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2007.02

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1 (of 24 ) 2007.2

## the Restek Advantage

2007.02

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# Retention Cross-over Phenomenon in Gas Chromatography—Can the Mystery be Revealed? Part 1

By Werner Engewald, Ph.D., Professor Emeritus, University of Leipzig, Institute of Analytical Chemistry, Leipzig, Germany; engewald@uni-leipzig.de



Have you ever faced changes in elution order after modifying the column temperature or the heating rate in the temperature program of the GC analysis of complex samples? This so-called cross-over phenomenon, which can lead to problems in peak identification, has been a well-known mystery in GC for decades.<sup>1</sup> But, so far, the physico-chemical background is still not well understood.

The cross-over phenomenon is very common when separating compounds with different functional groups on polar stationary phases. For example, we observed a reversal in the elution order for components like linalool and camphor on a polyethylene glycol column (Carbowax 20M) after changing the column temperature programming rate: at 5°C/min. linalool elutes before camphor but at 3°C/min. camphor will elute first. Effects like this are often observed when essential oils are analyzed or, to be more precise, when the GC methods are optimized. The reversal of the elution order is mainly explained as a result of the different temperature-dependencies of the intermolecular interactions, which are responsible for the retention: London-type dispersion forces and induction forces are independent of temperature, whereas the orientation forces and hydrogen bridge bonds depend strongly on the temperature (Figure 1).

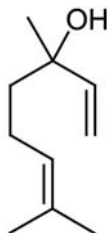
However, this explanation is only half the truth and we should examine the influence of column temperature on retention in some more detail. It is generally known that the column temperature is one of the two most important variables in GC (the other being of course the nature of the stationary phase). In partition GC, the effect of temperature on the solute partition coefficient  $K$  is given by the van't Hoff relationship  $\ln K = HS/RT_c + C$  (with  $HS$  being the molar heat of solution of solute). From this follows the fundamental correlation between column temperature  $T_c$  and retention factors:

$$\ln k' = HS/RT_c + C' - \ln \beta$$

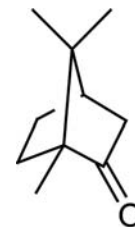
where  $k'$  is the retention or capacity factor ( $k' = t_R/t_M$ ) and  $\beta$  the column phase ratio. This equation indicates that the retention decreases logarithmically as the column temperature increases.

**Figure 1** Functional groups influence elution order.

Linalool (bp.: 199°C)



Camphor (bp.: 209°C)



Continued on page 23.



# Explaining the Small Particle Advantage

## Faster Sample Throughput on a 1.9 $\mu$ m Pinnacle™ DB HPLC Column

By Rick Lake, Pharmaceutical Innovations Chemist, Randy Romesberg, HPLC Innovations Chemist, and Becky Wittrig, Ph.D., HPLC Product Marketing Manager

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

Restek is pleased to introduce an exciting new addition to our family of HPLC columns—the 1.9 $\mu$ m Pinnacle™ DB small particle column. Intended for use in ultra-high pressure liquid separations, the 1.9 $\mu$ m Pinnacle™ DB column combines the benefits of a popular technique with the unmatched quality you expect from Restek. From the manufacturing of the base silica through the packing of the column, Restek performs and tightly controls every step in the manufacturing process, guaranteeing ruggedness and reliability. Here we discuss how and why small particle HPLC columns provide faster separations, and demonstrate the high efficiency, excellent peak symmetry, and rapid analysis times that can be achieved on the 1.9 $\mu$ m Pinnacle™ DB column. *Continued on page 4.*

## Explaining the Small Particle Advantage *(continued from page 3)*

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

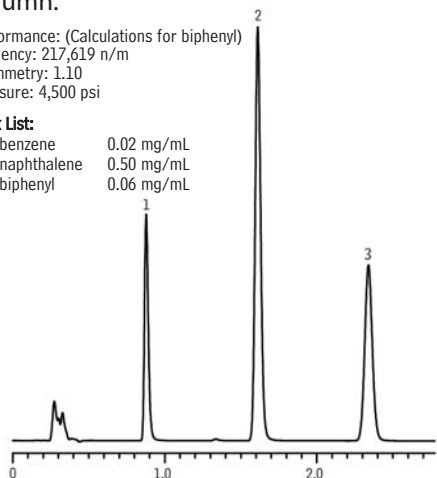
To demonstrate the substantial gain in sample throughput that is possible on a small particle column, we assayed a series of parabens under conditions that give comparable linear velocities on both a C18 column with conventional dimensions and on a 1.9µm Pinnacle™ DB C18 column (Figure 2B & C). Similar resolution was achieved in a much shorter analysis time on the 1.9µm Pinnacle™ DB C18 column. We also doubled the flow rate on the 1.9µm Pinnacle™ DB C18 column: the resolution and peak efficiencies again were comparable, but the analysis time was cut in half (Figure 2A). This illustrates the considerable effect that small particles can have on chromatographic separations; a much wider range of usable flow rates translates into significantly faster analysis times—in this case 10-fold faster, with no loss in chromatographic quality.

**Figure 1** Excellent peak symmetry and efficiency on a 1.9µm Pinnacle™ DB C18 column.

Performance: (Calculations for biphenyl)  
Efficiency: 217,619 n/m  
Asymmetry: 1.10  
Pressure: 4,500 psi

**Peak List:**

1. benzene 0.02 mg/mL
2. naphthalene 0.50 mg/mL
3. biphenyl 0.06 mg/mL



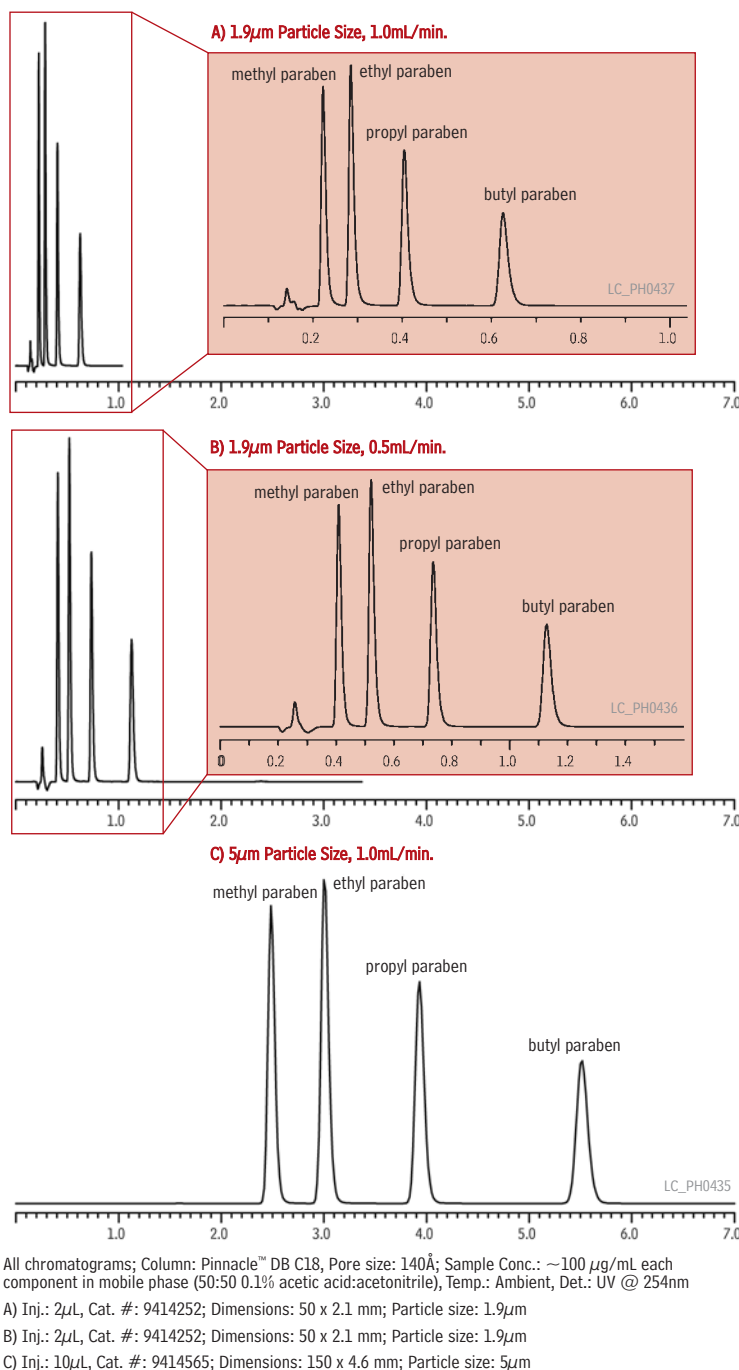
Sample: Inj.: 2µL, HPLC Reversed Phase Test Mix #1 (cat.# 35005),  
Sample diluent: water:methanol (25:75), Sample temp.: ambient,  
Column: Pinnacle™ DB C18, Cat. #: 9414252, Dimensions: 50 x 2.1mm,  
Particle size: 1.9µm, Pore size: 140Å, Mobile phase: water:acetonitrile  
(45:55), Flow: 0.4 mL/min., Temp.: 25°C, Det.: UV @ 254nm LC\_EX0427

**Table 1** 1.9µm Pinnacle™ DB C18 column offers the highest efficiency of all columns tested.

Column	Efficiency (n/m)	Pressure (psi)	Asymmetry
1.9µm Pinnacle™ DB	217,619	4,500	1.10
Competitor A	177,999	4,400	1.13
Competitor B	188,508	4,300	1.09

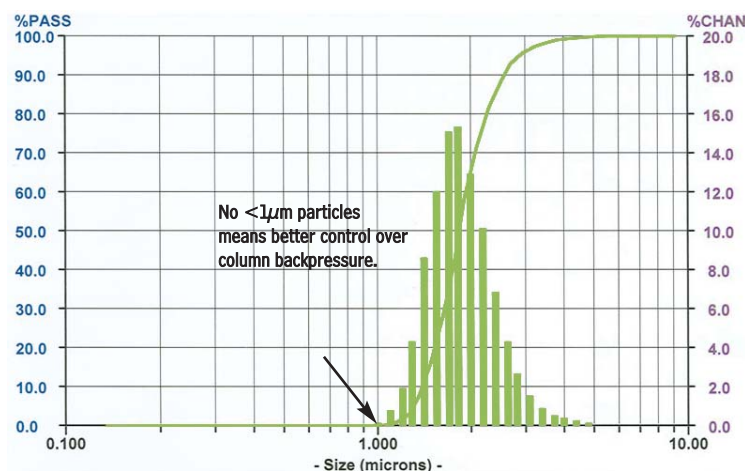
Data from the biphenyl peak of a reversed phase test mix.

**Figure 2** 1.9µm Pinnacle™ DB columns offer a wider range of usable flow rates, dramatically increasing sample throughput—with no loss in resolution.





**Figure 3** Pinnacle™ DB silica particle size distribution shows an exceptionally tight, symmetrical distribution around 1.9µm.



