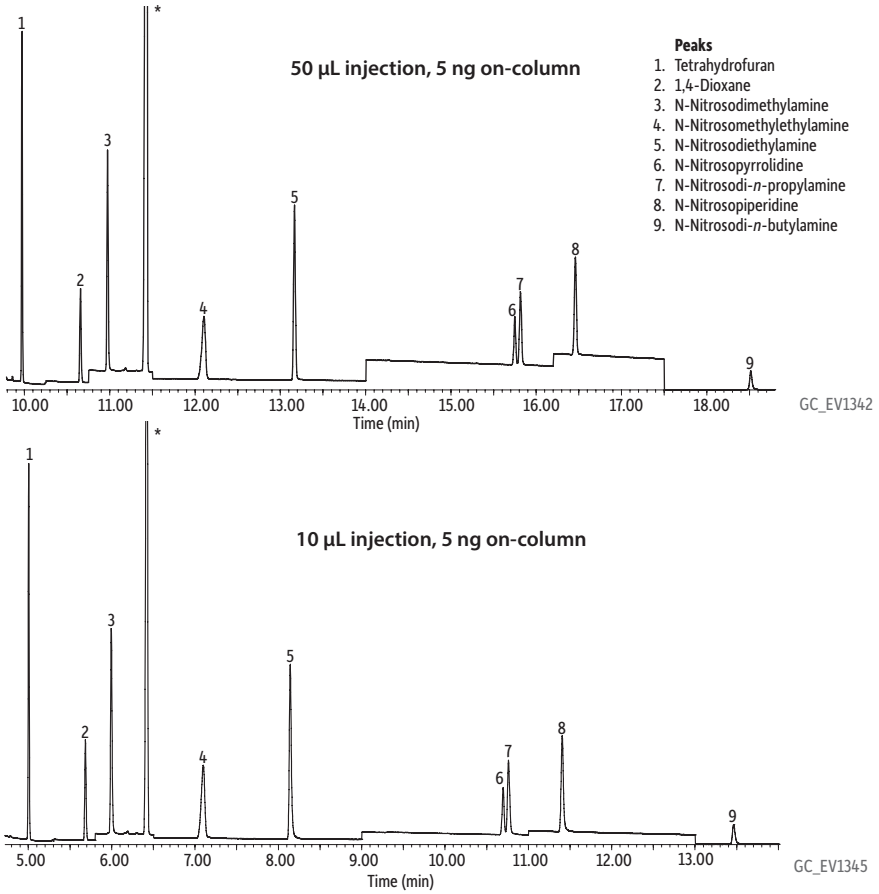
[3] J. Cochran, *The Solvent Effect in Concurrent Solvent Recondensation Large Volume Splitless Injection with Methylene Chloride – EPA Method 8270 Semivolatiles*, ChromaBLOGraphy, Restek Corporation, 2011 http://blog.restek.com/?p=1902 (accessed March 2, 2012).

