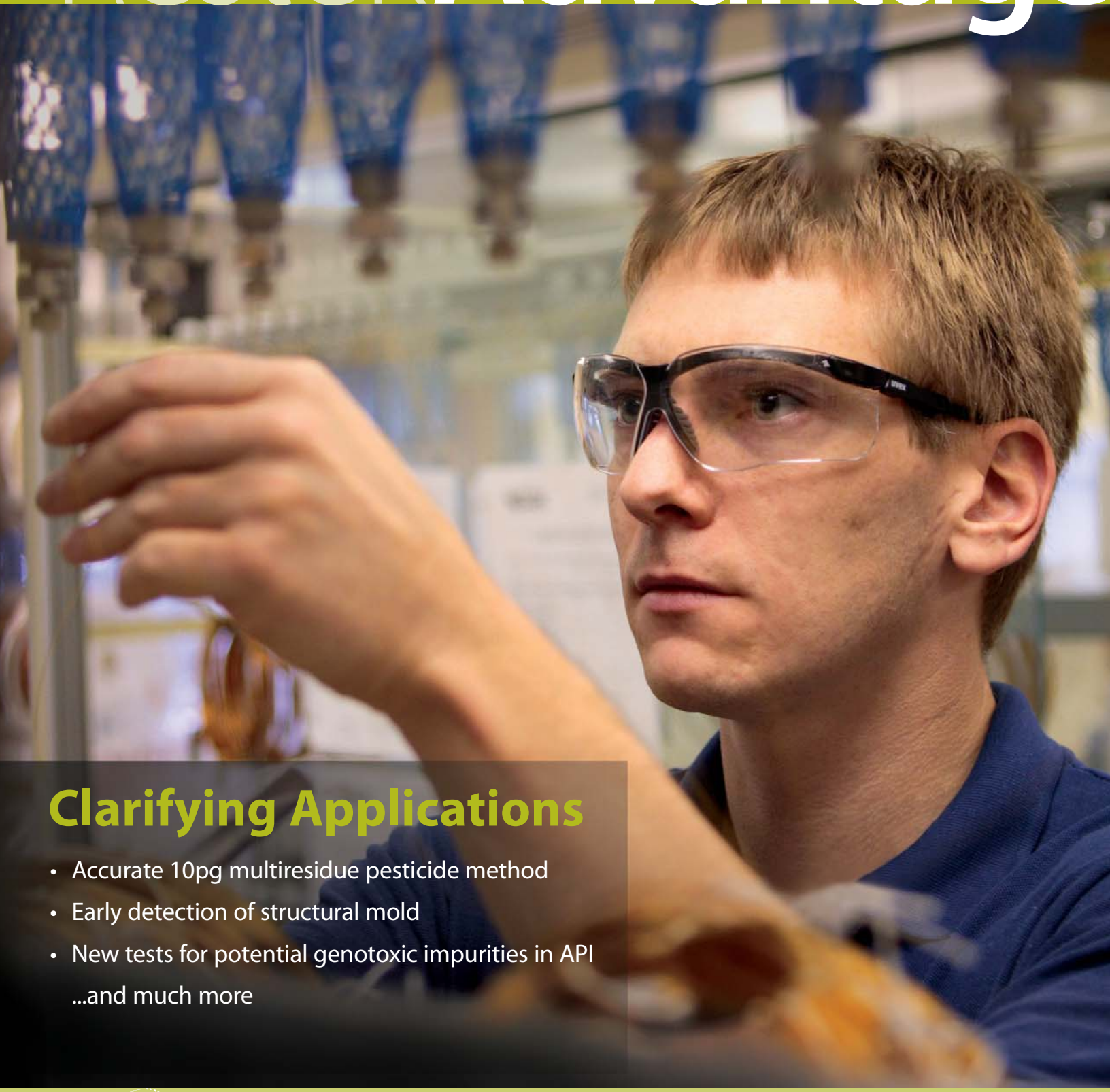
Restek Advantage

2008.03



Clarifying Applications

- Accurate 10pg multiresidue pesticide method
- Early detection of structural mold
- New tests for potential genotoxic impurities in API

...and much more

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in this issue

2008.03

Erratum:
In Advantage 2008.02, Figure 1 on page 19 was incorrect. The corrected figure can be seen at www.restek.com/aoi_fff_A016.asp

Editorial

Achieving Faster GC

Hans-Gerd Janssen, Ph.D., Unilever Food and Health Research Institute

Numerous articles have been published in the scientific literature regarding faster methods for gas chromatography (GC), yet confusion remains on how best to speed up separations. A significant source of this confusion is the fact that authors often neglect to define the terms "analysis speed" and "analysis time". Does the analysis time include sample preparation time? Or is it just the run time between injection and last time point on the chromatogram? Does it include reconditioning, paperwork, or interpretation? Is it the instrument time or the operator time? Numerous questions often are left unanswered and it is these questions that are to blame for the chaos in fast GC. Here I will try to clarify this confusion.

A chromatographic analysis consists of four steps: sample preparation, chromatographic separation, detection, and data interpretation. Clearly these steps are related and can not be considered in isolation. Changes in the sample preparation might affect the performance of the separation, and more sensitive and selective detectors may allow simpler sample preparation. It is these very strong interactions among the four steps that make it very difficult to describe the consequence of a change somewhere in the procedure on the total analysis time. The next problem to consider is the fact that the term "total analysis time" also is not very well defined. Is it the time-to-result for a sample, or is it the total operator time for the analysis of 100 samples divided by 100? Because of all this confusion, information from the literature on how to speed up GC analyses should be interpreted and used with great care. It is the author's sincere belief that these undefined terms have been, and still are, major obstacles, to the success of faster GC. People have tried solutions towards faster GC that too often did not work. This made people lose their confidence in fast GC. However, we should not forget there are almost 20 methods for speeding up a GC separation![1] If one selects the wrong route, all too often the conclusion is that fast GC does not work, rather than that the analyst was wrong in his or her selection. Fast GC works if—and only if—the correct route is selected. Doing that is much simpler than one might expect. Simple guidelines can be followed to select the best option, if we restrict ourselves to the chromatographic separation itself.

The selection of the best route to speed up a separation starts with an understanding of why a chromatographic separation takes time. The total time a chromatogram takes is the sum of all empty baseline segments plus the sum of the width of all baseline peaks. How can we minimize the total time? Very simple: Get rid of the baseline, only separate those peaks that need to be separated and make the peaks as narrow as possible. This sentence summarizes the three main routes to faster GC. In correct scientific terms, and in the correct order of implementation, one would describe them as 1) minimize resolution to a value just sufficient, 2) maximize the selectivity of the chromatographic system, and 3) implement a method that reduces analysis time while holding resolution constant.

If your chromatogram contains baseline or over-resolved peaks, the first step in making the separation faster is to eliminate this over-resolution. The options to do this include:

- shortening the column.
- working at an above optimum carrier gas velocity.
- increasing the initial temperature or the temperature programming rate.
- converting an isothermal separation to a programmed method.
- using flow programming.
- using a thinner film.

Only after having eliminated all baseline and situations of over-resolution should one continue to step 2. But more importantly, if one does not have baseline or over-resolved peaks, do not even consider using these options! Faster temperature programming has been described as a universal solution for faster GC. But if your chromatogram is full of peaks all just separated without any excess resolution, faster programming will ruin your

Eliminate Column Breakage in High Temperature Biodiesel Analysis

By Barry L. Burger, Petroleum Innovations Chemist, Jaap de Zeeuw, International GC Specialist

*Beat high temperature breakage with Restek **MXT®-Biodiesel TG columns**. More stable than fused silica, for accurate, reliable performance and longer column lifetime. Available with either factory- coupled or fully-integrated retention gaps.*

Restek has raised the bar with a new high-temperature MXT®-Biodiesel TG column line to complement our fused silica column line for biodiesel analysis. These new MXT®-Biodiesel TG columns are stable to 430°C and offer unique retention gap options that minimize dead volume and leaks. Choose either a 0.32mm column factory-coupled to a 0.53mm retention gap, or select a single unit 0.53mm column featuring Integra-Gap™, a built-in retention gap that eliminates the need for a connector. Both designs are extremely stable at high temperatures and produce fast elution times and sharp peaks for high molecular weight glycerides.

Eliminate Column Breakage in High Temperature Biodiesel Analysis

Unsurpassed Stability

The high temperature programs required for analysis of biodiesel oils (B100) by either ASTM D-6584 or EN-14105 methodology present a significant challenge to the analytical column. High-temperature fused silica tubing breaks down under these extreme conditions, but the metal MXT® tubing does not degrade, even at temperatures up to 430°C (Figure 1). This allows analysts to bake out any residue eluting after the triglycerides, preventing carryover without damaging the column.

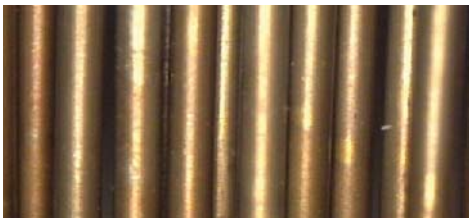
So how well do the MXT®-Biodiesel TG columns perform? We conducted a benchmarking experiment comparing an MXT®-Biodiesel TG column with an Integra-Gap™ retention gap to a high-temperature fused silica column which was coupled to a conventional 0.53mm retention gap. Methodology followed ASTM method D-6584, except the final temperature was modified to 430°C. Both columns were subjected to 100 temperature cycles up to 430°C and then derivatized B100 was injected to check column performance.

MXT®-Biodiesel TG columns are undamaged by the high temperatures required for biodiesel analysis and easily outperform high temperature fused silica columns.

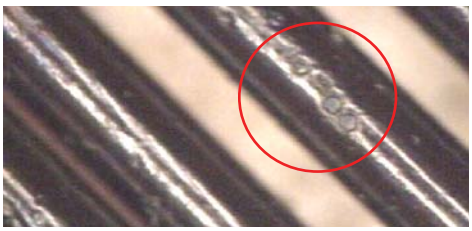
This evaluation was performed using a Shimadzu 2010 gas chromatograph equipped with a flame ionization detector, a model AOC 20i + S autosampler with a 10µL SGE syringe and 42mm 26-gauge needle, and a cold on-column programmable injector with a stainless steel injector insert. A Parker hydrogen generator supplied the carrier gas. Peak symmetry and retention time were evaluated as indicators of thermal stability.

Peak symmetry of butanetriol on a commercial high-temperature fused silica column deteriorates after just 20 injections, compared to the excellent symmetry that is maintained on the MXT®-Biodiesel TG column (Figure 2). In addition to peak shape, retention time stability was used to evaluate column performance. The decrease in retention time seen on the high-temperature fused silica column indicates the liquid phase is being lost (Figure 3). In contrast, the consistent retention times obtained on the MXT®-Biodiesel TG column demonstrate its stability. Practically, this translates into reliable performance and longer column lifetimes.

Figure 1 MXT®-Biodiesel TG columns are undamaged by high thermal cycles compared to high-temperature fused silica columns, which break down under the same conditions.



MXT®-Biodiesel TG columns are undamaged by high thermal cycles.



HT fused silica columns, labeled as stable to 430°C, show pitting and breakdown.

100 temperature cycles to 430°C totaling 500 minutes at maximum temperature.

Figure 2 Stable and consistent peak shape for the internal standard butanetriol gives you more accurate quantitation.

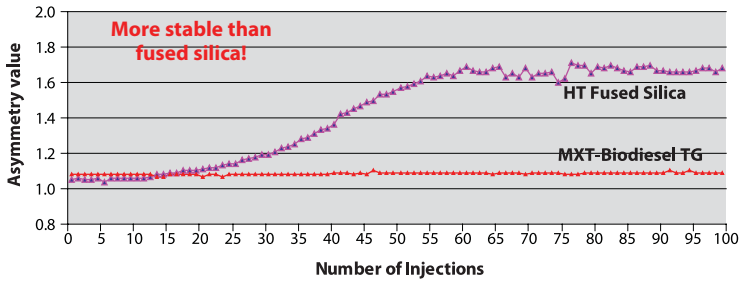
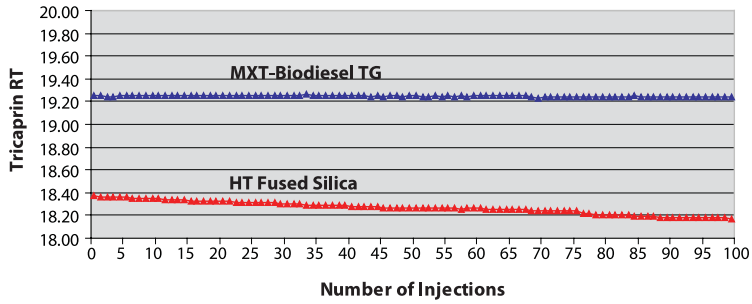


