

Determination of Omega-3 (n-3) and Omega-6 (n-6) Fatty Acid Composition in Evening Primrose Oil, Flax Seed Oil, Black Currant Oil, and Borage Oil

Essential fatty acids (EFAs) are polyunsaturated fatty acids (PUFAs) that the human body requires, yet cannot produce, and therefore must be obtained through dietary sources or nutritional supplements. α -Linolenic acid (LNA) and γ -Linolenic acid (GLA) are important Omega-3 (n-3) and Omega-6 (n-6) fatty acids. Accurate determination and quantitation of these EFAs, especially the separation of LNA and GLA, can be performed by capillary gas chromatography (GC). The FAMEWAX™ column is ideal to provide the composition of the EFAs found in evening primrose oil, flax seed oil, black currant seed oil, and borage oil.

Why are these fatty acids essential?

The two families of EFAs are the Omega-3 (n-3) series and the Omega-6 (n-6) series. The Omega-3 (n-3) series includes LNA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The Omega-6 (n-6) series includes linoleic acid (LA), GLA, dihommogamma-linolenic acid (DGLA), and arachidonic acid (AA).

These EFAs are nutrients that perform key functions in our bodies. For example, they determine membrane fluidity and reactivity, oxidation rate, metabolic rate, and energy production. In addition, they are a factor in maintaining body temperature, insulating nerves, and cushioning body tissue. EFAs also are precursors to prostaglandins, hormone-like substances that are critical to the body's overall health maintenance. Prostaglandins regulate blood pressure, blood clotting, stimulation of the immune system, and general regulation of heart, kidneys, liver, lungs, and brain. They are short-lived molecules and constantly need to be replenished. Without EFAs this can be impossible.

Background

EFAs are similar to vitamins in their importance to one's overall health. However, vitamins are required in small dietary quantities ($\mu\text{g}/\text{day}$), whereas EFAs are a macronutrient (i.e., necessary in g/day). A joint study released by the Food and Agriculture Organization and the World Health Organization recommends that at least 3% of our daily calorie intake be in the form of EFA.¹

Polyunsaturated oils, such as safflower, sunflower, and corn oil are good sources of LA. Once ingested, LA can be converted to the other Omega-6 acids: GLA, DGLA, and AA.

Green, leafy vegetables and flax oil are good sources of LNA. From the LNA provided in our diets, our bodies can produce the other Omega-3 (n-3) acids: EPA and DHA.

Unfortunately, one's diet may not be well-fortified with these food sources. Also, physiological conditions can inhibit the

conversion process of LA and LNA to the other essential Omega-3 and Omega-6 fatty acids.¹ Therefore, nutritional supplements can be used to help people attain the suggested daily intake. Evening primrose oil, flax seed oil, black currant seed oil, and borage oil are rich sources of these EFAs and are available in capsules as nutritional supplements.

A number of clinical conditions have been treated with oils rich in GLA. Oral dosages of evening primrose oil have been used to treat premenstrual tension, rheumatoid arthritis, breast disorders, and atopic eczema.²

Analysis

The oils were obtained from soft-gel capsules of evening primrose oil, flax seed oil, black currant oil, and borage oil. The fats were initially in the form of triglycerides. They were saponified into their free acids and esterified for better volatility and inertness by GC. To do this, 5mL of hexane and 250 μL of 2N KOH were added to 0.24g oil. The mixture was shaken for 2 minutes in a closed 20mL vial. After settling, the supernatant was injected.

In the 1980s, packed and capillary GC, as well as liquid chromatography (LC), were evaluated for the analysis of EFAs in evening primrose oil and soybean oil.² According to one reference, "gas chromatography using a capillary column (25m, Carbowax® 20 M) was the best tool for the separation of GLA (C18:3n6) and LNA (C18:3n3)."³

Based on this finding, we used a Restek column—the 30m, 0.25mm ID, 0.25 μm FAMEWAX™ column (cat.# 12497)—to analyze these oils. The FAMEWAX™ column contains a polyethylene glycol stationary phase, which is slightly more polar than the Stabilwax® column. The FAMEWAX™ column offers excellent selectivity and efficiency, not only to separate saturated (C16:0 and C18:0) and monounsaturated (C18:1n9) fatty acids from the Omega-3 and -6 fatty acids of interest, but also to resolve the isomers of linolenic acid (C18:3n3 and C18:3n6).

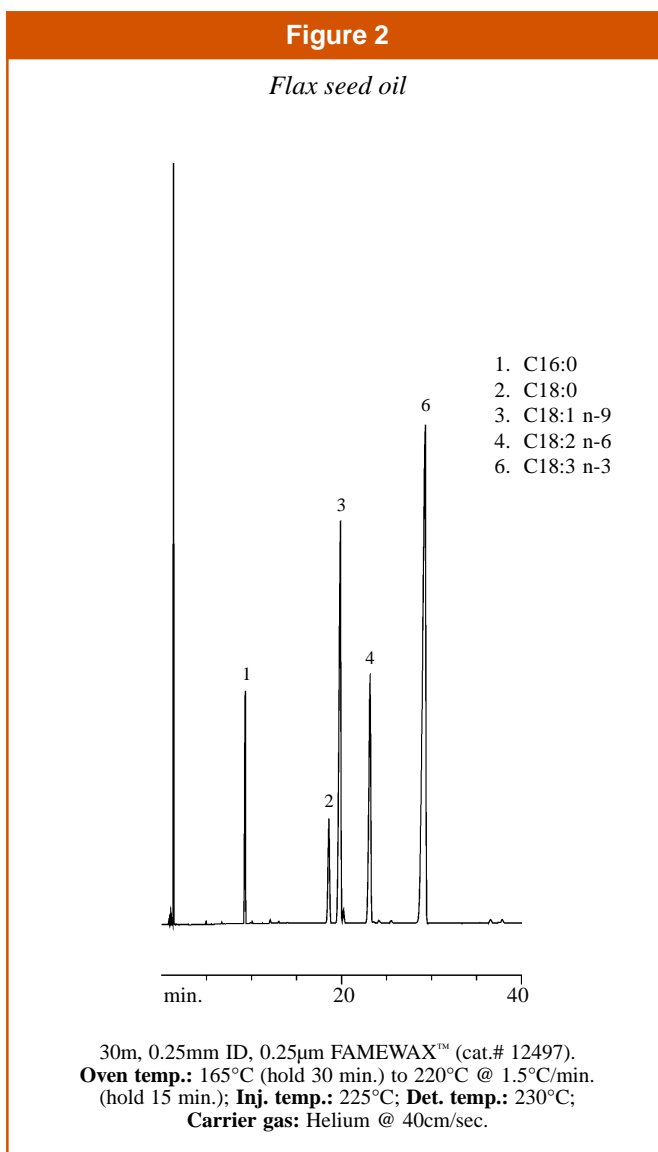
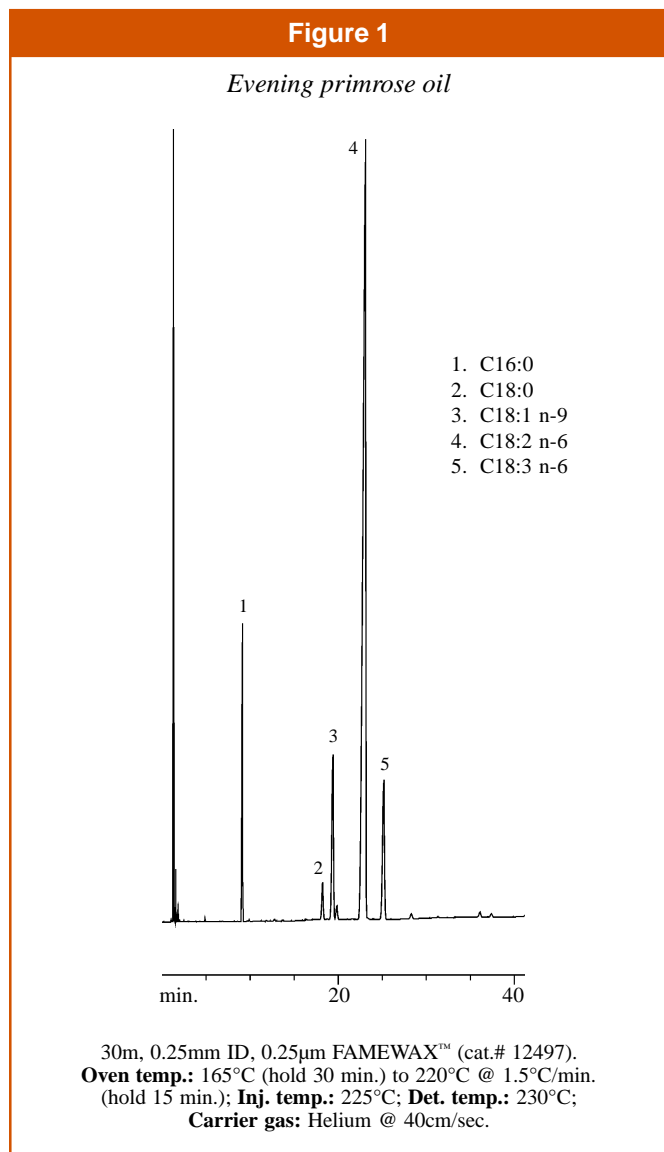
We used an HP 5890 GC with a flame ionization detector (FID) and a split/splitless injection port, used in the split mode, with a split vent flow of 40mL/min. The inlet liner was a deactivated 4mm ID split sleeve (cat.# 20781). The injector and detector ports were set at 225°C and 230°C, respectively. The oven temperature program was initially set at 165°C for the first 30 minutes, and then increased at a rate of 1.5°C/min to 220°C, where it remained for the last 15 minutes. The carrier gas was helium and the linear velocity of 40cm/sec. was measured at the initial temperature.

Results

Regarding the content of the Omega-3 and Omega-6 essential fatty acids in the four oils studied, all contain LA (18:2n6) (**Figures 1-4 and Table 1**). Evening primrose oil (**Figure 1**) contains the largest amount of LA and would be the best source. Flax seed oil (**Figure 2**) reveals a significant amount of LNA (C18:3n3), and would be the best source of this EFA. Only black currant seed oil (**Figure 3**) contains an approximately equal amount of both isomeric linolenic acids. All chromatograms, except that of flax seed oil, illustrate the presence of GLA (C18:3n6). In fact, borage oil (**Figure 4**) and black currant seed oil are significant sources of GLA.

Conclusion

Omega-3 (n-3) and Omega-6 (n-6) EFAs perform key functions in our bodies. Quantifying these compounds in nutritional supplements such as evening primrose oil, flax seed oil, black currant seed oil, and borage oil is successfully achieved using the FAMEWAX™ column. This column offers excellent efficiency and selectivity towards these polyunsaturated methyl esters, providing an accurate determination of the fatty acid profiles. Thus the FAMEWAX™ column is an excellent column choice for this and similar applications.



Achieve baseline resolution of complex polyunsaturated FAMES in significantly less time using the FAMEWAX™ column as compared to other Carbowax® columns!

Call 800-356-1688 or 814-353-1300, ext. 3, or your local Restek representative to order your FAMEWAX™ column today.

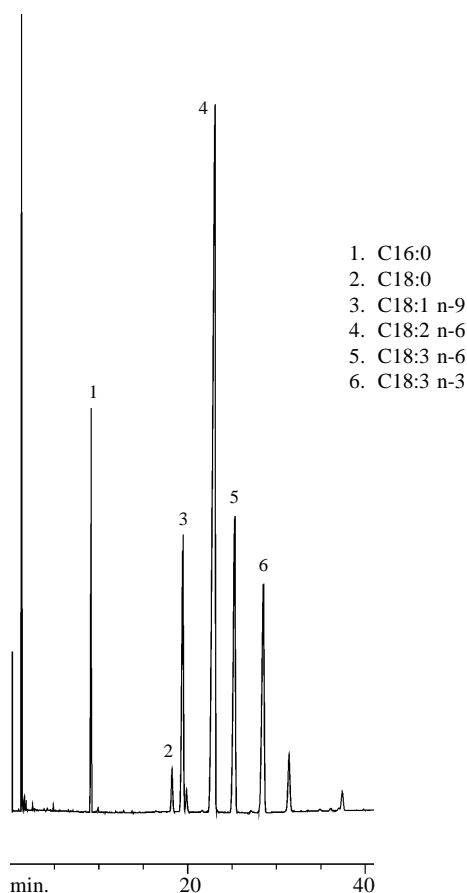
Table I

Composition of Omega-3 and Omega-6 EFAs in the four oils (% area).

	<i>C16:0</i>	<i>C18:0</i>	<i>C18:1n9</i>	<i>C18:2n6</i>	<i>C18:3n6</i>	<i>C18:3n3</i>
<i>Evening Primrose Oil</i>	6.5	1.8	8.6	73.5	8.7	n/a
<i>Flax Seed Oil</i>	4.9	5.2	23.7	15.2	n/a	50.1
<i>Black Currant Seed Oil</i>	6.7	1.6	11.3	47.1	15.3	13.1
<i>Borage Oil</i>	11.5	4.9	19.5	40.3	22.1	n/a

Figure 3

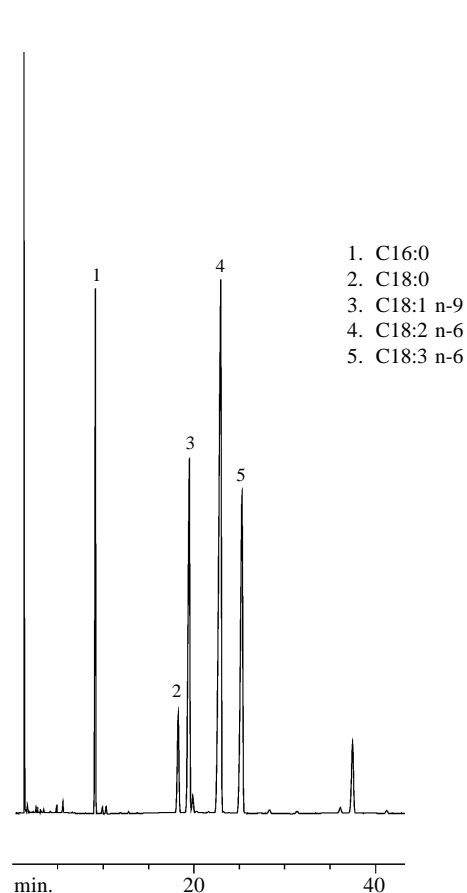
Black currant seed oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min.
 (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

Figure 4

Borage seed oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min.
 (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

References

1. Health and Healing News, "Evening Primrose Oil—Superfood for the '90s." <http://www.hhnews.com/epo.htm>
2. Robert A. Gibson, David R. Lines and Mark Neumann, "Gamma Linolenic Acid (GLA) Content of Encapsulated Evening Primrose Oil Products," *Lipids*, Vol. 27, no. 1 (1992).
3. M.S. Manku, "A Comparison of GLC and HPLC Methods for Determining Fatty Acid Composition of Evening Primrose and Soybean Oil," *Journal of Chromatographic Science*, Vol. 21, August (1983).

Product Listing

FAMEWAX™ Columns

- Ideal for FAME analysis.
- Similar to Omegawax™ columns.

ID	df (µm)	Stable to	30m
0.25mm	0.25	250°C	12497
0.32mm	0.25	250°C	12498
0.53mm	0.50	250°C	12499

Thermolite® Septa (green)

- Lowest bleed on FIDs, ECDs, and MSDs.
- Excellent puncturability.
- Preconditioned/ready to use.
- Does not adhere to hot metal surfaces.
- Usable to 340°C inlet temperatures.
- Packaged in non-contaminating tins.

Septum Diameter	25-pk.	50-pk.	100-pk.
9.5mm (3/8")	20359	20360	20361
10mm	20378	20379	20380
11mm (7/16")	20363	20364	20365
Shimadzu Plug	20372	20373	20374

Fatty Acid Methyl Ester Mixtures

Neat fatty acid methyl esters can be used to prepare specific mixtures not commercially available. These products are of the highest purity available. Each compound is packaged under a nitrogen blanket to ensure product stability. A Certificate of Analysis is provided with each ampul. *Packaged 100mg/ampul.*

Carbon No.	Compound	CAS#	cat.#
C14:0	methyl myristate	124-10-7	35045
C14:1Δ9 cis	methyl myristoleate	56219-06-8	35046
C15:0	methyl pentadecanoate	7162-64-1	35047
C16:0	methyl palmitate	112-39-0	35048
C16:1Δ9 cis	methyl palmitoleate	1120-25-8	35049
C17:0	methyl heptadecanoate	1731-92-6	35050
C18:0	methyl stearate	112-61-8	35051
C18:1Δ9 cis	methyl oleate	112-62-9	35052
C18:2Δ9, 12 cis	methyl linoleate	112-63-0	35053
C18:3Δ9,12,15 cis	methyl linolenate	301-00-8	35054
C19:0	methyl nonadecanoate	1731-94-8	35055
C20:0	methyl arachidate	1120-28-1	35056
C20:1Δ11 cis	methyl eicosenoate	2390-09-2	35057
C20:2Δ11,14 cis	methyl eicosadienoate	2463-02-7	35058
C20:3Δ11,14,17 cis	methyl eicosatrienoate	55682-88-7	35059
C20:4Δ5,8,11,14 cis	methyl arachidonate	2566-89-4	35060

High-Capacity Split Vent Trap

- Reduces the release of hazardous materials into the lab when using a split injection mode.
- Lasts one month or 1,500 injections.
- Connecting lines and mounting kit included.

Each	5-pk.
20698	20699

Autosampler Syringe 6-Packs for HP 7673 GCs

- Hamilton and SGE syringes are designed and tested to meet critical autosampler specifications.
- Needle point styles are developed to withstand multiple, fast septum injections.

Volume (µL)	Needle Term.	Gauge	Hamilton Restek cat.#	SGE Restek cat.#
5	ASN/F	23s	20170	24783
5	ASN/F	26s	21230	24782
5	ASN/F	23s-26s	24594	21214
10	ASN/F	23s	20169	24787
10	ASN/F	26s	24599	24786
10	ASN/F	23s-26s	24600	21215

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Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) Using Rtx®-5Sil MS and Rtx®-CLPesticides2 Capillary Columns

Analysis of polycyclic aromatic hydrocarbons (PAHs) is a very common method in environmental laboratories. US Environmental Protection Agency (EPA) Method 8100 requires gas chromatograph/fluorescence detection (GC/FID) to quantitate PAHs found in extracts from soil, water, or biological samples. This method requires the use of a dual-column system, because most samples often contain hydrocarbon interferences. Confirmational analysis increases the confidence of proper identification and quantitation of the PAHs. Good resolution is necessary for proper quantitation; the most difficult compound pairs to resolve are benzo(b)/benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene/dibenzo(a,h)anthracene. Short analysis time is another key consideration for most laboratories. By decreasing analysis time, sample throughput is increased and the lab benefits from a cost savings.

Primary Analysis

For this analysis, the primary analytical stationary phase is a 5%diphenyl/95%dimethyl-polysiloxane polymer. The Restek Rtx®-5Sil MS column is an equivalent phase and is recommended for this analysis (Fig. 1). While selectivity is similar to 5%diphenyl/95%dimethyl-polysiloxane columns, the proprietary silarylene stationary phase of the Rtx®-5Sil MS column is designed to produce very low bleed.

Confirmational Analysis

Confirmational analysis is a technique that requires two analytical columns of different selectivities, resulting in different retention

times of target or interfering compounds. These differences can improve quantitative and qualitative reliability through peak verification. The confirmational column recommended by Restek for this analysis is the Rtx®-CLPesticides2 column (Fig. 2). Quantitative reliability for this analysis is maintained because the stationary phases differ in selectivity, resulting in retention time shifts of both PAHs and interference compounds.

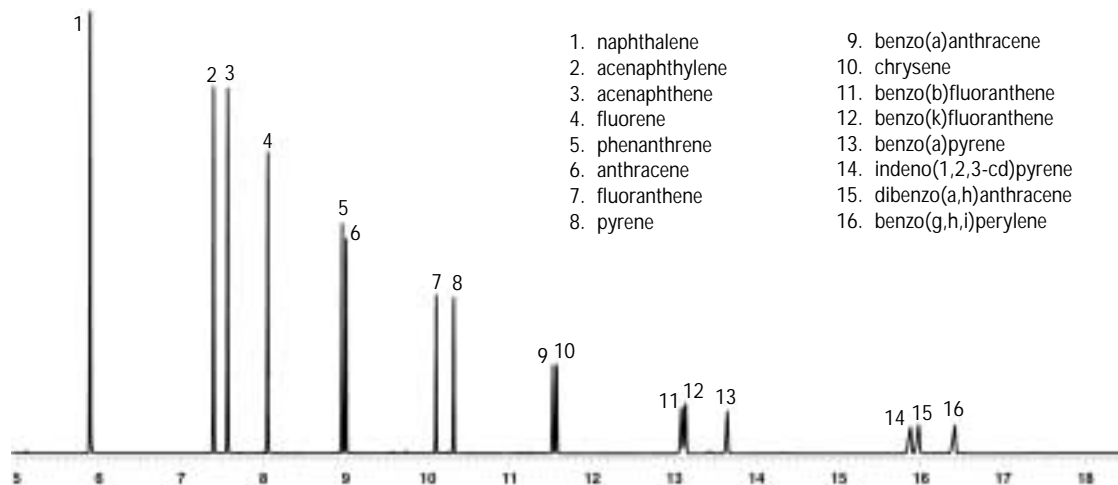
Resolution of PAHs

Resolution between benzo(b)fluoranthene and benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene is essential for quantitation when using an FID. To achieve excellent resolution of these peak pairs, the carrier gas, column flow rate, and temperature program must all be optimized. And, to achieve even better quantitative reliability, it is recommended to clean sample extracts following EPA Method 3630 (silica gel) prior to analysis.

Optimizing Carrier Gas Flows

The resolution of PAHs can be increased while reducing the overall analysis time by using hydrogen carrier gas at high flow rates. For this application hydrogen is a better choice than helium because it is more efficient at higher flow rates. And, if used in the constant flow mode, the best separation and fastest analysis time can be achieved. (Constant pressure mode is not recommended because the flow rate will decrease as the oven temperature is increased. This could result in a loss of resolution for the later eluting PAHs and a longer analysis time.)

Figure 1—The Rtx®-5Sil MS column exhibits excellent resolution of polycyclic aromatic hydrocarbons including benzo (b)/benzo (k)fluoranthene in less than 17 minutes.



30m, 0.25mmID, 0.25µm Rtx-5Sil MS (cat.# 12723); **Sample:** Method 610—Polynuclear Aromatic Hydrocarbons Mix (cat.# 31011); **Concentration:** 50ppm; **Solvent:** methylene chloride; **Sample size:** 1.0µl; **GC:** Thermo Trace 2000 Series; **Injector:** splitless @ 250°C; **Splitless hold time:** 2.0 min.; **Split vent flow:** 40cc/min.; **Carrier gas:** hydrogen (constant flow mode); **Column flow rate:** 4.0cc/min. @ 40°C; **Linear velocity:** 43cm/sec.; **Detector:** FID @ 340°C; **Make-up gas flow:** 40cc/min.; **Oven program:** 40°C (hold 2.0 min.) to 268°C @ 25°C/min. (hold 1.0 min.) to 330°C @ 5°C/min. (hold 10 min.)

The optimum carrier gas flow rate for the 30m, 0.25mm ID, 0.25µm Rtx®-5Sil MS column is less than 1mL/min. However, by increasing the flow rate to 4mL/min. for the analysis of PAHs, the separation of the isomer pairs is increased and the analysis time is reduced to less than 17 minutes (Figure 1). The Rtx®-CLPesticides2 confirmation column can separate these compounds under identical conditions (Figure 2). Again, the faster flow rate (4mL/min.) improves separation and reduces analysis time to less than 18 minutes.

Optimizing Temperature Program

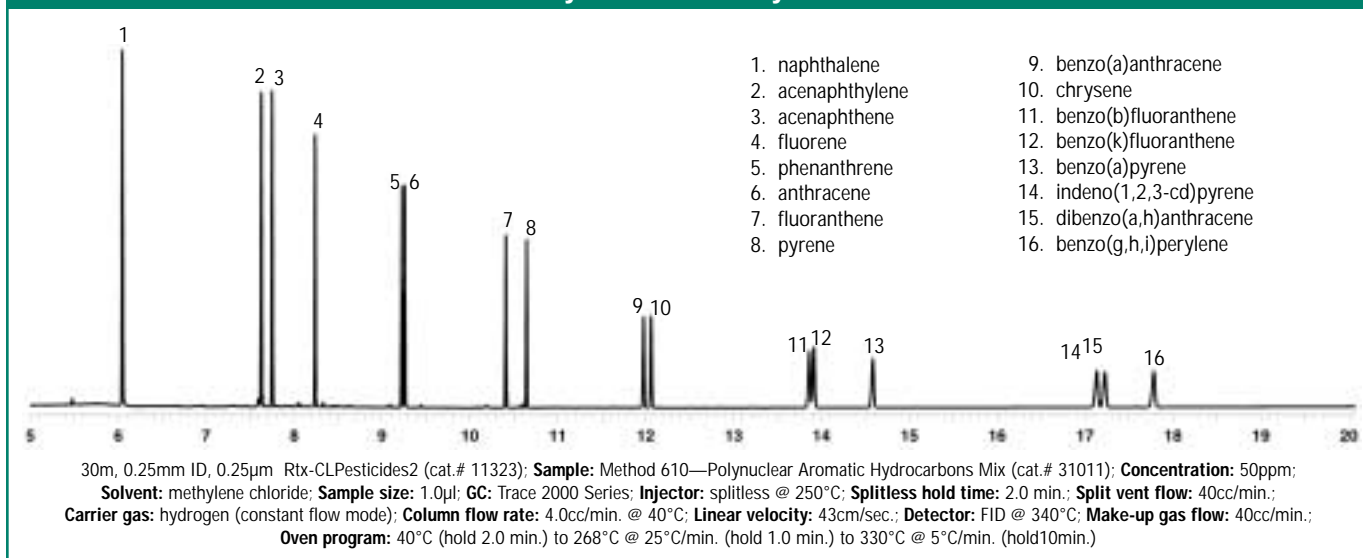
Optimizing the temperature program also contributes to better resolution of closely eluting peak pairs and shortens analysis times. The temperature program in Figures 1 and 2 achieves baseline resolution of indeno(1,2,3-cd)pyrene and

dibenzo(a,h)anthracene, and excellent resolution of benzo(b)fluoranthene and benzo(k)fluoranthene, while still keeping the analysis time under 18 minutes. Because the column flow rate and temperature program for both columns is the same, the analysis can be run simultaneously on the primary and confirmation columns.