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

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

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

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



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

New GC Method for Polycyclic Aromatic Compounds

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

By Jack Cochran, Julie Kowalski, and Amanda Rigdon

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

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

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

Speedy Sample Preparation Saves Time and Removes Matrix Interferences

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

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

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

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

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

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

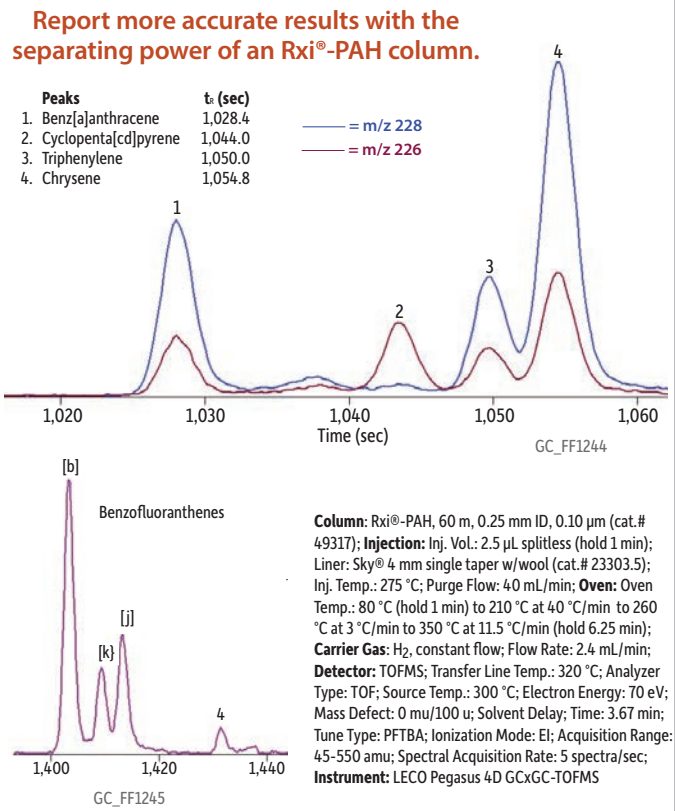
References

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

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

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

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



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

Fast, Simple Sample Preparation for PAHs in Mate Tea

Modified QuEChERS Extraction

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

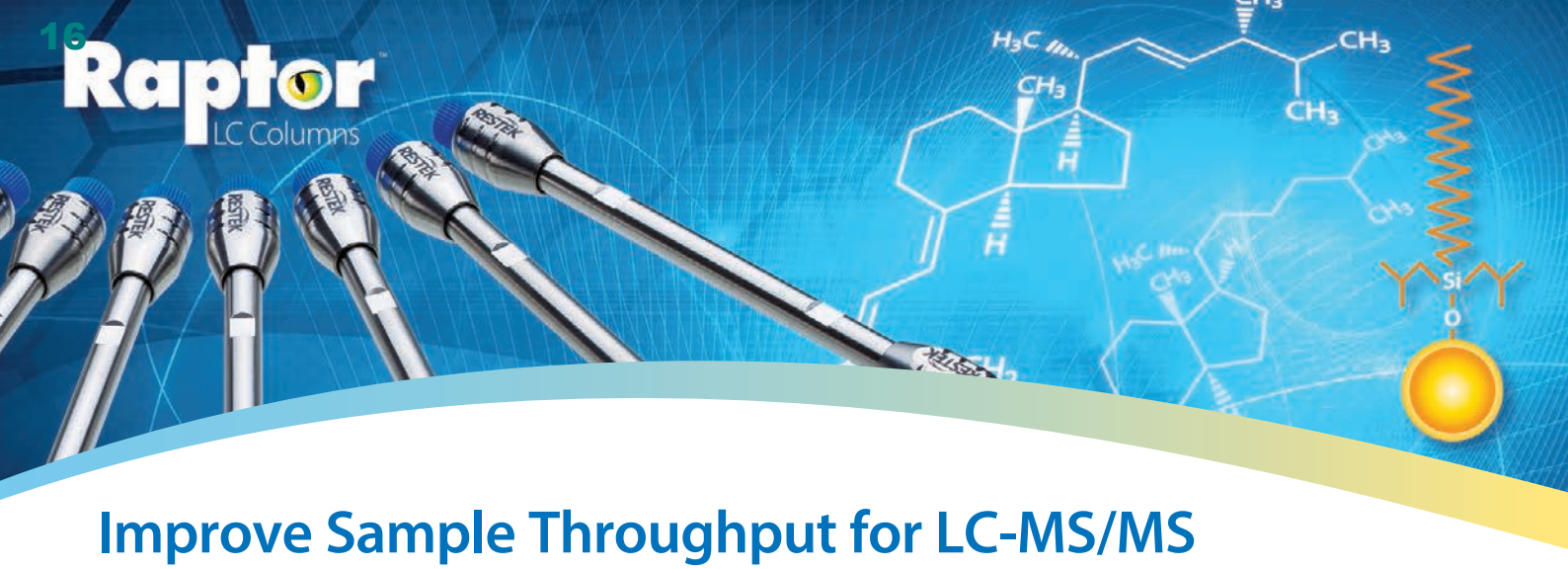
Silica SPE Cleanup

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

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

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

ND = not detected



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

With a New Raptor™ ARC-18 Column

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

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

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

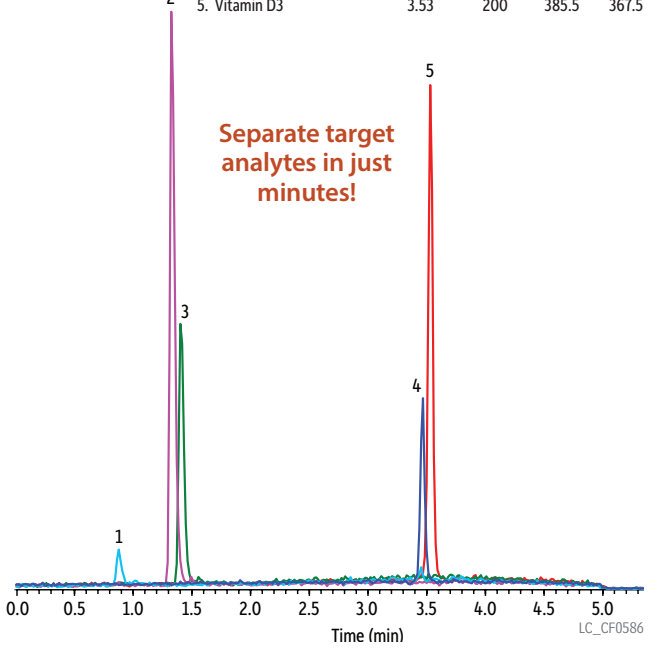
Separating fat-soluble vitamins by LC can be quite time-consuming, taking up to 20 minutes or longer by some methods. However, the new Raptor™ ARC-18 LC column can analyze these difficult compounds using reversed-phase chromatography (RPC) in less time than traditional columns, which helps increase sample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

Fast Analysis Times Improve Productivity

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

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

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



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

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

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

Table values are averages of replicate samples.