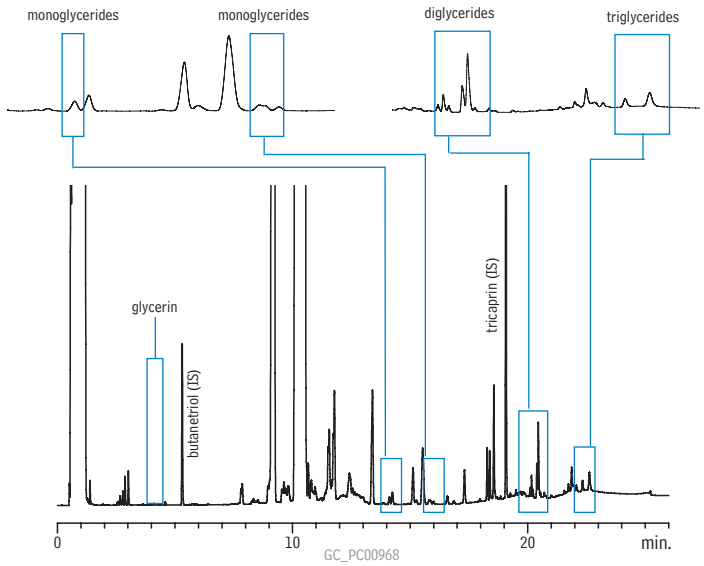
Figure 3 Retention time is stable on a metal MXT®-Biodiesel TG column, even after 100 cycles up to 430°C.



thank **you**

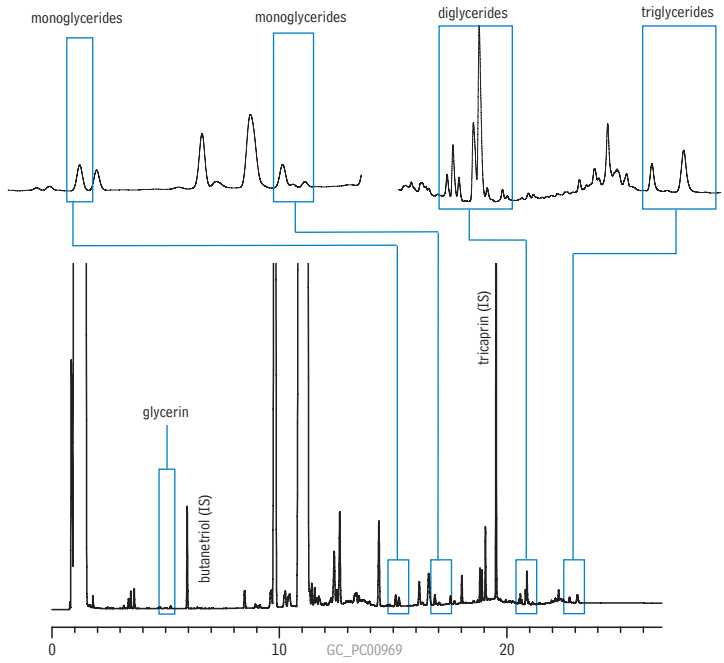
Instrument provided courtesy of Shimadzu **www.shimadzu.com**

Figure 4 Derivatized B100 samples resolve well on the 0.32mm MXT®-Biodiesel TG column, which is factory-coupled to a 0.53mm MXT® retention gap using an MXT® low-dead-volume.



Column: MXT®-Biodiesel TG, 15m, 0.32mm ID, 0.10µm (cat.# 70291) with a 2m x 0.53mm MXT® retention gap connected with an MXT™ low dead-volume connector (17m total length)
Sample: biodiesel (B100), derivatized; Inj.: cool on-column injection 1µL in heptane; Inj. temp.: oven track; Carrier gas: hydrogen, constant flow; Flow rate: 3mL/min.; Oven temp.: 50°C (hold 1 min.) to 180°C @ 15°C/min. to 230°C @ 7°C/min. to 380°C @ 30°C/min. (hold 5 min.); Det.: FID @ 380°C

Figure 5 Excellent chromatographic quality and resolution on the 0.53mm MXT®-Biodiesel TG analytical column with a built in Integra-Gap™ retention gap.



Column: MXT®-Biodiesel TG, 14m, 0.53mm ID, 0.16µm (cat.# 70289) with a 2m x 0.53mm with Integra-Gap™ retention gap (16m total length)
Sample: biodiesel (B100), derivatized; Inj.: cool on-column injection 1µL in heptane; Inj. temp.: oven track; Carrier gas: hydrogen, constant flow; Flow rate: 4mL/min.; Oven temp.: 50°C (hold 1 min.) to 180°C @ 15°C/min. to 230°C @ 7°C/min. to 380°C @ 30°C/min. (hold 5 min.); Det.: FID @ 380°C

Analytical Alternatives

Factory connected 0.32mm MXT®-Biodiesel TG columns & 0.53mm retention gaps

For accurate analysis of heavy triglycerides, on-column injection is required. ASTM D-6584 describes the use of a 0.32mm analytical column coupled with a 0.53mm retention gap. The 0.53mm ID retention gap allows the cool on-column technique to be used, but care must be taken to minimize dead volume and to establish a leak-tight connection. Restek's 0.32mm MXT®-Biodiesel TG columns are factory-coupled to a 0.53mm MXT® retention gap with an MXT® low-dead-volume connector, ensuring a leak-tight connection. Target analytes resolve well and the solvent and triglyceride peaks show excellent symmetry (Figure 4).

0.53mm MXT®-Biodiesel TG columns

The 0.53mm MXT®-Biodiesel TG columns are a simpler alternative to using a 0.32mm column coupled to a 0.53mm retention gap. Restek applied Integra-Gap™ technology to the 0.53mm MXT®-Biodiesel TG columns, eliminating the column coupling. These single unit leak-proof columns feature a built-in retention gap, reducing the risk of peak broadening and tailing. Chromatography from the 0.53mm MXT®-Biodiesel TG with Integra-Gap™ technology (Figure 5) is excellent and comparable to that obtained on the 0.32mm ID column in Figure 4.

Conclusion

As demonstrated, for high temperature GC analysis, the metal MXT®-Biodiesel TG column is a rugged column that withstands the harsh temperatures required for total residual glycerin analysis. The column has the resolution needed for accurate, reliable results and is more stable at high temperatures than competitive fused silica columns, leading to longer column lifetimes. To improve the reliability and robustness of your biodiesel analyses, try a Restek MXT®-Biodiesel TG column.

Product Listing

MXT®-Biodiesel TG Columns (Siltek® treated stainless steel)

Description	cat.#	price
14m, 0.53mm ID, 0.16 w/2m Integra-Gap	70289	
10m, 0.32mm ID, 0.10	70292	
10m, 0.32mm ID, 0.10 w/2m x 0.53mm retention gap	70290	
15m, 0.32mm ID, 0.10	70293	
15m, 0.32mm ID, 0.10 w/2m x 0.53mm retention gap	70291	

temp limits: -60 to 380/430°C *Total column length=16 meters.

Restek Tubing Scorer for MXT® Columns

Designed to make perfectly round cut every time!

Description	qty.	cat.#	price
Restek Tubing Scorer for MXT Columns (0.25-0.53mm ID & 0.5-0.8mm OD)	ea.	20523	
Replacement Scoring Wheel	ea.	20522	

Reliably Detect Pesticides Down to 10pg with Sensitive SIM GC/MS Multiresidue Method

*Market demands are increasing for multiresidue pesticide methods that are both sensitive and effective across a broad range of compound chemistries. The **Rxi®-5Sil MS** column gives accurate low level results for a wide variety of analytes in a single run.*

By Jason Thomas, Environmental Innovations Chemist

As labs operate in an extremely competitive market, the demand for more sensitive multiresidue pesticide methods is increasing. A GC/MS method is a logical choice, as this instrument provides a high degree of specificity, yet is relatively inexpensive and easy to operate, compared to LC/MS/MS, high resolution MS, or GC/MS/MS. However, to take full advantage of GC/MS, careful column selection is critical. The column used must be of the proper selectivity to separate compounds that share common spectra, and also exhibit a high degree of inertness and minimal bleed. Here we demonstrate the effectiveness of an Rxi®-5Sil MS column for low level analysis of a wide variety of pesticides differing in volatility, compound class, and degree of activity.

Excellent Response for Difficult Active Compounds

Column inertness, selectivity, and bleed are key considerations and often determine the success or failure of analytical runs. Inertness can be assessed through the behavior of active compounds, which often exhibit disproportionately poor responses at low concentrations. Although the compound list analyzed here contains many compounds with a high degree of activity, low level linearity (10-1,000ng/mL) was established with an r^2 value of 0.990 or above for many of these challenging compounds (Table I). In addition, the notoriously problematic compounds of EPA Method 8081, endrin and 4,4'-DDT, were among the least troublesome tested here, attaining values of 0.997 and 0.998, respectively. Note that standards were analyzed for this study and some compounds with r^2

Table I The Rxi®-5Sil MS column provides excellent linearity, and thus more accurate results, for a wide range of pesticide chemistries down to 10pg.

Compound	Retention time (min.)	Quant. ion	Qual. ion 1	Qual. ion 2	IS	r^2 (10-1,000 ppb)
methamidophos	5.77	141	95	94	BNB	0.997
dichlorvos	6.02	185	79	109	BNB	0.998
bromonitrobenzene (IS)	7.21	203	201	157	IS	—
mevinphos	8.26	192	127	109	BNB	0.995
acephate	8.30	136	95	94	BNB	0.982
o-phenylphenol	9.44	170	169	141	BNB	0.997
omethoate	10.23	156	110	109	BNB	0.976
dimethoate	11.77	125	143	93	BNB	0.981
pentachloronitrobenzene (IS)	12.13	295	249	237	IS	—
diazinon	12.45	179	304	137	PCNB	0.994
chlorothalonil	12.55	266	264	268	PCNB	0.983
vinclozin	13.48	285	198	212	PCNB	0.998
carbaryl	13.65	144	116	115	PCNB	0.996
metalaxyl	13.69	206	160	132	PCNB	0.997
dichlofluanid	14.17	123	167	224	PCNB	0.954
malathion	14.19	173	125	127	PCNB	0.992
thiabendazole I	15.34	201	202	174	PCNB	0.958
captan	15.34	79	119	149	PCNB	0.987
folpet	15.46	260	130	104	PCNB	0.964
imazalil	16.10	215	175	173	PCNB	0.982
myclobutanil	16.34	206	179	150	PCNB	0.973
endrin	16.82	265	279	317	PCNB	0.997
fenhexamid	17.79	177	179	97	PCNB	0.969
4,4'-DDT	17.79	237	235	165	PCNB	0.998
propargite	18.04	173	150	135	PCNB	0.999
triphenylphosphate (IS)	18.09	325	215	326	IS	—
iprodione	18.47	314	316	187	TPP	0.991
bifenthrin	18.64	181	166	165	TPP	0.998
fenpropathrin	18.82	265	208	181	TPP	0.985
dicofol	18.89	139	251	253	TPP	0.788
permethrin I	20.41	183	165	163	TPP	0.998
permethrin II	20.54	183	163	165	TPP	0.995
deltamethrin	22.87	253	251	181	TPP	0.995

Standard curve: 10, 25, 75, 150, 500, and 1,000 ng/mL mixed standards, single 1µL injections.

Figure 1 Outstanding peak symmetry for active compounds demonstrates column inertness (500pg).

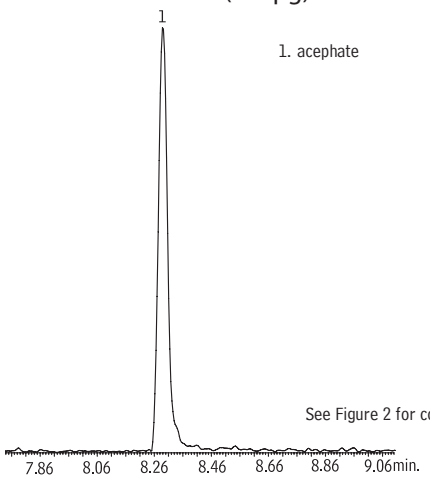
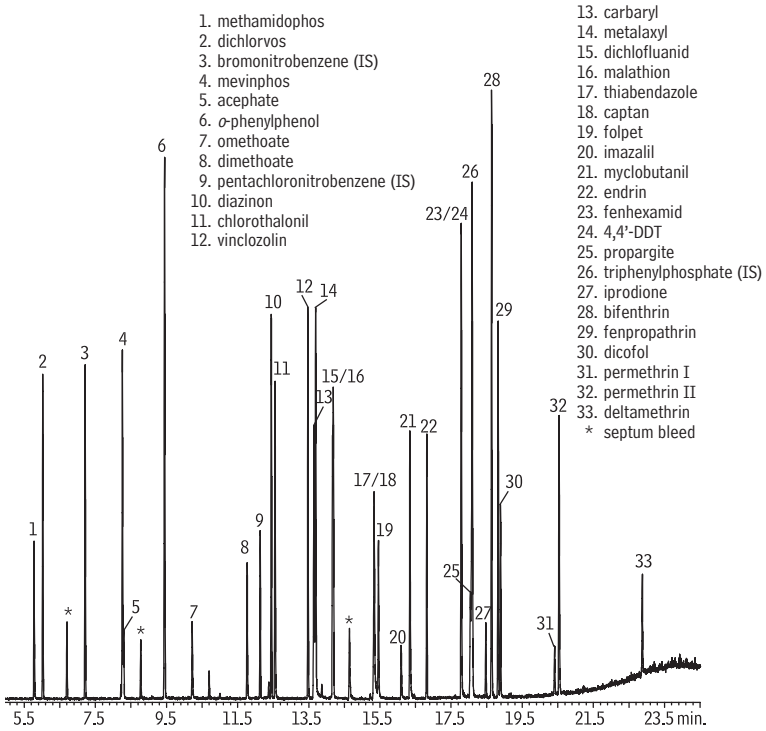


Figure 2 Use an Rxi®-5Sil MS column to easily resolve a broad range of pesticide chemistries in a single run (500pg).