Conclusion

PAH analysis by US EPA Method 8100 can be improved by choosing the appropriate analytical columns and by optimizing the temperature program, carrier gas, and column flow rates. When operating under the conditions listed for Figures 1 and 2, the Rtx®-5Sil MS and the Rtx®-CLPesticides2 columns yield excellent resolution and short analysis times for PAHs.

Figure 1—The Rtx®-CLPesticides column is an excellent confirmational column for polycyclic aromatic hydrocarbon analysis.



Rtx®-5Sil MS Columns (-60 to 330/350°C)

30m	0.25mm ID	0.25µm	cat.# 12723
30m	0.32mm ID	0.25µm	cat.# 12724

Rtx®-CLPesticides2 Columns (-60 to 310/330°C)

30m	0.25mm ID	0.20µm	cat.# 11323
30m	0.32mm ID	0.25µm	cat.# 11324

Method 610—Polynuclear Aromatic Hydrocarbons Mix

acenaphthene	chrysene
acenaphthylene	dibenzo(a,h)anthracene
anthracene	fluoranthene
benzo(a)anthracene	fluorene
benzo(a)pyrene	indeno(1,2,3-cd)pyrene
benzo(b)fluoranthene	naphthalene
benzo(k)fluoranthene	phenanthrene
benzo(ghi)perylene	pyrene

2,000µg/mL each in CH₂Cl₂, 1mL/ampul

	Each	5-pk.	10-pk.
	31011	31011-510	
w/data pack	31011-500	31011-520	31111

Inlet Liners

For Agilent GCs

2mm Splitless w/Wool	(2.0mm ID, 6.5mm OD, 78.5mm length)	
20712-200.1 (ea.)	20713-200.5 (5-pk.)	20714-200.25 (25-pk.)

4mm Splitless w/Wool	(4.0mm ID, 6.5mm OD, 78.5mm length)	
22400 (ea.)	22401 (5-pk.)	22402 (25-pk.)

For CE Instruments/ThermoQuest GCs TRACE™ Series

3mm Splitless w/Wool	(3.0mm ID, 8.0mm OD, 105mm length)	
20942-200.1 (ea.)	20943-200.5 (5-pk.)	20944-200.25 (25-pk.)



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Separating *m*- and *p*-Xylene Isomers by US EPA Method 8260 Using an Rtx®-200 GC Column

Xylenes are aromatic hydrocarbons that naturally occur in petroleum and coal tar; they also can be commercially derived from these substrates. Three xylene isomers exist: *meta*-, *para*-, and *ortho*-xylene, usually referred to as *m*-, *p*-, and *o*-xylene, respectively. Mixed xylenes produced from petroleum contain 20% *o*- and *p*-xylene, with 44% *m*-xylene.¹ The isomers of *m*- and *p*-xylene are difficult to resolve using gas chromatography (GC) and most capillary columns. Although limited data exists suggesting toxicological differences between *m*- and *p*-xylene, there is still interest in resolving them.

The US Environmental Protection Agency (EPA) does not require separation of the xylene isomers, but rather requests their calculation as totals or sums.² Some states such as New York have action limits based on *m*- and *p*-xylene separately. For example the action limit in drinking water is 5µg/L for *m*-xylene and 5µg/L for *p*-xylene. A drinking water sample that has 9µg/L of total xylenes could, in fact, exceed the limits by having 9µg/L of *m*-xylene and no *p*-xylene present in the sample.³ However, other states and agencies would consider the action limit for these two isomers as 10µg/L total.

A recent performance evaluation from New York state contained one of the isomers of xylene, which required the contracted environmental laboratories to determine actual concentrations of *m*- and *p*-xylene separately. The most common way to perform a GC separation of *m*- and *p*-xylene is by using a polyethylene glycol (PEG) stationary phase, such as the Restek Stabilwax® column. Chromatographically, baseline separation is possible; however, bleed levels are unacceptable for a mass spectrometer (MS) and sample matrices containing organic acids can contribute to bleed from the stationary phase.

The more ideal column choice for this particular separation is the Rtx®-200 column. The Crossbond® trifluoropropylmethyl polysiloxane stationary phase (**Figure 1**) features exceptionally low bleed at common volatile application working temperatures because its maximum operating temperature is 360° C.

The Rtx®-200 column provides unique separation of volatile organic compounds (VOCs) listed in US EPA Methods 524 and 8260 (**Figure 2**), making it the best column to separate xylene isomers for specific state

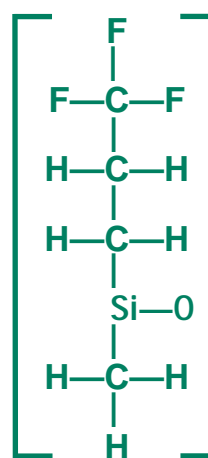
requirements (**Figure 3**). The Rtx®-200 column's only limitation is the resolution of the gases—peaks 2, 3, 5, and 6 (**Figure 2**).

This column also is a good choice for analyzing samples having complex matrices; analyses where coelutions of several compounds can make identification of tentatively identified compounds (TICs) nearly impossible on a “624” phase. Trifluoropropyl stationary phases like that of the Rtx®-200 column have a unique selectivity because of the electrophilic nature of the fluorine-containing polymer. This creates interactions with electron-rich molecules like ketones and halogenated compounds. This unique selectivity results in different elution orders and resolves compounds that phenyl, cyano and methyl phases cannot. In this analysis, the Rtx®-200 column can be used to confirm TICs and resolve multiple coelutions.

References

1. *Toxicological Profile for Total Xylenes*. Prepared by Clements Associates, Inc., under Contract No. 205-88-0608. Prepared for Agency for Toxic Substances and Disease Registry, US Public Health Services, Atlanta, GA. December 1990.
2. *US EPA Method 8000B, Rev. 2. Determinative Chromatographic Separations*. Page 7 Section 3.3.3. Washington, DC. December 1996.
3. *Consumer Confidence Report*. New York Water Service Corporation. 60 Brooklyn Avenue Merrick, New York, NY. September 1999.

Figure 1—Phase Structure
Rtx®/MXT®-200 trifluoropropylmethyl polysiloxane

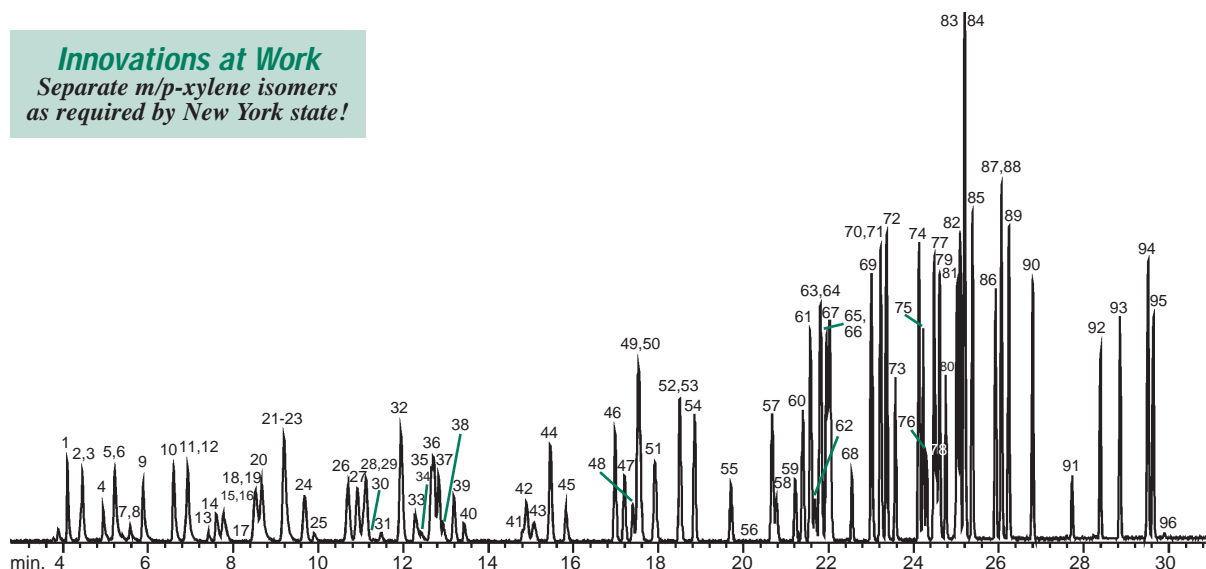


Polarity: selective for lone pair electrons.
Uses: environmental samples, solvents, Freon® samples, drugs, ketones and alcohols.

Figure 2

The Rtx[®]-200 column provides unique separation of the VOCs listed in US EPA Method 8260.

Innovations at Work
Separate *m/p*-xylene isomers
as required by New York state!



60m, 0.25 mm ID, 1.0µm Rtx-200 (cat.# 15056)
Compounds in at 10 ppb in 5mL of RO water.
Ketones, alcohols in at 2x (unless otherwise noted).

Concentrator: Tekmar LSC-3100 Purge and Trap
Trap: Vocarb 3000 (type K)
Purge: 11 min. @ 40mL/min. @ ambient temperature
Dry Purge: 1 min. @ 40mL/min.
Desorb Preheat: 245°C
Desorb: 250°C for 2 min., flow 10mL/min.
Bake: 260°C for 8 min.
Interface: transfer line 0.53mm ID Silcosteel MXT tubing

Oven Program: 40°C (hold 10 min.) to 100°C @ 6°C/min.
(hold 1 min.) to 210°C @ 30°C/min. (hold 7 min.)

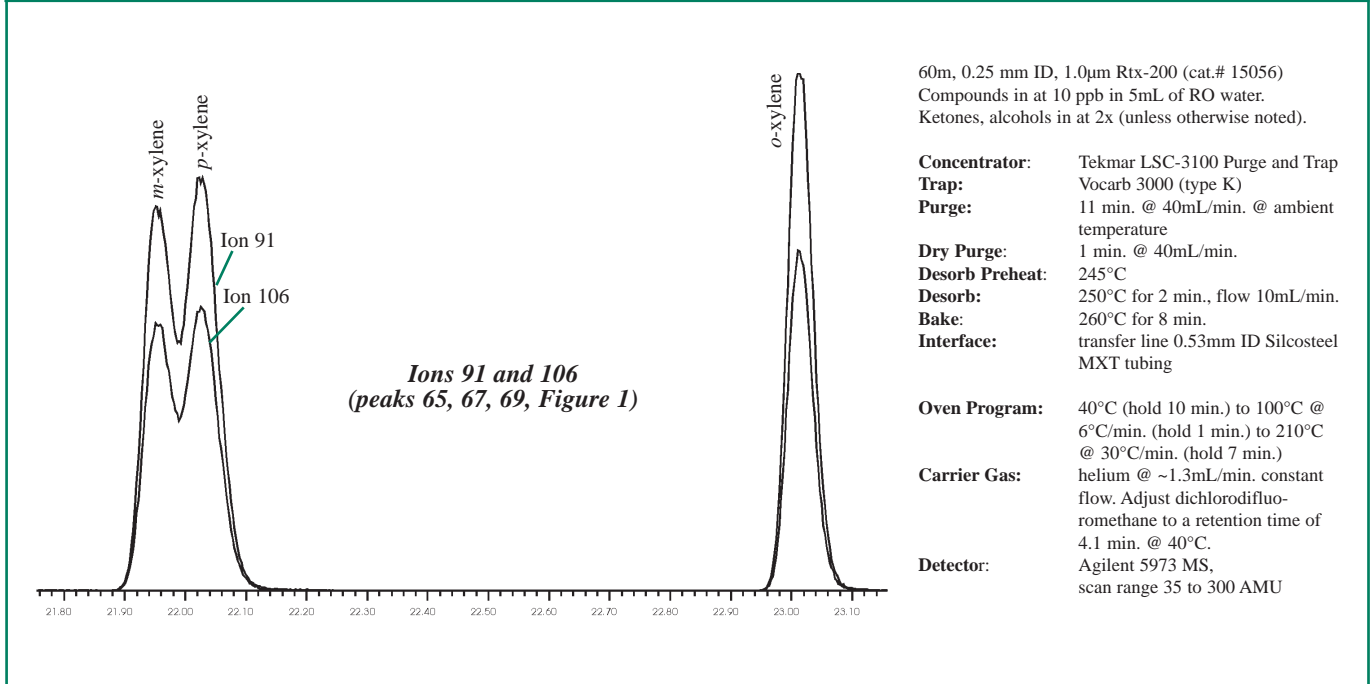
Carrier Gas: helium @ ~1.3mL/min. constant flow
Adjust dichlorodifluoromethane to a retention time of 4.1 min. @ 40°C.

Detector: Agilent 5973 MS, scan range 35 to 300 AMU

1. dichlorodifluoromethane	25. acetone	49. toluene	73. 1,1,2,2-tetrachloroethane
2. chloromethane	26. 2,2-dichloropropane	50. toluene-d8	74. <i>n</i> -propylbenzene
3. vinyl chloride	27. 1,1,1-trichloroethane	51. tetrachloroethene	75. bromobenzene
4. bromomethane	28. vinyl acetate	52. 1,1,2-trichloroethane	76. 4-bromo-1-fluorobenzene
5. chloroethane	29. 1,1-dichloropropene	53. <i>n</i> -propyl acetate	77. 1,3,5-trimethylbenzene
6. trichlorofluoromethane	30. isobutyl alcohol (500ppb)	54. <i>trans</i> -1,3-dichloropropene	78. pentachloroethane
7. carbon disulfide	31. acrylonitrile	55. 1,2-dibromoethane	79. 2-chlorotoluene
8. ethanol (2500ppb)	32. benzene	56. pyridine (250ppb)	80. 1,2,3-trichloropropane
9. 1,1-dichloroethene	33. <i>tert</i> -amyl-methyl ether	57. 1,3-dichloropropane	81. 4-chlorotoluene
10. methylene chloride	34. tetrahydrofuran	58. ethyl methacrylate	82. <i>tert</i> -butylbenzene
11. allyl chloride	35. 1,2-dichloroethane	59. bromoform	83. 1,2,4-trimethylbenzene
12. <i>trans</i> -1,2-dichloroethene	36. trichloroethene	60. 1,1,1,2-tetrachloroethane	84. <i>sec</i> -butylbenzene
13. <i>tert</i> -butyl alcohol (100ppb)	37. bromodichloromethane	61. ethylbenzene	85. <i>p</i> -isopropyltoluene
14. methyl <i>tert</i> -butyl ether	38. methyl acrylate	62. 4-methyl-2-pentanone	86. 1,3-dichlorobenzene
15. allyl alcohol (250ppb)	39. dibromomethane	63. chlorobenzene	87. 1,2-dichlorobenzene-d4
16. diisopropyl ether	40. ethyl acetate	64. chlorobenzene-D5	88. 1,4-dichlorobenzene
17. propargyl alcohol (500ppb)	41. 2-butanone	65. <i>m</i> -xylene	89. <i>n</i> -butylbenzene
18. 1,1-dichloroethane	42. 1,4-difluorobenzene	66. 2-picoline (250ppb)	90. 1,2-dichlorobenzene
19. ethyl- <i>tert</i> -butyl ether	43. pentafluorobenzene	67. <i>p</i> -xylene	91. 1,2-dibromo-3-chloropropan
20. chloroform	44. 1,2-dichloropropane	68. <i>n</i> -butyl acetate	92. hexachlorobutadiene
21. dibromofluoromethane	45. isopropyl acetate	69. <i>o</i> -xylene	93. 1,2,3-trichlorobenzene
22. <i>cis</i> -1,2-dichloroethene	46. <i>cis</i> -1,3-dichloropropene	70. styrene	94. naphthalene
23. bromochloromethane	47. dibromochloromethane	71. 2-hexanone	95. 1,2,4-trichlorobenzene
24. carbon tetrachloride	48. methyl methacrylate	72. isopropylbenzene	96. nitrobenzene (250ppb)

Figure 3

An extracted ion chromatogram shows the Rtx[®]-200 column is the best column choice to separate m/p-xylene isomers.



Product Listing:

Coiled Silcosteel[®] Tubing

Silcosteel [®] -Treated Welded/Drawn Grade 304 Stainless Steel Tubing	
<i>Sold by the foot—5 ft. minimum.</i>	
cat.#	ID, OD
20590	0.011" ID (0.28mm ID), 0.022" OD (0.56mm OD)
20591	0.021" ID (0.53mm ID), 0.029" OD (0.74mm OD)
20592	0.010" ID (0.25mm ID), 1/16" OD (1.59mm OD)
20593	0.020" ID (0.51mm ID), 1/16" OD (1.59mm OD)
20594	0.030" ID (0.76mm ID), 1/16" OD (1.59mm OD)
20595	0.040" ID (1.02mm ID), 1/16" OD (1.59mm OD)
<i>0.020" wall:</i>	
20596	0.085" ID (2.16mm ID), 1/8" OD (3.18mm OD)
<i>0.020" wall:</i>	
20597	0.210" ID (5.33mm ID), 1/4" OD (6.35mm OD)

Silcosteel [®] -Treated Seamless 316 Grade Stainless Steel Tubing	
<i>Sold by the foot—5 ft. minimum.</i>	
cat.#	ID, OD
<i>0.035" wall:</i>	
20598	0.055" ID (1.40mm ID), 1/8" OD (3.18mm OD)
<i>0.035" wall:</i>	
20599	0.180" ID (4.57mm ID), 1/4" OD (6.35mm OD)

Call for availability of lengths greater than 1000 ft.

*Metric conversion: 6 ft. (1.8m), 25 ft. (7.6m), 50 ft. (15.2m), 200 ft. (61m), >400 ft. (>122m)

Other lengths and sizes of Silcosteel[®] tubing are available on a custom basis!

Straight Silcosteel[®] Tubing

0.085" ID (2.16mm), 1/8" OD (3.18mm)		
Length	Individual	5-Pack
18" (457mm)	20575	20576

0.210" ID (5.33mm), 1/4" OD (6.35mm)		
Length	Individual	5-Pack
18" (457mm)	20577	20578

Minimum Bend Radius (dependent on OD)

OD	Min. Bend Radius
≤1/16"	1"
1/8"	2"
1/4"	4"

Column product listing continued on back.

Restek Australian Distributor • Chromalytic Technology
(03)9762 2034 • Fax (03) 9761 1169
• www.chromttech.net.au

Product Listing:

■ Rtx®-200 (Fused Silica)

(Crossbond® trifluoropropylmethyl polysiloxane) Stable to 360°

ID	df (µm)	temp. limits*	15-Meter	30-Meter	60-Meter	105-Meter
0.25mm	0.10	-20 to 320/340°C	15005	15008	15011	
	0.25	-20 to 320/340°C	15020	15023	15026	15029
	0.50	-20 to 310/330°C	15035	15038	15041	15044
	1.00	-20 to 290/310°C	15050	15053	15056	15059
0.32mm	0.10	-20 to 320/340°C	15006	15009	15012	
	0.25	-20 to 320/340°C	15021	15024	15027	15030
	0.50	-20 to 310/330°C	15036	15039	15042	15045
	1.00	-20 to 290/310°C	15051	15054	15057	15060
	1.50	-20 to 280/300°C	15066	15069	15072	15075
0.53mm	0.10	-20 to 310/330°C	15007	15010	15013	
	0.25	-20 to 310/330°C	15022	15025	15028	
	0.50	-20 to 300/320°C	15037	15040	15043	
	1.00	-20 to 290/310°C	15052	15055	15058	
	1.50	-20 to 280/300°C	15067	15070	15073	
	3.00	-20 to 260/280°C	15082	15085	15088	15091
ID	df (µm)	temp. limits	10-Meter	20-Meter	40-Meter	
0.18mm	0.20	-20 to 310/330°C	45001	45002	45003	
	0.40	-20 to 310/330°C	45010	45011	45012	

■ MXT®-200 (Silcosteel®)

(Crossbond® trifluoropropylmethyl polysiloxane) Stable to 360°

ID	df (µm)	temp. limits*	15-Meter	30-Meter	60-Meter
0.25mm	0.50	-20 to 330°C	75035	75038	
	1.00	-20 to 310°C	75050	75053	
0.53mm	1.00	-20 to 290/310°C	75052	75055	75058
	1.50	-20 to 280/300°C	75067	75070	75073
	3.00	-20 to 260/280°C	75082	75085	75088
ID	df (µm)	temp. limits*	10-Meter	20-Meter	40-Meter
0.18mm	0.20	-20 to 310/330°C	71881	71882	71883
	0.40	-20 to 310/330°C	71884	71885	71886

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

Trademarks: Stabilwax, Silcosteel, Rtx, Crossbond (Restek).
Vocarb, (Supelco), Freon (E.I. du Pont de Nemours & Co., Inc.)

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Techniques to Optimizing GC Analysis of Ethylene Glycol in Water

The analysis of ethylene glycol in water is a very common test in environmental laboratories. Many of these samples originate from water runoff at airports where ethylene glycol is used as a de-icing agent for airplanes during winter months. Because ethylene glycol is highly soluble in water, it is not easily concentrated by purge and trap. Therefore, the most frequently used sample introduction technique is direct aqueous injection. The direct aqueous injection of ethylene glycol can be challenging because, if not done properly, it can be difficult to attain reproducibility and good peak shape. The large expansion volume of water can cause backflash, carryover can cause inconsistent results, and excess water can extinguish the FID flame. These problems can prevent achieving the detection limit for ethylene glycol, which may vary in the 1-10ppm range.

Poor Peak Shape

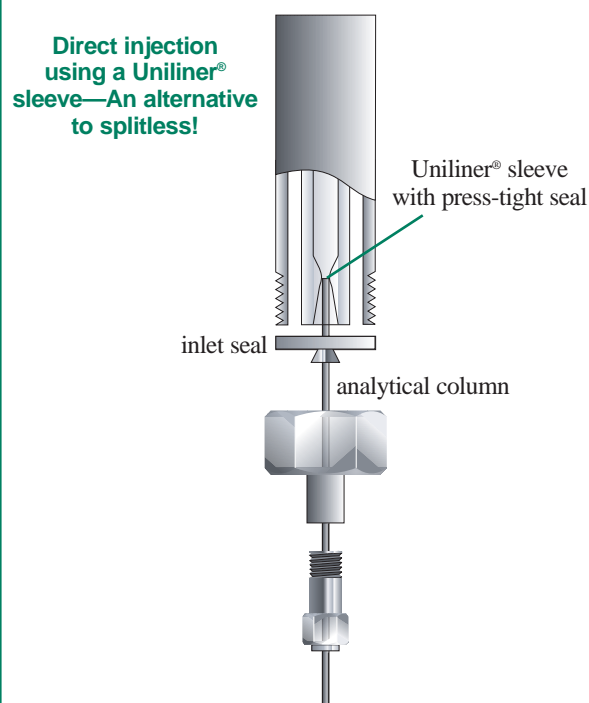
With a column head pressure of 10psig and an injection port temperature of 250°C, a 1µL injection of water will expand to 1420µL of vapor. This large vapor cloud exceeds the volume of most inlet liners, causing backflash. If backflash occurs, the vapor cloud can expand out of the liner and injection port and result in poor sample transfer onto the column. Also, the glycol compounds are not focused in a narrow band but, instead, are focused in the condensed water that beads onto the column walls, so the compounds of interest can elute as split peaks. This peak splitting effect is most apparent when performing a splitless injection because of the solvent focusing required. Split peaks and backflash compromise the analysis by causing irreproducible peak shapes.

One technique to reduce the effect of vapor expansion and poor solvent focusing is the use of a Uniliner® injection port sleeve. This sleeve forms a leak-free connection with the column end (Figure 1), thereby ensuring a complete sample transfer. Additionally, the Uniliner® sleeve requires operation at a higher pressure than traditional splitless liners, which forces the large vapor cloud to be focused into a narrow band when entering the column. This minimizes sample backflash and eliminates the need for solvent focusing. By using a Uniliner® sleeve, the aqueous ethylene glycol sample is completely vaporized and properly transferred to the column in a focused, narrow band, thereby achieving reproducible peak areas. Uniliner® sleeves are available for conversion of packed column injection systems and for split/splitless injection systems.

Sample Residue Carryover

Carryover is another problem associated with ethylene glycol analysis. When analyzing glycols, carryover can be caused by sample residue in the syringe being carried over from one injection to another. If the syringe is not properly cleaned between analyses, carryover will cause inconsistent results.

Figure 1
The Uniliner® sleeve forms a leak-free connection, minimizes backflash, and helps focus the sample.



Rinsing the syringe with either water or water/methanol (50:50) three to six times between each injection will eliminate sample residue and minimize the possibility of carryover.

FID Flameout

Column stationary phase choice is a critical consideration when analyzing glycols in water via direct injection. Water analyzed on a non-polar stationary phase, such as the Rtx®-1 column, or on a moderately polar stationary phase, such as the Rtx®-200 column, will cause the flame on the FID to be extinguished. This is because the water will not partition properly and will “bead up” on the phase, producing a large plug of water that passes through the detector and extinguishes the flame. The more commonly-used GCs will experience flameout under these circumstances while others will not.

To minimize the possibility of extinguishing the flame, select a polar stationary phase that is more compatible with water. The Stabilwax® stationary phase is one of the more polar phases, making it a good choice for water injections. It allows water to partition properly, which prevents it from beading up on the stationary phase and quenching the FID flame.

The Rtx®-Stabilwax® column can easily handle direct aqueous injections without showing any signs of degradation. Testing of the Rtx®-Stabilwax® column was performed by injecting 1µL of a water standard 100 times. Peak shape and response of ethylene and propylene glycol remained consistent throughout the analyses (**Figures 2 and 3**). The Rtx®-Stabilwax® column also allows sensitive detection of low ppm-levels of glycol compounds. Notice the 5ppm detection limit for ethylene glycol in water is easily achieved, and peak shape is maintained when compared to a 25ppm standard (**Figure 4**).

Conclusion

You can achieve better response and reproducibility for the GC analysis of ethylene glycol in water by using direct injection with a Uniliner® sleeve, a polar capillary column such as Stabilwax®, and multiple syringe washes between runs. Using these techniques can assist in attaining reproducible analyses with detection limits in the low ppm range.