Column	Target Particle Size (µm)	Actual Mean Particle Size (µm)	Standard Deviation	Particles present <1µm
1.9µm Pinnacle™ DB	1.9	1.952	0.437	No
Competitor A	1.7	1.993	0.529	No
Competitor B	1.8	1.832	0.468	Yes

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

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

### 1.9µm Pinnacle™ DB C18 HPLC Columns

#### Physical Characteristics:

particle size: 1.9µm  
pore size: 140Å  
carbon load: 11%

endcap: yes  
pH range: 2.5 - 10  
temperature limit: 80°C

#### 1.9µm Column, 2.1mm

	cat. #
30mm	9414232
50mm	9414252
100mm	9414212

## More Small Particles

For more information on the theory behind small particles, please refer to the complete article, “Explaining the Small Particle Advantage,” at [www.restek.com/pharmaceutical](http://www.restek.com/pharmaceutical)



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# Revised USP 467 Residual Solvent Method

## Satisfy New Method Requirements with Restek Columns and Standards

By Rick Lake, Pharmaceutical Innovations Chemist

- Overview of the new USP 30/NF 25 procedure.
- New reference standards - stock mixes, custom preparations.
- Optimize your testing within the constraints of the method.

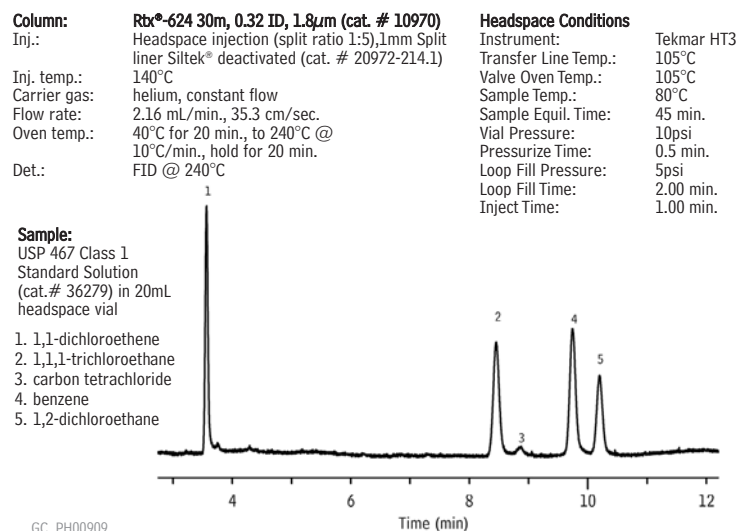
Organic volatile impurities (OVIs), commonly referred to as residual solvents, are trace level chemical residues in drug substances and drug products that are byproducts of manufacturing, or that form during packaging and storage. The United States Pharmacopeia recently revised the general chapter on residual solvent analysis, USP 467, to mirror the International Conference on Harmonization (ICH) guidelines. This revision, effective July 1, 2007, replaces previous methods that were not consistent with the ICH guidelines.

The revised procedure consists of a static headspace extraction coupled with a gas chromatographic separation and flame ionization detection (GC/FID), and is divided into two sections based on sample solubility – water soluble and water insoluble articles. Altogether, the test method consists of three separate procedures – A, B and C – that are designed to identify, confirm and quantify residual solvents in pharmaceuticals.

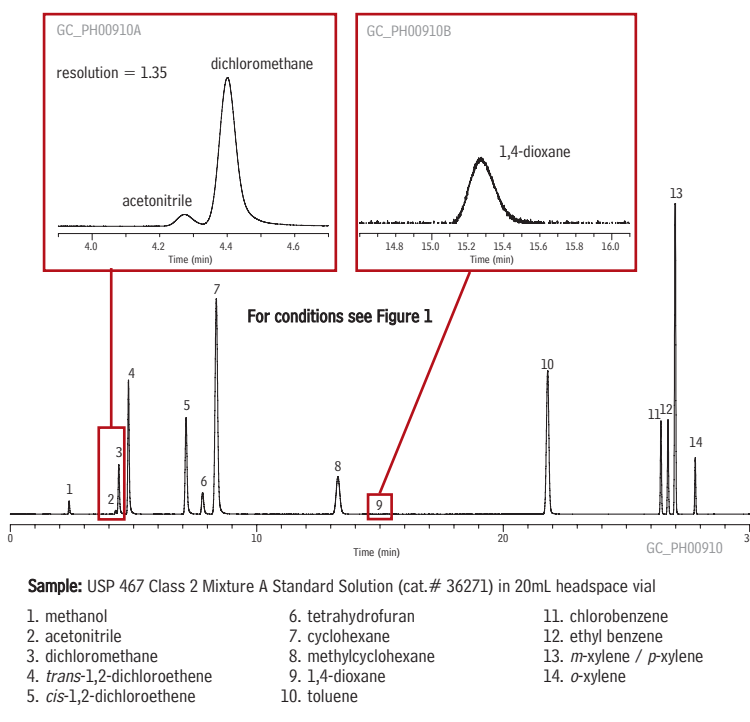
Procedure A is the first step in the identification process and is performed to screen samples for residual solvents. A series of residual solvent mixes, consisting of Class 1 and Class 2 mixes A and B, are analyzed along with the system suitability and test solutions on an Rtx®-624 column – equivalent to an Rtx®-1301 (G43) column (Figures 1-3). If a peak in the sample matches a retention time, and exceeds the response of the corresponding standard, the analyst proceeds to Procedure B for verification of the analyte.

Once a residual solvent is identified, Procedure B is performed to confirm analyte identity. We recommend a Stabilwax® (G16) capillary column as a confirmation column because it yields an alternate selectivity compared to an Rtx®-624 column or an Rtx®-1301 (G43) column. (See our OVI retention time index at [www.restek.com/ovi](http://www.restek.com/ovi)). The same reference mixes are analyzed with an acetonitrile/trichloroethylene system suitability solution. If a residual solvent is verified, Procedure C is used to quantify the analyte by comparison to a specific, individual standard for the analyte identified. For water-insoluble articles, the procedure is the same, except dimethylformamide and 1,3-dimethyl-2-imidazolidinone are used as the diluent and Class 2 Mix C (higher boiling point solvents mix) is analyzed as a reference solution.

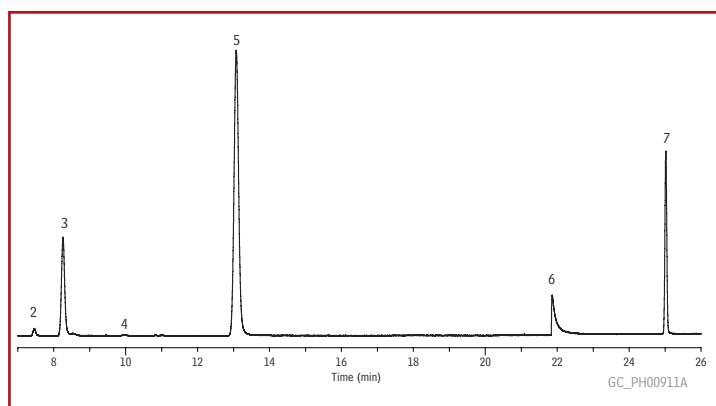
**Figure 1** USP Residual Solvent Class 1 standard solution on an Rtx®-624 (G43) column.



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



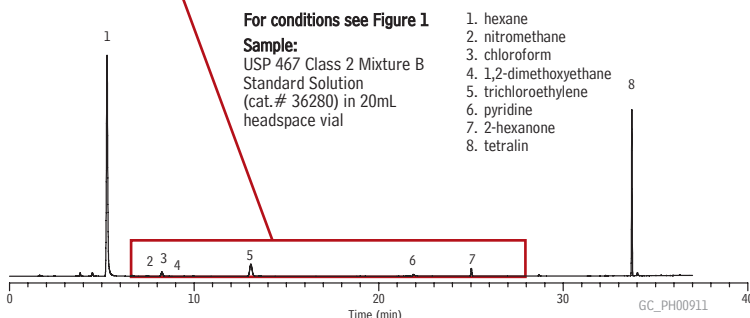
**Figure 3** USP Residual Solvent Class 2 Mixture B standard solution on an Rtx®-624 (G43) column.



For conditions see Figure 1

**Sample:**  
USP 467 Class 2 Mixture B  
Standard Solution  
(cat.# 36280) in 20mL  
headspace vial

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



Restek can supply all your USP 467 materials and can help you optimize your testing within the constraints of the method. Visit us on the web at [www.restek.com](http://www.restek.com) or contact our Technical Support team at 800-356-1688, ext.4, for solutions to your residual solvent testing needs and tips on optimizing your analysis.

#### Residual Solvents - Class 1

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

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

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

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

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

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

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

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

## did you know?

Restek offers a full day seminar on headspace analysis. Join us September 26, in Edison, NJ for a day of learning focused exclusively on headspace principles, techniques, and applications (cat.# 65563). To register, visit us online at [www.restek.com/seminar](http://www.restek.com/seminar)

#### Rtx®-624 (G43) Columns (fused silica)

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

ID	df (µm)	temp. limits	length	cat. #
0.32mm	1.80	-20 to 240°C	30-Meter	10970
0.53mm	3.00	-20 to 240°C	30-Meter	10971

#### Stabilwax® (G16) Columns (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	40 to 250°C	30-Meter	10624
0.53mm	0.25	40 to 250°C	30-Meter	10625

#### Siltek® 1mm Split Liners for Agilent GCs

Use this liner for increased sensitivity. Exclusive Siltek® deactivation makes liner inert to active sample components.

Benefits/Uses:	ID*/OD & Length (mm)	cat.# ea.	cat.# 5-pk.
for purge & trap inlet splitting or sample <1µL	1.0 ID 6.3 OD x78.5	20972-214.1	20973-214.5

\*Nominal ID at syringe needle expulsion point.

## for more info

- Technical poster:  
*Comprehensive Dual-Column Analysis of Residual Solvents in Water-soluble Articles Using Dynamic Headspace and Modular Accelerated Column Heating.*  
[www.restek.com/usp467](http://www.restek.com/usp467)
- A Technical Guide for Static Headspace Analysis Using GC, cat.# 59895A.
- OVI retention time index  
[www.restek.com/ovi](http://www.restek.com/ovi)

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We can supply all your residual solvent reference materials—For details, see our catalog or visit us online at [www.restek.com/standards](http://www.restek.com/standards).





## GC Inlet Liner Deactivations for Basic Drug Analysis

By Kristi Sellers, Clinical/Forensic Innovations Chemist, and Lydia Nolan, Innovations Chemist

- Base-deactivated inlet liners are inert to basic drugs, for greater responses.
- Inertness of Rtx®-5 Amine column is enhanced for basic compounds.
- Use this liner / column combination for the lowest %RSDs for basic drugs.

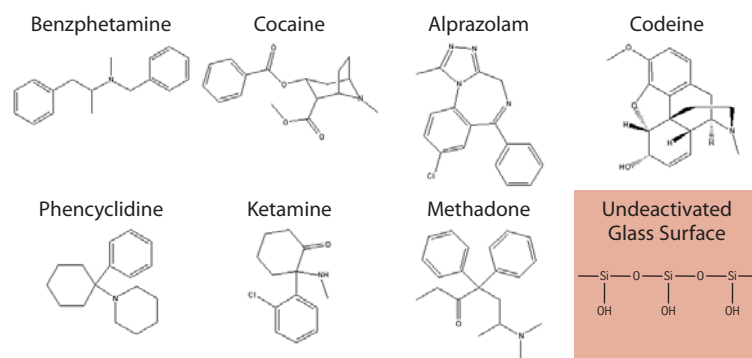
Clinical and forensic toxicologists are required to detect low levels of abused drugs in body fluids and confirm their presence by GC/MS. Typical limits of detection are 1-15ng/mL, depending on the sample matrix. For basic drugs (e.g., Figure 1), selecting the proper surface treatment for the GC inlet liner is important, because this parameter can affect responses. The surface of a glass inlet liner contains active silanol groups (Si-OH) that can act as electron pair acceptors, and react with nitrogen or oxygen electron pair donors in basic drug molecules (Figure 2).<sup>1</sup> These reactions usually are rapid and reversible, but they are expressed chromatographically as broad, tailing peaks and/or reduced responses. To eliminate these acid-base reactions, make chromatographic peaks sharp, Gaussian, and easy to integrate, and thereby help ensure reproducible and accurate responses, the -OH groups on the glass surface must be deactivated.