Column:	Rxi®-5Sil MS, 30m, 0.25mm ID, 0.25µm (cat.# 13623), with 5m x 0.25mm ID Rxi® deactivated guard tubing (cat.# 10029)
Instrument:	PerkinElmer Clarus 500 GC/MS
Sample:	1ppm each compound (custom mixes) 1ppm triphenylphosphate (cat.# 32281) 1ppm 1-bromo-2-nitrobenzene (cat.# 32279) 1ppm pentachloronitrobenzene (cat.# 32091)
Inj.:	0.5µL splitless (hold 1 min.), PSS Drilled Uniliner® (hole near bottom) inlet liner (cat.# 22989)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Linear velocity:	32cm/sec. @ 90°C
Oven temp.:	90°C (hold 1 min.) to 310°C @ 10°C/min. (hold 5 min.)
Det.:	MS
Transfer line temp:	300°C
Scan range:	50-350amu
Ionization:	EI
Mode:	scan

The inertness of the Rxi®-5Sil MS column ensures linear performance down to 10pg on-column, allowing more accurate low level quantification.

values less than 0.990, such as acephate, omethoate, and dicofol, show a more linear response when analyzed in matrix. As shown in Figure 1, the Rxi®-5Sil MS column is also highly inert, producing excellent peak shape even for difficult compounds such as acephate. The linearity, sensitivity, and inertness demonstrated here, make the Rxi®-5Sil MS column ideal for more accurate low level quantification of active compounds.

Low Bleed, High Selectivitity

Another crucial characteristic for multiresidue pesticide methods is column bleed. Minimizing bleed is critical in preventing interference with target compounds, even in SIM analysis, as some compounds may share ions and have similar bleed spectra. As shown in the TIC chromatogram in Figure 2, the ultra-low bleed of the Rxi®-5Sil MS column allows full scan analysis with minimal interference from column bleed. The Rxi®-5Sil MS column provides excellent separation for the wide range of chemistries tested and the column is also selective enough to easily separate isomers, such as permethrin I and II.

In summary, many of the difficulties associated with multiresidue methods are simplified by using the Rxi®-5Sil MS column. Its outstanding inertness, low bleed at high temperatures, and unique selectivity provide a robust capillary column with the sensitivity and longevity needed to address the tough challenges inherent to low level multiresidue pesticide analysis.

Product Listing

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623	

Rxi® Guard/Retention Gap Column

Nominal ID	Nominal OD	5-Meter
0.32mm	0.45 ± 0.04mm	10039

PSS Liners for PerkinElmer GCs

ID* x OD & Length (mm)	qty.	cat.#	price
PSS Drilled Uniliner (hole near bottom)			
20mm x 4.0mm x 86.2mm	5-pk.	22989	

PTV On-Column Liner Gives You Two Inlets in One

Why pay for a separate injection port when a simple liner change can convert your programmable temperature vaporization (PTV) inlet to allow for true cold on-column injections? Save time and money by using Restek's ***PTV On-Column Liner****.*

By Scott Grossman, Innovations Chemist, Jack Cochran, Director of New Business and Technology, and Jaap de Zeeuw, International GC Specialist

While PTV is popular internationally, it is an emerging technique in US laboratories and is expected to grow with the awareness of this versatile technique. Now, using a PTV On-Column liner, the capabilities of PTV can be expanded to include true on-column injections, which normally would have required a separate injection port. Why incur the additional expense of a separate injection port when the same results can be achieved with a simple liner change? Restek's PTV On-Column liner, available for Agilent PTVs and the Gerstel CIS4, allows you to perform true cold on-column injections with a PTV port, saving you money and retaining the versatility of the PTV inlet

A Simple Solution

Figure 1 illustrates how this liner works. A 0.53mm ID retention gap column is pressed into the bottom restriction of the liner, forming a leak-free seal between the retention gap's polyimide coating and the inner wall of the liner. The liner's top restriction guides a 26-gauge needle down into the 0.53mm ID retention gap, allowing samples to be injected directly on-column.

Protect Sensitive Compounds

By operating the inlet at low temperatures, an initial flash vaporization is eliminated, protecting thermally labile compounds. Injecting the sample directly into the column also helps avoid injection port activity issues and increases transfer of lower volatility compounds. Both of these features help decrease sample degradation, increase sensitivity, and improve reproducibility. Figure 2 illustrates the outstanding reproducibility that can be achieved with this liner using an example of explosives as probes. Absolute standard deviations were just 2.6% (500pg/µL nitroglycerin) and 1.5% (100pg/µL TNT) for relative peak areas over 5 replicate injections. Variation in realative area was similarly low for both compounds.

Using the PTV On-Column liner decreases sample degradation, increases sensitivity, and improves reproducibility.

Increase Injection Volume

An additional advantage of this liner configuration is the increased analytical sensitivity that can be obtained by injecting a larger sample volume. When the sample needs to be flash vaporized, sample volume expansion in the liner quickly becomes a concern, limiting injection volume to 1-2µL of sample. However, with cold on-column injections, larger sample volumes can be used because the solvent can be gradually vaporized and eluted before the analytes. Using a larger sample volume means more analyte is loaded on-column, giving greater overall sensitivity. The data in Figure 3 demonstrate the excellent linearity achievable using the PTV On-Column liner across a range of injection volumes. Instead of a traditional calibration curve that plots response vs. increasingly concentrated standards, this plot illustrates response vs. increasing volumes of the same standard, in effect producing the same result of more mass on-column. The correlation between peak area and injection volume (5 -100µL) was evaluated and r^2 values of

Figure 1 Use a PTV On-Column liner to perform cold on-column column injections in a PTV injection port.

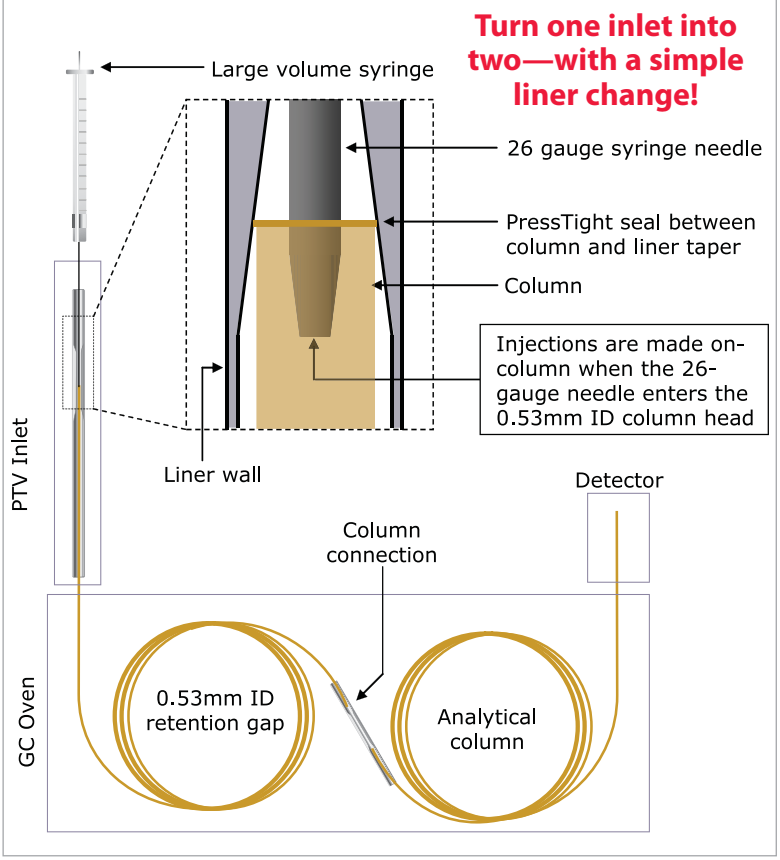
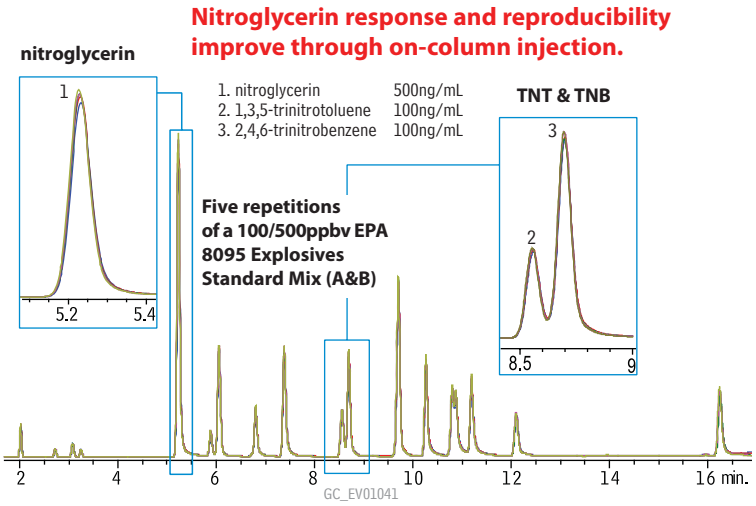


Figure 2 Increase reproducibility and sample integrity with a PTV On-Column liner.



Absolute area reproducibility improves for all compounds, and sensitive compound responses improve dramatically because of the lack of contact with the injection port.

nitroglycerin: Absolute Area % RSD = 2.6% • Relative Area % RSD = 1.6%

TNT: Absolute Area % RSD = 1.5% • Relative Area % RSD = 1.4%

Column: Rxi®-5ms, 6m, 0.53mm ID, 0.5µm (cat.# 563153) with 5m x 0.53mm IP guard tubing (cat.# 10045), connected using PTV On-Column liner (cat.# 24976); Sample: 8095 Calibration Mix A and 8095 Calibration Mix B diluted in acetonitrile; Inj.: PTV injection port splitless (15mL/min. @ 0.35 min.); Inj. temp.: 55°C to 285°C @ 10°C/min. (hold 10 min.); Carrier gas: helium, constant flow; Linear velocity: 60cm/sec. @ 300°C; Oven temp.: 50°C to 280°C @ 10°C/min. (hold 10 min.); Det.: µECD @ 300°C, nitrogen make-up gas @ 60mL/min.

0.9986 (TNB) and 0.9997 (TNT) were obtained. Note that a linear response is maintained—even for high injection volumes.

Why pay for two injection ports when a simple liner change gives you the benefits of having two inlets in one? Using a PTV On-Column liner saves you money and gives you flexibility in the lab. Use this liner and reliably perform true cold on-column injections with your PTV injection port.

Product Listing

PTV Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#	price
PTV On-Column Liner			
1.7mm x 3.0mm x 71mm	ea.	24976	
1.7mm x 3.0mm x 71mm	5-pk.	24977	

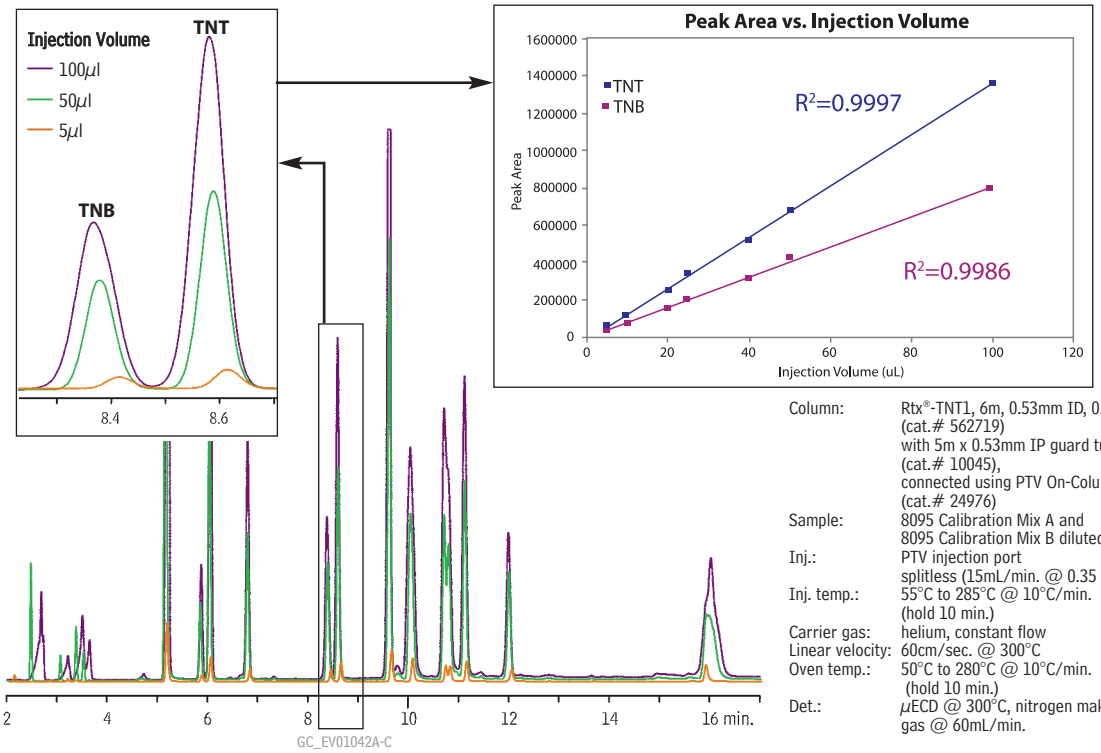
*Nominal ID at syringe needle expulsion point.

Get More!

Visit www.restek.com/environmental and download:

- Explosives and Brominated Flame Retardant Analysis by Gas Chromatography with a New On-Column Injector Liner for a Programmable Temperature Vaporizing Injector
- Using Guard Columns and Retention Gaps in GC

Figure 3 Improve sensitivity with larger injection volumes using a PTV On-Column liner.