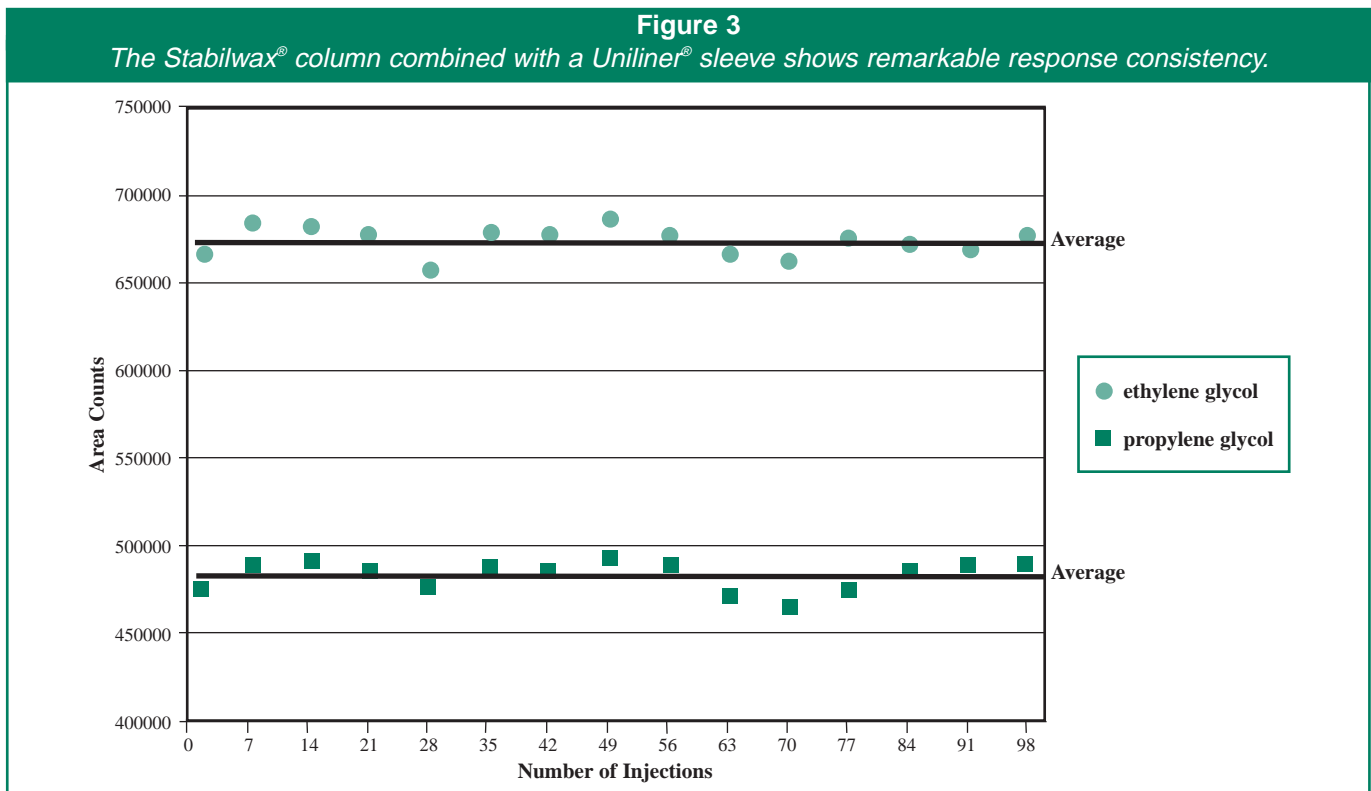
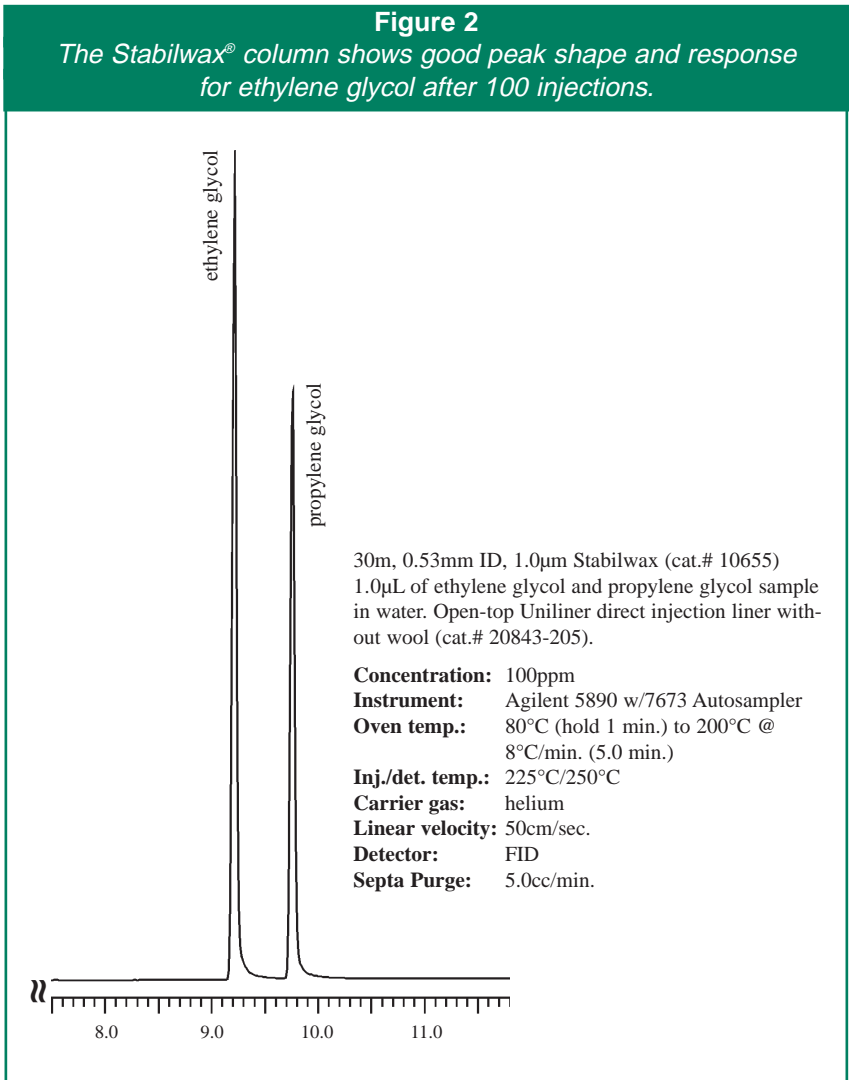


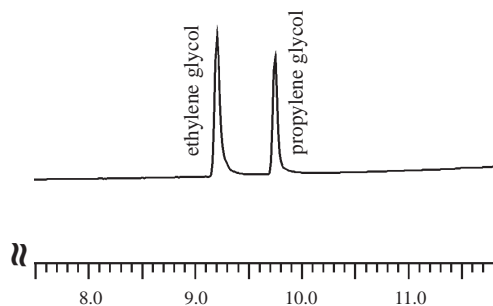
Figure 4

The Stabilwax[®] column can easily analyze 5ppm and 25ppm standards.

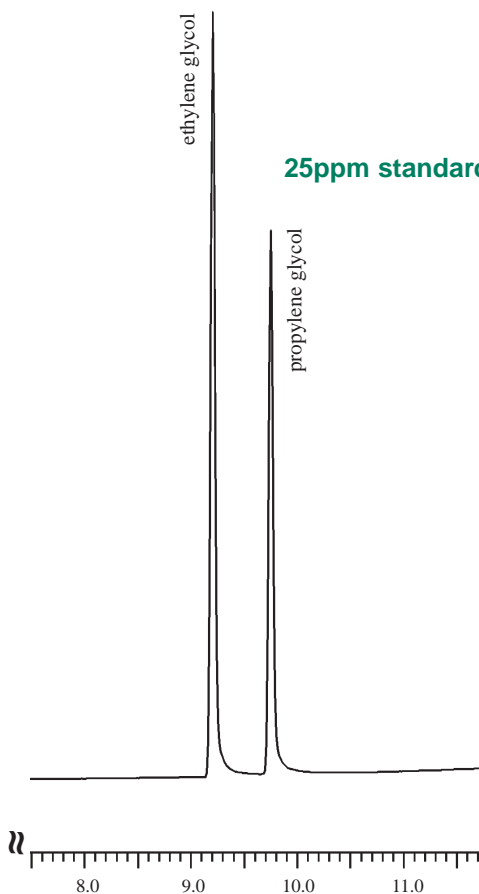
30m, 0.53mm ID, 1.0 μ m Stabilwax (cat.# 10655)
 1.0 μ L of ethylene glycol and propylene glycol sample
 in water. Open-top Uniliner direct injection liner with-
 out wool (cat.# 20843-205).

Concentration: 5ppm and 25ppm
Instrument: Agilent 5890 w/7673 Autosampler
Oven temp.: 80°C (hold 1 min.) to 200°C @
 8°C/min. (5.0 min.)
Inj./det. temp.: 225°C/250°C
Carrier gas: helium
Linear velocity: 50cm/sec.
Detector: FID
Septa Purge: 5.0cc/min.

5ppm standard



25ppm standard



Product Listing:

■ Rtx[®]-Stabilwax[®] Columns

30m	0.32mm ID	1.0 μ m	cat.# 10654
30m	0.53mm ID	1.0 μ m	cat.# 10655

■ Uniliner[®] Sleeves

Description	Column ID Inj. Mode	Each*	5-pack
<i>Uniliner[®] Sleeve</i> (large buffer volume chamber—85mm long for injections $\leq 4\mu$ L)	0.32 & 0.53mm DI only	20308	20309
	0.53mm DI or OC	20301	20305
<i>Cyclo-Uniliner[®] Sleeve</i> (for active dirty samples)	0.32 & 0.53mm DI only	20319	20320
<i>Open-Top Uniliner[®] Sleeve</i> (packed with fused silica wool)	0.32 & 0.53mm DI only	20315	20316
<i>Uniliner[®] Sleeve Adaptor</i> (required for installing Uniliner [®] sleeves in 1/4" injection ports)	includes a 1/4" SS nut and graphite ferrule, a 1/16" SS nut, and a 0.8mm ID graphite ferrule. For injection ports <8cm: cat.# 20310 ea. For injection ports 8-15cm: cat.# 20311 ea. For Shimadzu: cat.# 20312 ea.		

*Add the suffix "-205" to the catalog number to order without wool.

continued on back

Product Listing, continued:

■ Uniliner® Sleeves

Direct Injection Liners for Agilent & Finnigan GCs (0.32/0.53mm ID)		ID ^{***} /OD & Length (mm)	Each*	5-pack
	Benefits/Uses:			
<i>Uniliner</i> ^{®†}	trace, active samples, high recovery & linearity	4.0 ID, 6.3 OD x 78.5	20335	20336
<i>Cyclo-Uniliner</i> ^{®†}	trace, dirty, high MW active samples, high recovery & linearity	4.0 ID, 6.3 OD x 78.5	20337	20338
<i>Open-top Uniliner</i> [®] with Wool [†]	trace, dirty active samples, high recovery & linearity	4.0 ID, 6.3 OD x 78.5	20843	20844

Direct Injection Liners for Agilent NEW! 6890 GCs (0.32/0.53mm ID)		ID ^{***} /OD & Length (mm)	Each*	5-pack
	Benefits/Uses:			
<i>Drilled Uniliner</i> ^{®†}	allows direct injection when using an EPC-equipped GC	4.0 ID, 6.3 OD x 78.5	21054	21055

Direct Injection Liners for Varian GCs (0.32/0.53mm ID)		ID ^{***} /OD & Length (mm)	Each*	5-pack
	Benefits/Uses:			
<i>Uniliner</i> [®]	trace, active samples, high recovery & linearity	4.0 ID, 6.3 OD x 72	20345	20346
<i>Cyclo-Uniliner</i> [®]	trace, dirty, high MW, active samples, linearity	4.0 ID, 6.3 OD x 72	20347	20348
<i>Open-top Uniliner</i> [®] w/ Wool ^{**}	trace, dirty, active samples, high recovery & linearity	4.0 ID, 6.3 OD x 72	20845	20846

Direct Injection Liners for Shimadzu GCs (0.32/0.53mm ID)		ID ^{***} /OD & Length (mm)	Each*	5-pack
	Benefits/Uses:			
<i>128mm Uniliner</i> [®]	trace, active samples, high recovery & linearity	3.0 ID, 5.0 OD x 128	20872	20873
<i>128mm Cyclo-Uniliner</i> [®]	trace, dirty, high MW active samples, linearity	3.5 ID, 5.0 OD x 128	20874	20875
<i>99mm Uniliner</i> [®]	trace, active samples, high recovery & linearity	3.0 ID, 5.0 OD x 99	20876	20877
<i>99mm Cyclo-Uniliner</i> [®]	trace, dirty, high MW active samples, high recovery & linearity	3.0 ID, 5.0 OD x 99	20893	20894
<i>94mm Uniliner</i> [®] w/ Wool ^{**}	trace, dirty, high MW active samples, high recovery & linearity	3.0 ID, 5.0 OD x 94	21713	21719

Direct Injection Liners for Perkin-Elmer GCs (0.32/0.53mm ID)		ID ^{***} /OD & Length (mm)	Each*	5-pack
	Benefits/Uses:			
<i>Uniliner</i> [®]	trace, active samples, high recovery & linearity	3.5 ID, 5.0 OD x 100	20855	20856
<i>Cyclo-Uniliner</i> [®]	trace, dirty, active samples, linearity	3.5 ID, 5.0 OD x 100	20857	20858
<i>Auto SYS Open-top Uniliner</i> [®] w/ Wool ^{**}	trace, dirty, active samples, high recovery & linearity	4.0 ID, 6.2 OD x 92.1	20837	20838
<i>Auto SYS Cyclo-Uniliner</i> [®]	trace, dirty, high MW active samples, linearity	4.0 ID, 6.2 OD x 92.1	20839	20840

Split Liners for 5000-6000 Series GCs		ID ^{***} /OD & Length (mm)	Each*	5-pack
	Benefits/Uses:			
<i>Open-top Uniliner</i> [®] w/ Wool ^{**}	trace, dirty, active samples, high recovery & linearity	4.0 ID, 5.5 OD x 79.5	20841	20842

Direct injection Liners for 8000 & TRACE™ Series GCs		ID ^{***} /OD & Length (mm)	Each*	5-pack
	Benefits/Uses:			
<i>Uniliner</i> [®] w/ Wool	trace, active samples, high recovery & linearity	5.0 ID, 8.0 OD x 105	21005	21006

*Add the suffix "-205" to the catalog number to order without wool.

**These liners are packed with fused silica wool. To order glass wool instead, add the suffix "-202" to the liner's catalog number.

***Nominal ID at syringe needle expulsion point.

†These Uniliner® sleeves are for split/splitless injection ports.

Trademarks: Uniliner, Rtx, and Stabilwax (Restek). TRACE (ThermaQuest Corp.).

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Extraction of polynuclear aromatic hydrocarbons (PAHs) using Resprep™-18-47 SPE disks and Resprep™-6D extraction system

Sample Pretreatment

Allow 1L of deionized water to equilibrate to room temperature. Adjust sample pH to less than 2 with 6N HCl. Add 5mL of methanol and mix thoroughly. For QA/QC samples, spike with 1mL of a 2µg/mL solution for a final concentration in the water of 2ppb.

Apparatus Assembly

Assemble the Diskcover™-47 extraction disk holder with reservoir, making sure the Teflon® o-ring is in place. Also be sure to place the C18 disk in the Diskcover™-47 extraction disk holder *wrinkled side up*. Assemble the Resprep™-6D extraction system as detailed in its instruction sheet, along with the vacuum pump, vacuum line, and vacuum trap.

Disk Precleaning

Add 10mL of methylene chloride to the top surface of the disk and immediately draw it through under vacuum at 15 in. Hg. Continue to draw vacuum at 15 in. Hg for 5 minutes, removing all solvent.

Disk Conditioning

Add 10mL of methanol to the top surface of the disk and let stand for a few minutes. Draw the methanol through the disk until the methanol's top surface is just above the disk. **Do not allow any air to pass through the disk or reach the top surface of the disk.** Note: It is preferable to leave extra liquid above the disk rather than allow any air to contact the disk surface.

Sample Addition

Pour the sample into the Diskcover™-47 reservoir, adding it directly to the film of methanol left on the disk from the conditioning step. Adjust the vacuum to approximately 25 in. Hg.

Disk Drying

After the entire sample has been processed, draw air through the disk under vacuum to remove any residual water from the disk.

Analyte Elution

Release system vacuum. Insert the collection rack and vessels into the glass chamber, making sure to label each vessel with the corresponding Diskcover™-47. Reassemble the Resprep™-6D extraction system. Add 5mL methylene chloride directly to the sample bottle, and gently swirl to rinse all inner surfaces of the bottle. Transfer the methylene chloride to the disk using a glass pipette and rinse the reservoir sides in the process. Let the solvent stand for three minutes. Draw the solvent through the disk at 5 in. Hg. Repeat the bottle rinse and disk elution once more with a fresh aliquot of methylene chloride, combining all eluates in the collection tube.



Resprep™ sample preparation products allow complete, clean sample extraction for more accurate PAH analyses.

Table I

Accuracy/precision data from seven determinations of PAHs in distilled water at 2µg/L
using the Resprep™ -C18-47 glass fiber extraction disk.

Compounds	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Average	SD
naphthalene	65.7	78.8	73.7	67.7	73.5	80.8	83.0	74.7	6.5
2-methylnaphthalene	71.9	88.0	84.3	77.3	86.3	97.5	91.6	85.3	8.6
1-methylnaphthalene	75.2	90.9	86.1	81.2	87.2	99.5	96.2	88.0	8.4
biphenyl	77.1	95.0	90.5	84.1	91.5	103.2	102.1	91.9	9.3
2,6-dimethylnaphthalene	69.9	84.3	85.3	80.1	82.9	90.2	86.4	82.7	6.5
acenaphthylene	70.5	98.4	90.8	76.2	90.6	100.6	99.7	89.5	11.9
acenaphthene	83.5	102.6	94.9	90.4	97.2	104.3	106.2	97.0	8.2
2,3,6-trimethylnaphthalene	71.2	89.7	85.7	82.6	88.8	84.9	79.5	83.2	6.3
fluorene	83.8	109.0	106.8	95.6	100.9	107.4	107.3	101.5	9.1
phenanthrene	91.7	105.4	110.2	106.6	108.6	108.3	111.9	106.1	6.7
anthracene	81.5	102.0	89.3	77.7	79.4	94.2	98.6	89.0	9.7
2-methylphenanthrene	91.5	111.1	112.5	99.2	109.9	104.1	110.9	105.6	7.8
1-methylphenanthrene	98.3	112.7	112.0	104.6	108.5	107.1	110.1	107.6	5.0
3,6-dimethylphenanthrene	88.7	108.2	105.0	102.4	104.9	103.5	109.2	103.1	6.8
fluoranthene	94.3	112.9	114.6	106.7	111.0	106.6	115.2	108.8	7.3
pyrene	97.7	113.4	112.4	103.9	110.4	107.5	117.8	109.0	6.7
2,3-benzofluorene	101.1	108.3	100.7	91.1	96.3	100.2	106.8	100.6	5.9
benzo(a)anthracene	97.6	109.2	101.6	89.6	94.0	101.1	110.6	100.5	7.6
chrysene	106.9	110.5	103.8	93.8	97.7	104.1	112.7	104.2	6.7
benzo(b)fluoranthrene	95.0	107.1	109.4	99.2	97.4	100.5	116.1	103.5	7.6
benzo(k)fluoranthrene	106.8	107.1	108.4	96.1	99.8	99.7	110.0	104.0	5.3
benzo(e)pyrene	101.3	106.2	106.6	98.8	103.4	101.1	115.9	104.8	5.7
benzo(a)pyrene	101.4	107.2	100.0	88.2	90.4	99.2	105.2	98.8	7.1
perylene	100.9	109.2	97.3	86.1	90.2	96.8	105.7	98.0	8.1
9,10-diphenylanthracene	111.4	119.7	112.0	94.6	98.0	106.0	104.9	106.7	8.6
indeno(1,2,3-cd)pyrene	110.9	119.7	113.3	102.0	105.8	110.3	115.4	111.1	5.9
dibenzo(a,h)anthracene	118.3	125.5	112.4	94.4	107.3	108.3	115.6	111.7	9.8
benzo(g,h,i)perylene	123.4	129.5	119.8	103.2	113.4	113.7	121.3	117.8	8.5
Surrogate Standards									
naphthalene-d8	65.5	83.0	73.8	65.5	75.4	80.2	79.8	74.7	7.0
acenaphthene-d10	86.2	103.6	96.4	87.8	102.6	110.7	106.1	99.1	9.3
phenanthrene-d10	101.7	115.8	122.9	110.8	118.1	129.3	122.5	117.3	9.0
chrysene-d12	125.7	136.5	122.7	105.2	124.0	150.4	170.0	133.5	21.2
perylene-d12	109.7	118.0	105.7	89.1	100.2	105.0	119.4	106.7	10.4

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environmental

Fewer Coelutions and Faster GC Analysis of EPA Method 8095 Explosives Using the Rtx®-TNT and Rtx®-TNT2 Column Pair

The standard environmental test method for nitroaromatic, nitramine, and nitroester analysis has been US Environmental Protection Agency (EPA) Method 8330¹, which uses high performance liquid separation (HPLC) separation and ultra-violet (UV) absorption detection. However, this method suffers from high solvent usage, multiple coelutions, and long analysis times. Analysts have been interested in designing a GC method to overcome these disadvantages.

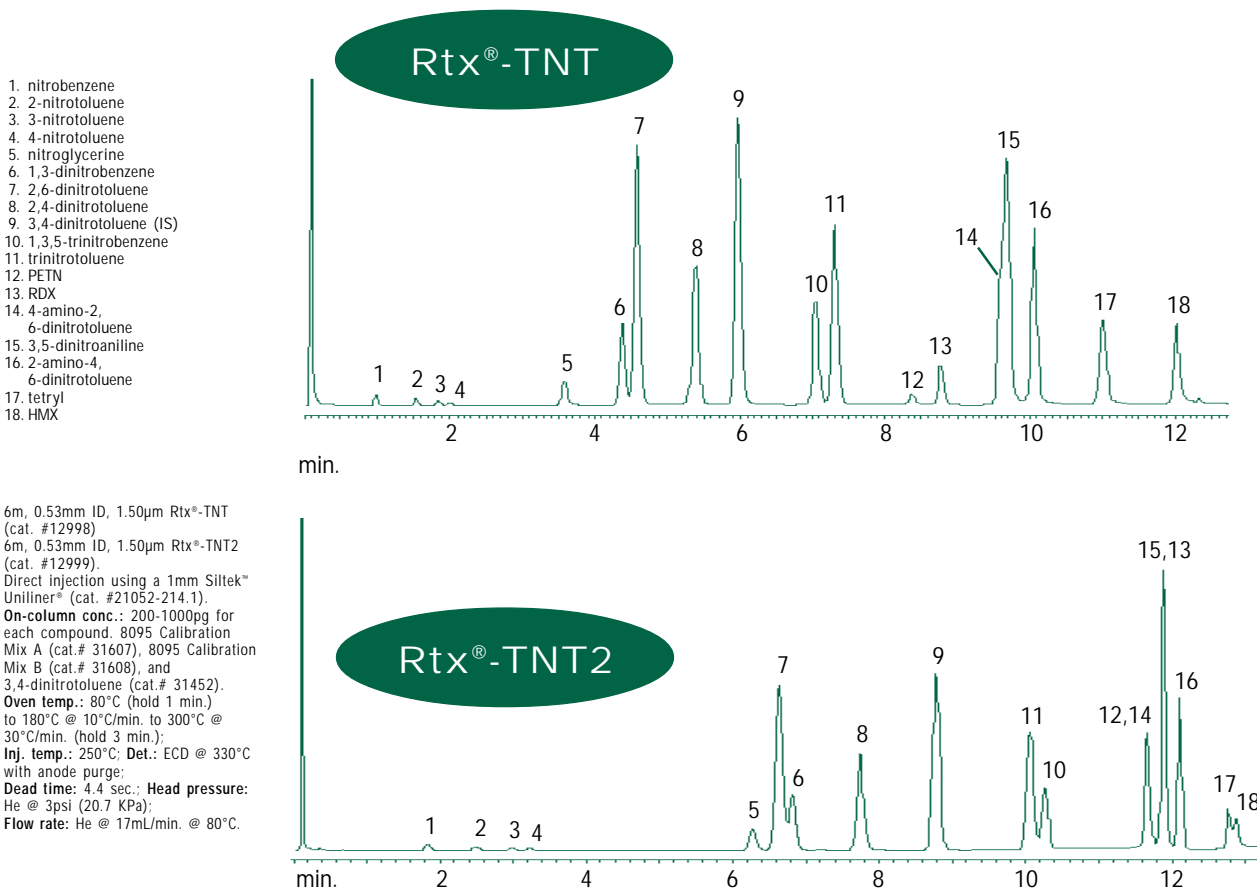
A gas chromatography/electron capture detection (GC/ECD) alternative to Method 8330 has been written as US EPA Method 8095². This GC/ECD method was developed at the U.S. Army Cold Regions Research and Engineering Laboratory³. The draft

of Method 8095 includes all Method 8330 target compounds, plus 3,5-dinitroaniline, nitroglycerine, and pentaerythritol-tetranitrate (PETN).

Restek has designed two new stationary phases specifically for Method 8095—the Rtx®-TNT and Rtx®-TNT2 columns. The TNT columns provide better resolution and higher thermal stability than any of the columns currently recommended in Method 8095. The Rtx®-TNT primary column and Rtx®-TNT2 confirmational column operate under identical GC oven temperature programs, allowing simultaneous dual-column confirmational analysis of all 16 nitroaromatic compounds (Figure 1).

Figure 1

Rtx®-TNT and Rtx®-TNT2 columns provide the best resolution of nitroaromatic explosive compounds in under 13 minutes.



environmental

Sample extraction & preparation

Water sample:

Method 8095 requires solid phase extraction disks or cartridges to extract analytes of interest from water samples, such as described in EPA Method 3535A. The extraction is performed using SDB/RPS-type disks or RDX-type cartridges. Please note that Method 8095 **does not** use the acetonitrile salting-out extraction procedure as described in Method 8330.

The use of SPE for explosives extraction requires extra drying time after the sample is loaded. Most explosive compounds have low water solubility, so the extra drying time helps their recovery into the acetonitrile elution solvent and reduces water content in the injected sample. Safety considerations prevent explosive extracts from further concentration by Kuderna-Danish concentrators. Extra drying time reduces the need for recalibration and maintenance when using water-sensitive electron capture detectors (ECD).

Soil sample:

In Method 8095, analytes of interest are extracted from soil and sediment samples using the same acetonitrile extraction with ultra-sonication procedure as described in Method 8330. Samples are air-dried at room temperature or cooler to a constant weight. Then the samples are screened with a colorimetric test (e.g., EPA Method 8515) to determine if they contain too much explosive residue to be ground safely with a mortar and pestle in preparation for the ultra-sonication extraction. If the sample contains less than 1% explosive residue, generally it is considered safe to be ground with a mortar and pestle. If so, 2g of finely ground soil are extracted using 10mL of acetonitrile in a cooled ultra-sonication bath for 18 hours. The acetonitrile extracts then are dried and filtered before analysis.