We evaluated several alternatives for deactivating inlet liners to determine the best deactivation chemistry for the analysis of basic drugs. Standards composed of the free base forms of the drugs shown in Figure 1 were prepared at concentrations of 5, 10, 25, 50, and 100 ng/mL for analysis on a 15m, 0.25mm ID, 0.25µm Rtx®-5 Amine column (5% diphenyl/95% dimethyl polysiloxane stationary phase). The analysis of these drug standards was repeated on a series of 4mm ID single gooseneck liners that had been treated with different deactivation techniques, as well as an untreated liner. Three replicate analyses were performed on each liner to determine which deactivation treatment offered the highest and most consistent response for these basic drugs.

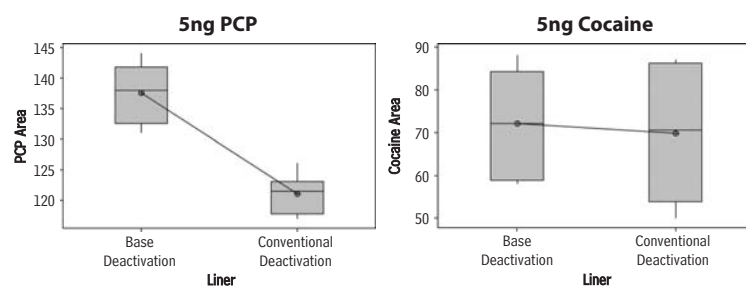
We used these results to generate box plots that display the range of data distribution, or variation – an indication of the reproducibility of the performance. We chose phencyclidine (PCP) and cocaine plots to represent the nitrogen-containing and nitrogen/oxygen-containing drugs, respectively (Figure 2). The line in each box indicates the mean response.

The data show that undeactivated liners and liners that received intermediate polarity treatment provided poorer responses or reproducibility, com-

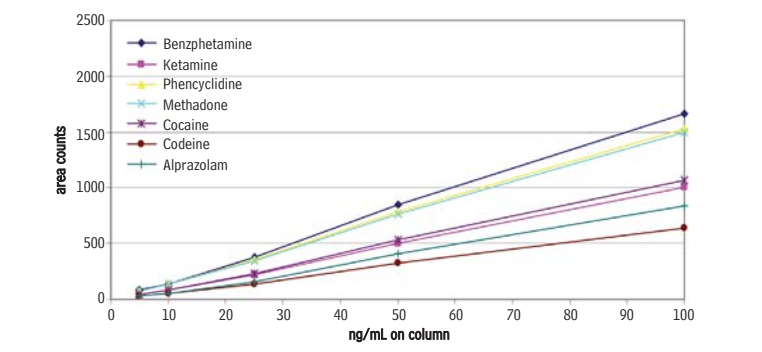
**Figure 1** Basic compounds can react with silanol groups on glass liner surfaces, causing poor chromatography.



**Figure 2** A base-deactivated inlet liner provides highest mean responses for PCP.



**Figure 3** Linearity plots for all drugs, analyzed using a base-deactivated inlet liner and an Rtx®-5 Amine column.





## Base Deactivated Inlet Liners for Basic Drug Analysis

For Agilent GCs	ea.	cat.# 5-pk.	25-pk.
Gooseneck Splitless, Base Deactivated (4.0mm ID* x 6.5mm OD x 78.5mm)	20798-210.1	20799-210.5	20800-210.25
Gooseneck Splitless, Base Deactivated w/ Base Deactivated Wool (4.0mm ID* x 6.5mm OD x 78.5mm)	20798-211.1	20799-211.5	20800-211.25
Split Straight, Base Deactivated w/ Base Deactivated Wool (4.0mm ID* x 6.3mm OD x 78.5mm)	20781-211.1	20782-211.5	20783-211.25
Cyclosplitter®, Base Deactivated (4.0mm ID* x 6.3mm OD x 78.5mm)	20706-210.1	20707-210.5	20708-210.25

\*Nominal ID at syringe needle expulsion point.

For liners for other instruments, refer to our catalog or website.

## Base-Deactivated Inlet Liners

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

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

## Base-Deactivated Wool

Ideal for amines and other basic compounds.

Description	qty.	cat.#
Base-Deactivated Wool	10 grams	20999

## Mini Wool Puller/Inserter

Insert and remove wool plugs easily.

Description	qty.	cat.#
Mini Wool Puller/Inserter	2-pk.	20114

## Inlet Liner Removal Tool

- Easily remove liner from injector—no more burned fingers.
- Made from high-temperature silicone.
- Won't chip or crack the liner.

Description	qty.	cat.#
Inlet Liner Removal Tool	3-pk.	20181

## Rtx®-5 Amine Columns (fused silica)

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

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 300/315°C	15-Meter	12320
0.25mm	0.25	-60 to 300/315°C	30-Meter	12323



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pared to base-deactivated or Siltek® treated liners, due to the acidic nature of the undeactivated glass surface or to a small but influential number of residual acidic sites remaining on the intermediate polarity deactivated surface.

Because the undeactivated liners and intermediate polarity treated liners exhibited either low mean response or high variation, we reanalyzed the data, excluding these treatments and comparing the remaining data (for base-deactivated liners and Siltek® treated liners) for responses and reproducibility. As shown by the examples in Figure 2, base-deactivated liners and Siltek® treated liners performed equally well for cocaine, but the base-deactivated liners yielded the best responses and reproducibility for PCP. Ultimately, a base-deactivated liner would give the best overall performance. Figure 3 shows the linearity plots for all analyzed drugs, obtained using a base-deactivated liner and an Rtx®-5Amine column. Low %RSD values for ketamine (3%), phencyclidine (2%), methadone (2%), cocaine (3%), codeine (5%), and alprazolam (12%) confirm the reproducibility of data obtained from this combination.

Because nitrogen- and oxygen-containing drugs react with silanol groups on glass surfaces, it is important to use properly deactivated glass inlet liners when analyzing these compounds by GC. This work demonstrates that a base-deactivated inlet liner, used in combination with a base-deactivated column, produces high and reproducible responses for basic drugs.

### Reference

1. Seyhan N. and D.C. Ege, *Organic Chemistry Health and Company*, 1984, pp.124-136.

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## Simple, Reliable HPLC Analyses of Organic Acids

### Using Water-Compatible Allure® or Ultra C18 Columns

Julie Kowalski, Ph.D., Innovations Chemist, and Becky Wittrig, Ph.D., HPLC Product Marketing Manager

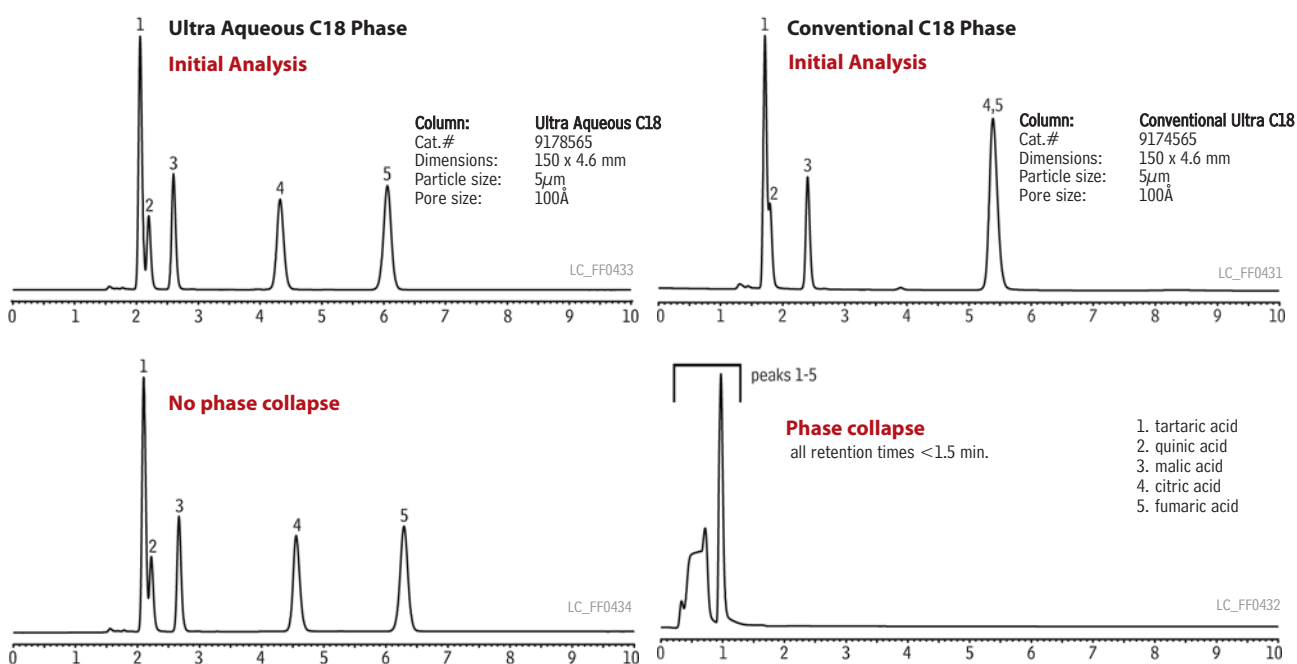
- Use 100% aqueous mobile phases without losing retention.
- Simple, isocratic method.
- Complete resolution of critical fruit juice organic acids, including quinic and tartaric acids.

Organic acids are common components in foods and beverages, and play a critical role in product characteristics like taste and aroma. They can be tested for in many food products including fruits, cheeses, and various beverages such as juices and wines. Organic acids can originate in the foods themselves (e.g. cranberries) or can be produced by food processing (e.g. alcoholic fermentation). A method that allows resolution of organic acids, as well as their quantification, can help determine product quality and authenticity.

Reversed phase HPLC coupled with UV-Vis detection is a popular technique for organic acid analysis. One common method, AOAC method 986.13, stipulates reversed phase HPLC using two C18 stationary phase columns in series. Because organic acids are low in molecular weight, and have polar functionalities, 100% aqueous buffer is needed for adequate retention. A low pH buffer is used to ensure that the organic acids remain protonated or neutral, thus allowing the best interaction between the organic acids and the C18 stationary phase. However, using a 100% aqueous mobile phase can cause the C18 chain in conventional C18 columns to collapse. Phase collapse results in loss of retention, and the column must be flushed with organic mobile phase, a time consuming step, to restore chain structure and column performance.

Three Restek columns – the Ultra Aqueous C18 column, the Allure® Aqueous C18 column, and the Allure® Organic Acids column – use aqueous-compatible C18 phases that do not exhibit phase collapse, even with 100% aqueous mobile phases. The advantage of using these columns is demonstrated in Figure 1 by the fast analysis of organic acids on a Shimadzu Prominence 20A system. Here, we compared the ability of the Ultra Aqueous C18 phase and a conventional C18 phase to withstand phase collapse. Figures 1A and 1B show that the Ultra Aqueous C18 phase resolves organic acids in a 100% aqueous mobile phase without loss of retention. In comparison, the conventional C18 phase shown in Figure 1C and 1D suffers a complete loss of retention following phase collapse when used under the same conditions. Thus, in an analysis that requires, or is improved by, a mobile phase with a high aqueous content, an Ultra Aqueous C18 column is the superior choice.