Column: Rtx®-TNT1, 6m, 0.53mm ID, 0.5µm (cat.# 562719) with 5m x 0.53mm IP guard tubing (cat.# 10045), connected using PTV On-Column liner (cat.# 24976)
Sample: 8095 Calibration Mix A and 8095 Calibration Mix B diluted in acetonitrile
Inj.: PTV injection port splitless (15mL/min. @ 0.35 min.)
Inj. temp.: 55°C to 285°C @ 10°C/min. (hold 10 min.)
Carrier gas: helium, constant flow
Linear velocity: 60cm/sec. @ 300°C
Oven temp.: 50°C to 280°C @ 10°C/min. (hold 10 min.)
Det.: µECD @ 300°C, nitrogen make-up gas @ 60mL/min.

Early Detection of Structural Mold with SilcoCan™ Air Sampling Canisters

*Early detection of mold growth in buildings is critically important to protecting human health and property values. Restek **SilcoCan™ canisters** allow low levels of mold to be detected in air samples—before it can be seen—providing an opportunity for structural repair and safer living conditions.*

By Silvia Martinez, Innovations Chemist

Mold growth in homes has been linked to serious human health and property value issues; thus, early detection is of increasing importance. Mold releases microbial volatile organic compounds (MVOCs) which can be sampled in air and identified by GC/MS analysis, even prior to visual detection methods. MVOCs attributed to fungal growth include terpenes, ethers, ketones, alcohols, aldehydes, aromatic and chlorinated hydrocarbons, sulfur-based compounds, and amines. These compounds are not unlike other volatile organic compounds commonly analyzed in environmental and industrial hygiene laboratories, and the same equipment can be used to collect, positively identify, and quantify MVOCs.

Due to the polar nature of many MVOCs, and the low concentrations found in early detection, a passivated, large volume collection device is needed for sampling. SilcoCan™ canisters are an excellent choice for sampling and analyzing MVOCs. The canister surface, passivated with a chemically bonded fused silica layer, has been shown to provide the stability and inertness needed for collecting and storing low level volatiles (ppbv) such as those analyzed by EPA methods TO-14A and TO-15, including sulfur-containing compounds and microbial VOCs. Here we show a successful application of highly inert SilcoCan™ canisters and GC/MS for monitoring low level mold growth in building structures.

Table I Boiling points of low volatility MVOCs.

MVOC	bp (°C)
1-octanol	194
isoborneol	212
α-terpineol	214
geosmin	270

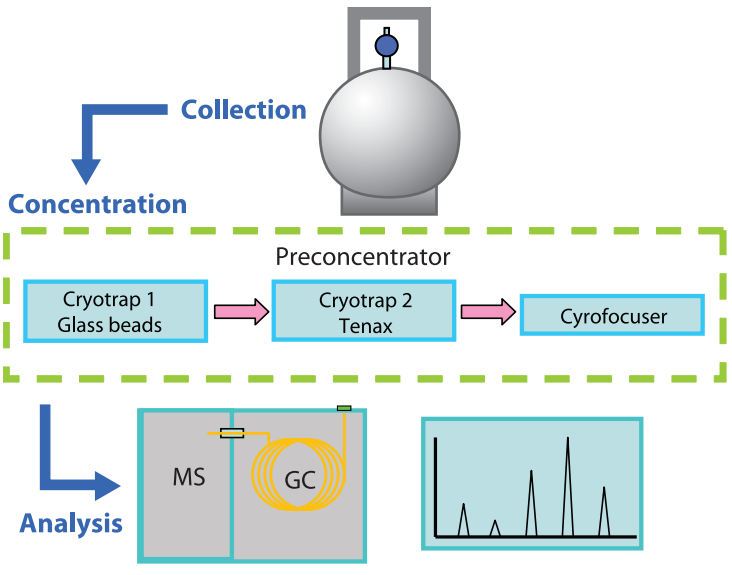
Sample Set-up

For our analysis, we began with standard solutions of 23 MVOCs in methanol at 100µg/mL. The compounds were separated by chemistry into four solutions to prevent degradation reactions: alcohols, ketones, 2-isopropyl-3-methoxypyrazine, and geosmin. After cleaning and evacuating a SilcoCan™ canister, 210µL of water were added to the canister to simulate natural humidity and aid recovery. After equilibration, 2µL of each solution were added to the canister. Finally, the canister was pressurized to 30psig with dry nitrogen to yield a final concentration of 2.2ng/200mL for each MVOC, or 1.4 to 3.8ppbv of each MVOC. (The final ppbv concentration is molecular weight-dependant.) To boost recoveries of the higher-boiling compounds, we used a Restek Air Canister Heating Jacket set to 75°C. The sample was heated to 75°C for 30 minutes prior to, and during testing. Boiling points of some of the lower volatility MVOCs are shown in Table I.

23 MVOCs Identified in Less than 30 Minutes

Sample analysis was conducted using standard air analysis equipment such as is used in environmental laboratories. In our case, we used a Nutech 8900DS autosampler and preconcentrator attached to an Agilent 6890/5973 GC/MS. Volatiles in the sample are concentrated by a cryogenic trap followed by an adsorbent trap, then cryofocused for injection into the GC/MS. Figure 1 shows a schematic of the sampling and preconcentration process. An Rxi®-1ms column was used to provide separation at the ultra-low bleed levels required for spectroscopic analysis. The MVOC sample was analyzed by concentrating 200mL of the 0.011ng/mL gaseous mix using a 1:1 split for only 1ng on column of each MVOC. The resulting chromatogram, shown in Figure 2, shows excellent peak response and resolution for the 23 compounds in less than 30 minutes.

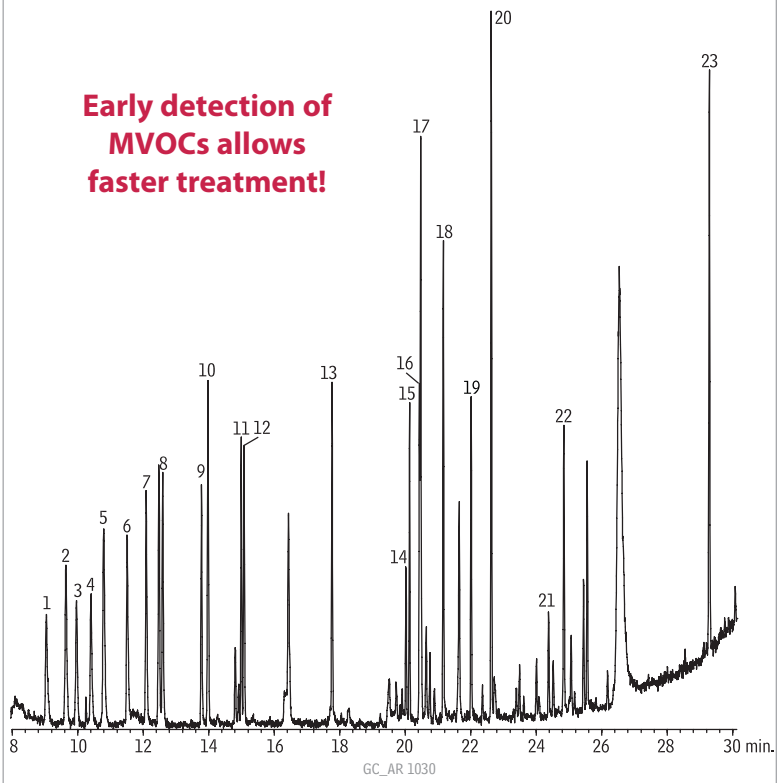
Figure 1 Sample set-up for low level MVOC analysis. Excellent response was seen, even for polar and high boiling point compounds.



Passivated SilcoCan™ canisters are ideal for sampling low concentrations of MVOCs. The inertness of these canisters provides an exceptional storage environment, particularly for polar and high boiling point compounds.

SilcoCan™ canisters easily provide the inertness and stability required for the collection, storage, and analysis of MVOCs, especially for polar and high-boiling compounds. Air sampling of MVOCs using SilcoCan™ canisters allows for early detection of fungal growth, providing an opportunity for structural treatments to eradicate damaging mold.

Figure 2 Detect low levels of structural mold using SilcoCan™ canisters for air sampling (1ng on-column).



Compound	Rt (min.)
1. 2-butanone	9.047
2. 2-methyl-furan	9.640
3. 3-methyl-furan	9.962
4. 2-methyl-1-propanol	10.405
5. 2-methyl-2-butanol	10.791
6. 1-butanol	11.506
7. 3-methyl-2-butanol	12.092
8. 2-pentanol	12.592
9. 2-methyl-1-butanol	13.779
10. dimethyl disulfide	13.979
11. 3-hexanone	14.994
12. 2-hexanone	15.080
13. 2-heptanone	17.767
14. 1-octen-3-ol	20.019
15. 3-octanone	20.133
16. 3-octanol	20.433
17. 2-pentyl-furan	20.476
18. 2-ethyl-1-hexanol	21.163
19. 1-octanol	22.013
20. 2-isopropyl-3-methoxypyrazine	22.628
21. isoborneol	24.379
22. α-terpineol	24.844
23. geosmin	28.347

Column: Rxi®-1ms, 60m, 0.25mm ID, 1.00µm (cat.# 13356)
Sample: microbial volatile organic compounds (MVOCs), 2ppbv, 60% RH
Inj.: 1.0µL split (split ratio 1:1), 1mm split inlet liner (cat.# 20972)
Inj. temp.: 200°C
Carrier gas: helium, constant flow
Flow rate: 1.5mL/min.
Oven temp.: 10°C (hold 1 min.) to 235°C @ 8°C/min.
Det: Agilent 6890/5973 GC/MS 5 min. solvent delay

Transfer line temp.: 260°C
Scan range: 35 to 350amu
Ionization: EI
Mode: scan
Other: Nutech 8900DS Preconcentrator Conditions:
Sample = 200mL from canister
Cryotrap1 = -160°C
Desorb = 20°C
Cryotrap2 = 20°C
Desorb = 200°C
Cryofocuser = 200°C
Desorb = 200°C

Product Listing

Rxi®-1ms Columns (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	1.00	-60 to 330/350°C	60-Meter	13356	

1mm Split Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#	price
1mm Split			
1.0mm x 6.3mm x 78.5mm	ea.	20972	

SilcoCan™ Air Monitoring Canisters

Ideal for low-level reactive sulfur (1-20ppb), TO-14A, or TO-15 compounds

Canisters are the gold standard for ambient VOC monitoring.

Description	Volume	qty.	cat.#	price
SilcoCan Canister, 1/4" Valve	1L	ea.	24180	561
SilcoCan Canister, 1/4" Valve	3L	ea.	24181	581
SilcoCan Canister, 1/4" Valve	6L	ea.	24182	602
SilcoCan Canister, 1/4" Valve	15L	ea.	24183	923

Air Canister Heating Jacket

The ultimate in controlled heating, for reliably cleaning your air canisters!

Description	qty.	cat.#	price
Air Canister Heating Jacket (110 volt)	ea.	24123	

*Not CE certified.

Prepare Samples in Half the Time Using a Fraction of the Solvent with dSPE

*Simplify and speed up sample preparation with **Resprep dSPE tubes**! Here we show the extraction and clean-up of pesticide residues from olive oil samples—twice as fast as GPC, with only a fraction of the solvent required for conventional SPE.*

By Michelle Misselwitz, Environmental Innovations Chemist, Julie Kowalski, Ph.D., Food Flavors, and Fragrances Innovations Chemist, Mark Crawford*, Applications Chemist, Michael Halvorson Ph.D.*, Senior Product Specialist, and Joan M. Stevens Ph.D.*, Applications Manager
*Gilson, Inc.

Olive oil is considered a healthy fat source and is a staple in many recommended diets. However, concerns about potentially negative health effects associated with pesticide residues have increased consumer interest in testing. While organophosporus pesticides are currently used in olive orchards to control pests, organochlorine pesticides are still tested for as persistent organic pollutants (residues), even though they are no longer in commercial use. There are several existing methods for measuring pesticide residues in olive oil, all of which involve sample extraction and clean-up.[1] The common goal of these methods is to remove lipids that are harmful to the analytical system.[2] Efficient sample clean-up procedures are critical to maximizing sample throughput and minimizing labor and material costs. Here we demonstrate the efficiency of a dSPE clean-up procedure, as well as the capabilities of both method-specific and general purpose analytical columns.

Simple Procedure Uses Half the Time and Minimal Solvent

Sample extraction and clean-up can be accomplished with gel permeation chromatography (GPC), solid phase extraction (SPE), or dispersive solid phase extraction (dSPE) methods. However the dSPE method shown here is much less expensive than GPC (which requires specialized equipment) and uses substantially less solvent than comparable GPC or SPE methods (Table I).[3] The method is simple to use and allows sample extraction and clean-up to be accomplished in half the time of other techniques (Table II).

Extraction and dSPE Clean-up for Pesticide Residues in Olive Oil

Test sample: A 1.5mL sample of commercially obtained virgin olive oil was spiked with a standard organochlorine pesticide mix. The spiked sample was processed as follows.

1. Dilute with 1.5mL hexane.
2. Add 6mL of acetonitrile (ACN).
3. Mix for 30 minutes on a shaker.
4. Allow layers to separate (approximately 20 minutes), then collect the top (ACN) layer.
5. Repeat the liquid-liquid extraction (steps 2-4) and combine both ACN extract layers.
6. Place 1mL of the combined ACN extract in a 1.5mL tube containing 150mg magnesium sulfate and 50mg PSA.
7. Shake the tube for 2 minutes.
8. Centrifuge at 3,000 U/min. for approximately 5 minutes.
9. Remove the top layer and inject directly into the gas chromatograph system.