Good Accuracy and Precision Ensure Reliable Results

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

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

Summary

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

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

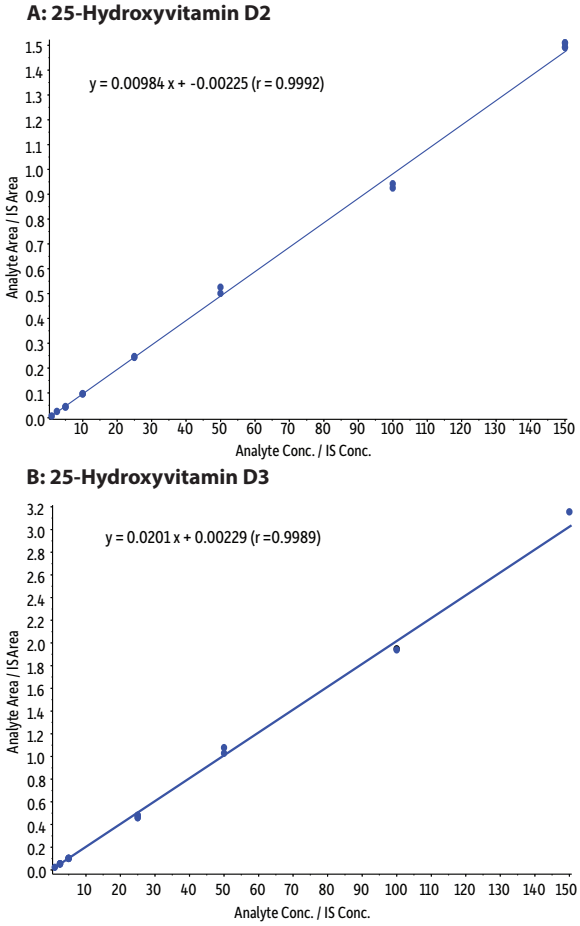
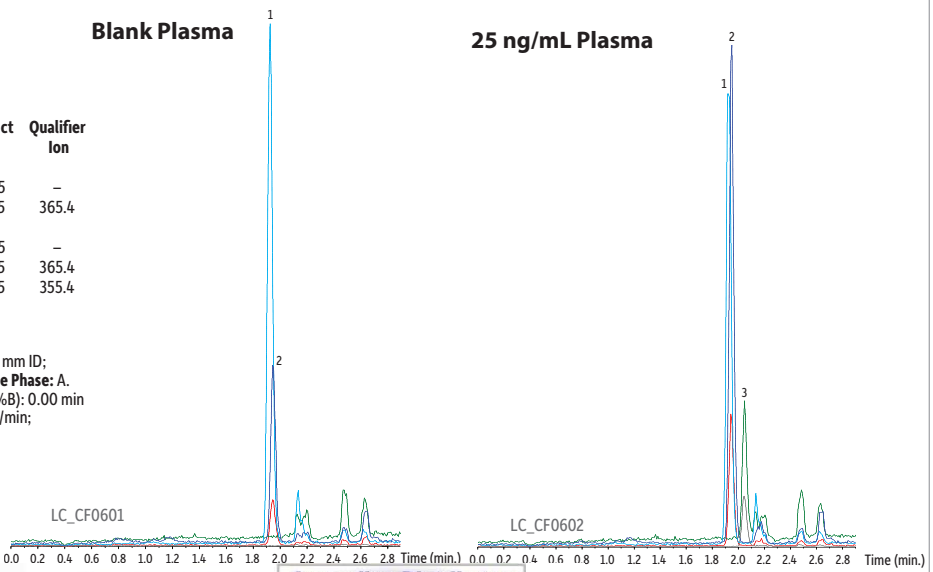
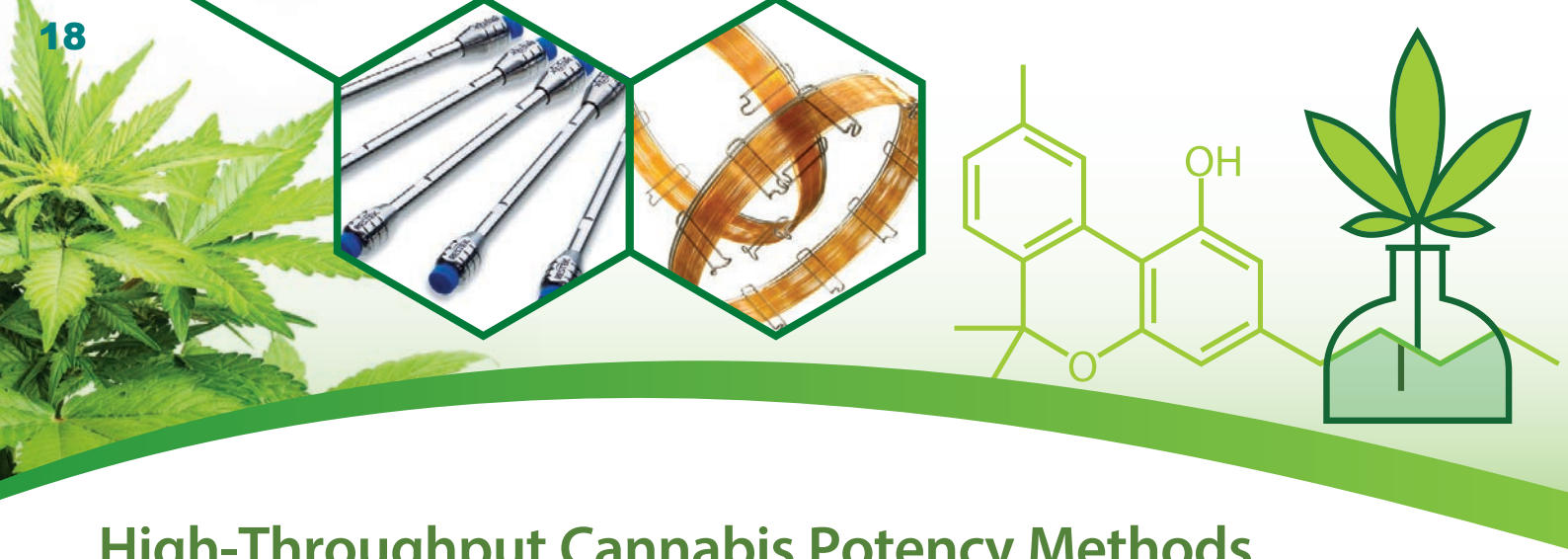


Figure 3: Good separation of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 from matrix components ensures more accurate results.

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

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





High-Throughput Cannabis Potency Methods for LC and GC Produce Results Quickly without the Cost of New Equipment

By Frances Carroll, Jack Cochran, and Amanda Rigdon

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

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

Analyze Cannabinoids at UHPLC Speed without Investing in New Equipment

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

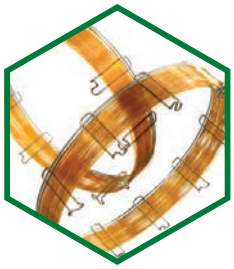
As shown in Figure 1, Restek has developed a fast analysis (3.8 min analysis [7 min total cycle time]) of cannabinoids that can be performed on any LC instrument. By utilizing Raptor™ column technology, you can obtain UHPLC speed without the capital investment. Also, we specifically chose simple, fast,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