**Figure 1** Restek's water-compatible C18 phase does not collapse in a 100% aqueous mobile phase, compared to a conventional C18 column which shows a complete loss of retention.



**Instrument:** Shimadzu Prominence 20A

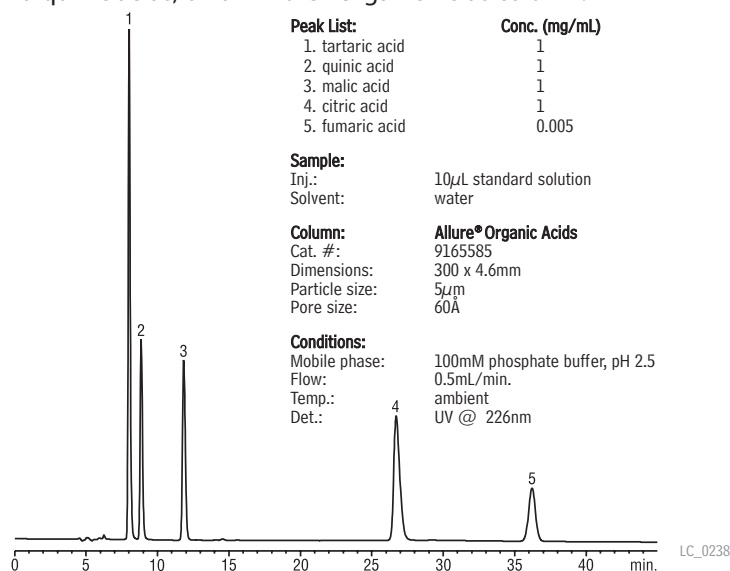
**Sample:** Inj.: 10µL; Conc.: 2000µg/mL each component except fumaric acid (10µg/mL) (Organic Acids Reference Mixture cat.# 35080); Sample diluent: deionized water

**Conditions:** Mobile phase: 20mM potassium phosphate (pH 2.5); Flow: 1.0mL/min.; Temp.: 30°C; Det.: UV @ 226nm

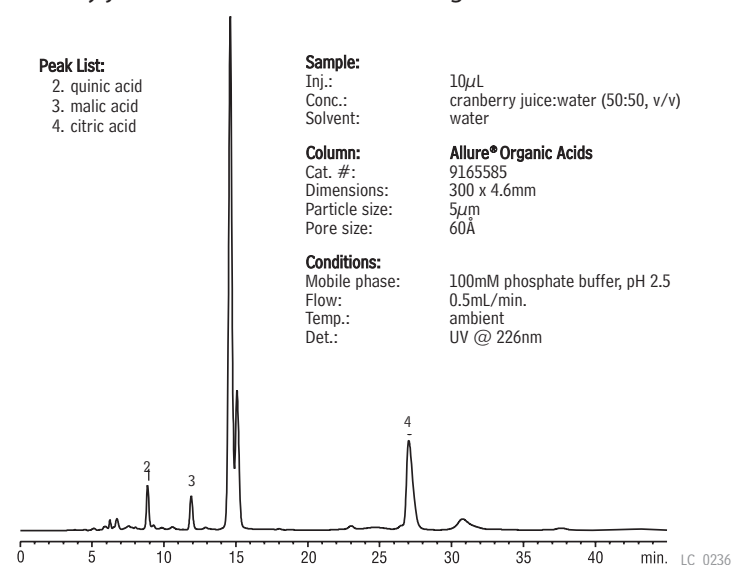
Phase collapse caused for experimental purposes by releasing column pressure



**Figure 2** Excellent resolution of organic acids, including tartaric and quinic acids, on an Allure® Organic Acids column.



**Figure 3** Sharp, easily differentiated organic acid profiles for cranberry juice cocktail on an Allure® Organic Acids column.



In analyses of organic acids, specifically, under high aqueous mobile phase conditions, the Allure® Organic Acids column is the column of choice. We have developed a method using a 300mm Allure® Organic Acids column to separate critical organic acids: tartaric, quinic, malic, citric and fumaric acids. This method calls for 100% aqueous mobile phase as recommended by AOAC method 986.13. The Allure® Organic Acids column is tested specifically for resolving critical organic acids. Figure 2 shows that tartaric and quinic acids are resolved to baseline; Figure 3 shows typical analyses under the conditions we recommend.

#### References

1. [http://www.restek.com/advantage/adv\\_2003\\_03\\_02a.pdf](http://www.restek.com/advantage/adv_2003_03_02a.pdf).
2. Official Methods of Analysis (2000). AOAC International, 17th edition, method # 986.13.
3. Manolaki, P. et al., Food Chemistry, 98 (2006), page 658-663.
4. Kafkas, E. et al., Food Chemistry, 97 (2006), page 732-736.

#### Fruit Juice Organic Acid Standard

citric acid	2000µg/ml	quinic acid	2000
fumaric acid	10*	tartaric acid	2000
malic acid	2000		
In water, 1mL/ampul			
cat. # 35080 (ea.)			
In water, 5mL/ampul			
cat. # 35081 (ea.)			

\*Fumaric acid is a trace impurity in malic acid, as well as an added component of the mix. The amount of fumaric acid in malic acid will not affect the stated concentration of malic acid, but can represent a significant and variable deviation from the low concentration of fumaric acid stated to be in the mix. All other components of the mix are at the specified concentration.

#### Allure® Organic Acids Column

5µm Column, 4.6mm	cat. #
150mm	9165565

#### Allure® Aqueous C18 Column

5µm Column, 4.6mm	cat. #
150mm	9168565

#### Ultra Aqueous C18 Column (USP L1)

5µm Column, 4.6mm	cat. #
150mm	9178565

#### for more info

For more information on our Allure® Aqueous C18, Ultra Aqueous C18 and Allure® Organic Acid columns, visit us online at [www.restek.com](http://www.restek.com).

#### ordering note

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

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# Separate Explosives and Propellant Residues

## Using Ultra C18 and Pinnacle™ II Biphenyl Columns

by Robert Freeman, Environmental Innovations Chemist

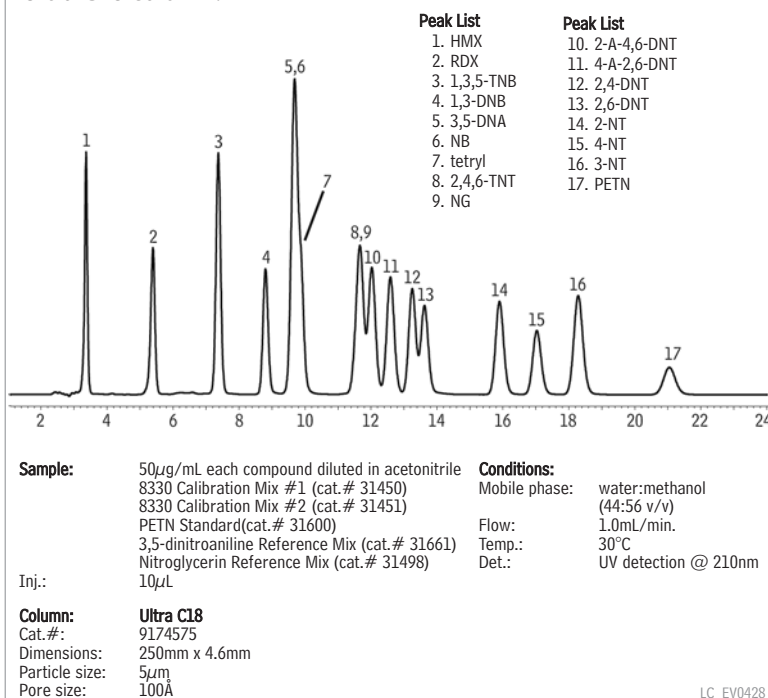
- Easily quantify and confirm new US EPA Method 8330B target analytes.
- Excellent resolution, improved accuracy.
- Simple, easy to use, isocratic method.

US EPA 8330, a test method for determining trace amounts of 14 nitramines and nitrate esters, was recently revised to include three new target analytes. The new method, EPA 8330B, includes nitroglycerin (NG), pentaerythritol tetranitrate, (PETN), and 3,5-dinitroaniline (3,5-DNA) and now covers 17 analytes that are commonly found in explosives and propellants residues. This method uses reversed phase HPLC and dual wavelength UV detection (210 & 254nm) in conjunction with a primary and a confirmation column.

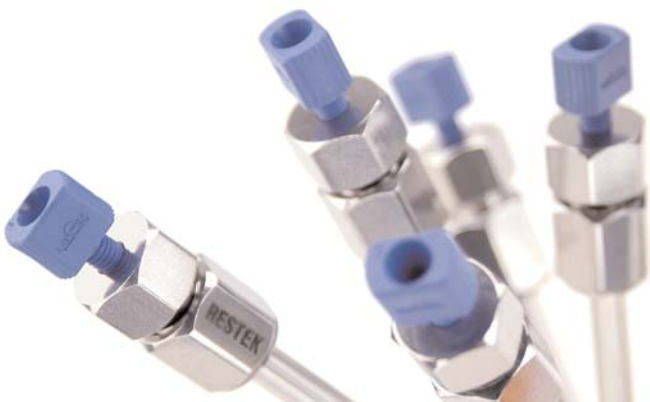
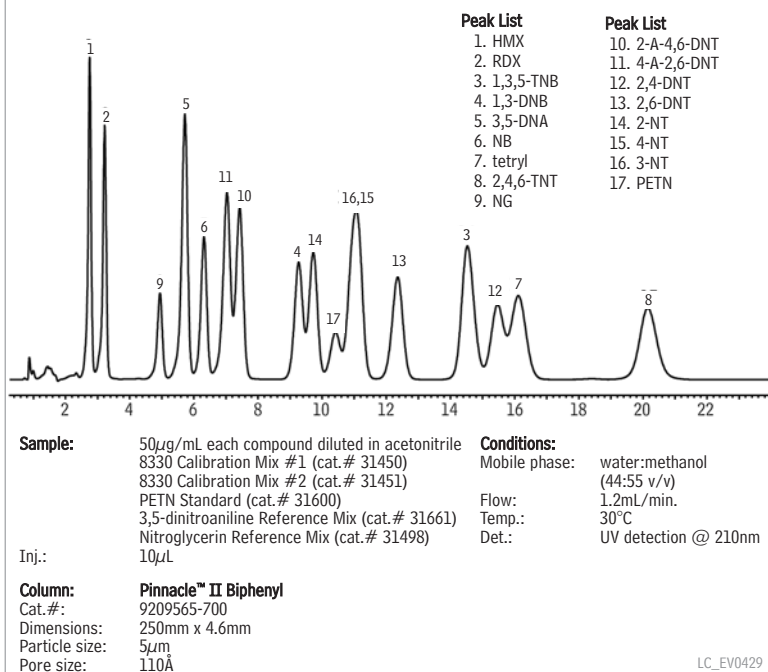
We recently assessed the performance of our current column offerings relative to the elution order and retention times of the new analytes in the revised method. Separations on all columns were accomplished using a simple, isocratic water:methanol mobile phase (Table 1). The primary and confirmation columns that we recommend for the EPA 8330 analysis are the Ultra C18 and Pinnacle™ II Biphenyl columns, respectively. Based on this work, we conclude this combination will work well for the revised method, EPA 8330B, as shown by the chromatograms in Figures 1 and 2. Both columns provide excellent resolution of the EPA 8330B analytes and their differing selectivity provides a true confirmation analysis.