Extracts were analyzed using both Rtx®-CLPesticides2 and Rxi®-5Sil MS columns (Figure 1). The Rtx®-CLPesticides2 column is a method specific column that resolves all compounds. The Rxi®-5Sil MS column is a general purpose column that has one coelution that can easily be extracted by a mass

Table I Resprep dSPE method uses 42% and 89% less solvent than SPE and GPC methods respectively.

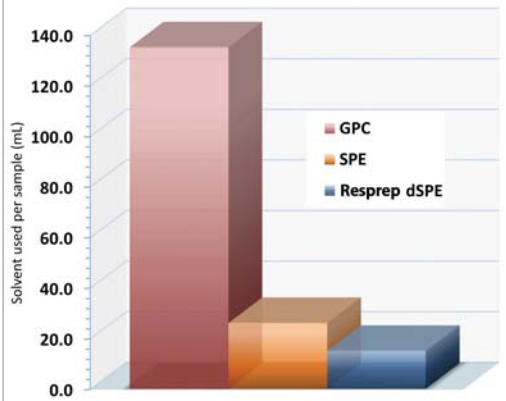


Table II Cut extraction/clean-up time by 50% using a Resprep dSPE method.

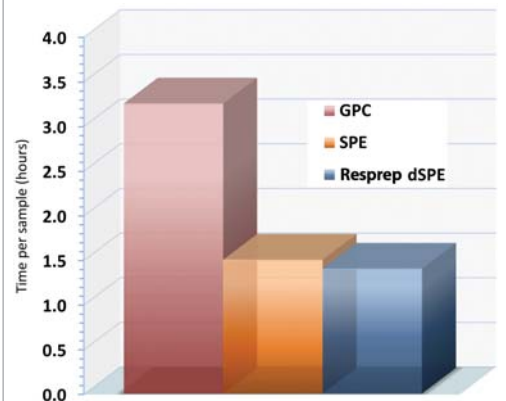
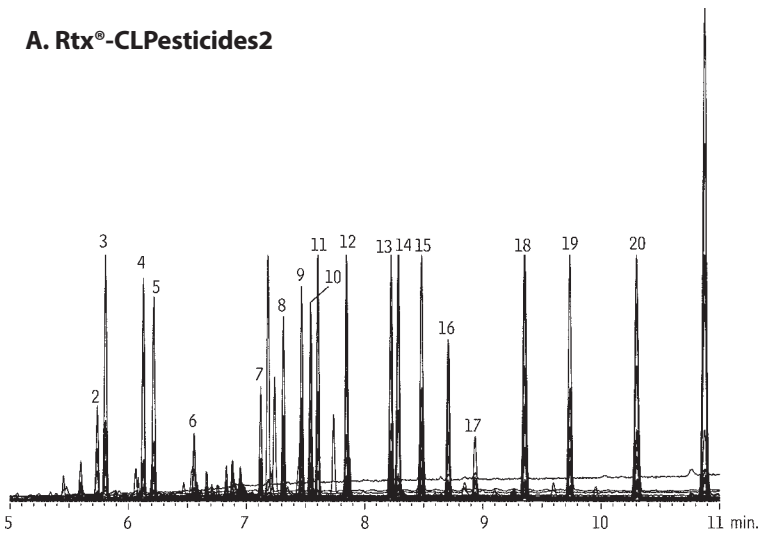


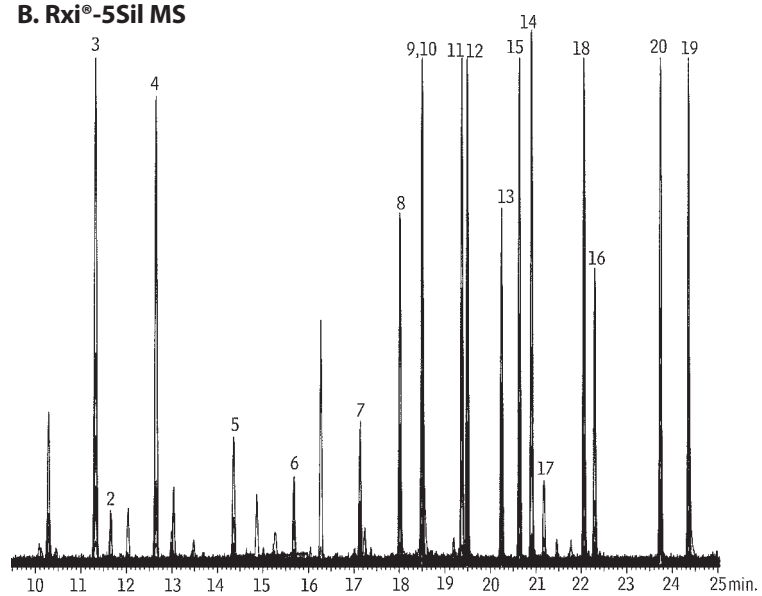
Figure 1 Chlorinated pesticide residues in olive oil are easily separated on either Rtx®-CLPesticides2 or Rxi®-5Sil MS columns.

A. Rtx®-CLPesticides2



GC_FF01043

B. Rxi®-5Sil MS



GC_FF01044

Column:	A. Rtx®-CLPesticides2, 30m, 0.25mm ID, 0.20µm (cat.# 11323) B. Rxi®-5Sil MS, 30m, 0.25mm ID, 0.25µm (cat.# 13623)
Sample:	10µg/mL Organochlorine Pesticide Mix AB # 3 (cat.# 32415) in olive oil
Inj.:	1µL, splitless (hold 0.5 min.), 3.5mm single gooseneck liner (cat.# 20962) packed with wool
Inj. temp.:	225°C
Carrier gas:	helium, constant flow
Flow rate:	1mL/min.
Oven temp.:	A. 140°C (hold 0.5 min.) to 268°C @ 20°C/min. to 290°C @ 3°C/min. to 330°C (hold 5 min.) @ 20°C/min. B. 130°C (hold 0.5 min.) to 330°C @ 5°C/min
Det:	MS
Transfer line temp.:	320°C
Ionization:	EI
Mode:	SIM

Compound	Quant. ion	Qual. ion 1	Qual. ion 2
*1. α-BHC	219	181	109
2. γ-BHC	219	181	109
3. β-BHC	219	181	109
4. δ-BHC	219	181	109
5. heptachlor	272	237	100
6. aldrin	263	293	220
7. heptachlor epoxide	263	237	81
8. δ-chlordane	272	237	65
9. α-chlordane	272	237	65
10. endosulfan I	195	207	241
11. 4,4'-DDE	246	318	176
12. dieldrin	79	263	277
13. endrin	263	281	81
14. 4,4'-DDD	235	165	199
15. endosulfan II	195	207	-
16. 4,4'-DDT	235	165	199
17. endrin aldehyde	67	250	345
18. endosulfan sulfate	272	229	239
19. methoxychlor	227	274	-
20. endrin ketone	67	317	281

* not present

spectrometer detector (MSD). Only α-BHC was not detected, a subject of further investigation, however either column can be used effectively. Recoveries of 70%-80% were obtained, levels comparable to conventional SPE—without the necessity of vacuum manifolds or high pressure systems. The GPC method attained recoveries of > 95%. However this method requires large amounts of solvent and takes over twice as long as other methods.

The dSPE method shown here is an efficient, cost-effective way to clean up chlorinated pesticide residues in olive oil. With good recoveries and minimal matrix interference, it is an easy way to reduce solvent usage, compared to conventional SPE, and is more cost-effective than GPC.

References
1. C. Lentza-Rizos, E.J. Avramides, Rev. Environ. Contam. Toxicol. 141 (1995) 111.
2. S. Cunha, S. Lehotay, K. Mastovska, J. Sep. Sci. 30 (2007) 620.
3. M. Crawford, M. Halvorson, J. Stevens, The Examination and Automation of GPC, SPE and QuEChERS Utilized in Extracting Pesticides from Olive Oil. HPLC 2008 poster presentation.

Product Listing

dSPE Tube for Clean-Up of Pesticide Residue Samples

Description	Material	Methods	qty.	cat#	price
2mL Microentrifuge Tubes for dSPE					
Resprep	150mg $MgSO_4$,	AOAC			
Q250	50mg PSA	2007.1	100-pk.	26124	

PSA—primary and secondary amine exchange material.

Organochlorine Pesticide Mix AB # 3

(20 components)

aldrin	dieldrin
α-BHC	endosulfan I
β-BHC	endosulfan II
δ-BHC	endosulfan sulfate
γ-BHC (lindane)	endrin
α-chlordane	endrin aldehyde
γ-chlordane	endrin ketone
4,4'-DDD	heptachlor
4,4'-DDE	heptachlor epoxide (isomer B)
4,4'-DDT	methoxychlor

2,000µg/mL each in hexane:toluene (1:1), 1mL/ampul
cat. # 32415 (ea.)

Rtx®-CLPesticides2 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.20	-60 to 320/340°C	30-Meter	11323	

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13623	

Prevent Fraud in Egg Pasta with Simple Analysis of Cholesterol and Glycerides

*Eliminate the uncertainty of using cholesterol alone to authenticate egg content. Determine both glycerides and cholesterol in a single run using an **Rtx®-65TG** column and get definitive, fraud-identifying results.*

By Julie Kowalski, Ph.D., Food Flavors, and Fragrances Innovations Chemist, Gary Stidsen, Product Marketing Manager, Daniele Naviglio*, Professor, Analytical Chemist, and Fabiana Pizzolongo*, Ph.D., Food Technologist

*Dipartimento di Scienza degli Alimenti – Università degli Studi di Napoli "Federico II" – Via Università, 100 - 80055 Portici (NA) – Italia

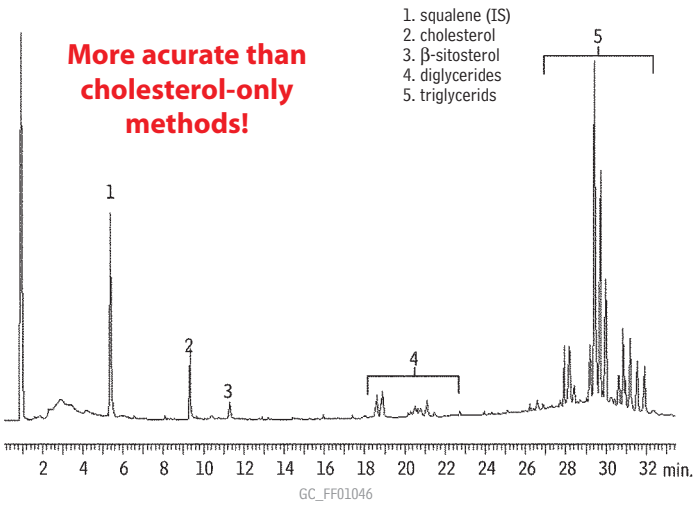
Eggs enhance the nutritional and commercial value of pasta, and thus many countries have established minimum egg content levels (based on either counts or weights) for pasta and other egg-containing products. Although egg content standards have been established, methods are not usually specified and a number of procedures may be applied. Cholesterol methods are often used to authenticate products claimed on the label to be made with eggs; however, since cholesterol can be added using non-egg sources, its presence alone is not a reliable marker of egg content. Also, even if egg is the source of the cholesterol in the product, it is difficult to correlate quantitatively to egg content levels, because the levels of cholesterol found naturally in eggs are highly variable. The method presented here allows the use of glycerides, in addition to cholesterol, to assess egg content in pasta. This method provides chromatographic separation of cholesterol, diglycerides, and triglycerides, allowing fraudulent (non-egg) sources of cholesterol to be easily and accurately determined, so qualitative and quantitative comparisons can be made.

Simple Extraction Method

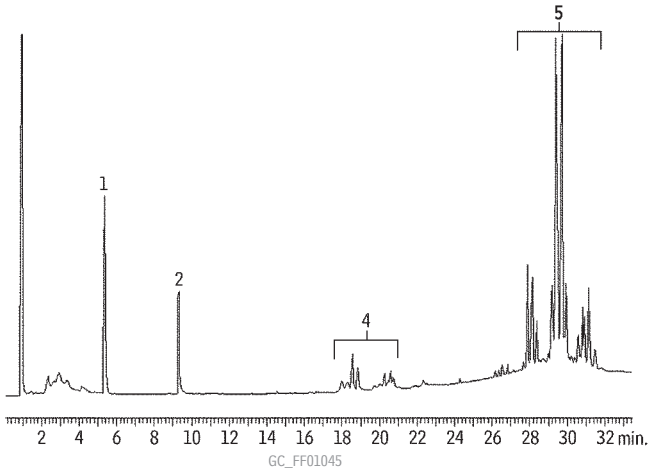
Current methods used for the extraction of fat from flour components generally involve either a 24-hour diethyl ether extraction or an 8-hour Soxhlet extraction. The extraction described here is rapid by comparison. In this simple procedure, fat is extracted from egg pasta dough and freeze-dried egg product by homogenizing the samples and pouring them into glass columns filled with sodium sulfate. The fat phase is eluted with 100mL diethyl ether and then evaporated with nitrogen. Approximately 50mg of the dried fat extract is then dissolved in 1mL internal standard solution (3,000 ppm squalene in diethyl ether). The extracted samples are analyzed by gas chromatography (GC) using an Rtx®-65TG column, which is specifically tested for triglyceride performance.