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

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

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

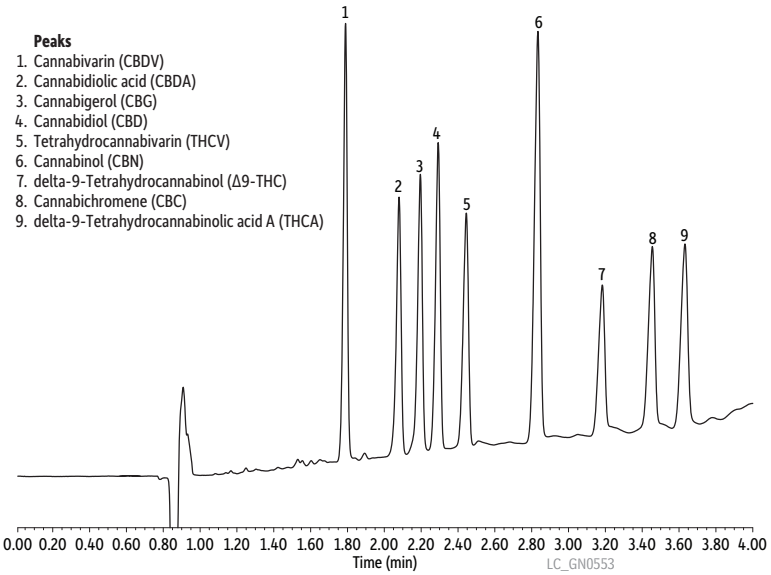
Whether you are using LC or GC for cannabis potency analysis, Restek can provide the products and expertise you need to obtain accurate results quickly. Use the methods shown here for analyzing the full spectrum of acid and neutral cannabinoids using LC with minimal capital investment, or get extremely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



tech **tip**

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

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

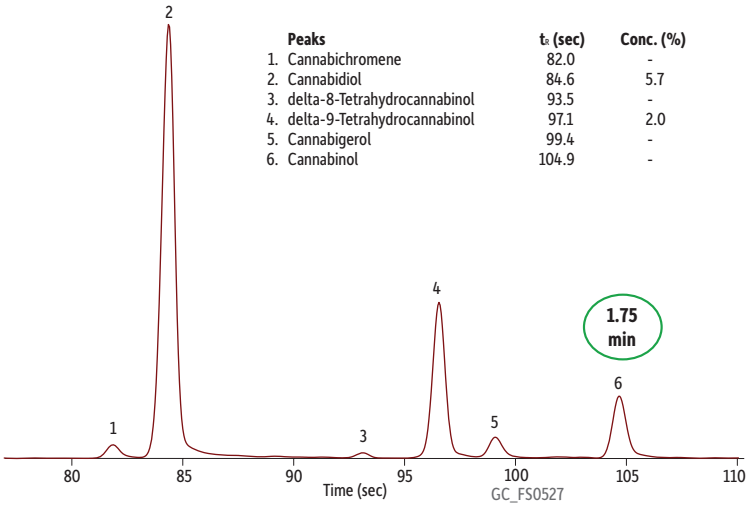


Column	Raptor™ ARC-18 (cat.# 9314A65)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	2.7 µm
Temp.:	50 °C
Sample	Cannabidiolic acid (cat.# 34094) Cannabigerol (cat.# 34091) Cannabidiol (cat.# 34011) Cannabinol (cat.# 34010) delta-9-Tetrahydrocannabinol (THC) (cat.# 34067) Cannabichromene (cat.# 34092)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