A main difference in sample preparation for GC is that the samples are not diluted 1:1 with reagent water prior to being injected, as is the case for HPLC samples.

Injection mode

Cool on-column injection:

While few laboratories routinely use a cool on-column injection port because of the perception of higher maintenance, it has several advantages over direct injection for the analysis of explosives. These advantages include complete sample transfer into the column via a highly inert pathway and reduced peak width.

Direct injection:

Most laboratories will analyze explosive samples by direct injection. Many direct injection parameters need to be optimized for successful analysis of these compounds. The injection port temperature must be determined carefully to provide good response for the thermally labile compounds, such as nitroglycerine, and sufficient vaporization for the high boiling compounds, such as HMX. For most analyses, an injection port temperature between 250–275°C is best. Restek offers a unique Siltek™-deactivated sleeve to meet the needs of explosive analysis by direct injection. The internal diameter (ID)

of the sleeve was reduced from the typical 4mm ID to a new 1mm ID (cat.# 21052-214.1). This reduction of dead volume in the injection port reduces peak broadening. Also, the Siltek™ deactivation process improves reproducibility, and most sample residue can be solvent-rinsed so the sleeve can be reused without having to be reactivated.

Carrier gas flow

Explosive analysis by GC works best when using very high carrier gas flows. While the low-boiling compounds (e.g., nitrotoluenes) would be analyzed well at normal carrier gas flow rates, the high-boiling compounds (e.g., HMX) are analyzed best with carrier gas flow rates five to seven times the normal linear velocity. Most laboratories will find a column head-pressure of 2 to 3psi is optimum for this analysis; it provides a linear velocity of 100 to 140cm/sec. or 12 to 17mL/min..

Oven temperature programming and solvent focusing

While the method recommends an initial oven temperature of 100°C, Restek discovered improved solvent focusing and decreased peak widths by using an initial oven temperature of 80°C with acetonitrile injections.

Column recommendation

Typically, a 100% dimethyl polysiloxane phase is used for the primary column, but it results in a complete coelution of PETN and RDX, and a partial coelution of 4-amino-2,6-dinitrotoluene and 3,5-dinitroaniline. A 50% cyanopropylmethyl/50% phenylmethyl polysiloxane phase typically is used for the confirmational column, but results in poor resolution of the early and late eluting compounds and requires slow ramps and long analysis times.

The new Rtx®-TNT column provides baseline resolution of PETN and RDX, and the Rtx®-TNT and TNT2 column pair provides resolution of the nitroaromatics in the fastest analysis time compared to the recommended column pair. The columns are optimized at a 6m length to minimize surface area and contact times of thermally liable explosives.

Analytical reference material considerations

Obtaining pure, neat compounds for the preparation of calibration standards can be very difficult. Some of these compounds are not available commercially at a high enough purity for accurate analytical results. These materials can contain desensitizing agents, such as beeswax, water, plasticizers, or other manufacturing by-products. Many commercially-available explosives are shipped wet and must be dried carefully before solution preparation.

To ensure the highest quality explosive standards, Restek chemists carefully purified or synthesized all of the compounds listed in Method 8095. All compounds used to prepare these standards have 98% purity or higher. Multiple analytical techniques, including GC, HPLC, GC/MS, FTIR, and DSC, are used to verify raw material purity.

Conclusions

Using GC/ECD for explosives analysis as per Method 8095 is now an excellent alternative to HPLC analysis. Careful consideration of sample extraction, preparation, and analytical techniques will ensure successful analysis of the explosives. Restek has optimized the column stationary phases and dimensions, injection technique, and analytical reference materials to help achieve the best resolution of nitroaromatic compounds in the fastest analysis time.

Product Listing

Rtx®-TNT & Rtx®-TNT2 Columns

Column	ID	df (µm)	Temp. Limits	6-Meter
Rtx®-TNT	0.53mm	1.50	-20 to 300/310°C	12998/3-pk.
Rtx®-TNT2	0.53mm	1.50	-20 to 300/310°C	12999/3-pk.

1mm Siltek™ Uniliner®

This Uniliner® inlet sleeve is recommended for the US EPA Method 8095 analysis of explosive extracts in acetonitrile by direct injection onto 0.53mm ID columns. In this application, analysts can expect reduced peak width as compared to the standard 4mm ID Uniliner® inlet sleeves.

1mm Siltek™ Uniliner® Inlet Sleeve

cat.# 21052-214.1 (ea.) cat.# 21053-214.5 (5-pk.)

RDX Sample Extraction Tubes

- Suitable for extraction of explosive compounds from water samples.
- Similar performance to Waters™ RDX cartridges. 6mL, 500mg, **cat.# 26093 (30-pk.)**

Siltek™ Guard Columns

- Revolutionary deactivation process lowers analyte break-down to less than 1%.
- Minimizes bleed.
- Ideal for chlorinated pesticide analysis.
- Analyze tough forensic samples quickly and accurately.

Siltek™ Guard Columns

Nominal ID	Nominal OD	5-Meter	10-Meter
0.25mm	0.37 ±0.04mm	10026	10036
0.32mm	0.45 ±0.04mm	10027	10037
0.53mm	0.69 ±0.04mm	10028	10038

O-Rings

Graphite:

Graphite o-rings have excellent thermal stability and can be used at injection port temperatures up to 450°C!

	Restek cat.#	
	10-Pk.	50-Pk.
6.3mm ID for split liners	20296	20297
6.5mm ID for splitless liners	20298	20299

See Standards on the
Back Page

Thermolite® Septa—Green

- Lowest bleed on FIDs, ECDs, & MSDs.
- Each batch is tested to ensure lowest bleed.
- Excellent puncturability.
- Preconditioned and ready to use.
- Usable to 340°C inlet temperatures.

Thermolite® Septa			
Septum Diameter	25-Pk.	50-Pk.	100-Pk.
9.5mm (³ / ₈ "	20359	20360	20361
10mm	20378	20379	20380
11mm (⁷ / ₁₆ "	20363	20364	20365
12.5mm (¹ / ₂ "	20367	20368	20369
17mm	20384	20385	20386
Shimadzu Plug	20372	20373	20374

Short-Cap, 9mm Screw-Thread Vials

Fit all 2.0mL, 12 x 32mm,

Crimp-Top Vial Based Autosamplers.

- Designed with a shorter screw cap to allow the robotic autosampler to function properly.
- Features a wider opening to prevent syringe problems.
- Dimensions are effectively identical to the crimp-top vial.
- We offer a variety of colors and liner material.

Description	100-Pk.	1000-Pk.
2.0mL Clear Vial*	21140	21141
2.0mL Amber Vial*	21142	21143
2.0mL Clear Vial**	21154	21155

* w/White Graduated Marking Spot

** without Graduated Marking Spot

References

1. US Environmental Protection Agency. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846 Update III, Office of Solid Waste, Washington, DC, 1997.
2. US Environmental Protection Agency. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846, Proposed Draft Update IVB, Office of Solid Waste, Washington, DC, 1999.
3. M. E. Walsh, T. Ranney. "Determination of Nitroaromatic, Nitramine, and Nitrate Ester Explosives in Water Using Solid-Phase Extraction and Gas Chromatography-Electron Capture Detection: Comparison with High-Performance Liquid Chromatography." *Journal of Chromatographic Science*, Vol. 36, pp. 406-416, August 1998.

EPA Method 8095

8095 Calibration Mix A

2-amino-4,6-dinitrotoluene	HMX
4-amino-2,6-dinitrotoluene	RDX
1,3-dinitrobenzene	tetryl
2,4-dinitrotoluene	1,3,5-trinitrobenzene
2,6-dinitrotoluene	2,4,6-trinitrotoluene

1,000µg/mL ea. in acetonitrile, 1mL/ampul

	Each	5-pk.	10-pk.
	31607	31607-510	
w/data pack	31607-500	31607-520	31707

8095 Calibration Mix B

3,5-dinitroaniline	1,000µg/mL
nitrobenzene	5,000
nitroglycerine	5,000
2-nitrotoluene	5,000
3-nitrotoluene	5,000
4-nitrotoluene	5,000
PETN	5,000

Prepared in acetonitrile, 1mL/ampul

	Each	5-pk.	10-pk.
	31608	31608-510	
w/data pack	31608-500	31608-520	31708

Matrix Spike Solutions

8095 Matrix Spike Mix A

2-amino-4,6-dinitrotoluene	200µg/mL
4-amino-2,6-dinitrotoluene	200
1,3-dinitrobenzene	200
2,4-dinitrotoluene	200
2,6-dinitrotoluene	200
HMX	2,000
RDX	200
Tetryl	200
1,3,5-trinitrobenzene	200
2,4,6-trinitrotoluene	200

Prepared in acetonitrile, 1mL/ampul

	Each	5-pk.	10-pk.
	31609	31609-510	
w/data pack	31609-500	31609-520	31709

8095 Matrix Spike Mix B

3,5-dinitroaniline	200µg/mL
nitrobenzene	1,000
nitroglycerine	1,000
2-nitrotoluene	1,000
3-nitrotoluene	1,000
4-nitrotoluene	1,000
PETN	1,000

Prepared in acetonitrile, 1mL/ampul

	Each	5-pk.	10-pk.
	31610	31610-510	
w/data pack	31610-500	31610-520	31710

8095 Surrogates

3,4-dinitrotoluene

1,000µg/mL in methanol, 1mL/ampul

	Each	5-pk.	10-pk.
	31452	31452-510	
w/data pack	31452-500	31452-520	31552

2-methyl-4-nitroaniline

1,000µg/mL in methanol, 1mL/ampul

	Each	5-pk.	10-pk.
	31612	31612-510	
w/data pack	31612-500	31612-520	31712



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The Rtx[®]-5Sil MS Column Provides the Best Resolution for Gasoline Range Organic (GRO) Compounds Listed in Alaska Method AK101AA

The Alaska Department of Environmental Conservation (ADEC) developed a new technique for the gas chromatographic (GC) analysis of gasoline range organic (GRO) compounds in soil, water, and waste water—Method AK101AA. This method quantitates aromatic and aliphatic compounds from C6 (hexane) to C10 (decane), and is capable of a higher level of accuracy over existing GRO methods. Restek's Rtx[®]-5Sil MS column is ideal for the analysis of GRO compounds, and specifically meets the requirements of Method AK101AA.

The key difference between AK101AA and other GRO methods that use photoionization detection/flame ionization detection (PID/FID) for differentiation of aliphatic and aromatic compounds, is that AK101AA uses the C9 alkyl benzenes (e.g., methyl ethylbenzenes) as target compounds in the calibration mixture. Using specific compound identification reduces error over other GRO methods that rely only on PID ranges for the determination of aromatic compounds. This can help determine the difference between highly degraded gasoline and light components of diesel fuels, such as kerosene and arctic fuel.

Method AK101AA prevents a high bias in reporting of aromatic compounds when they are in the presence of alkenes and alkynes. These are straight-chain unsaturated compounds that can give a false positive on the PID. Because all gasoline compounds respond on the FID, the total quantitation of gasoline is achieved with this detector, and the identification of single compounds are performed with the more selective PID detector. Method AK101AA also disregards analytes eluting before C6 because these pentanes and oxygenates have similar retention and are poorly resolved.

Peak List and Conditions for Figure 1

- | | |
|--|--------------------------------|
| 1. hexane | 10. 4-bromo-fluorobenzene (ss) |
| 2. benzene | 11. <i>n</i> -propylbenzene |
| 3. α,α,α -trifluorotoluene (ss) | 12. 1-ethyl-3-methylbenzene |
| 4. toluene | 13. 1-ethyl-4-methylbenzene |
| 5. ethylbenzene | 14. 1,3,5-trimethylbenzene |
| 6. <i>m</i> -xylene | 15. 1-ethyl-2-methylbenzene |
| 7. <i>p</i> -xylene | 16. 1,2,4-trimethylbenzene |
| 8. <i>o</i> -xylene | 17. decane |
| 9. isopropylbenzene | 18. 1,2,3-trimethylbenzene |

40m, 0.45mm ID, 1.50 μ m Rtx[®]-5Sil MS (cat.# 12798). Injection of 4-bromofluorobenzene (cat.# 30026); α,α,α -trifluorotoluene (cat.# 30048), decane, hexane, and Alaska aliphatic/aromatic GRO mix (cat.# 30461). **GC:** Finnigan 9001; **Column flow:** 9mL/min.; **Concentrator:** Tekmar LSC-3000 Purge & Trap, BTEX trap; **Interface:** direct with Siltek[™] transfer line; **Oven temp.:** 40°C (hold 2 min.) to 85°C @ 4°C/min. (hold 1 min.) to 225°C @ 40°C/min. (hold 2 min.); **Det.:** FID (280°C)/PID (200°C); **Make-up flow rate:** 15mL/min.

Figure 1

The Rtx[®]-5Sil MS column resolves the alkyl benzenes and all the branched aromatic compounds listed in the Alaska GRO Method AK101AA providing more accurate identification and quantitation.

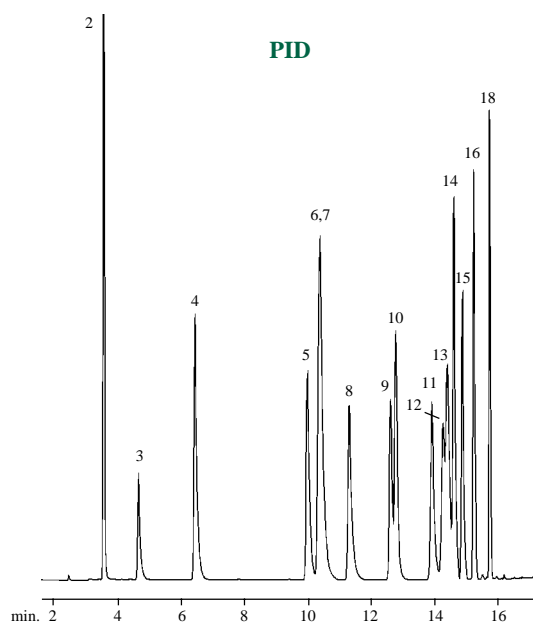
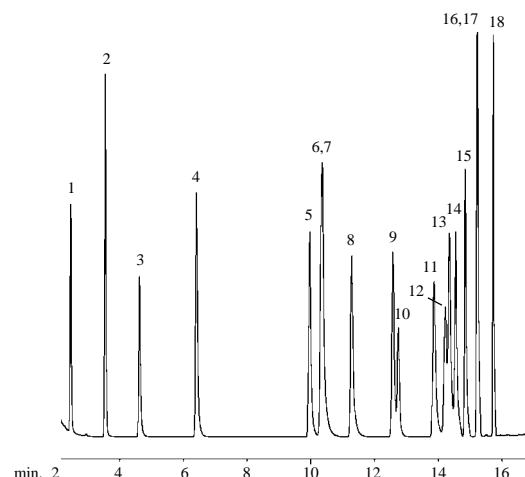
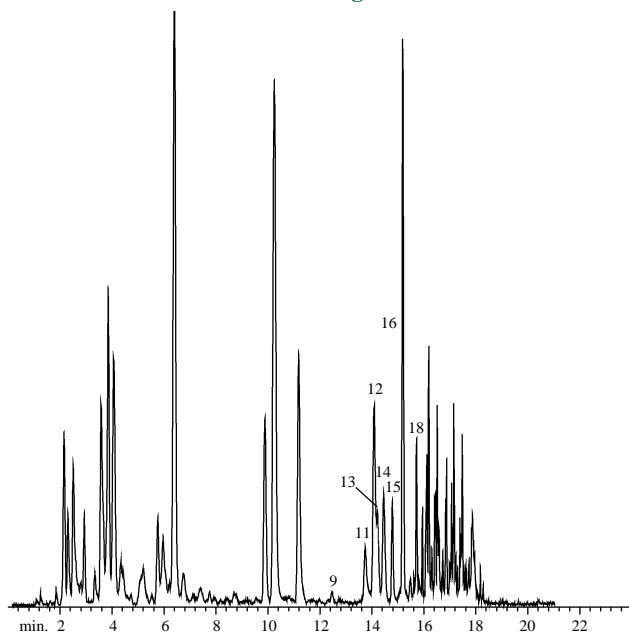

FID


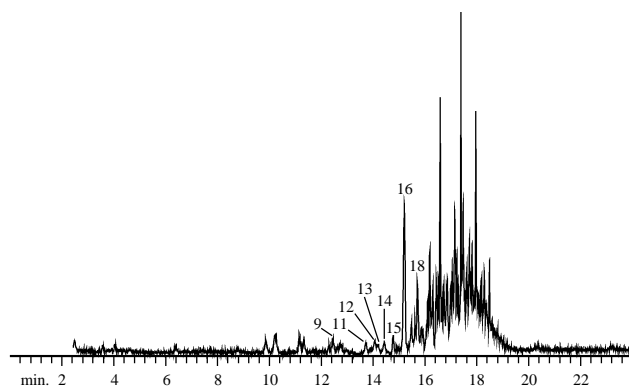
Figure 2

Comparing patterns and elution can help determine unweathered gasoline, kerosene, and diesel fuel.

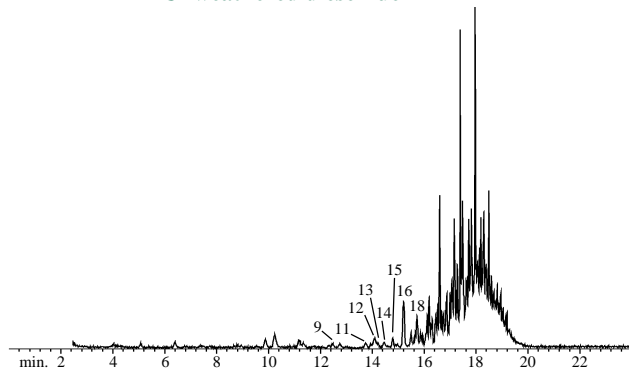
Unweathered gasoline



Unweathered kerosene



Unweathered diesel fuel



The Rtx[®]-5Sil MS column is capable of resolving all 13 aromatic compounds listed in the method. Columns commonly used for GRO analysis, such as the Rtx[®]-5 and Rtx[®]-502.2 columns, cannot adequately resolve the C9 alkyl benzenes. The Rtx[®]-5Sil MS phase design, column dimensions, and suggested GC conditions are optimized to provide the best possible resolution of the alkyl benzenes and all the branched aromatics listed in the Alaska method (Figure 1). Additionally, the 40m length and a 0.45mm internal diameter (ID) results in faster GC run-times, reduced cost, and lower column bleed. Bleed levels are exceptionally low even at temperatures up to 300°C.

The FID chromatogram in Figure 1 shows the elution of the AK101AA target compounds with the addition of two surrogates and two window markers— α,α,α -trifluorotoluene and 4-bromofluorobenzene, and C6 and C10, respectively. Two of the eight aromatic C9 compounds—1,2,4-trimethylbenzene and 1,2,3-trimethylbenzene—have longer retention times on the Rtx[®]-5Sil MS column than the C10 marker, thus these two analytes elute after decane. All the C9 aromatic compounds are included for analyte quantitation on the PID, whereas the FID uses the total gasoline range quantitation, which ends with C10.

Unweathered gasoline, kerosene, and diesel fuel were analyzed under the same conditions to illustrate the differences in their patterns and elution (Figure 2). These three different fractions of petroleum were analyzed using GC/mass spectrometry (MS), with the C9 aromatic compounds labeled. The distribution and concentration of the C9 aromatics is different depending on the following: degree of weathering, type of fuel, and source of the petroleum. It is important to run both weathered and unweathered fuels using PID/FID and your conditions to assist in determining the types of petroleum and degrees of weathering.

Figure 3 shows examples of the C9 aromatic compounds found in gasoline and kerosene, analyzed using GC/MS in selected ion monitoring (SIM) mode and scanning for M/Z 120. These alkyl-benzenes are the only compounds that share ions 105 and 120 in this region of the chromatogram. Concentrations of C9 compounds found in kerosene and gasoline differ by an order of magnitude; therefore, the standards were made in two different concentrations to produce a similar signal intensity on the MS system. The area of each C9 aromatic hydrocarbon was compared relative to the total area of all eight of these compounds in

Peak List and Conditions for Figure 2

- | | |
|--|--------------------------------|
| 1. hexane | 10. 4-bromo-fluorobenzene (ss) |
| 2. benzene | 11. <i>n</i> -propylbenzene |
| 3. α,α,α -trifluorotoluene (ss) | 12. 1-ethyl-3-methylbenzene |
| 4. toluene | 13. 1-ethyl-4-methylbenzene |
| 5. ethylbenzene | 14. 1,3,5-trimethylbenzene |
| 6. <i>m</i> -xylene | 15. 1-ethyl-2-methylbenzene |
| 7. <i>p</i> -xylene | 16. 1,2,4-trimethylbenzene |
| 8. <i>o</i> -xylene | 17. decane |
| 9. isopropylbenzene | 18. 1,2,3-trimethylbenzene |

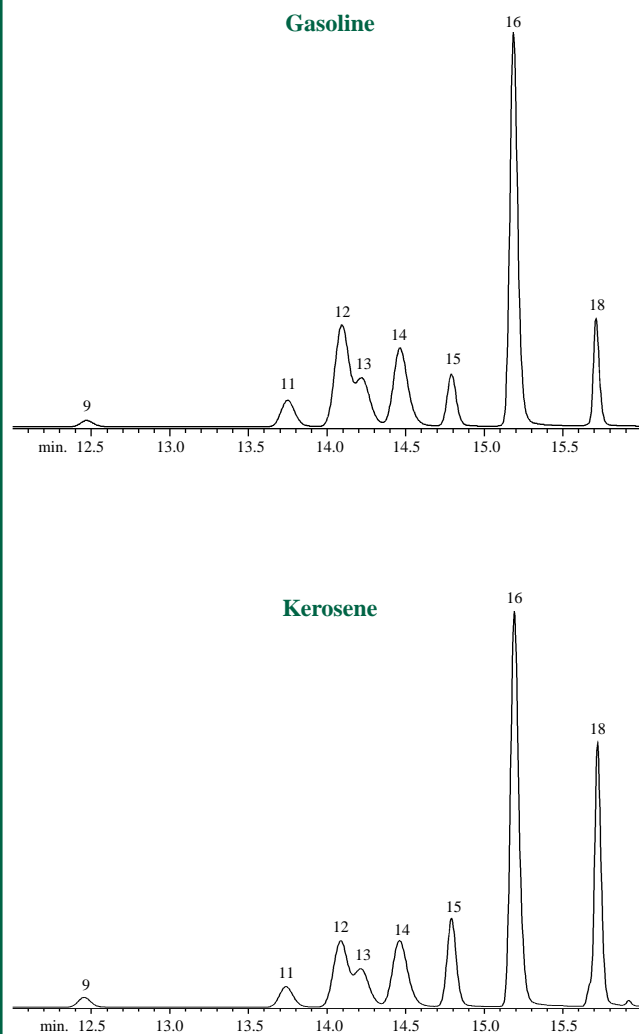
40m, 0.45mm ID, 1.50 μ m Rtx[®]-5Sil MS (cat.# 12798).

Injections of gasoline, kerosene, and diesel.

Column flow: 9mL/min.; **Concentrator:** Tekmar LSC-3000 Purge & Trap, BTEX trap; **Interface:** direct with Siltek™ transfer line; **Oven temp.:** 40°C (hold 2 min.) to 85°C @ 4°C/min. (hold 1 min.) to 225°C @ 40°C/min. (hold 2 min.); **Det.:** GC/MS TIC.

Figure 3

Distribution of C9 for varying types of petroleum are different, which can aid in fuel identification and quantitation.



Peak List and Conditions for Figure 3

- | | |
|--|--------------------------------|
| 1. hexane | 10. 4-bromo-fluorobenzene (ss) |
| 2. benzene | 11. <i>n</i> -propylbenzene |
| 3. α,α,α -trifluorotoluene (ss) | 12. 1-ethyl-3-methylbenzene |
| 4. toluene | 13. 1-ethyl-4-methylbenzene |
| 5. ethylbenzene | 14. 1,3,5-trimethylbenzene |
| 6. <i>m</i> -xylene | 15. 1-ethyl-2-methylbenzene |
| 7. <i>p</i> -xylene | 16. 1,2,4-trimethylbenzene |
| 8. <i>o</i> -xylene | 17. decane |
| 9. isopropylbenzene | 18. 1,2,3-trimethylbenzene |

40m, 0.45mm ID, 1.50 μ m Rtx[®]-5Sil MS (cat.# 12798).
Injections of gasoline and kerosene. **Column flow:** 9mL/min.;
Concentrator: Tekmar LSC-3000 Purge & Trap, BTEX trap;
Interface: direct with Siltek[™] transfer line; **Oven temp.:** 40°C
(hold 2 min.) to 85°C @ 4°C/min. (hold 1 min.) to 225°C @ 40°C/min.
(hold 2 min.); **Det.:** GC/MS SIM mode for ion 120 only.

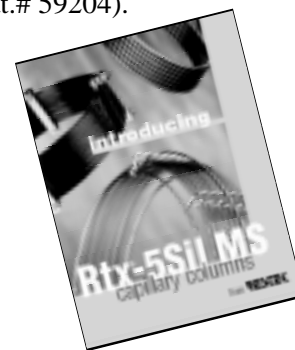
each chromatogram. 1-ethyl-2-methylbenzene and 1,2,3-trimethylbenzene distributions are different for gasoline and kerosene using our standards. Other slight differences were noted between 1-ethyl-3-methylbenzene and 1,3,5-trimethylbenzene requiring separation of the ethyl-methylbenzene isomers. It is important to stress that results may vary due to different fuel sources and the degree of weathering; the point is that the distribution of C9 for varying types of petroleum are different, and these differences can aid in fuel identification and quantitation.

The standards for Method AK101AA consist of a mixture of 13 aromatic compounds. Restek offers this mixture complete with quality assurance (QA) documentation and a Certificate of Analysis, which can be used for audits as well as for internal QA needs. Our Alaska GRO standards are made in the correct concentrations, ensuring accurate identification and quantitation of environmental samples.

Use of the Rtx[®]-5Sil MS column and the analytical method outlined in Method AK101AA will achieve the best possible resolution of the alkyl benzenes and all the listed branched aromatics.

for *more* info

Request the Rtx[®]-5Sil MS Capillary Columns flyer
(lit. cat.# 59204).