Figure 1 Easily detect fraud by comparing cholesterol and glyceride profiles in one run on the Rtx®-65TG column.

A. Extracted egg pasta fats



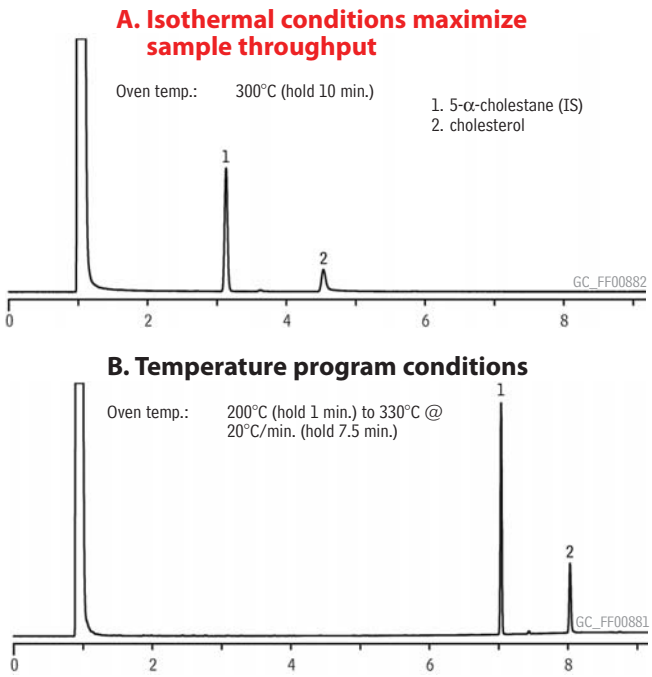
B. Extracted egg fats



Column:	Rtx®-65TG, 30m, 0.25mm ID, 0.10μm (cat.# 17008)
Sample:	A. 50μg/mL fat extract from egg pasta in diethyl ether solution with 3,000ppm squalene (IS) B. 50μg/mL fat extract from egg in diethyl ether solution with 3,000ppm squalene (IS)
Inj.:	0.5μL, split (1:80), 70°C (hold 12 sec.) at 99°C up to 370°C (hold 5 min.)
Carrier gas:	hydrogen
Flow rate:	1.5mL/min.
Oven temp.:	220°C (hold 2.0 min.) to 360°C @ 5°C/min. (hold 5 min.)
Det:	FID @ 370°C

Fraudulent label claims of egg content in egg pasta can be detected more accurately by evaluating glycerides and cholesterol, compared to analyzing cholesterol alone. This simple method determines both in a single run.

Figure 2 5-minute run times benefit cholesterol methods requiring high sample throughput instead of source confirmation.



Column:	Rxi®-5ms, 15m, 0.25mm ID, 0.25μm (cat.# 13420)
Sample:	1,000μg/mL cholesterol in DMF, 1,000μg/mL 5-α-cholestane in hexane; 25ng cholesterol, 150ng 5-α-cholestane on column
Inj.:	1.0μL, split (20:1), single gooseneck inlet liner w/wool (cat.# 22405)
Inj. temp.:	250°C
Carrier gas:	helium, constant pressure (9.7psi @ 200°C)
Linear velocity:	24cm/sec.
Oven temp.:	see above
Det.:	FID @ 340°C

Easy Identification of Fraudulent Product

Excellent chromatographic separation of cholesterol, squalene, diglycerides, and triglycerides was obtained (Figure 1). Once separated, these fractions can be used to confirm the addition of egg fat by comparing the glyceride profiles of the egg pasta extract with those from the egg sample. Egg pasta products adulterated with non-egg sources of cholesterol will not show comparable patterns. Note, while cholestane often is used as an internal standard in cholesterol testing, the use of squalene instead in this method is advantageous as it allows both cholesterol and the glyceride profiles to be analyzed. Squalene is highly stable and similar to cholesterol, but the compounds are well-resolved on the Rtx®-65TG column. Cholestane is not sufficiently separated from cholesterol on this polar phase, however, for methods that recommend cholestane, separations can be accomplished on the less polar Rxi®-5ms column (Figure 2). In fact, for methods with a goal of high throughput cholesterol determination, rather than source authentication, using the Rxi®-5ms column under isocratic conditions can cut analysis time by nearly 50%.

In summary, estimating cholesterol in food products is often part of the authentication testing of label claims regarding egg content. However, the presence of cholesterol in a product may be due to a non-egg source, and the natural variability of cholesterol levels in eggs further complicates quantitative conclusions. The method shown here simplfies fraud detection by incorporating glyceride testing. Easy comparision of the chromatographic profiles of egg and egg product (pasta) samples can be made using an Rtx®-65TG column, which is specifically tested to assure excellent separations and a reliable performance for glycerides.

Product Listing

Rtx®-65TG Columns (fused silica)

(Crossbond® 65% diphenyl/35% dimethyl polysiloxane)

ID	df (μm)	temp. limits	length	cat. #	price
0.25mm	0.10	40 to 370°C	30-Meter	17008	

Rxi®-5ms Columns (fused silica)

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

ID	df (μm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	15-Meter	13420	

Fast Screening and Confirmation of Gamma-Hydroxybutyrate (GHB) in Urine

Maximize your analytical options with this versatile GHB extraction method. No derivatization means faster sample preparation. Extracts are amenable to both liquid injection GC/FID and headspace GC/MS methods.

By Amanda Rigdon, Pharmaceutical Innovations Chemist and Kristi Sellers, Clinical/Forensic Innovations Chemist

Gamma-hydroxybutyrate (GHB) and its precursor, gamma-butyrolactone (GBL), are controlled substances associated with drug-facilitated sexual assault. Criminal cases often hinge on lab results, which can include screening urine samples and then quantifying GHB using GC/MS. In its native state, GHB is extremely difficult to chromatograph and must be analyzed as a trimethylsilyl derivative or converted to GBL. The headspace (HS) procedure described here (adapted from an FBI Chemistry Unit method) eliminates time-consuming derivatization.[1] This procedure reduces sample preparation time and minimizes both column contamination from derivatization reagents and contamination from sample matrix caused by liquid injections.

Eliminate Derivatization and Reduce System Contamination

Samples were spiked in urine and extracted according the procedure in Table I, using alpha-methylene-gamma-butyrolactone (AMGB) as an internal standard. GHB is converted to GBL with sulfuric acid, eliminating the need for derivatization (Figure 1). Note the unconverted sample shows comparable levels of GBL and AMGB, whereas GBL levels in the converted sample are significantly higher, due to the conversion of GHB to GBL.

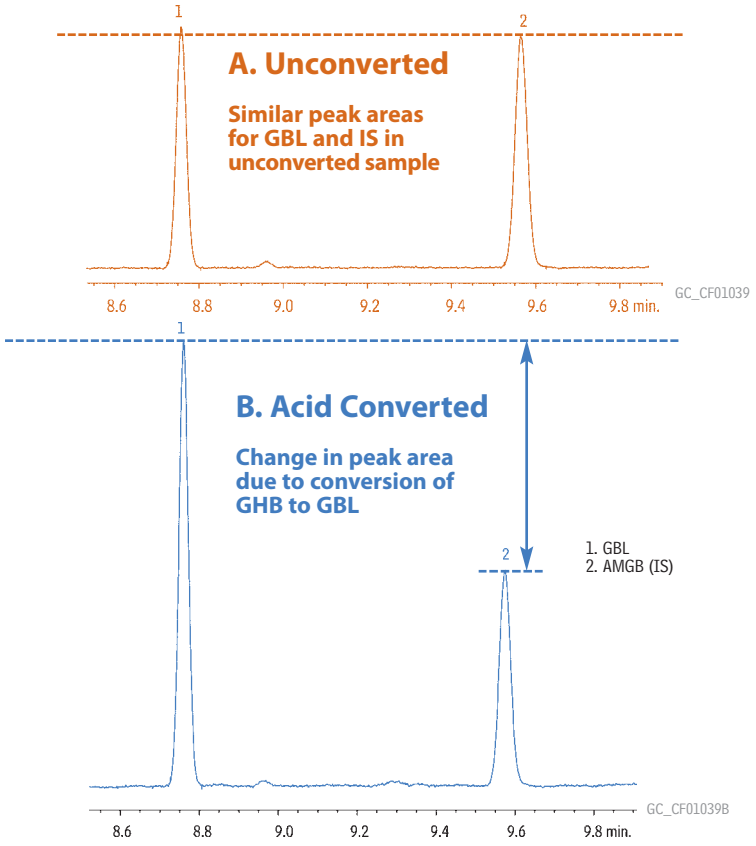
Reliably Screen Samples Using Existing Blood Alcohol Testing Set-Up

Headspace injections (using the total vaporization technique) of the final urine extracts were screened by GC/FID using an Rtx®-BAC1 column in a blood alcohol headspace GC system. This system is com-

Table I Extraction procedure for GHB and GBL.

1. Label two screw top test tubes per specimen. One for total GHB, the other for GBL only.
2. Add 1mL of sample (urine) to each tube.
3. Add 50µL of AMGB to each tube.
4. Add 150µL concentrated sulfuric acid only to tubes used for analysis of total GHB.
5. Vortex all tubes and allow them to sit 5 minutes.
6. Add 5mL methylene chloride to each tube. Shake 10 minutes to extract.
7. Centrifuge samples at 3,000 rpm for 5 minutes.
8. Transfer bottom (methylene chloride) layer to a clean test tube for drying.
9. Concentrate samples to ~100µL at 30°C under nitrogen.
10. For headspace analysis, inject 15µL of sample into a capped headspace vial. Or, for liquid injection, transfer extract to a limited volume insert.

Figure 1 GHB can be converted to GBL for analysis, saving time by eliminating derivatization.



Column:	Rtx®-BAC1, 30m, 0.32mm ID, 1.8µm (cat.# 18003)	Headspace conditions:	Equilibration temp.: 100°C Equilibration time: 10 min. Injection volume: 1mL
Sample:	50µg/mL GHB, GBL, and AMGB (IS) in urine A: unconverted B: converted with sulfuric acid	Oven temp.:	50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.)
Inj.:	1mL headspace injection, split (10:1), 1mm split inlet liner (cat.# 20972)	Det.:	FID @ 240°C
Inj. temp.:	200°C	Hydrogen:	40mL/min.
Carrier gas:	helium, constant flow	Air:	400mL/min.
Flow rate:	1.0mL/min.	Makeup:	40mL/min.

Sample: urine spiked with 50µg/mL each GHB, GBL, and AMGB (IS), extracted according to procedure in Table I, and analyzed using headspace (total vaporization technique).

This versatile extraction and headspace method improves lab efficiency and reduces both contamination and matrix effects by eliminating the need for derivatization and liquid injections.

Figure 2 GHB (analyzed as GBL) confirmation method calibration curve for headspace GC/MS analysis (10-300µg/mL in urine).

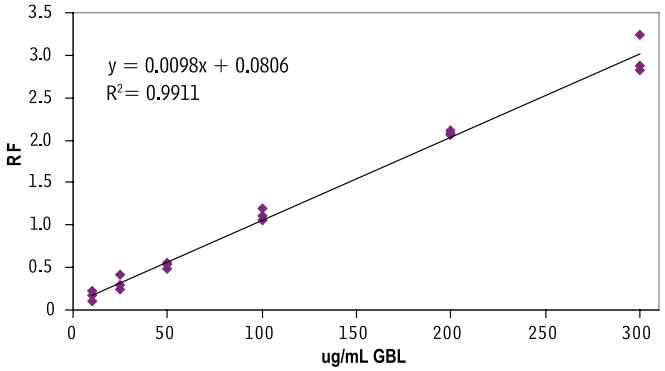
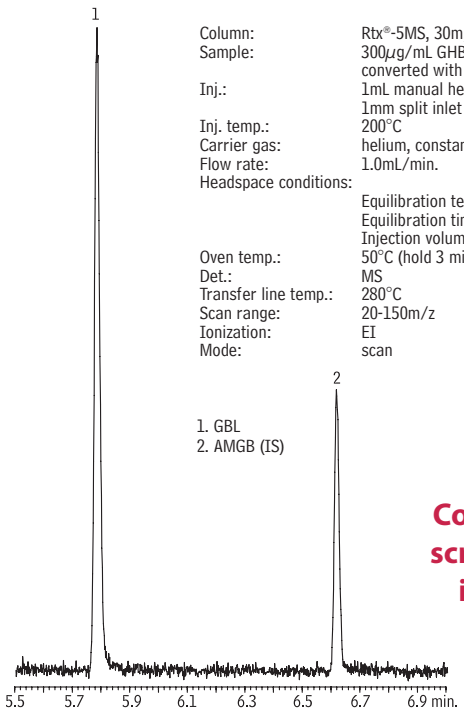


Figure 3 Confirmation headspace GC/MS analysis of 300µg/mL converted GHB (analyzed as GBL) standard in urine.



Column:	Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample:	300µg/mL GHB, 50µg/mL AMGB in urine, converted with sulfuric acid
Inj.:	1mL manual headspace injection, split (10:1), 1mm split inlet liner (cat.# 20972)
Inj. temp.:	200°C
Carrier gas:	helium, constant flow
Flow rate:	1.0mL/min.
Headspace conditions:	Equilibration temp.: 100°C Equilibration time: 10 min. Injection volume: 1mL
Oven temp.:	50°C (hold 3 min.) to 150°C @ 20°C/min. (hold 7 min.)
Det.:	MS
Transfer line temp.:	280°C
Scan range:	20-150m/z
Ionization:	EI
Mode:	scan

Confirm positive screening results in 7 minutes!

monly used in clinical/forensic labs, eliminating the need for additional equipment. Excellent linear response was obtained from both unconverted (r^2 = 0.9992, 10-100µg/mL 4-point curve) and converted GHB in matrix (r^2 = 0.9910, 20-200µg/mL 4-point curve) with AMBG at 50µg/mL.