As an alternative to the Ultra C18/Pinnacle™ II Biphenyl combination, a Pinnacle™ II C18 column and a Pinnacle™ II Cyano column work well together as a primary-confirmation column set. Another column of interest is the Allure® Biphenyl column. A high organic mobile phase was required on this column but the analysis was completed in approximately six minutes (Table 1).

**Figure 1** Excellent resolution of EPA 8330B target analytes on the Ultra C18 column.



**Figure 2** Alternate selectivity of EPA 8330B analytes on the Pinnacle™ II Biphenyl column confirms compound identity.





**Table 1** Retention times for EPA 8330B analytes on various Restek columns.

- new target analytes are shown in red
- highlighted cells indicate coelution.

H <sub>2</sub> O:MeOH	Primary Columns		Confirmation Columns		
	50:50	44:56	45:55	50:50	20:80
Flow	1.5 mL/min	1.0 mL/min	1.2 mL/min	1.5 mL/min	1.5 mL/min
Analytes	Pinnacle™ II C18	Ultra C18	Pinnacle™ II Biphenyl	Pinnacle™ II Cyano	Allure® Biphenyl
HMX	2.29	3.38	2.76	18.65	1.61
RDX	3.63	5.41	3.22	9.38	1.75
1,3,5-TNB	4.89	7.39	14.54	4.78	5.69
1,3-DNB	5.94	8.82	9.26	4.59	3.92
3,5-DNA	6.63	9.71	5.73	6.34	2.30
tetryl	6.97	9.71	16.12	11.47	4.42
NB	6.97	9.88	6.31	3.80	2.79
2,4,6-TNT	8.23	11.69	20.17	5.94	6.22
NG	8.23	11.69	4.94	8.52	1.98
2-A-4,6-DNT	8.94	12.05	7.43	7.24	2.50
4-A-2,6-DNT	8.94	12.61	7.02	6.34	2.41
2,6-DNT	9.73	13.27	12.36	5.10	4.09
2,4-DNT	9.73	13.64	15.46	5.58	5.14
2-NT	11.92	15.92	9.73	4.38	3.40
4-NT	12.76	17.05	11.07	4.38	3.72
3-NT	13.74	18.32	11.07	4.38	3.73
PETN	16.13	21.08	10.43	17.24	2.67

**Ultra C18 Column (USP L1)**

5µm Column, 4.6mm	cat. #
250mm	9174575
250mm (with Trident™ Inlet Fitting)	9174575-700

**Pinnacle™ II Biphenyl Column (USP L11)**

5µm Column, 4.6mm	cat. #
150mm	9209565
150mm (with Trident™ Inlet Fitting)	9209565-700

**ordering note**

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

**8330 Calibration Mix #1** (7 components)

1,3-dinitrobenzene	RDX
2,4-dinitrotoluene	1,3,5-trinitrobenzene
HMX	2,4,6-trinitrotoluene
nitrobenzene	
1,000µg/mL each in acetonitrile, 1mL/ampul	
	cat. # 31450 (ea.)

**8330 Calibration Mix #2** (7 components)

2-amino-4,6-dinitrotoluene	3-nitrotoluene
4-amino-2,6-dinitrotoluene	4-nitrotoluene
2,6-dinitrotoluene	tetryl
2-nitrotoluene	
1,000µg/mL each in acetonitrile, 1mL/ampul	
	cat. # 31451 (ea.)

**Single-Component Explosives Reference Mixes**

Volume is 1mL/ampul unless otherwise noted. Concentration is µg/mL unless otherwise noted.

3,5-dinitroaniline	ACN	1,000	31661
nitroglycerin	M	1,000	31498
PETN (pentaerythritol tetranitrate)	M	1,000	31600

ACN = acetonitrile

M = methanol

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# Fast, Accurate FAMES Analyses of Biodiesel Fuel

Using a Stabilwax® Capillary GC Column

By Barry L. Burger, Petroleum Innovations Chemist

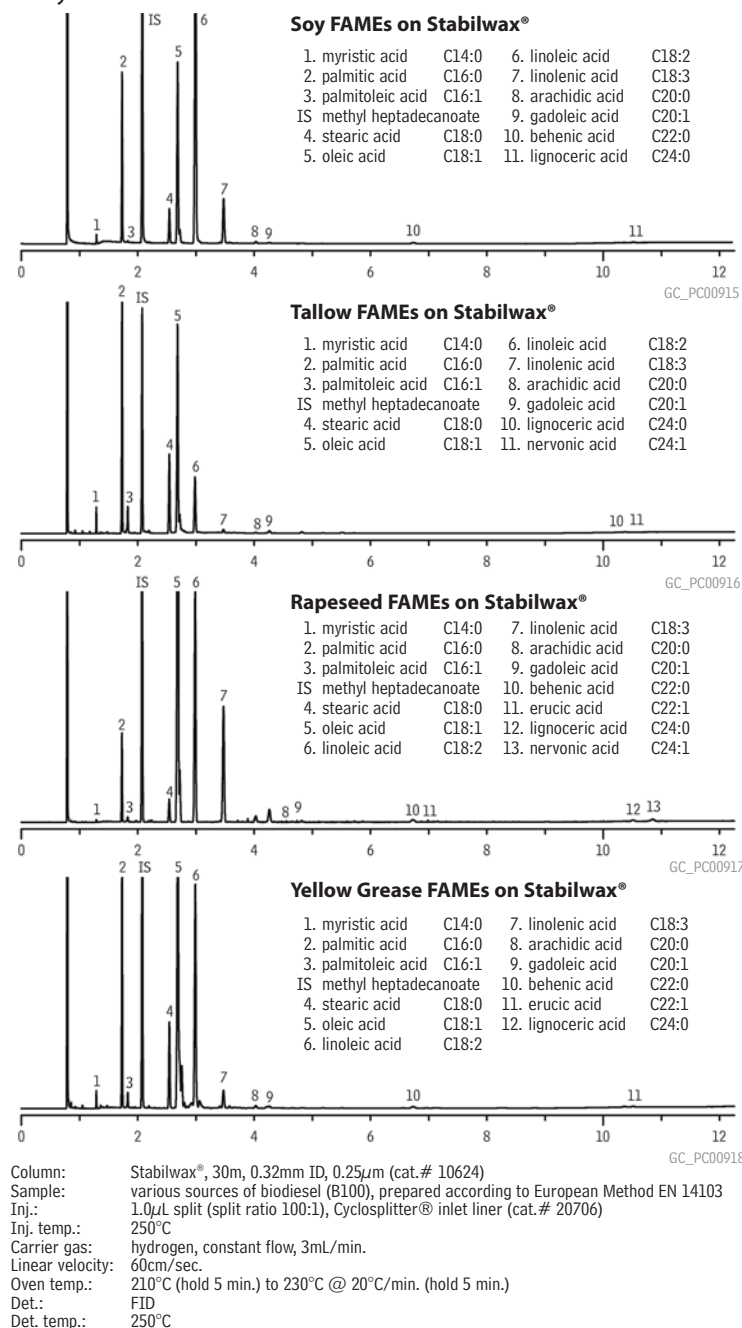
- Stable baselines, excellent peak symmetry, baseline resolution of all compounds.
- Analysis complete in less than 11 minutes using hydrogen.
- All RSD% values less than 1%.

A Stabilwax® fused silica GC column affords excellent peak symmetry, resolution, and reproducibility for determining the fatty acid methyl ester (FAME) and linolenic acid methyl ester content in B100 biodiesel fuel, using European standard method EN 14103. The chromatograms and quantified data shown here were generated from four different sources of biodiesel fuel, and meet or exceed the method criteria.

As biodiesel fuel continues to stimulate interest worldwide as an energy source, several gas chromatographic methods have been developed to determine the quality of B100 fuel. European standard method EN 14103 is used for determining the FAME and linolenic acid methyl ester content, European standard method EN 14105 and ASTM standard method D-6584-00e1 are used for determining free and total glycerin, and European standard method EN 14110 is used for determining residual methanol. Method EN 14103 permits the analyst to assure the B100 product is greater than 90% fatty acid methyl esters (m/m) and the linolenic acid content is between 1% and 15% (m/m). The analysis is appropriate for FAME compositions between C14:0 and C24:1.

In evaluating the suitability of the Stabilwax® column for quantifying FAMES and linolenic acid methyl ester by method EN 14103, we prepared reference standards from each of the four B100 fuel sources – soy, tallow, rapeseed, and yellow grease (Table 1) – by weighing 250mg of the source material into a 10mL vial, then adding 5mL of a 10mg/mL solution of internal standard methyl heptadecanoate. (Avoid allowing the samples to stand longer than 12 hours, or quantification will be inaccurate.) We installed the 30m x 0.32mm ID x 0.25µm Stabilwax® column (cat.# 10624) in an Agilent 6890 instrument equipped with a split/splitless injector, a flame ionization detector, and ChemStation software. To obtain the fastest analysis, without sacrificing resolution, we selected hydrogen as the carrier gas, supplied from a Parker Balston hydrogen generator.

**Figure 1** Stable baselines, excellent peak symmetry, and rapid, baseline resolution of all compounds characterize FAMES analyses on a Stabilwax® column.





**Table 1** Sources of FAMES in B100 biodiesel fuel (% m/m).

		Soy	Tallow	Rapeseed	Yellow Grease
Myristic acid	C14:0	0.21	1.7	0.11	0.68
Palmitic acid	C16:0	11.24	25.5	4.1	16.35
Palmitoleic acid	C16:1	0.2	3.27	0.27	1.23
Stearic acid	C18:0	4.04	14.41	1.8	9.32
Oleic acid	C18:1	21.93	40.34	58.57	47.8
Linoleic acid	C18:2	53.84	12.02	22.2	20.01
Linolenic acid	C18:3	7.29	0.99	13.26	2.93
Arachidic acid	C20:0	0.36	0.4	0.79	0.46
Gadoleic acid	C20:1	0.26	1.03	1.79	0.39
Behenic acid	C22:0	0.45		0.57	0.44
Erucic acid	C22:1			0.13	0.23
Lignoceric acid	C24:0	0.16	0.34	0.3	0.24
Nervonic acid	C24:1		0.17	0.54	

**Table 2** Relative standard deviations for FAMES do not exceed 1% in analyses on a Stabilwax® column (n = 3).

		Soy	Tallow	Rapeseed	Yellow Grease
Myristic acid	C14:0	0.33	0.42	0.24	0.36
Palmitic acid	C16:0	0.04	0.06	0.02	0.04
Palmitoleic acid	C16:1	0.23	0.17	0.19	0.09
Stearic acid	C18:0	0.05	0.02	0.13	0.19
Oleic acid	C18:1	0.02	0.3	0.2	0.25
Linoleic acid	C18:2	0.25	0.41	0.11	0.22
Linolenic acid	C18:3	0.13	0.16	0.07	0.14
Arachidic acid	C20:0	0.3	0.37	0.23	0.31
Gadoleic acid	C20:1	0.33	0.28	0.37	0.41
Behenic acid	C22:0	0.28		0.29	0.17
Erucic acid	C22:1			0.21	0.26
Lignoceric acid	C24:0	0.53	0.14	0.1	0.33
Nervonic acid	C24:1		0.55	0.83	

**Parker Hydrogen Generators**

- Selectable delivery pressure: 0–100psig.
- High hydrogen purity—99.9995%—for better chromatography.
- No high-pressure cylinders—greater convenience and improved lab safety.