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

Detector	UV/Vis @ 220 nm
Instrument	HPLC

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



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

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

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

By Corby Hilliard and Amanda Rigdon

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

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

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

Minimize Downtime with Virtually Particle-Free PLOT Column Performance

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

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

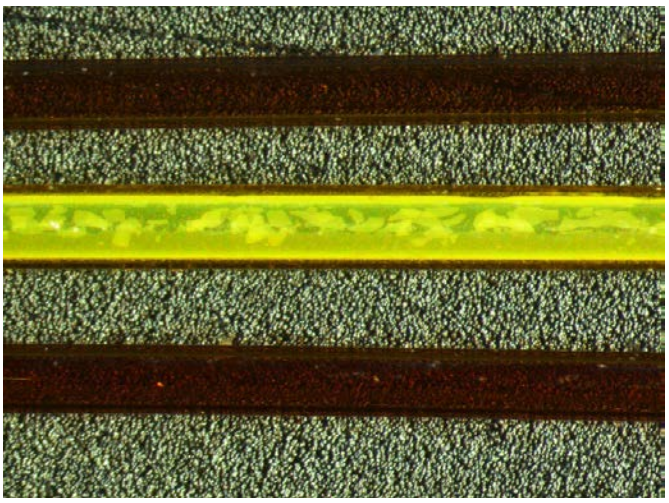
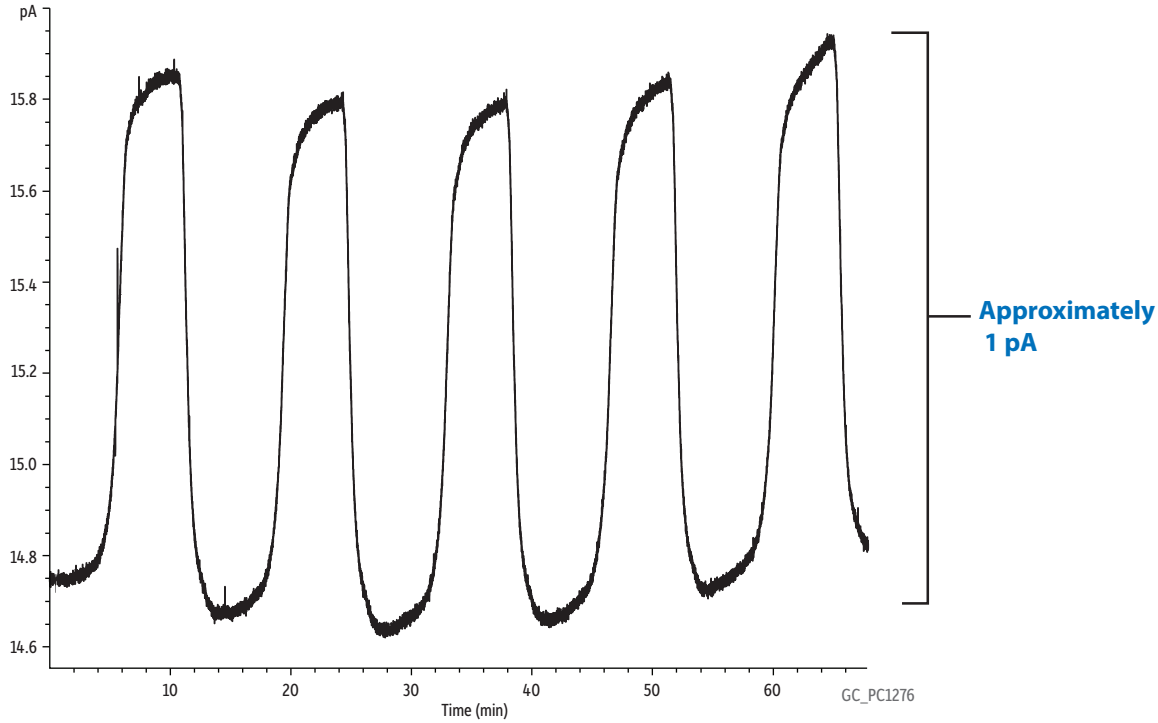
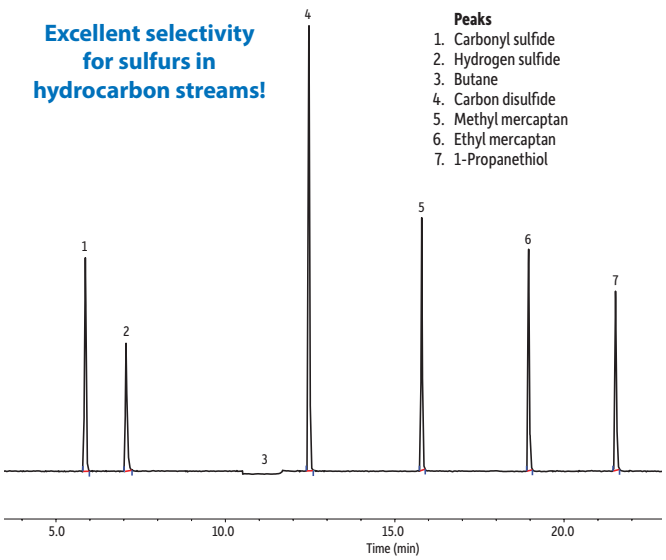


Figure 2: The Rt®-Silica BOND PLOT column shows no large particle spikes, even with temperature and pressure variation.



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

Figure 3: Sulfur Compounds in Butane.



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

Versatile Column for Many Applications

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

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

Rt®-Silica BOND Columns (fused silica PLOT)

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

Innovators in Chromatography

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

The Role of Selectivity in Liquid Chromatography Method Development

By Kevin A. Schug, Ph.D.

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

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

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

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

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

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

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

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

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

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

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