Fast, Definitive Confirmation Analysis by Headspace GC/MS

Positive screening results were quickly confirmed on an Rtx®-5MS column by headspace GC/MS; several quantification and qualifier ions were identified for each compound (GBL: 42, 56, 86; AMBG: 40, 68, 98). Again, excellent linearity was achieved (Figure 2) and analysis time was less than 7 minutes (Figure 3).

In summary, the versatile extraction and headspace method shown here saves lab time and minimizes contamination by eliminating the need for derivatization and by reducing matrix effects. Rapid screening is accomplished on commonly used blood alcohol GC columns, allowing labs to reduce costs by using existing equipment. Confirmation testing using the Rtx®-5MS column, provides the definitive results needed in court with a fast analysis time of less than 7 minutes.

References
1. M.A. LeBeau, M.A. Montgomery, M.L Miller, S. G. Burmeister, J. Anal. Toxicol. 24 (2000) 421.

Product Listing

Rtx®-BAC1 Columns (fused silica)

ID	df (µm)	temp. limits	length	cat. #	price
0.32mm	1.80	-20 to 240/260°C	30-Meter	18003	

Rtx®-5MS—Low-bleed GC/MS Columns (fused silica)

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

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623	

Exempted Drug of Abuse Reference Materials

Concentration is µg/mL. Volume is 1mL/ampul.

Compound	CAS#	Solvent Code	Conc.	cat.#	price
GHB					
γ-butyrolactone (GBL)	96-48-0	ACN	1,000	34077	
α-methylene-γ-butyrolactone (AMGBL)	547-65-9	ACN	1,000	34079	

ACN=acetonitrile

1mm Split Liners for Agilent GCs

ID* x OD & Length	qty.	cat.#	price
1mm Split 1.0mm x 6.3mm x 78.5mm	ea.	20972	

Beyond C18—Increase Retention of Hydrophilic Compounds Using Biphenyl Columns

*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.*

By Amanda Rigdon, Pharmaceutical Innovations Chemist and Rick Lake, Pharmaceutical Market Development Manager

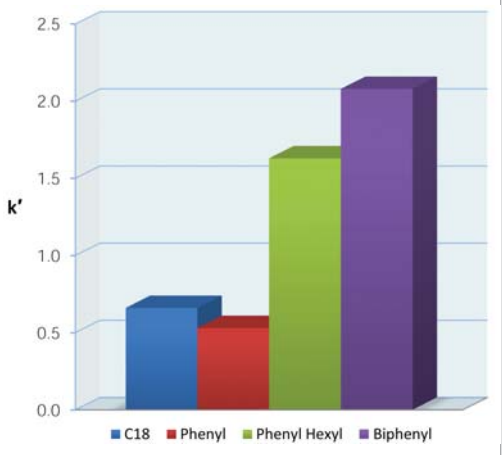
Many drug classes include compounds with aromatic ring structures, some of which also contain a sulfone or sulfoxide group. Both sulfur groups have dipole moments, adding a hydrophilic character to compounds containing these functional groups. The analysis of hydrophilic compounds on a traditional alkyl column (e.g., C18) can be problematic, since alkyl columns depend on hydrophobic (dispersive) interactions for retention. Since the sulfone and sulfoxide groups contain π 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.

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 π-π interactions. Although the phenyl stationary phase also allows for the use of π-π 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).

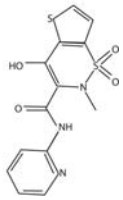
The improved retention for hydrophilic aromatics shown here is due to the unique π-π interaction retention mechanism of the Biphenyl phase. This mechanism is particularly useful for analysis of sulfone- and sulfoxide-containing drug compounds, which are not easily retained on alkyl or phenyl phases. 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.

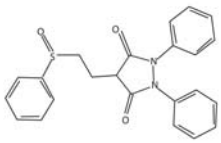


Biphenyl columns are much more effective than alkyl, phenyl, or phenyl hexyl phases when increased retention of hydrophilic aromatics is desired.

Figure 2 Only the Biphenyl phase retains both test probes to k' > 2, the level recommended to ensure separation from unretained matrix contaminants.



2. tenoxicam



3. sulfinpyrazone

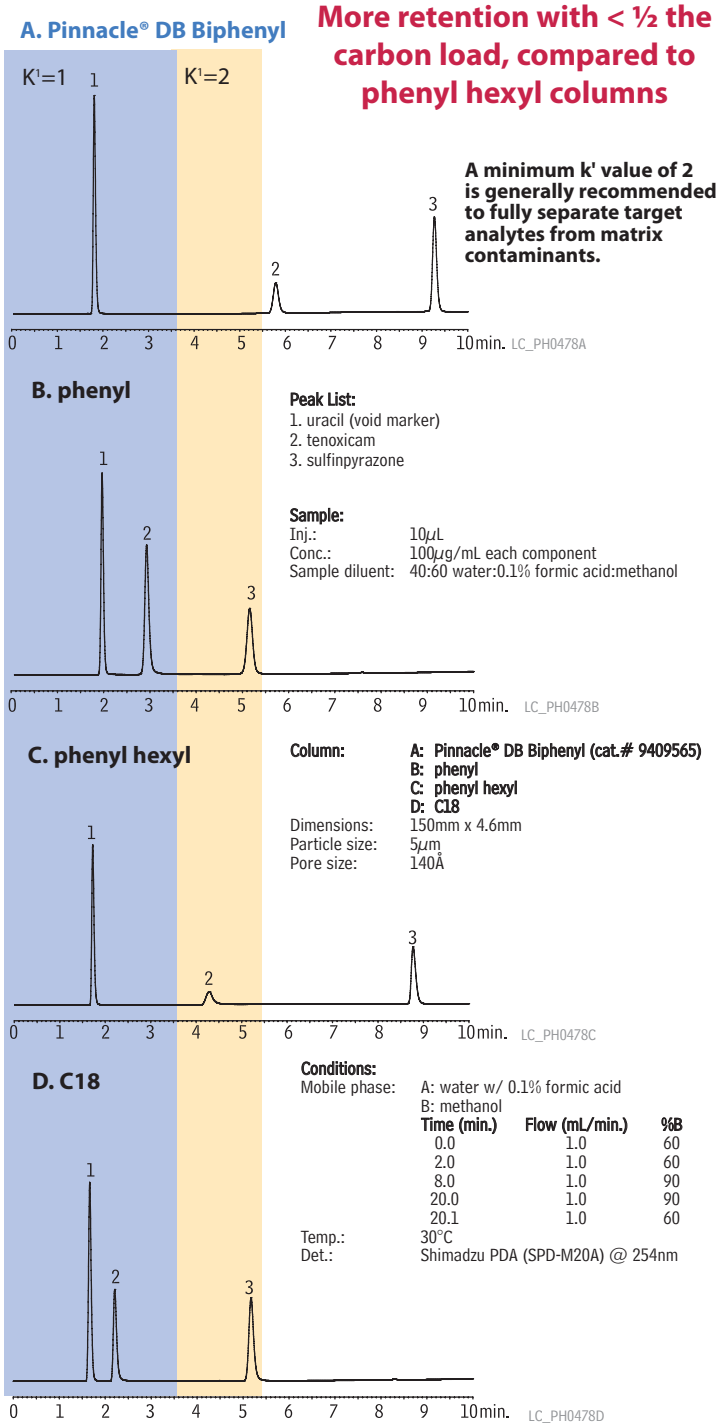


Table I Biphenyl columns show improved retention of sulfone- and sulfoxide-containing aromatic drugs.

K' Value	Biphenyl	Phenyl hexyl	Phenyl	C18
Tenoxicam	2.23	1.39	0.637	0.235
Sulfinpyrazone	4.18	3.90	1.88	1.89

Product Listing

Pinnacle® DB Biphenyl Columns (USP L11)

particle size: 1.9µm, 3µm or 5µm, spherical
pore size: 140Å
carbon load: 8%
endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

3µm Column, 1.0mm	cat. #	price
30mm	9409331	
50mm	9409351	
100mm	9409311	
150mm	9409361	
3µm Column, 2.1mm	**cat. #**	**price**
30mm	9409332	
50mm	9409352	
100mm	9409312	
150mm	9409362	
3µm Column, 3.2mm	**cat. #**	**price**
30mm	9409333	
50mm	9409353	
100mm	9409313	
150mm	9409363	
3µm Column, 4.6mm	**cat. #**	**price**
30mm	9409335	
50mm	9409355	
100mm	9409315	
150mm	9409365	
5µm Column, 1.0mm	**cat. #**	**price**
30mm	9409531	
50mm	9409551	
100mm	9409511	
150mm	9409561	
200mm	9409521	
250mm	9409571	
5µm Column, 2.1mm	**cat. #**	**price**
30mm	9409532	
50mm	9409552	
100mm	9409512	
150mm	9409562	
200mm	9409522	
250mm	9409572	
5µm Column, 3.2mm	**cat. #**	**price**
30mm	9409533	
50mm	9409553	
100mm	9409513	
150mm	9409563	
200mm	9409523	
250mm	9409573	
5µm Column, 4.6mm	**cat. #**	**price**
30mm	9409535	
50mm	9409555	
100mm	9409515	
150mm	9409565	
200mm	9409525	
250mm	9409575	

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Two Options for Analyzing Potential Genotoxic Impurities in Active Pharmaceutical Ingredients

*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 both sulfonate esters and alkyl halides on the **Rtx®-200 column**.*

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.

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 in API. Some of these compounds are potentially genotoxic impurities (PGIs) and may raise concern about cancer and/or birth defects. Because of the toxicity of these compounds, it is essential that they be controlled to low levels in API after synthesis. In January of 2007, the European Medicines Agency (EMEA) released guidance on acceptable limits of PGIs in APIs (Guideline on the Limits of Genotoxic Impurities (EMEA/CHMP/QWP/251344/2006)). Developing new methods for sensitive detection of impurities is an increasingly active area of research across the pharmaceutical industry.

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

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

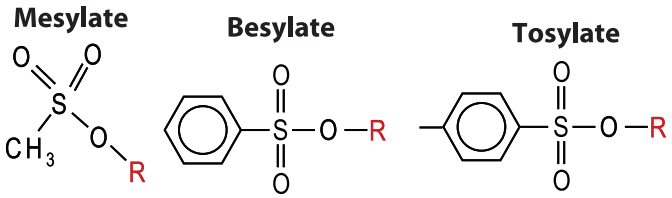
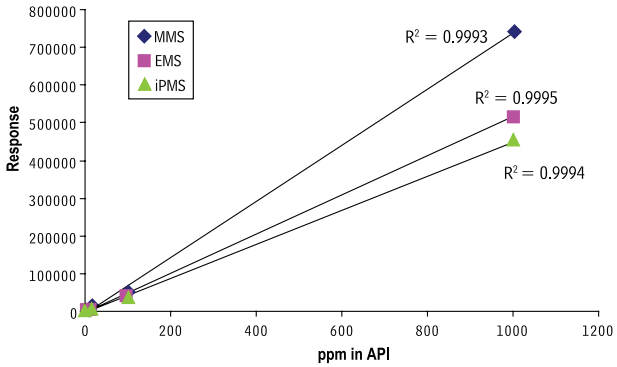


Figure 2 Linearity of fast GC/MS analysis for selected sulfonate esters.



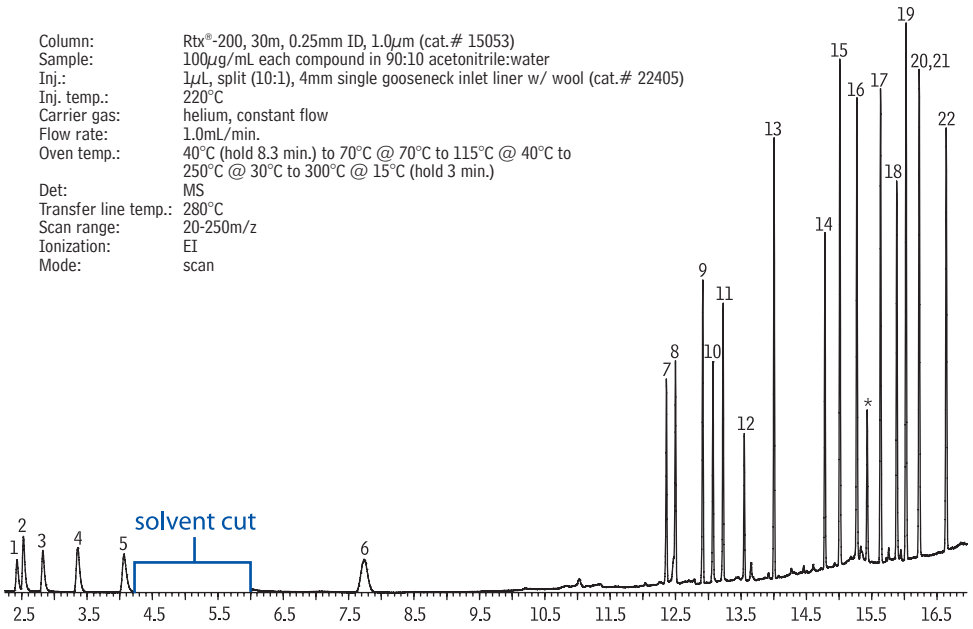
Four structural classes of PGIs are discussed in this article. The first three classes, known collectively as sulfonate esters, include mesylates, besylates, and tosylates (Figure 1). These alkylating sulfonic acid esters may form when sulfonic acid reacts with an alcohol solvent. The first three classes 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.