Product Listing

Rtx[®]-5Sil MS Columns

ID	df (µm)	Stable to	30m	40m	60m
0.45mm	1.50	300/320°C	—	12798	—

Siltek™ Guard Columns

nominal ID	nominal OD	5-meter	10-meter
0.25mm	0.37 ± 0.04mm	10026	10036
0.32mm	0.45 ± 0.04mm	10027	10037
0.53mm	0.69 ± 0.04mm	10028	10038

Alaska UST Method AK101AA

benzene	toluene
ethylbenzene	1,2,3-trimethylbenzene
1-ethyl-2-methylbenzene	1,2,4-trimethylbenzene
1-ethyl-3-methylbenzene	1,3,5-trimethylbenzene
1-ethyl-4-methylbenzene	<i>o</i> -xylene
isopropylbenzene	<i>m</i> -xylene
<i>n</i> -propylbenzene	<i>p</i> -xylene

1,000µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30461	30461-510	
w/data pack	30461-500	30461-520	30561

4-bromofluorobenzene

2,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30026	30026-510	
w/data pack	30026-500	30026-520	30126

10,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30082	30082-510	
w/data pack	30082-500	30082-520	30182

α,α,α-trifluorotoluene

2,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30048	30048-510	
w/data pack	30048-500	30048-520	30148

10,000µg/mL in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30083	30083-510	
w/data pack	30083-500	30083-520	30183

Unleaded Gasoline Composite

From samples of regular- and premium-grade unleaded gasoline from three sources, blended to form a composite sample.

2,500µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30081	30081-510	
w/data pack	30081-500	30081-520	30181

50,000µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30205	30205-510	
w/data pack	30205-500	30205-520	30305

50,000µg/mL ea. in P&T methanol, 5mL/ampul.

	each	5-pack	10-pack
	30206	30206-510	
w/data pack	30206-500	30206-520	30306

Suitable for Matrix Spikes and Laboratory Control Samples



WA VPH Marker Standard

decane (C10)	octane (C8)
dodecane (C12)	pentane (C5)
hexane (C6)	toluene
1-methylnaphthalene	1,2,3-trimethylbenzene
naphthalene	

1,000µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30450	30450-510	
w/data pack	30450-500	30450-520	30550

WA VPH Standard

benzene	octane (C8)
decane (C10)	pentane (C5)
dodecane (C12)	toluene
ethylbenzene	1,2,3-trimethylbenzene
hexane (C6)	<i>m</i> -xylene
1-methylnaphthalene	<i>o</i> -xylene
methyl- <i>tert</i> -butyl ether	<i>p</i> -xylene
naphthalene	

1,000µg/mL ea. in P&T methanol, 1mL/ampul.

	each	5-pack	10-pack
	30451	30451-510	
w/data pack	30451-500	30451-520	30551

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Rtx[®]-CLPesticides and Rtx[®]-CLPesticides2 Columns: The Ideal Confirmational Pair for Analyzing Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are a group of industrial organochlorine chemicals that have become a major environmental concern. Since the 1950's, over one million metric tons of PCBs have been produced. They are very persistent in the environment and bioaccumulate in living systems. All PCBs are practically insoluble in water, but they are soluble in hydrophobic media like fats or oily substances. They were used commercially because they are chemically inert liquids and are difficult to burn; they have low vapor pressures, are inexpensive to make, and are excellent electrical insulators. As a result, they were used extensively as coolant fluids in transformers and capacitors; and later as plasticizers, de-inking solvents, heat transfer fluids in machinery, and water-proofing agents, among other uses.

Because of their persistence and their solubility in fatty tissue, PCBs in food chains undergo biomagnification. Strong heating of PCBs in the presence of oxygen can lead to the formation of polychlorodibenzofurans (PCDF), which are structurally and toxicologically similar to dioxins. Commercial PCB mixtures (e.g., Aroclor[®] mixtures) contain small amounts of PCDF as a result of the synthesis. PCB mixtures are not highly toxic, but toxicity due to the PCDF concentration has caused concern.

Certain PCB congeners can be highly toxic; toxicity depends on where the chlorine substitution resides on the biphenyl molecule. The congeners without chlorine substitution on the ortho positions are the most toxic. These are termed "coplanar PCBs" because the phenyl rings can maintain a planar geometry to each other. This makes these compounds "dioxin-like," and the most toxic of these PCB congeners is one-tenth the toxicity of the 2,3,7,8-tetrachloro dibenzo dioxin. The coplanar PCBs also have been implicated as endocrine disruptors.¹

It is important, therefore, when designing a PCB analysis method to determine if the separation will be by specific congener (for toxicity) or by commercial Aroclor[®] mixture. The commercial synthesis of PCBs results in chlorination of the biphenyl molecule, and this reaction produces a mixture of many of the 209 congeners of the PCB family.

Naming of the specific congeners follows the positional numbering shown in Figure 1. Because the IUPAC names for these compounds are long, the congeners are normally referred to by their IUPAC number or BZ number as defined by Ballschmitter and Zell.² The exact proportions of congeners in the Aroclor[®] mixtures depends on the ratio of chlorine to biphenyl, the reaction time, and the temperature. Although many of the PCB compounds are solids, the mixtures usually are liquids or low-melting-point solids. Commercially, the PCB compounds were not isolated. Instead, they were sold as partially separated mixtures, with the average chlorine content in different products ranging from 21% to 68%. These Aroclor[®] mixtures are, therefore, composed of a number of individual PCB congeners and have a characteristic profile depending on the percent of chlorine substitution. The Aroclor[®] mixtures are named by the number of carbons (12), followed by the weight % of chlorine (42). Thus Aroclor[®] 1242 represents a mixture of PCB compounds with an average weight percent of 42.

There are 9 common Aroclor[®] mixtures: 1221, 1232, 1242, 1248, 1254, 1260, 1262, 1268, and 1016. Aroclor[®] 1016 does not follow the same naming sequence, and appears chromatographically similar to 1242. Samples from contamination sites often are quantitated and reported as concentration of PCBs, i.e., as Aroclor[®] mixtures. This analysis requires the individual PCB Aroclor[®] mixtures to be analyzed as standards, then the sample extract chromatograms are compared to the standards to qualitatively identify the Aroclor[®] mixtures. Once this identification has been made, the quantitation can be performed by selecting five of the largest peaks and treating them as individual compounds, then reporting the average concentration.

Due to the unreactive nature of the PCBs, instrument conditions and column choice is less critical than when analyzing chlorinated pesticides. When choosing columns, it is important to select stationary phases that have low bleed and high thermal stability; allowing the columns to be baked out at the end of the run to prevent carryover from one injection to the next. Because many instruments used for the analysis of PCBs also may be used for pesticide and herbicide analyses, the column pair of choice is the Rtx[®]-CLPesticides and Rtx[®]-CLPesticides2 columns. This column pair provides excellent separation of the pesticide and herbicide compounds, low bleed, high thermal stability, and they are designed to compliment each other for primary column analysis and secondary column confirmation.

Figure 2 shows the chromatograms obtained for seven commercial Aroclor[®] mixtures injected on the Rtx[®]-CLPesticides column. Figure 3 shows the chromatograms for the same mixtures injected on the Rtx[®]-CLPesticides2 column.

Table 1 (on back) lists the retention times for the individual 209 PCB congeners on these same stationary phases. The analysis of PCBs by congener requires each peak to be treated like an individual component; making a standard curve for each of the congeners of interest. While many laboratories are interested in the analysis of PCBs by congener, most do not need, or desire, to analyze all 209. For this reason, the retention table is listed, and conditions may be modified to better suit the particular separation in your laboratory. If you have questions regarding the analysis of PCBs by congener, contact Restek's technical service team at 800-356-1688 or 814-353-1300, ext. 4, or contact your local Restek representative.

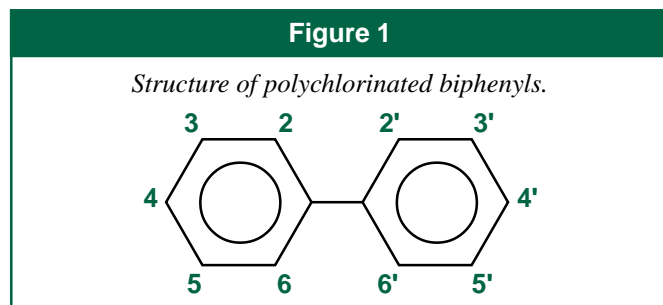
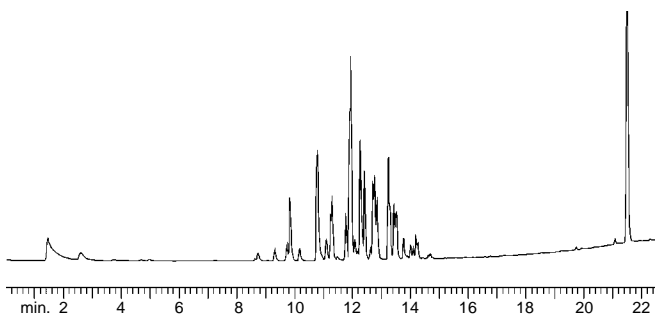


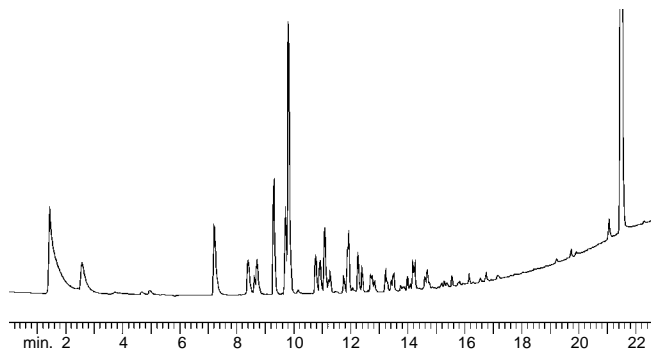
Figure 2

Aroclor® standards run on the Rtx®-CLPesticides column at 320ppb.

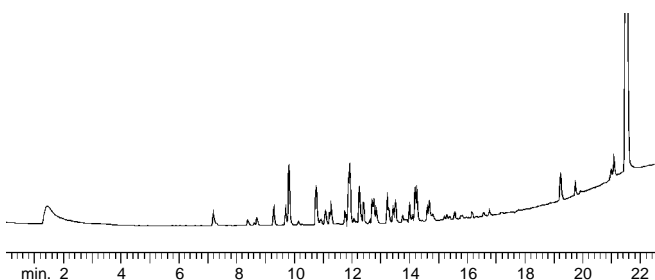
Aroclor® 1016



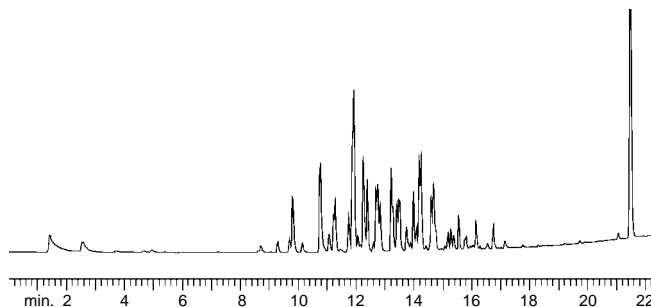
Aroclor® 1221



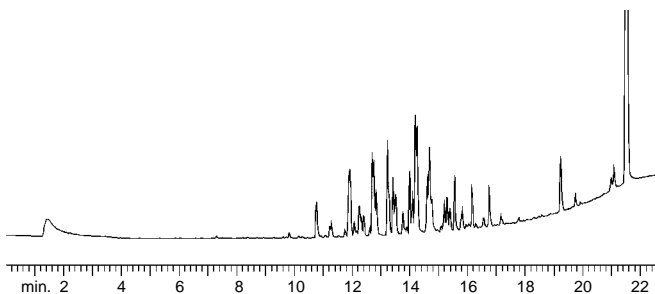
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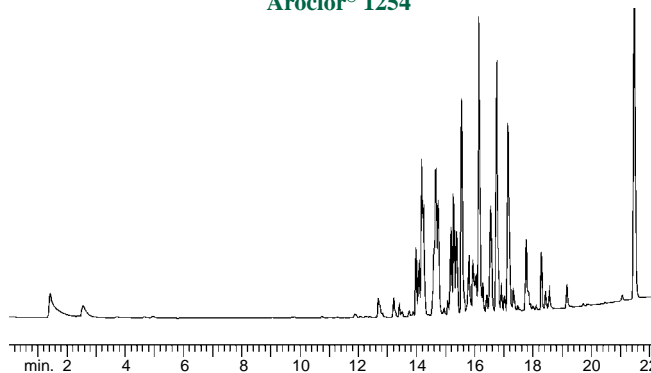
Aroclor® 1242



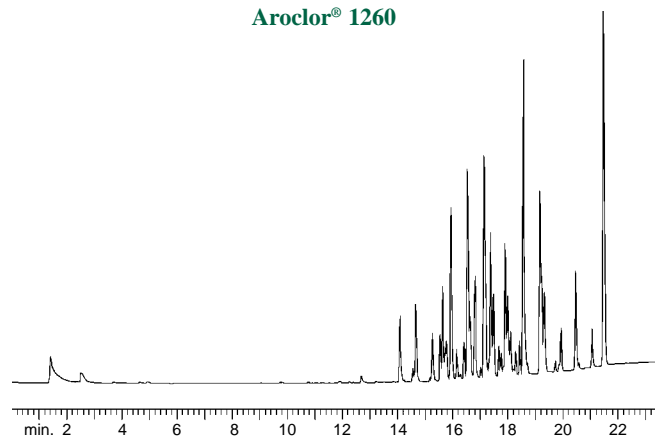
Aroclor® 1248



Aroclor® 1254



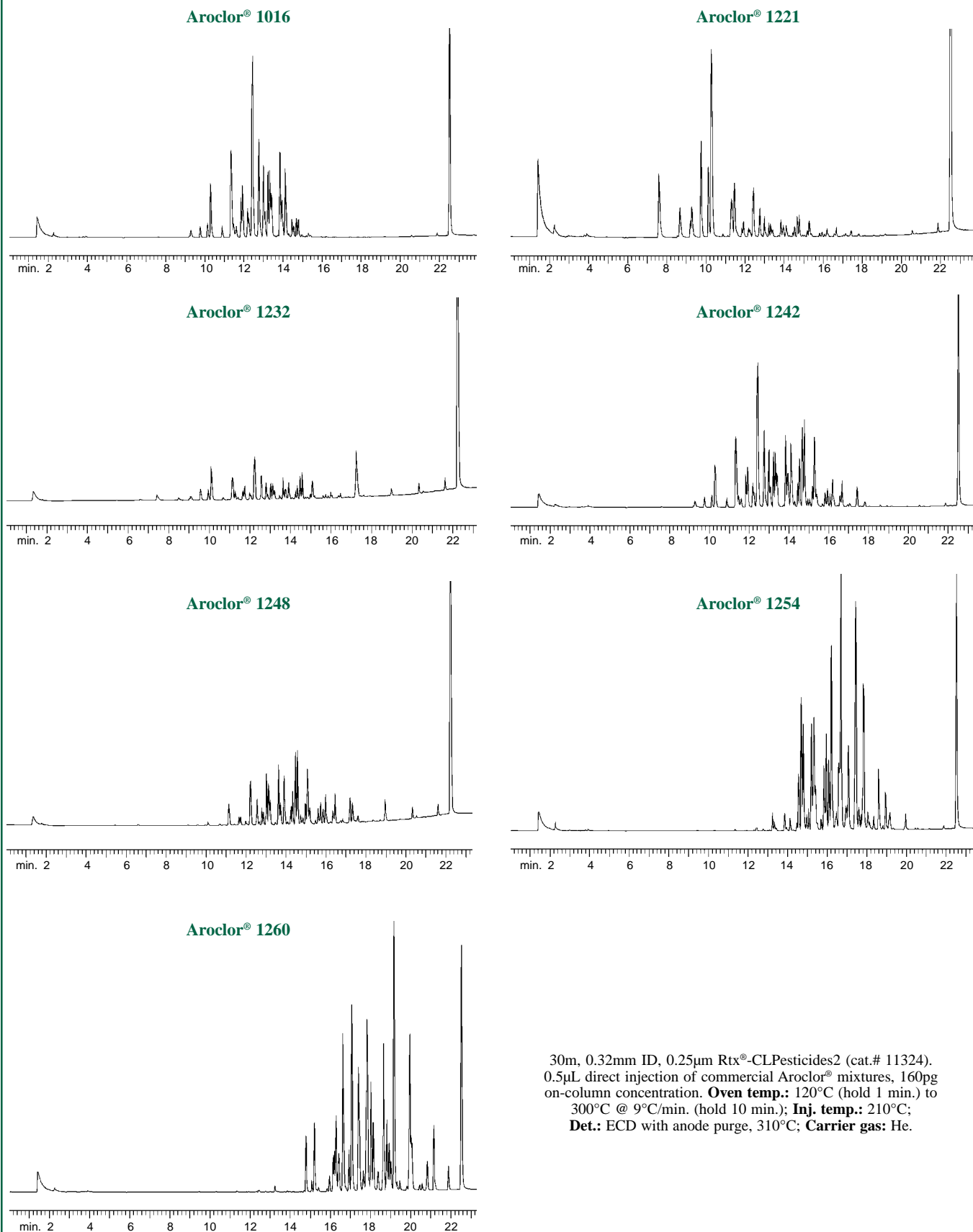
Aroclor® 1260



30m, 0.32mm ID, 0.50µm Rtx®-CLPesticides (cat.# 11139).
0.5µL direct injection of commercial Aroclor® mixtures, 160pg
on-column concentration. **Oven temp.:** 120°C (hold 1 min.) to
300°C @ 9°C/min. (hold 10 min.); **Inj. temp.:** 210°C;
Det.: ECD with anode purge, 310°C; **Carrier gas:** He.

Figure 3

Aroclor® standards run on the Rtx®-CLPesticides2 column at 320ppb.



30m, 0.32mm ID, 0.25µm Rtx®-CLPesticides2 (cat.# 11324).
0.5µL direct injection of commercial Aroclor® mixtures, 160pg
on-column concentration. **Oven temp.:** 120°C (hold 1 min.) to
300°C @ 9°C/min. (hold 10 min.); **Inj. temp.:** 210°C;
Det.: ECD with anode purge, 310°C; **Carrier gas:** He.

Table I—PCB Congener Retention Time Data

Rtx®-CLP		Rtx®-CLP2		Rtx®-CLP		Rtx®-CLP2		Rtx®-CLP		Rtx®-CLP2		Rtx®-CLP		Rtx®-CLP2	
PCB	RT	PCB	RT	PCB	RT	PCB	RT	PCB	RT	PCB	RT	PCB	RT	PCB	RT
IUPAC#	(min)	IUPAC#	(min)	IUPAC#	(min)	IUPAC#	(min)	IUPAC#	(min)	IUPAC#	(min)	IUPAC#	(min)	IUPAC#	(min)
1	13.36	1	14.33	52	25.24	73	26.47	155	28.50	88	29.89	135	31.90	147	33.60
2	ND	2	ND	65	25.27	52	26.56	66	28.54	155	30.07	139	32.10	82	33.69
3	15.86	3	16.61	43	25.29	46	26.70	80	28.77	91	30.22	149	32.22	108	33.75
4	16.57	10	17.79	62	25.30	43	26.71	55	28.90	55	30.39	140	32.35	107	33.79
10	16.38	4	17.94	73	25.33	49	26.73	60	29.28	92	30.54	124	32.37	139	33.80
7	17.84	7	18.98	49	25.34	75	26.79	92	29.28	101	30.81	108	32.37	149	33.86
9	17.86	9	18.99	39	25.34	38	26.86	84	29.34	90	30.85	107	32.43	123	33.89
6	18.73	6	19.80	75	25.40	65	26.87	89	29.42	60	30.91	123	32.49	106	33.96
5	18.91	8	20.13	47	25.48	47	26.88	90	29.44	56	30.96	106	32.51	118	34.01
8	18.96	5	20.21	48	25.52	62	26.92	56	29.48	113	30.96	118	32.65	140	34.06
19	19.64	14	20.56	104	25.53	48	26.93	101	29.51	99	31.07	142	32.67	133	34.25
14	19.80	30	21.17	38	25.78	104	27.31	150	29.60	84	31.16	188	32.68	165	34.31
30	19.82	19	21.36	44	26.32	35	27.61	99	29.68	89	31.26	143	32.68	143	34.36
18	21.00	11	21.93	59	26.34	59	27.80	113	29.76	79	31.28	134	32.69	188	34.39
17	21.06	12	22.24	42	26.44	44	27.82	152	30.01	119	31.42	131	32.88	134	34.46
11	21.10	13	22.34	35	26.56	72	27.85	112	30.02	150	31.43	114	32.93	161	34.49
24	21.27	18	22.40	64	26.71	42	28.00	119	30.08	112	31.46	184	33.00	142	34.54
12	21.31	17	22.46	96	26.71	37	28.07	109	30.10	109	31.58	133	33.03	146	34.57
13	21.34	15	22.66	41	26.86	68	28.11	116	30.18	78	31.83	165	33.19	114	34.60
27	21.67	24	22.88	37	26.89	103	28.38	79	30.27	83	31.84	146	33.30	131	34.68
15	21.68	27	23.00	72	26.90	71	28.40	145	30.29	111	31.87	161	33.30	184	34.73
32	22.01	32	23.49	103	26.97	64	28.41	83	30.33	152	31.89	122	33.40	153	34.86
16	22.11	23	23.63	71	26.98	41	28.50	86	30.46	116	32.00	132	33.55	122	34.88
54	22.36	16	23.65	68	27.00	57	28.61	117	30.49	148	32.04	153	33.56	168	34.98
23	22.37	34	23.69	100	27.22	100	28.70	97	30.56	86	32.07	179	33.74	127	35.11
29	22.52	29	23.78	57	27.44	96	28.72	115	30.57	97	32.13	168	33.75	132	35.46
34	22.69	26	24.27	40	27.44	67	28.83	148	30.69	120	32.14	105	33.95	141	35.60
50	23.01	54	24.37	67	27.61	40	29.10	136	30.72	117	32.19	176	34.07	105	35.61
26	23.15	25	24.39	61	27.73	58	29.13	78	30.73	125	32.19	141	34.15	179	35.63
25	23.20	50	24.55	63	27.76	63	29.18	87	30.79	145	32.20	127	34.16	137	35.96
31	23.46	31	24.74	94	27.78	61	29.29	125	30.84	115	32.26	137	34.34	176	36.01
28	23.56	28	24.81	58	27.92	94	29.30	111	30.91	87	32.40	186	34.39	130	36.20
21	23.79	21	25.32	93	28.00	74	29.37	85	30.98	81	32.43	130	34.58	160	36.27
53	23.85	53	25.44	74	28.00	80	29.42	154	31.07	154	32.45	160	34.66	163	36.37
51	24.04	33	25.48	98	28.07	121	29.47	81	31.10	85	32.65	163	34.80	164	36.39
33	24.22	20	25.53	102	28.13	98	29.68	120	31.13	136	32.74	138	34.81	186	36.41
20	24.23	51	25.68	88	28.16	102	29.68	110	31.38	110	32.95	158	34.90	178	36.44
45	24.33	36	25.85	95	28.22	70	29.70	151	31.60	77	33.06	164	34.93	138	36.48
22	24.53	22	25.97	70	28.39	93	29.72	144	31.79	151	33.14	178	34.99	158	36.50
46	24.93	45	26.12	76	28.44	76	29.74	77	31.79	144	33.35	166	35.15	175	36.73
69	24.96	69	26.21	121	28.46	95	29.86	82	31.87	135	33.44	175	35.22	182	36.90
36	25.05	39	26.39	91	28.47	66	29.88	147	31.89	124	33.56	129	35.23	187	36.91

30m, 0.32mm ID Rtx®-CLPesticides (cat.# 11139) and Rtx®-CLPesticides2 (cat.# 11324) columns.
Oven temp.: 100°C (hold 1 min.) to 290°C @ 4°C/min.; **Inj. temp.:** 210°C; **Det. temp.:** 310°C; **Carrier gas:** He @ 36cm/sec.

References (not available from Restek):

- 1 Environmental Chemistry, Colin Baird, W.H. Freeman and Co., 1998, pp. 337-353.
- 2 Ballschmiter, K, and Zell, M., Fresenius Z. Anal. Chem., 302, 20, (1980)

Product Listing

Rtx®-CLPesticides Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.25	340°C	11120	11123
0.32mm	0.50	340°C	11136	11139
0.53mm	0.50	340°C	11137	11140
ID	df (µm)	Stable to	10m	20m
0.18mm	0.18	340°C	42101	42102

Rtx®-CLPesticides2 Columns

ID	df (µm)	Stable to	15m	30m
0.25mm	0.20	340°C	11320	11323
0.32mm	0.25	340°C	11321	11324
0.53mm	0.42	340°C	11337	11340
ID	df (µm)	Stable to	10m	20m
0.18mm	0.14	340°C	42301	42302

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Siltek™ Deactivation Delivers Inertness to Analyte Breakdown and Reactivity, and Durability to Physical and Chemical Challenges

A common concern in gas chromatographic (GC) analyses is the interaction of analytes with active surfaces in the GC pathway. The injection port is the first source of active sites, often leading to adsorption and breakdown of analytes. However, not all analyses are affected by reactivity within the injection port. Hydrocarbons, typically, are not susceptible to adsorption or breakdown. In contrast, active compounds such as pesticides, drugs, phenols, amines, and alcohols, which are often injected via splitless mode, are more prone to these problems. With a splitless injection, carrier gas flow rate through the liner is very slow, increasing the sample residence time in the injector and the chance for reactivity. Complete and effective liner deactivation is crucial to minimize available active sites and ensure repeatable results.

Restek has designed Siltek® deactivation to deliver both enhanced inertness and durability. Gas chromatography accessories coated with Siltek® deactivation provide durability for matrices of extreme pH or high-temperature applications.