Description	Capacity	qty.	cat.#
Hydrogen Generator A9090	90cc/min.	ea.	22033
Hydrogen Generator A9090 with European Power Cord	90cc/min.	ea.	22033-551
Hydrogen Generator A9150	160cc/min.	ea.	22034
Hydrogen Generator A9150 with United Kingdom Power Cord	160cc/min.	ea.	22034-550
Hydrogen Generator B9200	250cc/min.	ea.	22035
Hydrogen Generator B9400	500cc/min.	ea.	22036
<b>Replacement Components for Hydrogen Generators (for all models listed above)</b>			
Replacement Deionizer Bag		2-pk.	21670
Replacement Desiccant Cartridge		ea.	21671

Figure 1 shows, for each source material, the analysis to FAME C24:1 is completed in less than 11 minutes. Particularly notable are the stability of the baselines, the excellent peak symmetry, and baseline resolution of all compounds of interest. Table 2 summarizes the RSD% values for the FAMES measurements, all of which are less than 1%.

A 30m x 0.32mm ID x 0.25µm Stabilwax® column, used with hydrogen carrier gas, permits high speed analysis and ensures precise data acquisition for accurate quantification of C14:0-C24:1 FAMES and linolenic acid methyl ester.

**Stabilwax® Column (fused silica)**

(Crossbond® Carbowax® polyethylene glycol)

ID	df (µm)	temp. limits	length	cat. #
0.32mm	0.25	40 to 250°C	30-Meter	10624

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## Assure Accurate Sampling and Reliable Sample Purity

### Restek Sampling System Treatments Prevent Adsorption, Protect Components

By Gary Barone, Manager, Restek Performance Coatings

- Quantify active compounds (e.g., sulfur, mercury, NO<sub>x</sub>) at parts-per-billion levels.
- Corrosion protection equal to specialty alloys – at lower cost.
- Assemble a new system from treated stock, or treat an existing system.

When surface activity or corrosion are a concern, solutions must be engineered. Restek Performance Coatings offers a family of surface treatments that address reactivity and corrosion over a wide spectrum of applications. These treatments reduce process upsets, reduce capital costs, and reduce maintenance costs.

#### Accurate sampling with Siltek®/Sulfinert® tubing and fittings.

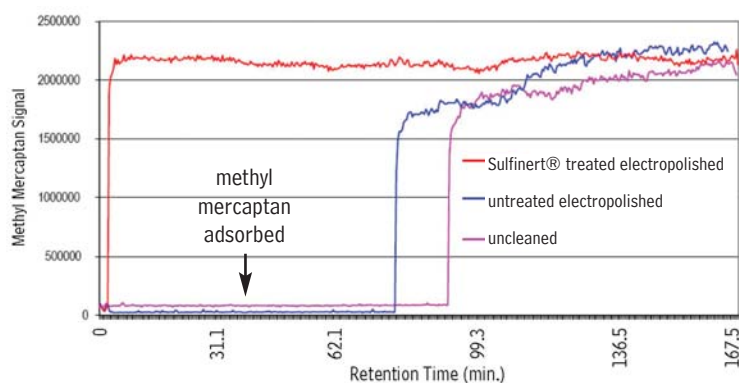
Adsorption problems in sample pathways often can be traced to the tubing and fittings used to transfer the sample to the analytical instrument. Always use treated tubing and fittings for applications involving active compounds. To ensure maximum inertness and minimal surface area, use Siltek®/Sulfinert® treated electropolished tubing. Figure 1 shows uptake and release curves for 500ppbv of methyl mercaptan, an active sulfur compound, in a gas stream passing through a variety of tubing substrates. Siltek®/Sulfinert® treated tubing reduces uptake by orders of magnitude, relative to untreated stainless steel tubing.

#### Reduce maintenance cost, extend system life with Silcosteel®-CR tubing and fittings.

In corrosive environments, Silcosteel®-CR treatment is an excellent alternative to expensive alloys. Silcosteel®-CR treatment extends component life while reducing the frequency of preventive maintenance and ensuring the purity of the process or sample stream. Silcosteel®-CR improves corrosion resistance by up to 10X over untreated 316 stainless steel (Figure 2).

Figure 3 shows the results of a 4000-hour salt spray test on Silcosteel®-CR treated 316L stainless steel and untreated 316L stainless steel. The Silcosteel®-CR treated material exhibited virtually no change. Silcosteel®-CR treatment has extended the life of process systems in oil and gas production, oil refining, petrochemical processing, aerospace equipment, food and beverage processing, and laboratory testing. Figure 4 shows Silcosteel®-CR treatment can reduce the overall lifetime cost of a typical process system by hundreds of thousands of dollars. While the initial cost of an unprotected stainless steel system is lower than that of a comparable Silcosteel®-CR system, the overall lifetime cost, considering replacement cost due to corrosion, is nearly double that of a Silcosteel®-CR treated system. High performance alloy systems offer superlative corrosion performance, but the initial material cost can be up to six times that of a stainless steel system.

**Figure 1** Sulfinert® treated electropolished seamless stainless steel tubing does not adsorb methyl mercaptan (500ppbv).



#### simply the best

Restek-treated electropolished tubing is the best tubing choice when purity, inertness, or reproducibility are concerns.



Top: electropolished finish, surface roughness average number: 5-10. Bottom: conventional finish, surface roughness average number: 23-27.

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### Treat the entire sample pathway for maximum benefit.

#### Fittings

Connections can be a source of adsorption and sample loss, and there is benefit to employing Restek surface treatment on many of these components. In corrosive environments, Silcosteel®-CR treatment will extend the useful life of system fittings, as well as tubing. We offer extensive lines of treated Swagelok® and Parker fittings, in sizes from 1/16" to 3/8".

#### Valves

The sample flow path through a valve can prolong contact between the sample stream and the valve components. Restek surface treatments have been applied to many valve geometries, to eliminate adsorption to bodies, stems, diaphragms, or other components.

#### Filters

Frits and other filtering devices trap particles and prevent them from entering the analytical instrument, but they also very effectively adsorb active components in sample streams. Their large surface areas can increase sample/system contact by orders of magnitude. Siltek®/Sulfinert® treatment of frits and filters creates an inert flowpath. Our chemical vapor deposition technology ensures the treatment penetrates even the smallest pores in sintered metal frits.

#### Sample Vessel Equipment

Restek treated sampling containers prevent active components from adsorbing to vessel, valve, or outage tube surfaces. We offer a complete line of high pressure sampling equipment for applications involving liquefied petroleum gases, ethylene, natural gas, or propylene.

#### Sampling Probes

An untreated probe contributes to the active surface area in the system, and this should be considered when identifying potential adsorption sites during active stream transfer.

#### Heated Transport Lines

Active compounds in the sample quickly can be adsorbed onto the hot tubing in a heated "trace line". Restek surface treatment prevents adsorption of active compounds.

#### Summary

Surface treatments from the Restek Performance Coatings group prevent adsorption of active compounds or corrosion in process systems, and always should be considered in applications in which active or corrosive streams are to be sampled, transferred, or analyzed.

#### Siltek®/Sulfinert® Treated Coiled 316L Grade Stainless Steel Tubing

ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	> 400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22508				
0.180" (4.57mm)	1/4" (6.35mm)**	22509				
0.277" (7.04mm)	3/8" (9.52mm)***	22914				

#### Silcosteel®-CR Treated Coiled 316L Grade Stainless Steel Tubing

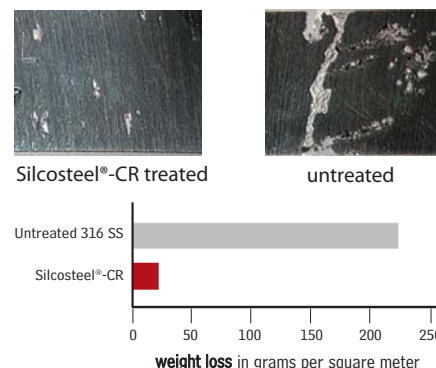
ID	OD	cat.#	5-24 ft.	25-199 ft.	200-399 ft.	> 400 ft.
0.055" (1.40mm)	1/8" (3.18mm)**	22896				
0.180" (4.57mm)	1/4" (6.35mm)**	22897				
0.277" (7.04mm)	3/8" (9.52mm)***	22915				

#### Siltek®/Sulfinert® Treated Coiled Electropolished 316L Grade Stainless Steel Tubing

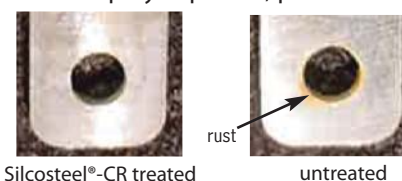
ID	OD	cat.#	5-24 ft.	25-99 ft.	100-299 ft.	> 300 ft.
0.085" (2.16mm)	1/8" (3.18mm)*	22538				
0.180" (4.57mm)	1/4" (6.35mm)**	22539				

\*0.020" wall thickness    \*\*0.035" wall thickness    \*\*\*0.049" wall thickness

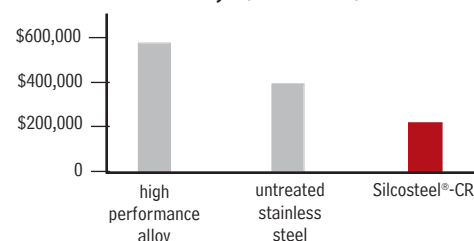
**Figure 2** Silcosteel®-CR resists pitting and crevice corrosion when exposed to ferric chloride, per ASTM G48, B.



**Figure 3** Silcosteel®-CR treated stainless steel shows no sign of attack after 4000-hour salt spray exposure, per ASTM B117.



**Figure 4** Silcosteel®-CR demonstrates significant cost savings, compared to untreated stainless steel or alloys (US dollars).



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Visit us at [www.restekcoatings.com](http://www.restekcoatings.com) for:

- Siltek®/Sulfinert® treated and Silcosteel®-CR treated Swagelok® and Parker fittings
- Siltek®/Sulfinert® treated and Silcosteel®-CR treated valves
- Siltek® treated in-line filters
- Sulfinert® treated Swagelok® sample cylinders
- Sulfinert® treated Alta-Robbins sample cylinder valves
- Additional treated stainless steel tubing
  - Siltek®/Sulfinert® treated, electropolished, 316L grade
  - Siltek®/Sulfinert® treated, 316L grade
  - Siltek®/Sulfinert® treated, 304 grade
  - Silcosteel®-CR treated, electropolished, 316L grade
  - Silcosteel®-CR treated, 316L grade





## Peak Performers

### Introduction to Pressure Regulators

by Donna Lidgett, GC Accessories Product Manager



#### General Purpose or Analytical?

General-purpose regulators usually are best suited for applications involving gases that are less than 99.995% pure: pneumatically-actuated valves and autosamplers, blanketing, inert atmospheres, and any other application not directly integrated with analytical data production. General purpose regulators have nylon-reinforced neoprene diaphragms that provide very good pressure control but are prone to air and moisture diffusion and hydrocarbon off-gassing.

Analytical regulators are recommended for applications in which maintaining the purity of a gas or mixture is the overriding concern, i.e., for applications requiring gases that are greater than 99.995% pure. They are commonly used in analytical labs. Analytical regulators have stainless steel diaphragms for pressure control. Stainless steel is not subject to the diffusion and off-gassing associated with neoprene diaphragms, and is easily purged of atmospheric contaminants when put into service.

#### Dual- or Single-Stage?

Dual-stage regulators reduce the source pressure to outlet pressure in two steps. The first stage reduces the inlet pressure to about three times the maximum working pressure. Outlet pressure regulation is controlled by the second stage and is set through an adjusting knob. This two-step regulation is highly recommended for services requiring a near constant delivery pressure as the source pressure decays, including chromatographic analyses.