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. The use of a thin film Rxi®-5Sil MS column allows for speedy analysis of these active 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. A linearity study performed by Merck shows that this method is linear for sample concentrations from 1ppm to 1,000ppm in API (Figure 2). Depending on the dose of API to the patient, it may be necessary to detect levels of impurities as low as 1 ppm in order to meet EMEA requirements. The 1ppm spike represents the threshold for toxicological concern (TTC) as set by the EMEA for PGIs.

Figure 3 Small, polar alkyl halides are well-retained on the fluorinated Rtx®-200 column, as are less volatile sulfonate esters.



Option 2: Comprehensive PGI Method

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 a 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 3). 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 (Figure 4). 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.

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.

Product Listing

Rtx®-200 Columns (fused silica)

(Crossbond® trifluoropropylmethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.20	-20 to 310/330°C	20-Meter	45002	
0.25mm	1.00	-20 to 290/310°C	30-Meter	15053	

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.18	-60 to 330/350°C	20-Meter	43602	

Reduce Downtime with Robust Lipidomics Method

*Why lose days to downtime? Restek columns, such as the 10,000 injection **Rxi®-5ms** column shown here, are rugged and built for consistent long-term performance.*

By Julie Kowalski, Ph.D., Innovations Chemist, and John Hanley Jr.*, Ph.D., Platform Development Manager
*Lipomics Technologies

Lipidomic studies of cholesterol synthesis, absorption, and excretion, provide information central to the investigation of cardiovascular disease and other disorders. High-throughput methods are critical to lipidomics and are used to screen thousands of samples in order to identify biomarkers and clinical diagnostics with significant predictive power. Labs can save days of downtime by using an Rxi®-5ms column in assays similar to our test method for cholesterol and low-level sterol metabolites.

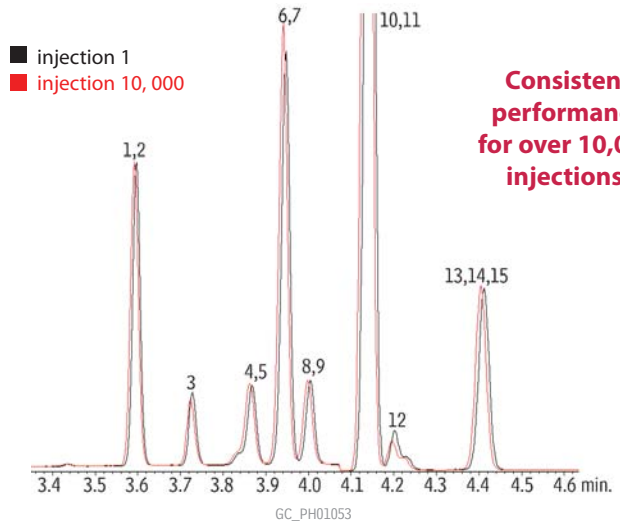
Here, extremely reproducible results were obtained using an Rxi®-5ms column, which gave highly consistent separations—even after 10,000 injections (Figure 1). In our method, biological samples were treated to form trimethylsilyl derivatives. Two injections were made: one to quantify minor sterols using a 10:1 split, and another, using a 100:1 split, to analyze cholesterol. To achieve maximum sensitivity for low-level sterols, multiple SIM retention time windows were set up.

Reduced downtime for column changes and revalidation significantly increased lab productivity.

Stable retention times are critical to our testing program as revalidation is required if significant drift occurs. Revalidation requires days of downtime because inter-day variability must be assessed. The Rxi®-5ms column was chosen for this method, in part, because its long lifetime and stable performance reduced the number of column changes and revalidations, resulting in more days of productive analyses.

We found the performance of the Rxi®-5ms column to be remarkably consistent and reliable for high-throughput testing. The Rxi®-5ms column should be considered by labs running similar lipidomic methods that would benefit from a highly reproducible performance—or by any lab interested in reducing downtime and increasing productivity.

Figure 1 Stable, highly reproducible results on the Rxi®-5ms column mean less downtime and more productive days.



Compound:	m/z
1. coprostanol	370.40
2. d5-cholestanol (IS)	220.20
3. 7α-hydroxycholesterol	456.40
4. cholestanol	306.30
5. d5-epicholestanol (IS)	220.20
6. 7-dehydrocholesterol	325.25
7. desmosterol	343.25
8. lathosterol	458.35
9. d4-lathosterol (IS)	462.40
10. campesterol	382.35
11. 4-cholestenone	382.30
12. stigmasterol	394.15
13. lanosterol	393.35
14. β-sitosterol	396.35
15. d7-β-sitosterol (IS)	403.45

Column:	Rxi®-5ms, 30m, 0.25mm ID, 0.25µm (cat.# 13423)
Sample:	lipid plasma extract as trimethylsilyl derivatives
Inj.:	1µL, split (10:1), 4mm gooseneck inlet liner w/wool
Inj. temp.:	310°C
Carrier gas:	helium, constant flow
Flow rate:	1.0mL/min.
Oven temp.:	250°C (hold 1 min.) to 320°C @ 30°C/min. (hold 1.6 min.)
Det:	MS
Transfer line temp.:	330°C
Ionization:	EI
Mode:	SIM

Product Listing

Rxi®-5ms Columns (fused silica)

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

ID	df (µm)	temp. limits	length	cat. #	price
0.25mm	0.25	-60 to 330/350°C	30-Meter	13423	

Editorial

Achieving Faster GC

Continued from page 2

separation (and another disappointed user is born!). Please also bear in mind that the above options will reduce all baseline segments in your chromatogram to the same extent. So, if you have over-resolution throughout your chromatogram except for one critical peak pair that is just barely resolved, forget about these options. In general however, all of the above options are low-risk options that could be tried before moving on to the more elaborate steps discussed below.

Now that you have eliminated all the empty parts of the baseline you can move to step 2, maximizing the selectivity of the system. Selectivity is the ability to distinguish between compounds. This can be done through the separation or through detection (once the method for sample preparation has been selected). Options for improving selectivity include:

- using a more selective stationary phase or coupled columns.
- using conventional 2-dimensional or comprehensive 2-dimensional GC.
- using selective detection, with mass spectrometry (MS) being particularly attractive.
- backflushing.

Because the above options are all rather expensive and require special instruments and expertise, the only really widely used option is the use of MS detection. Indeed MS is a marvellous way to get selectivity in an easy and quick way.

You have now gone through the two initial steps of speeding up your method. You have selected a system that offers you the required resolution, yet not more resolution than really needed. If the analysis time in this "minimum acceptable resolution" situation still exceeds the desired or permitted time, options that reduce the analysis time at constant resolution should be exploited. Possibilities include:

- reducing the column inner diameter.
- using hydrogen as the carrier gas.
- appling vacuum-outlet conditions.
- using turbulent flow conditions.

Of these options the first two always work; however, vacuum operation only works if you have a separation on a short wide-bore column, and turbulent flow operation in practice is of little use.

Mea culpa, with more than 20 papers published on fast GC, I have also contributed to the chaos in faster GC. I hope the above discussion helps resolve at least part of the confusion. Faster GC is possible, it is always possible, and the need for it is actually still increasing as a result of recent trends in process control and high-throughput experimenting.

1. P. Korytár, H.-G. Janssen, E. Matisová, U.A.Th. Brinkman, Trends in Analytical Chemistry 21 (2002) 558-572.

Hans-Gerd Janssen received his Ph.D. in analytical chemistry from Eindhoven University in 1991. After having worked at Eindhoven as an associate professor for eight years, he joined Unilever Research to work as the group leader for chromatography and mass spectrometry. In 2004, Hans-Gerd was appointed part-time professor at the University of Amsterdam, focusing on biomacromolecular separations.

Restek On-the-Road

Tradeshow Schedule

October, 2008

Show: **2008 NIH Research Festival Exhibit**
Date: Oct. 16-17
Location: National Institutes of Health, Bethesda, MD

Show: **Society of Forensic Toxicologists (SOFT)**
Date: Oct. 27-31
Location: Arizona Grand Hotel, Phoenix, AZ

Show: **COLACRO XII**
Date: Oct. 28-30
Location: Florianopolis Convention Center, Florianopolis, Brazi

November, 2008

Show: **2008 AAPS Annual Meeting & Expo**
Date: Nov. 16-20
Location: Georgia World Congress Center, Atlanta, GA

Show: **Eastern Analytical Symposium (EAS)**
Date: Nov. 17-20
Location: Garden State Convention Center, Somerset, NJ

Show: **Symposium on Air Quality Methods & Technology**
Date: Nov. 3-6
Location: Chapel Hill, NC

Show: **LC/MS Montreux Symposium**
Date: Nov. 12-14
Location: Montreux Convention Center, Montreux, Switzerland

January, 2009

Show: Gulf Coast Conference
Date: Jan. 20-21
Location: Moody Gardens Convention Center, Galveston, TX

Seminar Schedule

Date	Cat. #	City	State
Petrochemical Seminar			
10/27	65746	Corpus Christi	TX
10/29	65747	Houston	TX
10/31	65748	Oklahoma City	OK
Comprehensive HPLC			
11/3	65749	Seattle	WA
11/5	65750	San Francisco	CA
11/7	65751	San Jose	CA

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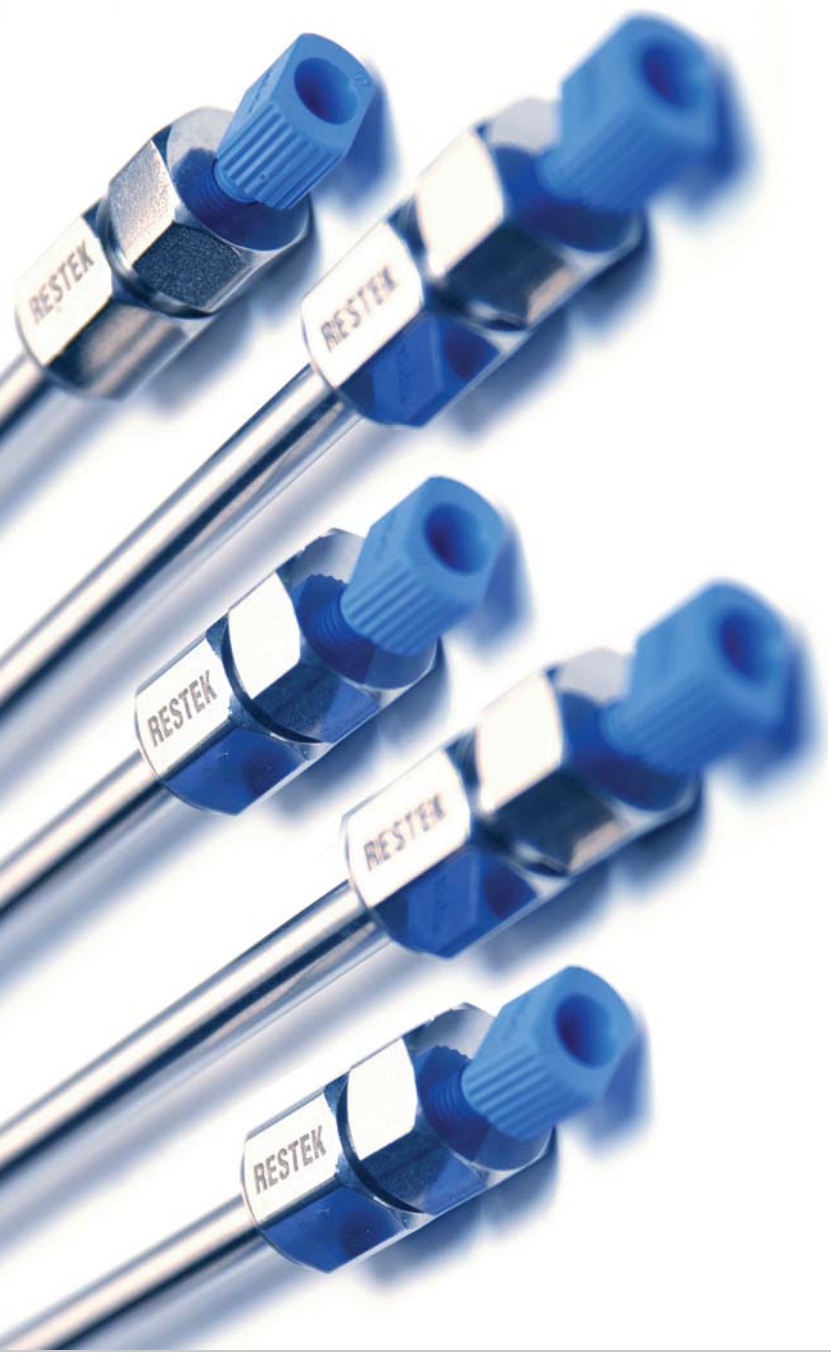

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- Optimized for UHPLC, fully scalable to HPLC

Pinnacle® II Biphenyl
- Restek manufactured silica
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Allure® Biphenyl
- High purity, high surface area silica
- Excellent choice for maximum retention and LC/MS

Ultra Biphenyl
- High purity, midrange surface area silica
- The workhorse—reliable performance for a broad range of applications

Viva Wide Pore Biphenyl
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