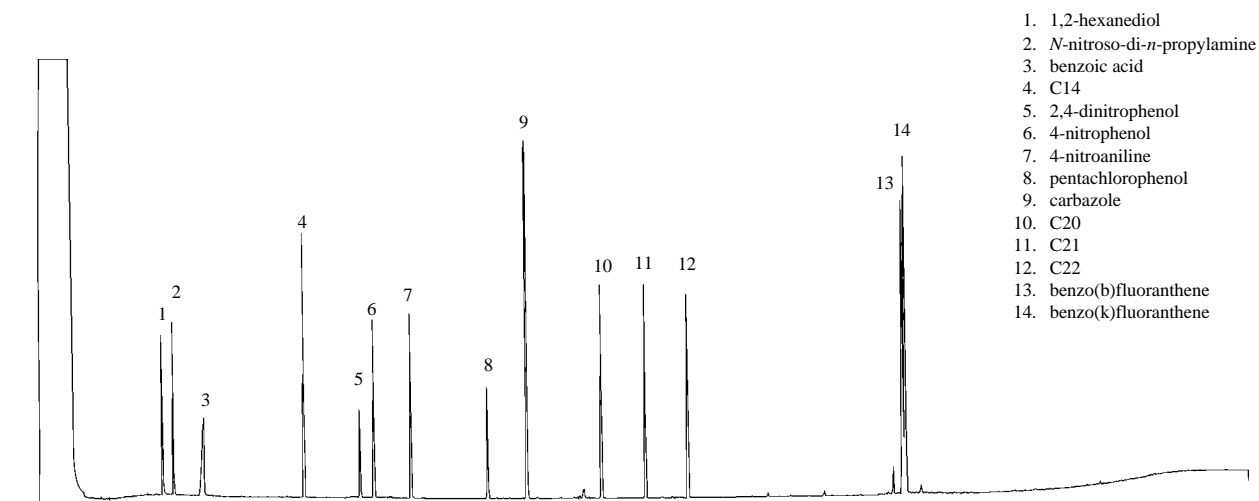
Inertness

Semivolatile analysis places extreme demands on the GC system. One key to successfully analyzing semivolatiles is having the capability to handle basic and acidic compounds in the GC system. The analytical column must provide selectivity for both classes without resulting in poor peak shape. Additionally, liner deactivation is critical to analytical success because the vaporized sample comes in contact with the inlet liner first.

The Restek XTI test mix was chosen to evaluate the inertness of a Siltek™-deactivated liner. This mix contains both acidic and basic probes, some of which are pollutants monitored in US Environmental Protection Agency (EPA) Method 8270 (4-nitroaniline, *N*-nitroso-di-*n*-propylamine, 2,4-dinitrophenol, pentachlorophenol, benzoic acid, benzo(b)- and benzo(k)fluoranthene). A splitless injection of the XTI mix with an on-column concentration of 4-10ng shows an excellent response for all of the probes, including the active compounds dinitrophenol, 1,2-hexanediol, and benzoic acid (Figure 1).

Figure 1

Siltek™-deactivated liner shows excellent inertness for acidic and basic probes.



30m, 0.25mm ID, 0.25µm XTI®-5 (cat.# 12223) with a Siltek™-deactivated 4mm splitless single gooseneck sleeve (cat.# 20798-214.1). **Oven temp.:** 40°C (hold 2 min.) to 100°C @ 30°C/min., to 180°C @ 9°C/min., to 330°C @ 30°C/min. (hold 10 min.); **Inj. temp.:** 250°C; **Det.:** 330°C; **Carrier gas:** He.

Thermal Stability

To test the durability of the Siltek® liner deactivation, two sources of stress were investigated—high inlet temperature over a period of 10 days and repeated exposure to aqueous injections of low, then high pH. High inlet temperatures can promote degradation of the deactivation layer by causing it to bake or bleed off of the liner. In the first study, a baseline splitless XTI injection was performed, and response factors (relative to C14) were calculated. The injection port was then set at 330°C overnight and another XTI injection was made. This process was continued for 10 days. After 10 days at 330°C, the Siltek® deactivation retained its integrity, achieving essentially unchanged response factors, even for the critical probes (Figure 2).

Resistance to Chemical Attack

For the next durability study, a Siltek®-deactivated liner was repeatedly exposed to aqueous HCl injections, pH 1.4. The ability to withstand low pH aqueous samples is important with environmental applications that require acidification of the matrix. Very low or very high pH samples can cause pinpoint holes in the deactivation layer that will eventually undercut the layer and strip it away. For this study, a baseline XTI injection was made via direct injection and relative response factors were calculated. In the direct injection mode, a leak-free connection is formed in the liner, minimizing sample exposure within the injection port. Ten microliters of the pH 1.4 sample were injected, followed by a direct injection of the XTI test mixture.

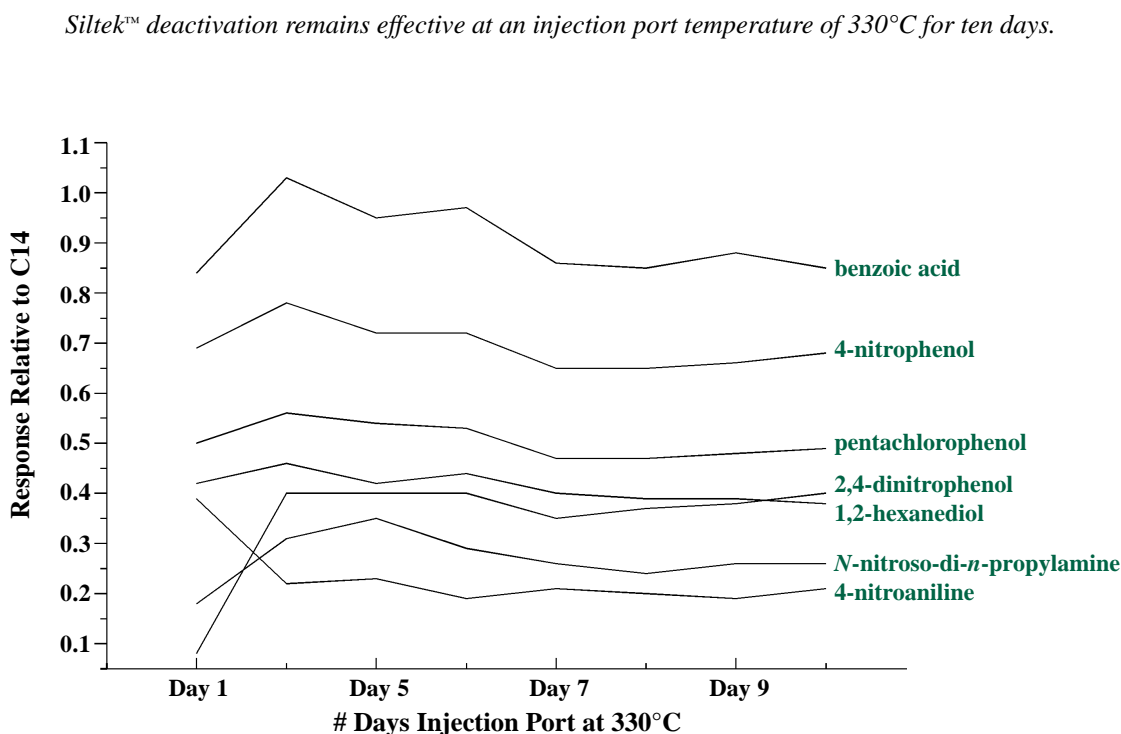
This cycle continued until a total of 180µL were injected (Figure 3). Key probes, such as 2,4-dinitrophenol (DNP), pentachlorophenol (PCP), *N*-nitroso-di-*n*-propylamine (*n*-propylamine), and 1,2-hexanediol (diol) retained their responses up to at least 120µL injected.

The experiment was repeated with an identical set-up using aqueous NH₄OH injections, pH 10.1 (Figure 4). Under these demanding conditions, the response for the XTI compounds was consistent for 70 injections. As expected, the response of the acidic compounds began to decrease with repeated injections but many compounds continued to have excellent response for more than 120µL injections.

Siltek® deactivation offers both inertness and resistance to temperature and pH extremes within a GC system. It is available as a deactivation for fused silica guard columns and inlet liners.

**For more information on Siltek™—
the next generation of deactivation,
please request our Siltek™ Benefits
brochure (cat.# 59803).**

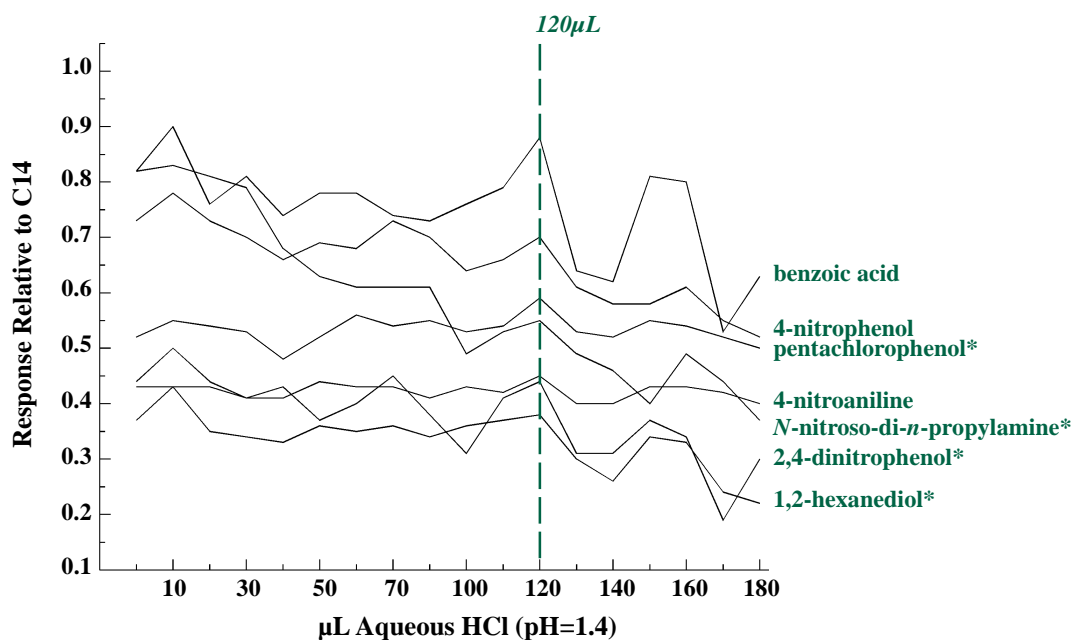
Figure 2



30m, 0.25mm ID, 0.25µm XTI®-5 (cat.# 12223) with a Siltek™-deactivated splitless sleeve (cat.# 20798-214.1). Oven temp.: 40°C (hold 2 min.) to 190°C @ 6°C/min., to 330°C @ 30°C/min. (hold 10 min.); Inj. temp.: 330°C; Det.: 330°C; Carrier gas: He.

Figure 3

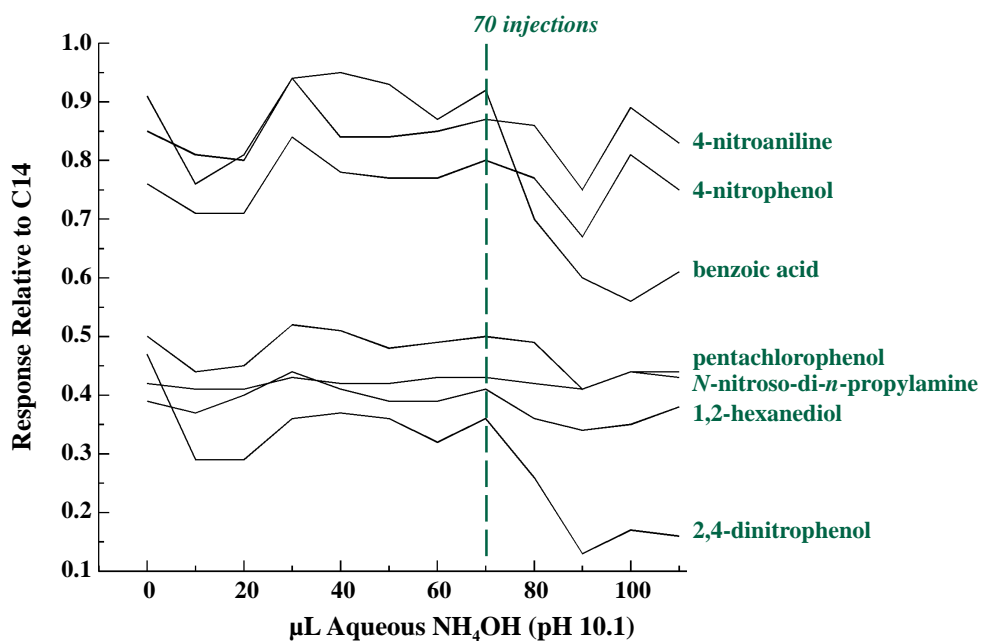
Difficult probes* retain their response on Siltek™ deactivation up to 120µL of an aqueous solution of pH 1.4.



30m, 0.25mm ID, 0.25µm XTI®-5 (cat.# 12223) with a Siltek™-deactivated 4mm open-top Uniliner® w/o wool (cat.# 20843-214.1).
Oven temp.: 40°C (hold 2 min.) to 190°C @ 6°C/min., to 330°C @ 30°C/min. (hold 10 min.); Inj. temp.: 250°C; Det.: 330°C; Carrier gas: He.

Figure 4

Response for XTI compounds on Siltek™ deactivation was consistent for 70 injections of an aqueous solution at pH 10.1.



30m, 0.25mm ID, 0.25µm XTI®-5 (cat.# 12223) with a Siltek™-deactivated 4mm open-top Uniliner® w/o wool (cat.# 20843.214.1).
Oven temp.: 40°C (hold 2 min.) to 190°C @ 6°C/min., to 330°C @ 30°C/min. (hold 10 min.); Inj. temp.: 250°C; Det.: 330°C; Carrier gas: He.

Product Listing

XTI[®]-5 Columns

ID	df (µm)	Temp. limits	15m	30m
0.25mm	0.25	-60 to 360°C	12220	12223
	0.50	-60 to 330/350°C	12235	12238
	1.00	-60 to 325/350°C	12250	12253
0.32mm	0.25	-60 to 360°C	12221	12224
	0.50	-60 to 330/350°C	12236	12239
	1.00	-60 to 325/350°C	12251	12254
0.53mm	0.50	-60 to 330/360°C	12237	12240
	1.00	-60 to 325/350°C	12252	12255
	1.50	-60 to 310/330°C	12267	12270

Inlet Liners for HP GCs

Liner type	ID/OD/Length (mm)	ea.	5-pk.	25-pk.
4mm split w/wool	4.0/6.3/78.5	20781	20782	20783
2mm splitless	2.0/6.5/78.5	20712	20713	20714
4mm splitless	4.0/6.5/78.5	20772	20773	20774
4mm gooseneck	4.0/6.5/78.5	20798	20799	20800
4mm double gooseneck	4.0/6.5/78.5	20784	20785	20786
Cycloplitter [®]	4.0/6.3/78.5	20706	20707	20708

Inlet Liners for Varian GCs

Liner type	ID/OD/Length (mm)	ea.	5-pk.	25-pk.
2mm splitless	2.0/6.3/74	20721	20722	20723
4mm splitless	4.0/6.3/74	20904	20905	20906
0.5mm SPI	0.53/4.6/54	20775	20776	20777
0.8mm SPI	0.80/4.6/54	20778	20779	20780
SPI with buffer	2.4/4.6/54	20850	20851	20852

Siltek[™]-Deactivated Guard Columns

Nominal ID	Nominal OD	5m	10m
0.25mm	0.37 ± 0.04mm	10026	10036
0.32mm	0.45 ± 0.04mm	10027	10037
0.53mm	0.69 ± 0.04mm	10028	10038

Siltek[™]-Deactivated Press-Tight[®] Connectors

Type	Qty.	cat.#
straight	25-pk.	20449
angled 'Y'	3-pk.	20469

For other Siltek[™]-deactivated Press-Tight[®] connectors, add suffix "-266" to the catalog number.

Siltek[™]-Deactivated Inlet Liners

Siltek [™]	Siltek [™] with Siltek [™] -deact. wool	Siltek [™] with CarboFrit [™]	Qty.
-214.1	-213.1	-216.1	each
-214.5	-213.5	-216.5	5-pk.
-214.25	-213.25	-216.25	25-pk.

For Siltek[™]-deactivated liners, add the corresponding suffix number to the liner's catalog number.

Siltek[™]-Deactivated Glass Wool

Qty.	cat.#
10 grams	21100

**For more information on Siltek[™]—
the next generation of deactivation,
please request our Siltek[™] Benefits
brochure (cat.# 59803).**

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The Institute for Nutraceutical Advancement (INA) Validates GC Methods for Saw Palmetto Using Rtx®-5 and Stabilwax® Columns

History of the INA Methods Validation Program

Consumer demand for natural products and dietary supplements has grown exponentially, with increasing amounts of botanical materials being used in the manufacture of a large variety of products. As the supplies and number of suppliers multiply, the consistency of raw materials has become an issue for virtually every major player in the natural products industry. Even companies with conscientious and responsible quality control procedures have found it difficult to ensure consistency in their products due to the lack of any published standards for analysis.

These issues, along with other science and market-based factors, led 29 companies to come together in an international effort to validate and make available analytical methods that will meet the demand for global consistency in the testing of botanicals.

The effort is called the Methods Validation Program, or MVP, and it is the first project for the newly formed Institute for Nutraceutical Advancement (INA). INA is a non-corporate division of Denver-based Industrial Laboratories, an independent laboratory that provides analytical and consulting services to the natural products industry.

The INA MVP is being developed under the direction of a broad range of representatives from within the natural products industry, including suppliers, manufacturers, retailers, marketing companies, a grower and an independent laboratory. Companies from both the United States and Europe are represented. In addition, ten major natural products organizations, including the Food and Drug Administration (FDA), have accepted seats on the INA MVP Advisory Committee as a way of ensuring that the process is inclusive. (Additional information is available at <http://www.nutraceuticalinstitute.com/whoweare.>)

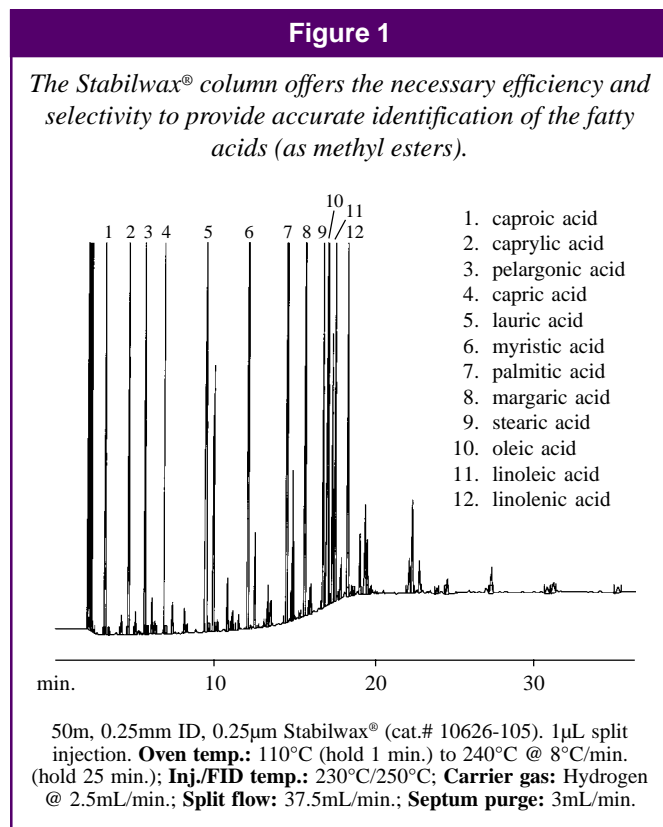
All currently validated methods from the INA can be viewed at their website: <http://www.nutraceuticalinstitute.com/methods>. Although many use high performance liquid chromatography (HPLC), two methods involve gas chromatography (GC) for the analysis of fatty acids and sterols in saw palmetto. This fruit contains several principles thought to have physiological activity, including fatty acids such as caproic, capric, lauric, myristic, oleic, palmitic, stearic, and 1 to 2% essential oils. Furthermore, saw palmetto contains phytosterols and high molecular weight polysaccharides such as β -sitosterol, β -sitosterol 3-O- β -D-glucoside, campesterol, stigmasterol. Purified ethanolic or CO₂ extracts of saw palmetto usually contain 70 to 80% free fatty acids. The fatty acids present are in several forms: free fatty

acids, fatty acid esters of the fatty alcohols, and fatty acid esters of the phytosterols. This oil is commonly blended with excipients to form a dry powder at 30% free fatty acids concentration.

Determination of Fatty Acids in *Serenoa Repens* (Saw Palmetto or Sabel) by GC

This assay can be used to determine fatty acid distribution in saw palmetto fruit, oil extract, and blended powders. Determination is performed using GC, after transesterification of the triglycerides into the methyl esters occurs. For more specific information on the method itself and all procedures involved, please refer to <http://www.nutraceuticalinstitute.com/methods/fattyacids.html>

The fatty acids from saw palmetto are separated in Figure 1, which was obtained using a Restek Stabilwax® column and a Shimadzu GC-14A GC, with split injection and a flame ionization detector (FID). The Stabilwax® column offers the necessary efficiency and selectivity to provide accurate identification of the fatty acids (as methyl esters).

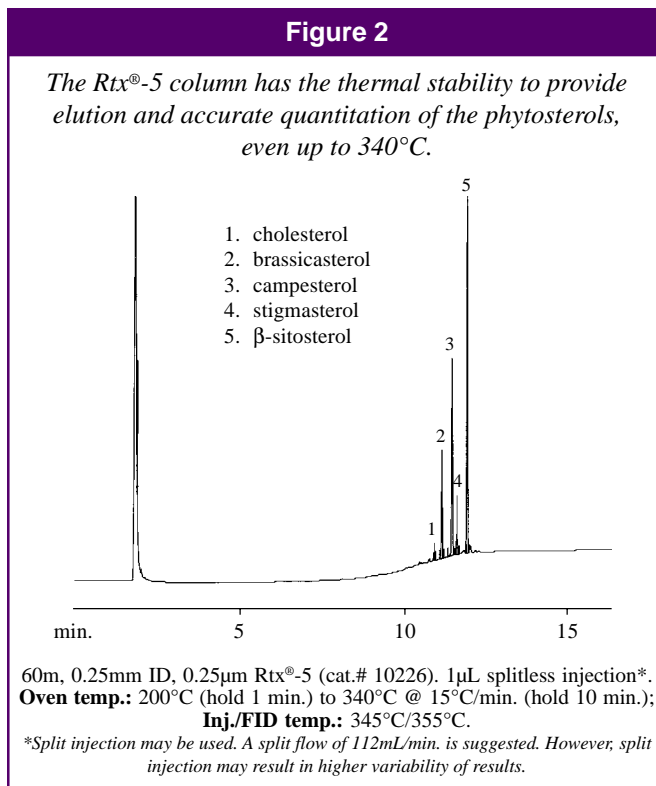


Determination of Sterols in *Serenoa Repens* (Saw Palmetto or Sabel) by GC

This assay can be used to determine stigmasterol, campesterol, brassicasterol, and β -sitosterol in saw palmetto fruit, oil extract, and blended powders. Determination is performed using GC after hydrolysis, saponification, and derivatization. For more specific information on the method itself and all procedures involved, please refer to <http://www.nutraceuticalinstitute.com/methods/sterols.html>

The sterols from saw palmetto are shown in Figure 2, which was obtained by using a Restek Rtx[®]-5 column and a Hewlett Packard 5890 Series II GC equipped with an FID and an autosampler. The Restek Rtx[®]-5 column contains a 5% diphenyl/95% dimethyl polysiloxane phase, and has the thermal stability to provide elution and accurate quantitation of the phytosterols, even up to 340°C.

Special thanks to Dr. Mark Lange, Director, and to Kathryn Bass, Marketing Director, of MVP for allowing us to print this material. Much of this text has been directly downloaded from the INA website.



Product Listing

Stabilwax[®] Columns

ID	df (μ m)	Stable to	50m
0.25mm	0.25	250°C	10626-105

Inlet Sleeves for HP GCs

Description	ID/OD/Length	ea.	5-pk.	25-pk.
2mm Splitless	2.0/6.5/78.5mm	20712	20713	20714
4mm Splitless	4.0/6.5/78.5mm	20772	20773	20774

Rtx[®]-5 Columns

ID	df (μ m)	Stable to	60m
0.25mm	0.25	360°C	10226

Inlet Sleeves for Shimadzu GCs

Description	ID/OD/Length	ea.	5-pk.	25-pk.
99mm Split	3.5/5.0/99mm	20860	20861	20862
Cyclosplitter [®]	3.5/5.0/99mm	20870	20871	—

For more information on Restek's Stabilwax[®] and Rtx[®]-5 GC columns, please request our informative Fast Facts Flyers (Lit. cat.# 59316 for Stabilwax[®] and Lit. cat.# 59310 for Rtx[®]-5).

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pharmaceutical

GC Analysis of Organic Volatile Impurities According to USP <467> Supplement Two of USP 24-NF 19, effective August 1, 2000

A new test for the gas chromatographic (GC) analysis of Organic Volatile Impurities (OVI) in pharmaceutical products was published in the Third Supplement to the US Pharmacopoeia (USP) XXII-NF XVII, which became effective November 15, 1990. Since its original appearance in the USP, this testing protocol

has undergone many revisions and additions.¹⁻⁶ The most recent of which was published as USP 24, effective January 1, 2000.⁷ The biggest change was to the limit test concentrations, which now match the European Pharmacopoeia (EP) concentrations and the ICH guidelines for the five USP <467>-regulated solvents (Table I).^{8,9}

NEW!
Table I

Limit Test Concentrations for USP <467>

benzene*	2ppm
chloroform	60ppm
1,4-dioxane	380ppm
methylene chloride	600ppm
trichloroethene	80ppm

* Testing for benzene only required when specified in the individual monograph.

USP issued an in-process revision announcing that the limit test for benzene is not required unless a specific limit for benzene is included in the individual drug monograph.¹⁰ The revision was needed because Methods I and V were unable to detect benzene at 2ppm. Currently, Method IV is the only method that detects benzene at 2ppm. This became official in Supplement Two of USP 24-NF 19¹¹, effective August 1, 2000. USP has officially removed the limit test requirements for benzene from any article specified to be tested by <467> for organic volatile impurities, except where a specific limit for benzene is in the individual monograph. It is anticipated that USP will make more revisions to <467> during 2001.

Figure 1 shows an analysis using USP <467> Method I on a G27 analytical column with a phenylmethyl guard column. Please note that the sample preparation used in this analysis deviates from the method-specified 1:50 dilution in distilled water. A 1:10 dilution in distilled water was used to obtain a detectable amount of benzene by direct injection.