Single-stage regulators perform the same function as dual-stage regulators, but in a single step down from source pressure to outlet pressure. For this reason, the outlet pressure cannot be as accurately maintained as the source pressure decays. We highly recommend that single-stage regulators be used only in circumstances in which the operator can monitor and adjust the regulator as needed, when the regulator is supplied with a nearly constant source pressure, or when additional pressure regulation is supplied downstream.

#### Brass or Stainless Steel?

Analytical regulators made from brass bar stock provide optimum performance for most analytical applications. Brass provides excellent strength and cleanliness and the machined bar stock design has less dead volume than forged-body regulators, making purging of atmospheric contaminants faster and more assured.

Regulators with stainless steel bodies were designed for delivering corrosive gases that would be incompatible with brass. With the advent of semiconductor manufacturing and high sensitivity analytical techniques, stainless steel also has proven to be a better surface for removing "sticky" atmospheric contaminants that interfere with detectors downstream. Unless these regulators are used in an all-stainless-steel system that incorporates welded tubing and special fittings, and in which rigorous cleaning and proper gas management are practiced, the extra expense relative to brass is not justified.

#### Overview of Restek's Brass and Stainless Steel Body Ultra-High-Purity Regulators

These regulators feature metal-to-metal seals throughout for long-term leak-tightness, and a metal diaphragm outlet valve ensures gas purity. Each regulator is helium leak-test-certifiable to  $1 \times 10^{-8}$  scc/sec. and is fully assembled and tested for your convenience. 100psig maximum delivery pressure supports pressure controlled operation. Maximum inlet pressure is 3000psig. Brass bar stock construction minimizes dead volume. Stainless steel construction is more easily purged of atmospheric contaminants, and is more resistant to attack from dry corrosive gases.

#### Ultra-High-Purity Stainless Steel Body Regulators

These regulators are the standard for ultra-high-purity and corrosion-resistant pressure regulation. They are more easily purged of atmospheric components, compared to brass regulators, making them ideal for the most demanding applications. Regulation performance is equal to our brass body regulators. For use in all-stainless steel systems where welded tubing and special fittings are used, and rigorous cleaning and proper gas management are practiced.

#### Dual-Stage Ultra-High-Purity Stainless Steel Regulators

- Most stable outlet pressure control throughout the life of a high-pressure gas cylinder.
- Secondary pressure regulation not needed.

Outlet pressure: 0 to 100psig  
 Outlet gauge: 30" – 0 to 200psig  
 Inlet gauge: 0 to 4000psig  
 Outlet assembly: diaphragm valve, 1/4" tube fitting

Fitting	qty.	cat.#
CGA 580 (N <sub>2</sub> , He, Ar)	ea.	20662
CGA 350 (H <sub>2</sub> , P <sub>2</sub> )	ea.	20663
CGA 590 (Air)	ea.	20664



### Single-Stage Ultra-High-Purity Stainless Steel Regulators

- Use when there is secondary pressure regulation downstream.
- Identical gas purity protection as with our dual-stage regulators.

Outlet pressure: 0 to 100psig  
 Outlet gauge: 30" – 0 to 200psig  
 Inlet gauge: 0 to 4000psig  
 Outlet assembly: diaphragm valve, 1/4" tube fitting

Fitting	qty.	cat.#
CGA 580 (N <sub>2</sub> , He, Ar)	ea.	20665
CGA 350 (H <sub>2</sub> , P <sub>2</sub> )	ea.	20666
CGA 590 (Air)	ea.	20667



### Dual-Stage Ultra-High-Purity Chrome-Plated Brass Regulators

- Oxidation-resistant, chrome-plated.
- Most stable outlet pressure control throughout the life of a high-pressure gas cylinder.
- Secondary pressure regulation not needed.
- Most widely used regulator.
- Less internal volume than stainless steel regulators.

Outlet pressure: 0 to 100psig  
 Outlet gauge: 30" – 0 to 200psig  
 Inlet gauge: 0 to 4000psig  
 Outlet assembly: diaphragm valve, 1/4" tube fitting

Fitting	qty.	cat.#
CGA 580 (N <sub>2</sub> , He, Ar)	ea.	21667
CGA 350 (H <sub>2</sub> , P <sub>2</sub> )	ea.	21668
CGA 590 (Air)	ea.	21669



### Single-Stage Ultra-High-Purity Chrome-Plated Brass Regulators

- Oxidation-resistant, chrome-plated.
- Use when there is secondary pressure regulation downstream.
- Identical gas purity protection as with our dual-stage regulators.

Outlet pressure: 0 to 100psig  
 Outlet gauge: 30" – 0 to 200psig  
 Inlet gauge: 0 to 4000psig  
 Outlet assembly: diaphragm valve, 1/4" tube fitting

Fitting	qty.	cat.#
CGA 580 (N <sub>2</sub> , He, Ar)	ea.	20646
CGA 350 (H <sub>2</sub> , P <sub>2</sub> )	ea.	20647
CGA 590 (Air)	ea.	20648

### Ultra-High-Purity Chrome-Plated Brass Line Regulator

- Oxidation-resistant, chrome-plated.
- Use where you need to reduce the line pressure by 20psi or more.
- Same purity protection as high-pressure cylinder regulators.

Inlet connections: 1/4" FPT

Outlet assembly: 1/4" FPT port

Fitting	Outlet Gauge	Outlet Pressure	qty.	cat.#
1/4" female NPT ports*	30" - 0 to 100psig	0-50psig	ea.	21666
1/4" female NPT ports*	30" - 0 to 200psig	0-100psig	ea.	22452

\*Order appropriate male connector, pipe-to-tube fittings.

### Male Connector, Pipe-to-Tube Fittings

Fitting Type	Size (inches)	Parker #	Similar to Swagelok®	Brass		Stainless Steel	
				qty.	cat.#	qty.	cat.#
Male Connector	1/4" to 1/4" NPT	4 MSC 4N	400-1-4	10-pk.	21842	2-pk.	21942
Male Connector	1/8" to 1/4" NPT	2 MSC 4N	200-1-4	10-pk.	21844	2-pk.	21944
Tube End Reducer	1/4" tube to 1/8"	4 TUR 2	200-R-4	5-pk.	21834	2-pk.	21934



male connector



tube end reducer

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## Preventing Septum Problems

By Donna Lidgett, GC Accessories Product Marketing Manager and Scott Grossman, GC Accessories Chemist

- Avoid extraneous peaks with proper septum handling & maintenance.
- Handy size chart & septum choice guidelines.
- Optimize performance by choosing the right septum for the job.

All septa, regardless of their composition, puncturability, or resistance to thermal degradation, will be a source of problems if they are mis-handled or used inappropriately. Poor septum choice and improper treatment can significantly compromise both qualitative and quantitative analytical results. Proper septum choice and careful handling can minimize septum bleed and septum coring, two of the most common septum problems that affect chromatography.

Septum bleed occurs when volatiles from the septum (e.g., silicone oils, phthalates) enter the column and then elute, creating elevated baselines (for isothermal analyses), baseline disturbances, or extraneous (but consistent) peaks in the chromatogram. Either baseline rise or extraneous peaks can interfere with identification and quantification of target analytes. This problem is prevalent in temperature-programmed analyses, because the septum volatiles collect on the column during the oven cool-down and initial hold periods.

To avoid septum bleed, either condition your septum prior to running your analysis, or use a pre-conditioned septum that is ready for immediate use. All Restek septa are preconditioned and ready to use. Allowing the septum to condition at operating temperatures for a few hours is an excellent way to assure optimum performance. Also always use clean forceps or wear clean powderless latex gloves, or cotton gloves when handling septa. Do not handle them with bare fingers or with powdered latex gloves since contaminants such as finger oils, perfumes, make-up, fingernail polish, skin creams, hand soaps, and talcum can be absorbed into the septum and bleed out during analysis.

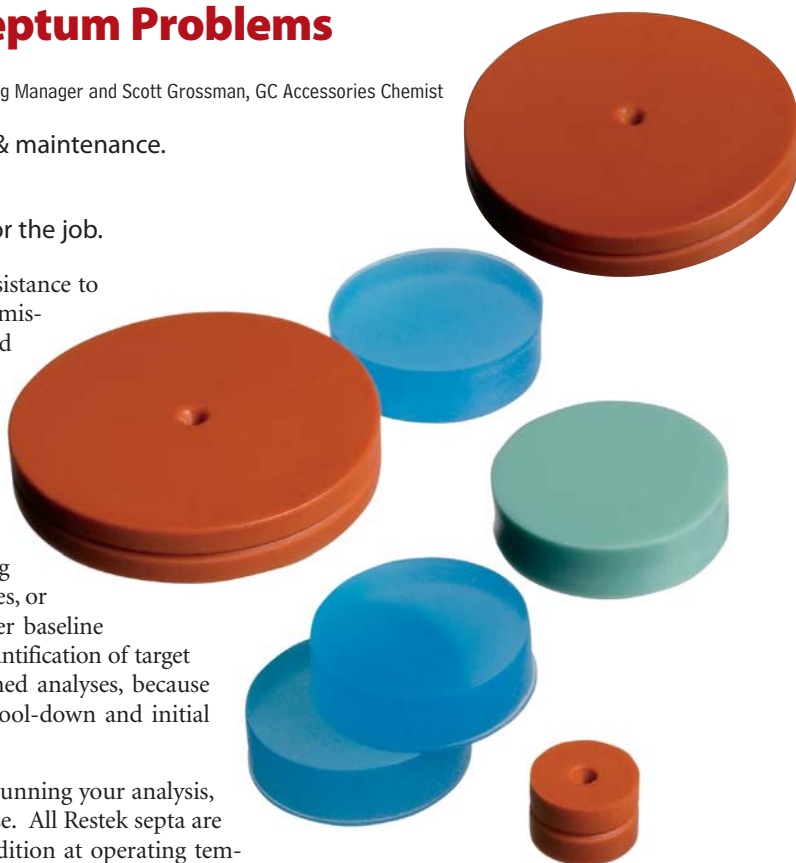
Septum coring is another common problem that can diminish chromatographic performance. Coring occurs when the septum has been punctured too many times, the needle is damaged, or the wrong needle tip type is used. In these cases, small particles may be cored from the body of the septum and fall into the inlet liner. Once in the liner, they are subjected to higher temperatures, causing the release of septum volatiles which are swept into the column and can appear on the chromatogram (see "How Hot is Your Septum?" on page 22).

To prevent septum coring, always follow the septum and instrument manufacturers' installation recommendations and take care not to over-tighten the septum nut. Over-tightening the septum nut invariably reduces septum lifetime by increasing coring and splitting. Routinely replacing your septum and inspecting your syringe needle (manual or autosampler) for tip damage also help prevent septum damage.

Softer septa, such as Ice-Blue™ septa, are less likely to core than firmer septa. However, softer septa usually have a lower maximum operating temperature than firmer septa, so consider your method requirements carefully before deciding to switch. Changing syringe needle styles also can help reduce coring. For example, a point-style #2 needle (beveled point) is much more likely to cause coring (especially when the tip has become bent or dull) than a point-style #5 needle (conical needle with side-port).

A septum that can be penetrated cleanly and easily by the needle is less prone to coring and has a longer life. Moreover, consistent injections made through such a septum help ensure accurate results. The soft silicone rubber from which all Restek septa are manufactured is specially formulated for chromatographic performance, which ensures our septa are easy to puncture. However, in cases in which a small degree of pliability is sacrificed for high-temperature optimization, the CenterGuide™ dimple will help guide the syringe, for clean, consistent injections, minimizing septum coring.

Careful consideration of instrument and method requirements should dictate your septum choice, but proper handling and maintenance are the keys to minimizing septum damage and maximizing the accuracy of your analyses. Restek offers septa for all major brands of gas chromatographs and injectors. Use our handy septum size chart to determine the septum diameter for your instrument or contact us at **1-800-356-1688 (ext. 4)** to discuss your application.





**Restek Septa**

- Precision molding assures consistent, accurate fit.
- Ready to use
- Do not adhere to hot metal surfaces.
- Packaged in non-contaminating glass jars.

Septum Diameter	25-pk.	50-pk.	100-pk.
<b>Thermolite® Septa</b>			
5mm (3/16")	27120	27121	27122
6mm (1/4")	27123	27124	27125
7mm	27126	27127	27128
8mm	27129	27130	27131
9mm	27132	27133	27134
9.5mm (7/8")	27135	27136	27137
10mm	27138	27139	27140
11mm (7/16")	27141	27142	27143
11.5mm	27144	27145	27146
12.5mm (1/2")	27147	27148	27149
17mm	27150	27151	27152
Shimadzu Plug	27153	27154	27155
<b>IceBlue™ Septa</b>			
9mm		27156	27157
9.5mm (7/8")		27158	27159
10mm		27160	27161
11mm (7/16")		27162	27163
11.5mm		27164	27165
12.5mm (1/2")		27166	27167
17mm		27168	27169
Shimadzu Plug		27170	27171
<b>BTO® Septa</b>			
5mm CenterGuide™		27100	27101
6mm (1/4")		27102	27103
9mm CenterGuide™		27104	27105
9.5mm (7/8")		27106	27107
10mm		27108	27109
11mm (7/16") CenterGuide™		27110	27111
11.5mm CenterGuide™		27112	27113
12.5mm (1/2") CenterGuide™		27114	27115
17mm CenterGuide™		27116	27117
Shimadzu Plug		27118	27119



## HANDY septum size chart

Instrument	Septum Diameter (mm)	Instrument	Septum Diameter (mm)
<b>Agilent (HP)</b>		<b>Pye/Unicam</b>	
5880A, 5890, 6890,		All models	7
6850, PTV	11	<b>Shimadzu</b>	
5700, 5880	9.5/10	All models	Plug
On-Column Injection	5	<b>SRI</b>	
<b>Thermo Scientific</b>		All models	Plug
TRACE™ GC	17	<b>Tracor</b>	
GCQ w/TRACE™, PTV	17	54011.5	
8000 series	17	550,560	9.5
<b>Finnigan (TMQ)</b>		220,222	12.5
GC 9001	9.5	<b>Varian</b>	
GCQ 9.5		Injector type:	
QCQ™ 9.5		Packed column	9.5/10
TRACE™ 2000	9.5	Split/splitless	
<b>Gow-Mac</b>		1078/1079	10/11
6890 series	11	1177 9	
All other models	9.5	1075/1077	11
<b>PerkinElmer</b>			
Sigma series	11		
900,990	11		
8000 series	11		
Auto SYS™	11		
Auto SYS™ XL	11		

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## How Hot Is Your Septum?

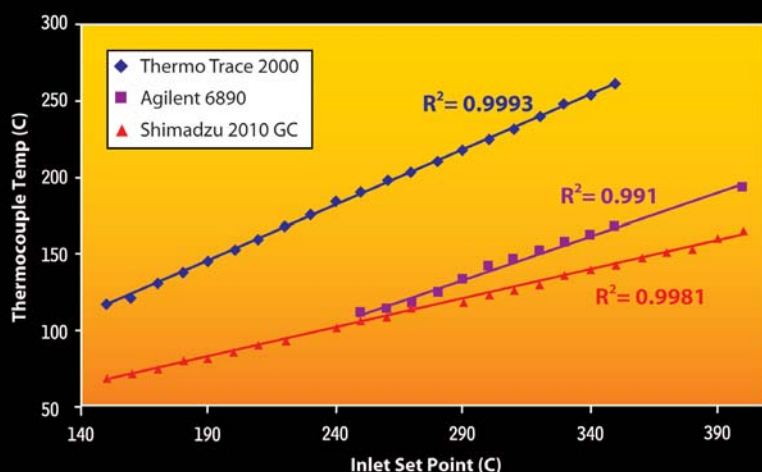
By Scott Grossman, GC Accessories Chemist

Different septa brands are given a single, maximum operating temperature based on their performance in a specific instrument inlet, not the actual temperature that the septum can withstand and still function properly. Understanding how different inlets influence the actual temperature at the septum can help prevent problems such as sticking. The temperature at the septum is affected by the heating element and the overall inlet design, which varies significantly among manufacturers. To illustrate this, we placed a thermocouple at the bottom of the septum in several instruments and compared the actual temperature to the inlet set point. The resulting data demonstrate that for any given setting the temperature at the septum is lower than the set point, but the degree of difference, or gradient, varies among instruments (Figure 1).

There are distinct advantages and disadvantages associated with different temperature gradients that should be considered. Inlets with a larger gradient (cooler septum compartment) typically experience fewer problems with septa sticking. In contrast, inlets with a smaller gradient (hotter septum compartment) are more prone to septa sticking, but have the advantage of a more evenly heated inlet and thus more uniform sample vaporization. Uniform vaporization reduces analyte discrimination, the bias against higher boiling point (i.e. higher molecular weight) compounds in favor of lower boiling point compounds that occurs when compounds are not vaporized with equal efficiency.

Operators of instruments that have a smaller temperature gradient should consider using septa that are rated for the highest possible temperature and setting the inlet at the lowest permissible temperature. Low bleed BTO® septa are one of the best choices for temperature resistance, and have the added benefit of a needle guide, which increases septum lifetime (see "Preventing Septum Problems" on page 20 for more information on septum selection and care). Understanding how your inlet temperature setting relates to the actual temperature at the septum allows you to control bias, avoid septum problems, and better understand your results.

**Figure 1** Septum temperature differs from inlet temperature set point; the degree of difference varies by manufacturer.



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## Retention Cross-over Phenomenon in Gas Chromatography- Can the Mystery be Revealed? Part 1

Continued from page 2

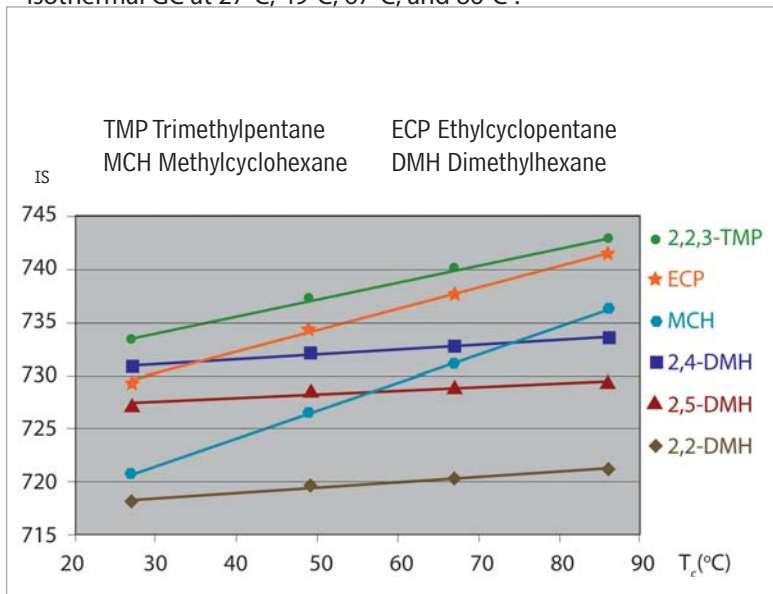
Therefore, the dependence of the retention time upon column temperature is usually expressed graphically as the log of the retention parameter (net retention time  $t'_R$  or retention factor  $k'$  or retention index  $I$ ) vs.  $T_c$  or  $1/T_c$ , where  $T_c$  is the absolute column temperature. In many cases, the plots are linear over the temperature range employed and, furthermore, the lines are approximately parallel to each other indicating that there is little change in selectivity by changing the column temperature in isothermal mode. This is valid for chemically similar compounds. But closer inspection reveals that some lines diverge slightly in their slope and even cross each other (Figure 2).<sup>2</sup> The practical implication is coelution of the two compounds at the temperature where the lines intersect. By further changing the column temperature the compounds are again separated but in reverse elution order. As mentioned above, this kind of behavior is often experienced when compounds of different chemical nature are analyzed on moderate to highly polar stationary phases.

But not only compounds with different functional groups will behave this way! In the next issue of the Restek Advantage, you will see examples of aliphatic versus cyclic compounds or cyclic compounds differing in their ring number, and the cross-over effect on non-polar columns.

### References:

- 1 Mehran M. et al., HRC, 14 (1991) 745 – 750.
- 2 Hively, R.A. and R.E. Hinton, J. Gas Chromatogr. 6 (1968) 203 – 217.

**Figure 2** Retention indices on squalane (IS) as a function of  $T_c$  for isothermal GC at 27°C, 49°C, 67°C, and 86°C<sup>2</sup>.



**more to reveal!**

See the next issue of the Restek Advantage for Part 2 of Retention Cross-over Phenomenon in Gas Chromatography- Can the Mystery be revealed?

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## Tradeshows Schedule

We'd be happy to talk with you at any of the following meetings or shows. We'll post our booth numbers as they become available to us.

### June, 2007

Date	June 3-7
Show	55th ASMS Conference on Mass Spectrometry
Location	Indiana Convention Center, Indianapolis, IN
Date	June 4-7
Show	30th International Symposium on Capillary Chromatography (ISCC)
Location	Dalian World Expo Centre, Dalian, P.R. China (Booth 80)
Date	June 11-14
Show	Metabolomics Society 3rd Annual Conference
Location	Renold Building, The University of Manchester, Manchester, UK
Date	June 17-21
Show	HPLC 2007
Location	International Convention Centre, Ghent, Belgium

### July, 2007

Date	July 17-19
Show	Semicon West 2007
Location	Moscone Center, San Francisco, CA
Date	July 22-25
Show	Florida Pesticide Residue Workshop (FPRW)
Location	TradeWinds Island Grand, St. Pete Beach, FL

### August, 2007

Date	August 19-23
Show	ACS 234th National Meeting & Exposition
Location	Boston, MA
Date	August 20-24
Show	National Environmental Monitoring Conference (NEMC)
Location	Hyatt Regency, Cambridge, MA
Date	August 26-31
Show	T2007 - TIAFT (International Conference (NEMC))
Location	Seattle Sheraton, Seattle, WA

For latest updates, see our Tradeshows Calendar at [www.restek.com/ontheroad](http://www.restek.com/ontheroad).

## Seminar Schedule

Date	Cat. #	City	State
<b>GC Hands-On Maintenance and Troubleshooting</b>			
6/13	65552	Lafayette Hill	PA
<b>Comprehensive Capillary GC</b>			
6/12	65551	Lafayette Hill	PA
6/15	65553	Wilmington	DE
6/19	65554	Cleveland	OH
6/20	65555	Buffalo	NY
6/22	65556	Pittsburgh	PA
7/23	65560	Idaho Falls	ID
7/25	65561	Boise	ID
7/26	65562	Spokane	WA
<b>Comprehensive HPLC</b>			
7/9	65557	Chicago	IL
7/11	65558	Madison	WI
7/13	65559	Kansas City	MO

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