Table II

USP <467> Methods and corresponding chromatographic systems

Method I

G27 with 5m phenylmethyl guard column (5% phenyl/95% methyl polysiloxane) 30m, 0.53mm ID, 5.0µm (Rtx®-G27 column, cat.# 10279-126)

Sample Introduction: Direct Aqueous Injection

Method IV

G43 (6% cyanopropylphenyl/94% dimethylpolysiloxane) 30m, 0.53mm ID, 3.0µm (Rtx®-G43 column, cat.# 16085)

Sample Introduction: Static Headspace

Method V

G43 with 5m phenylmethyl guard column (6% cyanopropylphenyl/94% dimethylpolysiloxane) 30m, 0.53mm ID, 3.0µm (Rtx®-G43 column, cat.# 16085-126)

Sample Introduction: Direct Aqueous Injection

Method VI

Choice of 9 columns, depending on monograph
Sample Introduction: Direct Aqueous Injection

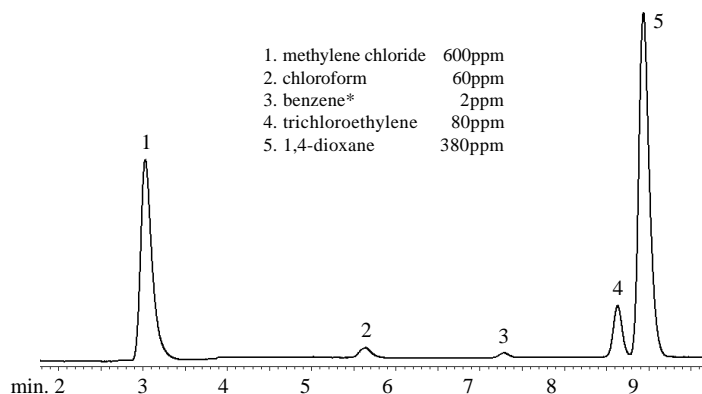
Method for Coated Tablets

0.2% polyethylene glycol, MW 1500 (G39) on graphitized carbon (S7) (0.2% Carbowax® 1500 on 80/100 CarboBlack™ C packed column, cat.# 80122)

Sample Introduction: Static Headspace

Figure 1

A minor modification of the dilution concentration for Method I allows analysis of 2ppm benzene using an Rtx®-G27 column.



Sample Preparation: 1:10 dilution of cat.# 36007 in distilled water (this deviation from the method's 1:50 dilution was needed to obtain a detectable benzene peak).

30m, 0.53mm ID, 5.0µm Rtx®-G27 with 5m phenylmethyl Integra-Guard™ (cat.# 10279-126).

Open temp.: 35°C (hold 5 min.) to 175°C @ 8°C/min., to 260°C @ 35°C/min. (hold 16 min.); **Inj. port:** Uniliner® direct injection sleeve 70°C; **Inj. size:** 1µL; **Det. temp.:** 260°C; **FID sensitivity:** 1 x 10⁻¹² AFS; **Carrier gas:** helium, 4.1psi constant pressure, 35cm/sec. set @ 35°C.

* Testing for benzene only required when specified in the individual monograph.

USP also has clarified that a 5m phenylmethyl guard column is not needed for the Method IV, headspace analysis.¹⁰ **Figure 2** shows an analysis using Method IV at the revised concentrations, the method-specified sample preparation procedure, a G43 analytical column, and no guard column.

The chromatogram in **Figure 3** shows an analysis using USP 24 <467> Method V on a G43 analytical column with a phenylmethyl guard column and, once again, a 1:10 dilution in order to obtain a detectable benzene peak.

USP made changes in 1997 to overcome the difficulties resulting from unregulated solvents coeluting with regulated solvents, and thereby causing over-representation of their concentrations using GC/flame ionization detection (FID) methods.¹² GC/mass spectrometry (MS) or a second, validated column having a dif-

ferent stationary phase may be used to confirm the presence of the coeluting unregulated solvent and report the correct concentration of regulated solvent. **Figures 4, 5, and 6** show commonly-used pharmaceutical processing solvents and their different elution orders on G27, G43, and Stabilwax® columns. Their usefulness is proven as secondary columns for confirmational analysis.

Restek will continue to review changes to pharmaceutical OVI testing. We have developed two new products to meet this most current change. These products—USP <467> Calibration Mix #4 and Mix #5 (cat.# 36006 dissolved in methanol, and cat.# 36007 dissolved in dimethylsulfoxide, respectively) are now available. **For more information regarding these applications, please call Restek's technical service at 800-356-1688 or 814-353-1300, ext. 4, or your local Restek representative.**

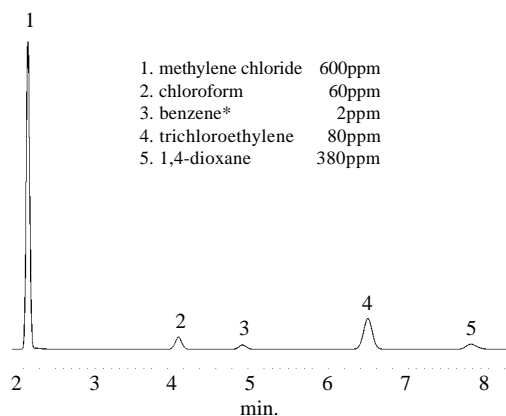
References

1. M.S. Bergren and D.W. Foust, "Comments on USP General Chapter, Organic Volatile Impurities <467>, and Associated Monograph Proposals," *Pharmacopoeial Forum*, May/June 1991, Vol. 17, No. 3, pp. 1963-1968.
2. J.A. Krasowski, H. Dinh, T.J. O'Hanlon, R.F. Lindauer, "Comments on Organic Volatile Impurities, Method I, <467>," *Pharmacopoeial Forum*, May/June 1991, Vol. 17, No. 3, pp. 1969-1972.
3. Pharmacopoeial Forum, March/April 1991, Vol. 17, No. 2, p. 1653.
4. Fifth Supplement, USP-NF, Organic Volatile Impurities <467>, Nov. 15, 1991, pp. 2706-2708.
5. "Organic Volatile Impurities <467>," *Pharmacopoeial Forum*, May-June 1993, Vol. 19, No. 3, pp. 5335-5337.
6. Pharmacopoeial Forum, September/October 1992, Vol. 18, No. 5, p. 4028.
7. USP 24/NF 19, <467> Organic Volatile Impurities, (1877-1878).
8. "ICH Harmonized Tripartite Guideline, Impurities: Guideline for Residual Solvents," *The Fourth International Conference on Harmonization*, July 17, 1997.
9. European Pharmacopoeia, Supplement 1999, pp. 14-15, 208.
10. Pharmacopoeial Forum, November - December 1999, Vol. 25, Number 6, (9223 - 9224).
12. Sixth Supplement, USP-NF, Organic Volatile Impurities <467>, May 15, 1997, pp. 3766-3768.
11. Supplement Two, USP 24/NF 19, August 1, 2000.

These references are not available from Restek.

Figure 2

The Rtx®-G43 column provides the resolution and detection limits needed for USP 24th edition <467> revised limit test concentrations in USP Method IV.



Sample Preparation: 100µL of cat.# 36007 in 5mL distilled water, 1 gram sodium sulfate in a 20mL headspace vial.

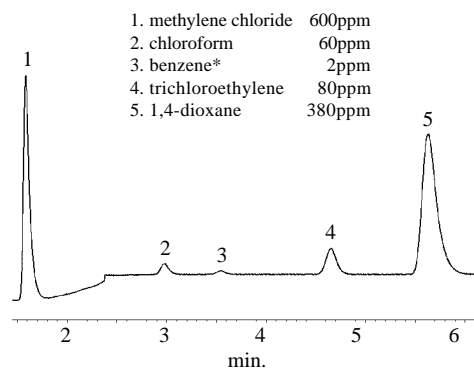
30m, 0.53mm ID, 3.0µm Rtx®-G43 (cat.# 16085)

Oven temp.: 40°C (hold 20 min.) to 240°C @ 35°C/min. (hold 20 min.);
Inj. temp.: 140°C, 1mm split sleeve (cat.# 20916); **Det. temp.:** 260°C;
FID sensitivity: 1.25 x 10⁻¹¹ AFS; **Carrier gas:** helium, 3.5psi constant pressure, 35cm/sec. set @ 40°C; **Split ratio:** 2:1; ThermoQuest HS 2000 Headspace Autosampler Vial 80°C, 60 min. shaker on.

* Testing for benzene only required when specified in the individual monograph.

Figure 3

Achieve analysis of 2ppm benzene for Method V using 1:10 dilution and an Rtx®-G43 column.



Sample Preparation: 1:10 dilution of cat.# 36007 in distilled water (this deviation from the methods 1:50 dilution was needed to obtain a detectable benzene peak).

30m, 0.53mm ID, 3.0µm Rtx®-G43 with 5m phenylmethyl Integra-Guard™ (cat.# 16085-126). ThermoQuest Trace 2000 Series. Uniliner® direct injection sleeve.

Oven temp.: 40°C isothermal (hold 20 min.) to 240°C @ 35°C/min. (hold 20 min.); **Inj. temp.:** 140°C; **FID sensitivity:** 260°C, 1 x 10⁻¹¹ AFS; **Carrier gas:** 4.1psi helium @ 35°C/sec.; **Det. temp.:** 260°C

* Testing for benzene only required when specified in the individual monograph.

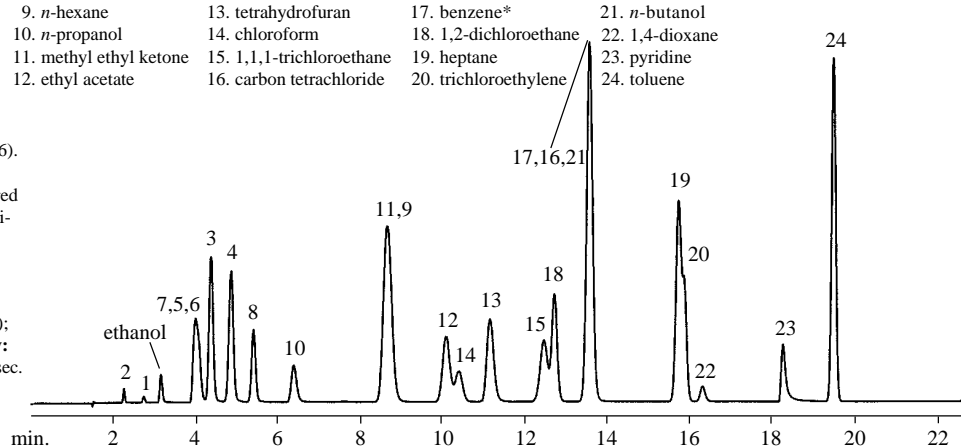
Figure 4

The Rtx[®]-G27 column resolves many solvents commonly used in pharmaceutical processing.

- | | | | | | |
|-----------------------|-----------------------|-------------------------|---------------------------|------------------------|-----------------------|
| 1. ethylene oxide | 5. acetone | 9. <i>n</i> -hexane | 13. tetrahydrofuran | 17. benzene* | 21. <i>n</i> -butanol |
| 2. methanol | 6. isopropanol | 10. <i>n</i> -propanol | 14. chloroform | 18. 1,2-dichloroethane | 22. 1,4-dioxane |
| 3. diethyl ether | 7. acetonitrile | 11. methyl ethyl ketone | 15. 1,1,1-trichloroethane | 19. heptane | 23. pyridine |
| 4. 1,1-dichloroethene | 8. methylene chloride | 12. ethyl acetate | 16. carbon tetrachloride | 20. trichloroethylene | 24. toluene |

30m, 0.53mm ID, 5.0µm Rtx[®]-G27 with 5m phenylmethyl Integra-Guard™ (cat.# 10279-126). Headspace injection of 24 common residual solvents for pharmaceutical processing. Prepared to equal about 500ppm in the bulk pharmaceutical. Samples shaken and heated at 90°C for 15 minutes, 1mL headspace injection.

Oven temp.: 35°C (hold 10 min.) to 100°C @ 5°C/min., to 240°C @ 25°C/min. (hold 5 min.);
Inj./det. temp.: 220°C/240°C; **FID sensitivity:** 1.05 x 10⁻¹¹ AFS; **Carrier gas:** helium, 35cm/sec. set @ 35°C; **Split ratio:** 2:1.



* Testing for benzene only required when specified in the individual monograph.

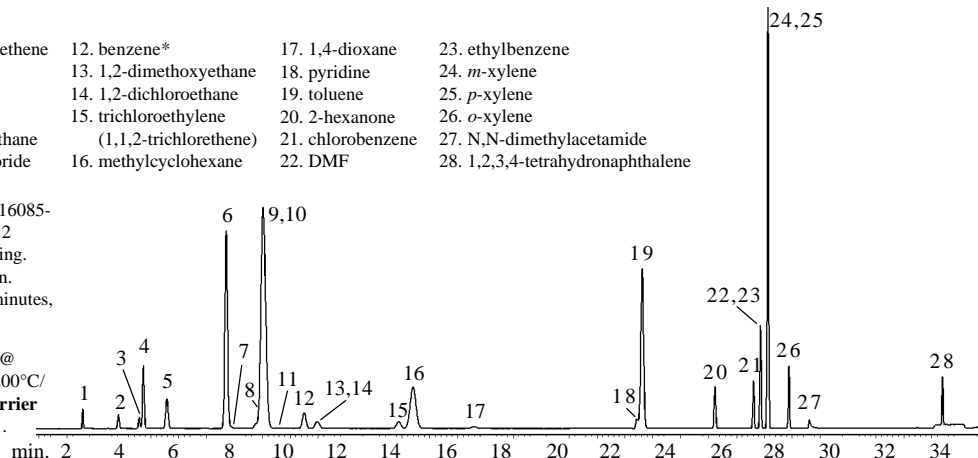
Figure 5

The Rtx[®]-G43 column shows excellent resolution of commonly-used pharmaceutical processing solvents. European Pharmacopoeia Class 1 and Class 2 compounds at the regulation limit concentration.

- | | | | | |
|---|-----------------------------------|---|-------------------|-----------------------------------|
| 1. methanol | 6. <i>cis</i> -1,2-dichloroethene | 12. benzene* | 17. 1,4-dioxane | 23. ethylbenzene |
| 2. 1,1-dichloroethene | 7. nitromethane | 13. 1,2-dimethoxyethane | 18. pyridine | 24. <i>m</i> -xylene |
| 3. acetonitrile | 8. chloroform | 14. 1,2-dichloroethane | 19. toluene | 25. <i>p</i> -xylene |
| 4. methylene chloride (dichloromethane) | 9. cyclohexane | 15. trichloroethylene (1,1,2-trichloroethene) | 20. 2-hexanone | 26. <i>o</i> -xylene |
| 5. hexane (C6) | 10. 1,1,1-trichloroethane | 16. methylcyclohexane | 21. chlorobenzene | 27. N,N-dimethylacetamide |
| | 11. carbon tetrachloride | | 22. DMF | 28. 1,2,3,4-tetrahydronaphthalene |

30m x .53mm ID x 3.0mm Rtx[®]-G43 (cat.# 16085-126). Headspace injection of 28 Class 1 and 2 residual solvents for pharmaceutical processing. Prepared at the regulatory limit concentration. Samples shaken and heated at 80°C for 15 minutes, 1mL headspace injection.

Oven temp.: 40°C (hold 20 min.) to 240°C @ 10°C/min. (hold 20 min.); **Inj./det. temp.:** 200°C/250°C; **FID sensitivity:** 1.1 x 10⁻¹¹ AFS; **Carrier gas:** hydrogen @ 35cm/sec.; **Split ratio:** 2:1.



* Testing for benzene only required when specified in the individual monograph.

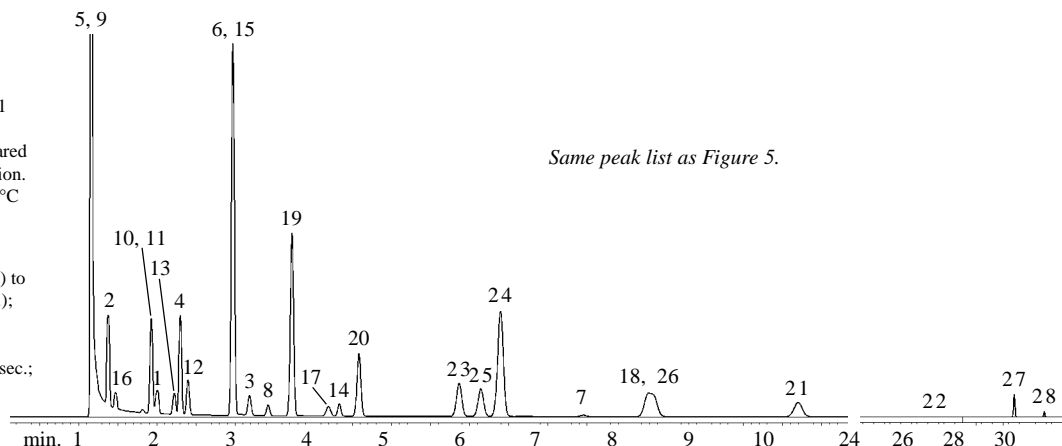
Figure 6

The Stabilwax[®] column makes an excellent confirmation column for commonly-used pharmaceutical processing solvents. European Pharmacopoeia Class 1 and Class 2 compounds at the regulation limit concentration.

30m x .53mm ID x 0.5µm Stabilwax[®] (cat.# 10640-126). Headspace injection of 28 Class 1 and 2 residual solvents for pharmaceutical processing. Prepared at the regulatory limit concentration. Samples shaken and heated at 80°C for 15 minutes, 1mL headspace injection.

Oven temp.: 50°C (hold 20 min.) to 165°C @ 6°C/min. (hold 20 min.);
Inj./det. temp.: 200°C/250°C;
FID sensitivity: 1.1 x 10⁻¹¹ AFS;
Carrier gas: hydrogen @ 35cm/sec.;
Split ratio: 2:1

Same peak list as Figure 5.



* Testing for benzene only required when specified in the individual monograph.

Product Listing

Rtx®-G27 with 5m phenylmethyl Integra-Guard™ (5% phenyl/95% methyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	5.00	-60 to 270/290°C	10279-126

Rtx®-G43 with 5m phenylmethyl Integra-Guard™ (6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	3.00	-20 to 240°C	16085-126

Rtx®-G43 USP <467> Method IV (6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	Temp. Limits	30-Meter
0.53mm	3.00	-20 to 240°C	16085

Stabilwax® Columns

ID	df (µm)	Temp. Limits	30-Meter
0.32mm	0.25	40 to 250/260°C	10624
0.53mm	0.50	40 to 250/260°C	10640

CarboBlack™ Packed Columns

Description	Length	OD	ID	cat.#
0.2% Carbowax® 1500 on 80/100 CarboBlack™ C	2m	1/8"	2mm	80122-*

*Please call for cat.# suffix to specific GC column configuration.

USP <467> Calibration Mix #2

benzene	100µg/mL
chloroform	50
1,4-dioxane	100
methylene chloride	500
trichloroethene	80

Prepared in methanol, 1mL/ampul

each	10-pk.
36002	36102

USP <467> Calibration Mix #3

benzene	100µg/mL
chloroform	50
1,4-dioxane	100
methylene chloride	500
trichloroethene	100

Prepared in DMSO, 1mL/ampul

each	10-pk.
36004	36104

USP <467> Calibration Mix #4

benzene	2µg/mL
chloroform	60
1,4-dioxane	380
methylene chloride	600
trichloroethene	80

Prepared in methanol, 1mL/ampul

each	10-pk.
36006	36106

USP <467> Calibration Mix #5

benzene	2µg/mL
chloroform	60
1,4-dioxane	380
methylene chloride	600
trichloroethene	80

Prepared in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36007	36107



USP <467> Calibration Mix #6

chloroform	60µg/mL
1,4-dioxane	380
methylene chloride	600
trichloroethylene	80

Prepared in methanol, 1mL/ampul

each	10-pk.
36008	36108



USP <467> Calibration Mix #7

chloroform	60µg/mL
1,4-dioxane	380
methylene chloride	600
trichloroethylene	80

Prepared in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36009	36109

USP <467> Method—ethylene oxide standard

500µg/mL in dimethylsulfoxide, 1mL/ampul

each	10-pk.
36005	36105

**Custom USP 24-NF 19 mixtures
are available on request.**

Restek Trademarks: CarboBlack, Integra-Guard, Press-Tight, Rtx, and Stabilwax.
Other Trademarks: Carbowax (Union Carbide Corp.)

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Bonded PLOT Columns for Dual-Column GC Analysis of Gases and Volatiles

Porous Layer Open Tubular (PLOT) columns have been used widely in the gas chromatography (GC) analysis of gases and volatile compounds. Their strong retention and unique selectivity for these analytes of interest make them ideal for a variety of environmental and petrochemical applications. They are more sensitive and efficient, and provide faster analyses of gases and volatiles than packed or gas-liquid columns.

Restek's bonded **Rt-Msieve™ 5A** PLOT column, made from molecular sieve 5A, provides the most effective separation of all the permanent gases and is capable of separating critical pairs such as He/Ne and Ar/O₂ to baseline at 30°C or above. But, this column is very reactive and retentive for CO₂, resulting in poor chromatographic peak shape.

Restek's bonded porous polymer-based **Rt-QPLOT™** column provides tremendous opportunities for tuning chromatographic selectivities in the analysis of gaseous and volatile hydrocarbons, and various solvents. It is very sensitive for CO₂, but does not separate the permanent gases as well as the molecular sieve 5A. Therefore, coupling the Rt-Msieve™ 5A and Rt-QPLOT™ columns dramatically enhances the separation effectiveness for the analysis of permanent gases and CO₂. Two segments of Rt-QPLOT™ column (50cm each, cut from the end of the analytical column cat.# 19716) were connected to a 30m x 0.32mm Rt-Msieve™ 5A column and a 30m x 0.53mm Rt-QPLOT™ column in parallel using two universal Press-Tight® 'Y' connectors (Figure 1).

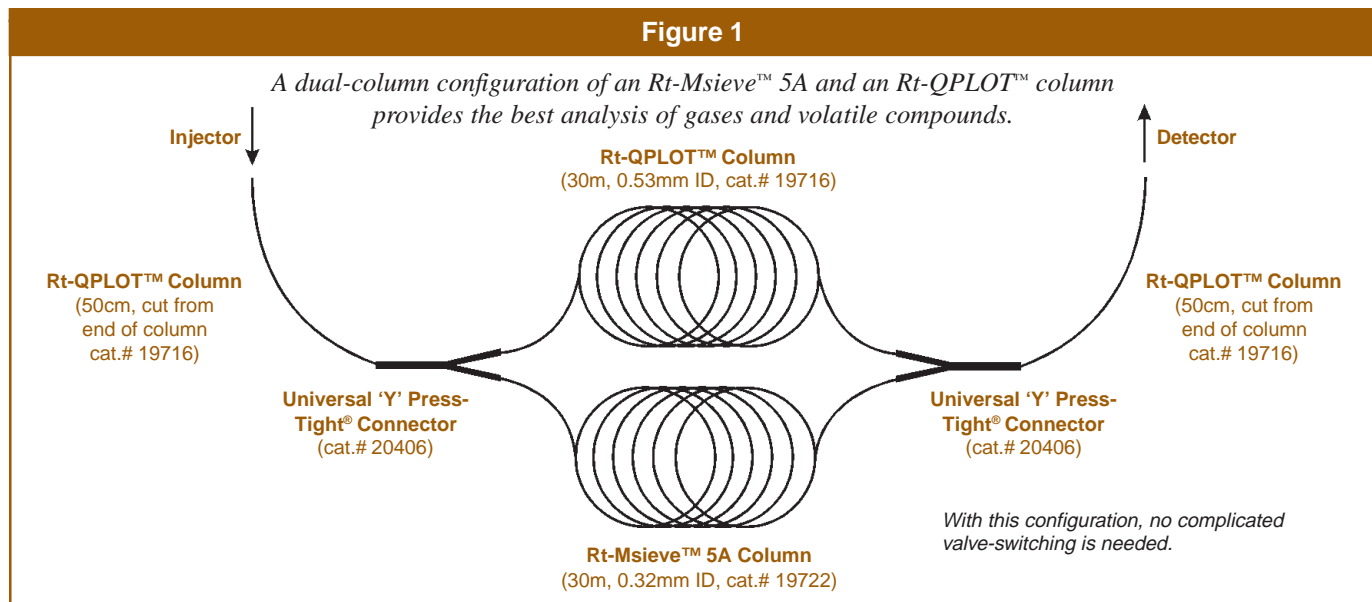
Using this configuration at an oven temperature of 30°C, all the gases are well separated in a single run (see Figure 2 on back). Under the listed analysis conditions, all gases including CO₂

elute from the Rt-QPLOT™ column and reach the detector first. Components not separated by the Rt-QPLOT™ column (i.e., He, Ar, O₂, N₂, CH₄, and CO) are separated by the Rt-Msieve™ 5A column and reach the detector next. It should be noted that Ar and O₂ are baseline resolved, and that CO elutes after 18 minutes with good peak shape.

Quantitation of this analysis can be done a couple of ways: The external standard approach assumes that the sample split at the head of the two columns is consistent from run to run, which is common practice for dual-column methods. The analytical system is initially calibrated with known standards and the subsequent sample areas are compared to the calibration curve. The concentrations of the compounds are reported directly from the calibration curve.

Another method is to report the compounds using area percent. In order to obtain accurate results, the area counts of the compounds eluting from both columns can be normalized.

The mixture of He, Ar, O₂, N₂, CH₄, CO, and CO₂, is impossible to completely separate using any single column, unless cryogenic cooling is applied. Multiple separation mechanisms are needed to pull these compounds apart at normal analysis conditions. This Applications Note focuses on a solution to this difficult analysis using Restek's bonded PLOT columns.



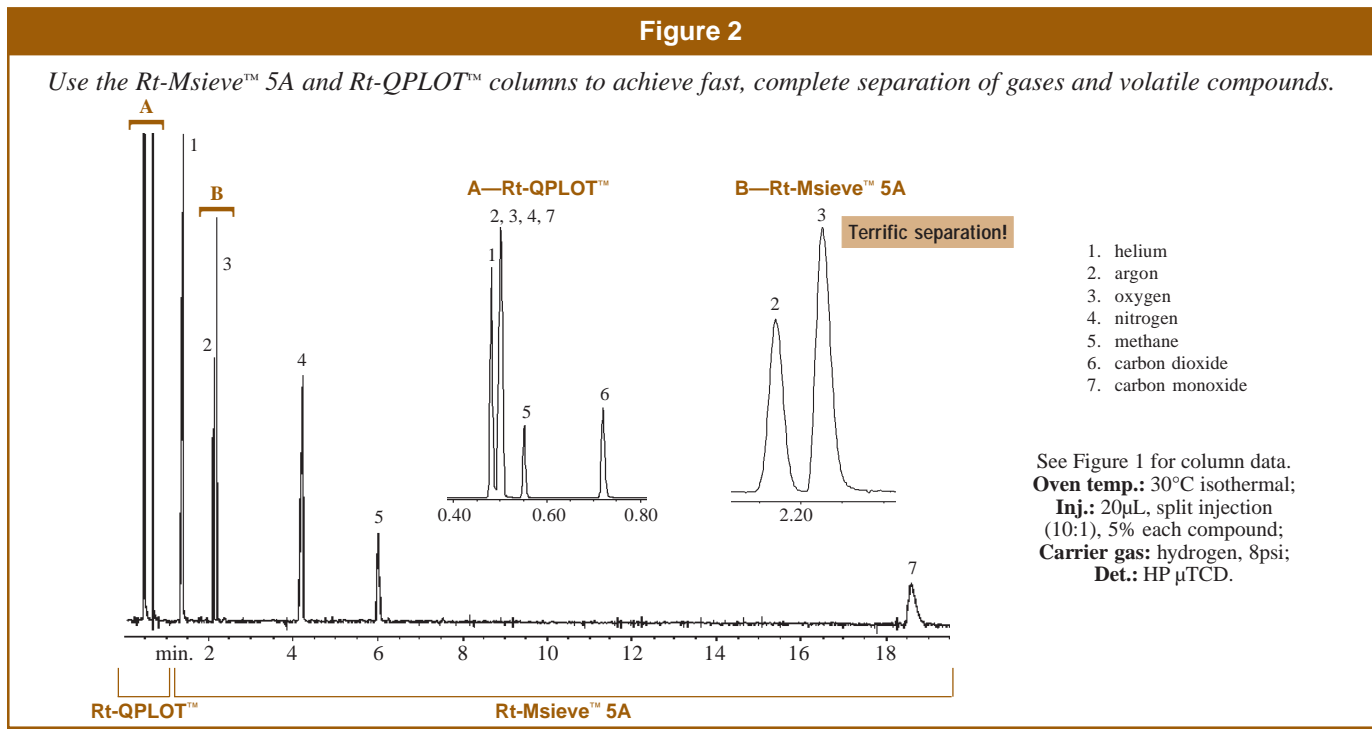
Because the sample splits prior to the two analytical columns, the CO₂ area count from the Rt-QPLOT™ column must be normalized to the other compounds eluting from the Rt-Msievie™ 5A column. One way to normalize the area counts is to use methane as an internal reference compound. Divide the CO₂ area by the methane area obtained from the Rt-QPLOT™ column. The peak areas of the compounds eluting from the Rt-Msievie™ 5A column are divided by the associated methane area. The normalized areas from both columns then can be combined to calculate percent levels for each of the compounds.

A second method to normalize the CO₂ response on the Rt-QPLOT™ column to the areas of the compounds on the Rt-Msievie™ 5A column is to use the summation of the permanent gas (He, Ar, O₂, N₂, and CO) areas from each column. The relationship is:

$$\frac{(\text{area of CO}_2 \text{ from the Rt-QPLOT}^\text{TM} \text{ column})}{(\text{total area perm. gases from the Rt-QPLOT}^\text{TM} \text{ column})} \times \frac{(\text{total area of perm. gases from the Rt-Msievie}^\text{TM} \text{ 5A column})}{(\text{relative area CO}_2 \text{ on the Rt-Msievie}^\text{TM} \text{ 5A column})} =$$

Using the relative area of CO₂ with the other compound areas on the Rt-Msievie™ 5A column, the percent levels of the compounds can be calculated.

The analysis of permanent gases including CO₂ has previously required valve switching with two column systems. Now, with Restek's innovative analytical approach using the Rt-Msievie™ 5A and the Rt-QPLOT™ columns in parallel, this analysis can be accomplished without cooling or complicated valve switching and with less expensive equipment.



Product Listing

Rt-Msievie™ 5A Columns				
ID	df (µm)	Temp. Limits	15m	30m
0.32mm	30	up to 300°C	19720	19722
0.53mm	50	up to 300°C	19721	19723

Rt-QPLOT™ Columns				
ID	df (µm)	Temp. Limits	15m	30m
0.32mm	10	up to 250°C	19717	19718
0.53mm	20	up to 250°C	19715	19716

Universal 'Y' Press-Tight® Connectors	
20405, each	20406, 3-pk.

Universal Angled 'Y' Press-Tight® Connectors	
20403, each	20404, 3-pk.

Restek Trademarks: Press-Tight, Rt-Msievie, Rt-QPLOT.

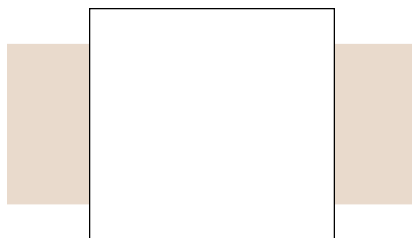
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Applications note

cat.# 59566

Analyze Fixed Gases Using the New Rt-Msieve™ 13X PLOT Column



After ten minutes of aggressive sonication, the molecular sieve particles remain intact.

- Unique selectivity of Rt-Msieve™ 13X improves overall analysis.
- Immobilized to eliminate particle generation.
- Columns can be reactivated after water contamination.
- Guaranteed column-to-column reproducibility.
- Available in 0.53mm and 0.32mm IDs.

Until recently, the only way to achieve rapid separations of fixed gases was the use of molecular sieve packed and micropacked columns. Traditional Molecular Sieve 5Å Porous Layer Open Tubular (PLOT) columns have been useful, but the extended retention and broadened peak width of carbon monoxide has been unavoidable. Restek has developed the Rt-Msieve™ 13X PLOT column to improve the analysis of fixed gases.

Fast and efficient analysis of fixed gases

The Rt-Msieve™ 13X combines the efficiency of traditional molecular sieve PLOT columns with the unique selectivity of 13X molecular sieve. **Figure 1** shows the rapid and efficient analysis of the permanent gases on the 30m, 0.32mm ID Rt-Msieve™ 13X. Baseline separation of

all compounds is achieved in just over 2 minutes. **Figure 2** shows the same analysis using the 15m, 0.32mm ID Rt-Msieve™ 13X PLOT column with complete resolution in 1.5 minutes.

Unique selectivity of Molecular Sieve 13X material

Until now, only Molecular Sieve 5Å PLOT columns have been available. With the Rt-Msieve™ 13X PLOT columns, the separation of nitrogen and methane is increased while overall analysis time is decreased by reducing the retention of carbon monoxide.¹ The 13X molecular sieve also produces a narrower peak shape for carbon monoxide allowing for lower levels of detection. **Figure 3** shows the analysis of the permanent gases on a Molecular Sieve 5Å PLOT column. While the 5Å PLOT column provides good resolution, the peak shapes are broadened, thus decreasing the minimum detection limit approximately ten-fold.

Resists particle generation

PLOT columns are prepared by coating a thick film of very small particles on the inside column wall. A major drawback of PLOT columns is particle generation caused by vibration or pressure surges. The material in the Rt-Msieve™ 13X has been immobilized by a process unique to Restek to minimize any particle generation. This immobilization is stable for applications where column flow rates are disrupted during valve switching or backflushing operations. Non-immobilized PLOT columns will damage or clog valves, causing expensive repairs and down time.

Available in 0.53 and 0.32mm IDs

The Rt-Msieve™ 13X is available in two configurations to satisfy a wide variety of applications. Use the 30m, 0.53mm ID Rt-Msieve™ 13X for most applications and

when using on-line analyzers. The 0.53mm ID Rt-Msieve™ 13X PLOT columns offer the flexibility and increased capacity many analysts require. For increased efficiency and low flow applications, Restek offers the 30m, 0.32mm ID Rt-Msieve™ 13X. The 0.32mm ID Rt-Msieve™ 13X PLOT is ideal for portable analyzers having limited gas supplies where low carrier gas flow is essential. For decreased analysis times, 15-meter versions are available for both IDs.

Columns can be reactivated

Molecular sieves are very hydrophilic and will adsorb any water present in the sample. Water contamination will have detrimental effects on separations causing, 1) the carbon monoxide peak shape to deteriorate and, 2) a reduction in overall resolution. Rt-Msieve™ 13X PLOT columns can be reactivated after water contamination by conditioning at 300°C under dry carrier gas flow, thus extending column lifetime.

Column-to-column reproducibility guaranteed

All Rt-Msieve™ 13X PLOT columns are tested with a mixture of permanent gases. Columns must pass rigorous specifications for efficiency and strict retention time criteria. This stringent testing insures analysts of column-to-column and run-to-run reproducibility.

The resolution of permanent gases can be improved and the overall analysis time can be reduced using the new Rt-Msieve™

Questions?

Call Restek's technical service staff
at

800-356-1688, ext. 4.

13X PLOT columns. The immobilized particles minimize potential damage to valves and reduce detector noise. These columns are available in 0.53mm ID for increased capacity or in 0.32mm ID for reduced carrier gas consumption.

Rigorous testing guarantees the performance of all Rt-Msieve™ 13X columns.

1. Cowper, C.J., DeRose, A.J., *The Analysis of Gases by Chromatography*, Pergamon Press, 1983.

Product Listing

Rt-Msieve™ 13X Columns

15m (cat.#) 30m (cat.#)

0.32mm ID 19707 19705

0.53mm ID 19708 19706

MXT®-Msieve 13X Columns (Silcosteel®)

15m (cat.#) 30m (cat.#)

0.53mm ID 79708 79706

Rt-Msieve™ 5A Columns

15m (cat.#) 30m (cat.#)

0.32mm ID 19720 19722

0.53mm ID 19721 19723

Rt-S PLOT Columns (fused silica)

15m (cat.#) 30m (cat.#)

0.32mm ID 19711 19710

0.53mm ID 19713 19712

Rt-Q PLOT Columns (fused silica)

15m (cat.#) 30m (cat.#)

0.32mm ID 19717 19718

0.53mm ID 19715 19716

MXT®-Q PLOT Columns (Silcosteel®)

15m (cat.#) 30m (cat.#)

0.53mm ID 79715 79716

Rt-Alumina™ Columns (fused silica)

30m (cat.#) 50m (cat.#)

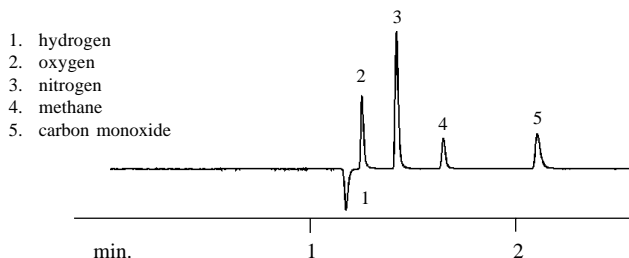
0.53mm ID 19700 19701

30m (cat.#) 60m (cat.#)

0.32mm ID 19702 19703

Figure 1

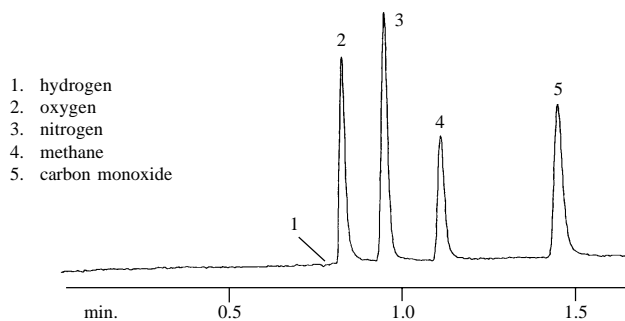
Resolve permanent gases in just over 2 minutes with a 30-meter Rt-Msieve™ 13X PLOT column.



30m, 0.32mm ID Rt-Msieve™ 13X PLOT column (cat.#19705). 15µl split injection of permanent gases (hydrogen spiked). **Oven temp:** 40°C isothermal; **Inj./det. temp:** 200°C/200°C; **Detector:** microcell TCD; **Carrier gas:** helium; **Linear velocity:** 44cm/sec. set @ 40°C (2cc/min.); **Det. sensitivity:** 50m V full scale; **Split ratio:** 15:1.

Figure 2

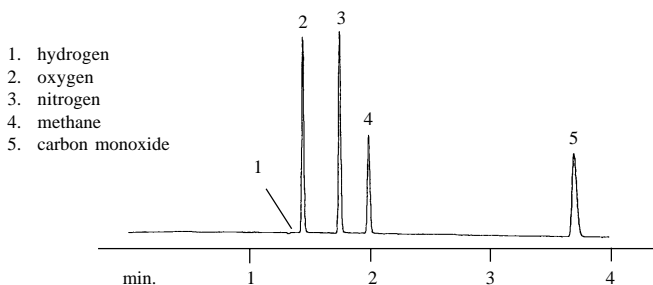
Resolve permanent gases in 1.5 minutes with a 15m Rt-Msieve™ 13X PLOT column.



15m, 0.32mm ID Rt-Msieve™ 13X PLOT column (cat.#19707). 20µl split injection of permanent gases. **Oven temp:** 40°C isothermal; **Inj./det. temp:** 200°C/200°C; **Detector:** microcell TCD; **Carrier gas:** helium; **Linear velocity:** 32cm/sec. set @ 40°C (1.5cc/min.); **Det. sensitivity:** 50m V full scale; **Split ratio:** 15:1.

Figure 3

The Molecular Sieve 5Å column produces broader peak shapes and longer retention times for carbon monoxide than the Rt-Msieve™ 13X.



30m, 0.32mm ID Molecular Sieve 5Å PLOT column. 20µl split injection of permanent gases. **Oven temp:** 40°C isothermal; **Inj./det. temp:** 200°C/200°C; **Detector:** microcell TCD; **Carrier gas:** helium; **Linear velocity:** 39cm/sec. set @ 40°C (1.85cc/min.); **Det. sensitivity:** 50m V full scale; **Split ratio:** 15:1.

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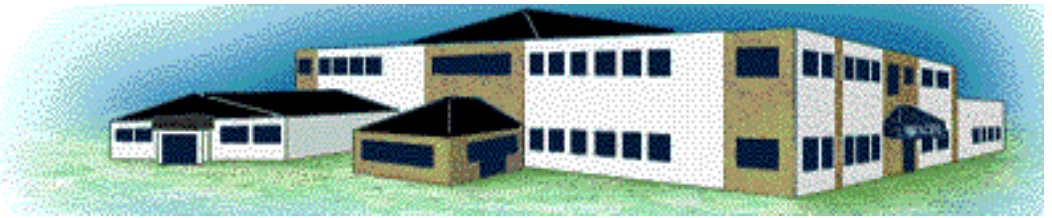
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Environmental Applications Note #59559: Optimizing the Analysis of Chlorophenoxy Herbicides

Chlorine-substituted phenoxyacetic acids, such as 2,4-D, MCPA, and 2,4,5-T [2,4-dichlorophenoxy acetic acid, (4-chloro-2-methylphenoxy) acetic acid, and 2,4,5-trichlorophenoxy acetic acid respectively], were introduced as selective weed killers in the 1940s. Due to their growth-regulating and herbicidal activities against broadleaf weeds, they have been commonly used for weed control on cereal crops, grasslands, and lawns. 2,4-D and 2,4,5-T also were used as the primary defoliant agents in Agent Orange during the Vietnam War. Today, chlorophenoxy herbicides still are used as commercially available lawn weed killers.¹

Chlorophenoxy herbicides are applied as either esters or salts, which are easily metabolized by plants. The esters are oil soluble, but also can be applied as emulsions in water. The salts typically are highly soluble in water and are used as aqueous concentrates. Because the chlorophenoxy herbicides are spread on top of the soil or grass and then leach into the ground, there is great potential for groundwater contamination. Chlorophenoxy herbicides readily degrade in the environment and for many years were not considered an environmental or public concern. However, potential hazards to public health and environmental quality led to the development of US Environmental Protection Agency (EPA) methods for the analysis of these herbicides. US EPA Methods 615 (municipal/industrial wastewater) and 8151 (solid waste) were developed to monitor chlorophenoxy herbicides in environmental samples.^{2,3}

Analysis of chlorophenoxy herbicides using gas chromatography (GC) is difficult. In their free acid form, these herbicides have limited volatility and are prone to irreversible adsorption. To overcome this problem, they are most frequently analyzed as methyl esters. Because these herbicides can be applied as several different types of esters or as a salt, they must first be converted to the free acid form, then derivatized into methyl esters for GC analysis. Methylation increases herbicide volatility and overcomes matrix interferences of herbicides extracted from soil. Despite this derivatization step, analysts still can experience the problems of poor resolution, matrix interference, and peak misidentification.

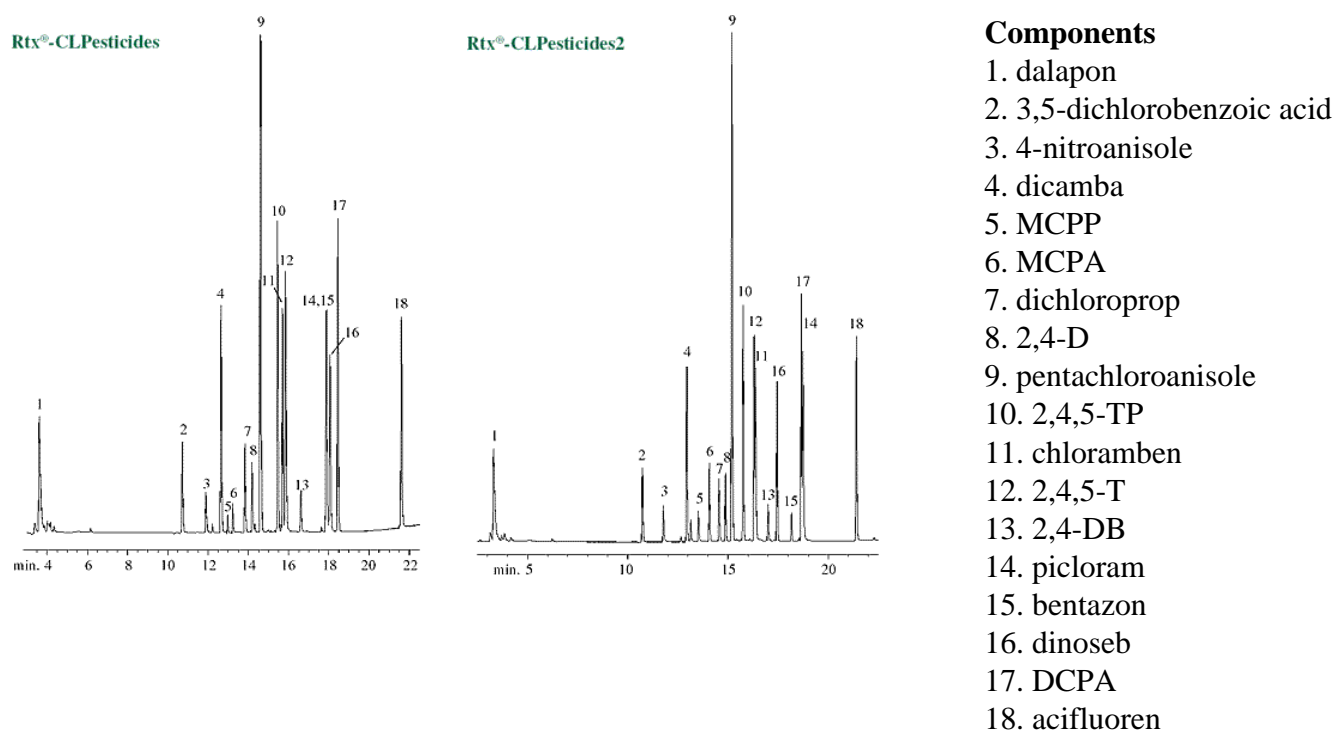
This analysis has been performed on a number of different GC column stationary phases, many of which suffer from slow analysis time, low thermal stability, or coelutions. In addition, many laboratories perform this analysis with the same instrument used for chlorinated pesticide analysis (US EPA 8081). The Rtx®-CLPesticides and Rtx®-CLPesticides2 columns provide unsurpassed separation of pesticides

and herbicides, while allowing high-temperature operation to facilitate the removal of contaminants from the system by programming to 300°C or higher.

Figure 1 shows the separation of the method 8151 target compounds on the [Rtx®-CLPesticides](#) and the [Rtx®-CLPesticides2](#) columns for simultaneous confirmation. These columns were connected using a glass "Y" connector (cat. # 20403) and a 5m guard column (cat. # 10044) installed into a single injection port. The injection port was configured with a direct injection liner (cat. # 20335), which improves the inertness and sample transfer of a splitless injection.

Figure 1: The Rtx®-CLPesticides and Rtx®-CLPesticides2 columns give good resolution for all 18 chlorophenoxy herbicides in a single injection.

[Click chromatograms to enlarge](#)



Conditions

30m, 0.32mm ID, 0.50µm, Rtx®-CLPesticides column (cat.# 11139) and 30m, 0.32mm ID, 0.25µm Rtx®-CLPesticides2 column (cat.# 11324). 1.0µL direct injection of chlorophenoxy herbicides using a Uniliner® sleeve (cat.# 20335), on-column concentration 200-20,000pg/mL. Oven temp.: 80°C (hold 1 min.) to 300°C @ 10°C/min. (hold 10 min.); Inj. / det. temp.: 200°C/300°C; Carrier gas: helium; Inlet pressure: 12.5psi set @ 80°C; ECD sensitivity: 60kHz.

In summary, by configuring your instrument as described and using the Rtx®-CLPesticides and Rtx®-CLPesticides2 columns, the separation of the chlorophenoxy herbicides can be obtained on both columns simultaneously. The surrogates are not shown in these chromatograms because many laboratories use a variety of compounds as surrogates and internal standards for this analysis. The common compounds (1,4-dichlorobenzene, DCAA, and 4,4'-dibromooctafluorobiphenyl) are baseline resolved on both of these columns, so you may use either of them without compromising the chromatography. This same

instrument configuration is also optimal for the analysis of chlorinated pesticides and PCBs.

Product Listing

Rtx®-CLPesticides Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.25	-60 to 310/330°C	11120	11123
0.32mm	0.50	-60 to 310/330°C	11136	11139
0.53mm	0.50	-60 to 310/330°C	11137	11140

Rtx®-CLPesticides2 Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.20	-60 to 310/330°C	11320	11323
0.32mm	0.25	-60 to 310/330°C	11321	11337
0.53mm	0.42	-60 to 310/330°C	11324	11340

Universal Angled "Y" Press-Tight® Connectors

20403 (ea.) 20404 (3-pk.)

Universal "Y" Press-Tight® Connectors

20405 (ea.) 20406 (3-pk.)

Phenylmethyl Deactivated Guard Columns

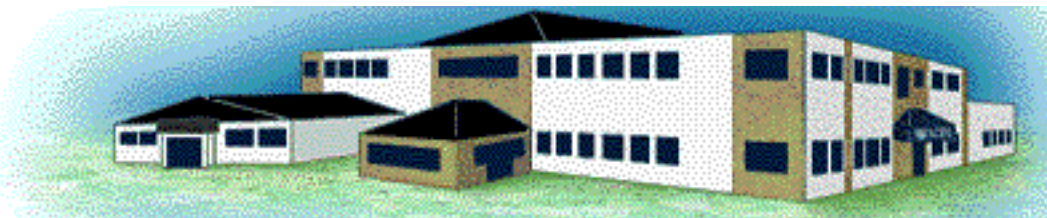
ID	length	cat.#
0.25mm	5m	10043
0.32mm	5m	10044
0.53mm	5m	10045

References

1. Kaufman, D.D., Kearney, P.C., Herbicides, Vol. 1, *Chemistry, Degradation, and Mode of Action*, 2nd Edition, Marcel Dekker, Inc., New York and Basel.
2. US EPA, *Organic Chemical Analysis of Municipal and Industrial Wastewater*; Method 615, "Determination of Chlorinated Herbicides in Industrial and Municipal Wastewater."
3. US EPA, *SW-846 Test Methods for Evaluating Solid Waste, 3rd Edition, Final Update 1*; Method 8150A, "Chlorinated Herbicides by Gas Chromatography."

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Environmental Applications Note #59562: Florisil® SPE Cleanup for Organochlorine Pesticides & PCBs

This procedure is adapted from the US Environmental Protection Agency (EPA) Contract Laboratory Program, Statement of Work for Organic Analyses, Multimedia, Multi-Concentration, OLM04.0. It is not intended to be a replacement or substitute for the official procedure. Please refer directly to the published procedure for additional information.

Preparing for Sample Cleanup

A. Determine the size of Florisil® solid phase extraction (SPE) tube needed for sample cleanup. If the gas chromatography (GC) autosampler can operate reliably with 1mL of sample extract, then a 500mg tube (cat.# 24031 & 24032) is used and the required final volume is 1mL. If the GC autosampler requires a larger sample volume, prepare 2mL of sample extract using a 1g tube (cat.# 24034). Manual injection requires only 1mL final extract and a 500mg tube. See [Tips for Better Results](#) below.

B. Every lot of Florisil® adsorbent must be quality control-tested for activity level before using for sample cleanup. Add 0.5mL of 2,4,5-trichlorophenol solution (0.1µg/mL in acetone) and 0.5mL of Standard Mixture A (midpoint concentration) to 4mL of hexane. Reduce the final volume to 0.5mL using nitrogen. Place mixture onto the top of a washed Florisil® cartridge, and elute it with 9mL of hexane:acetone (90:10) v/v. Use two additional 1mL hexane rinses to ensure quantitative transfer of standard from the Florisil® tube. Reduce the final volume to 1mL using nitrogen, and analyze the solution by GC/electron capture detector (ECD) using at least one of the GC columns specified for sample analysis. Determine the recovery and percent recovery for each analyte. The check sample must be analyzed on a GC/ECD and meet the initial calibration and calibration verification technical acceptance criteria. The Florisil® lot is acceptable if all pesticides are recovered at 80% to 120%, if the recovery of 2,4,5-trichlorophenol is less than 5%, and if no peaks interfering with the target analytes are detected.

Procedure for Sample Cleanup Using Florisil® SPE Tubes

1. Attach the vacuum manifold (cat.# 26077 or 26080) to a vacuum pump, install a trap between the

manifold and the vacuum source. Adjust the vacuum pressure in the manifold to <20" Hg. Place one Florisil® tube onto the vacuum manifold for each sample extract.

2. Prior to cleanup of samples, the tubes must be washed with hexane:acetone (90:10). This is accomplished by placing the tube on the vacuum manifold, pulling a vacuum, and passing at least 5mL of the hexane:acetone solution through the tube. While the tubes are being washed, adjust the vacuum applied to each tube so that the flow rate through each cartridge is approximately equal. Do not allow the cartridges to dry after they have been washed.
3. After the tubes on the manifold are washed, the vacuum is released, and a rack containing labeled 10mL collection vessels is placed inside the manifold.
4. After the collection vessels are in place, restore the vacuum to the manifold. A volume of extract equal to the required final volume (1 or 2mL)* from each sample, blank or matrix spike is transferred to the top frit of the appropriate Florisil® tube. Use a syringe or volumetric pipette to transfer the extract to the cleanup tube.

*This volume must equal the final volume after Florisil® cleanup.

5. The pesticides and PCBs in the extract concentrates then are eluted through the column with 8mL of hexane:acetone (90:10) and are collected into the 10mL collection vessels that are held in the vacuum manifold rack.
6. Transfer the elute in each collection vessel to a clean, appropriate vessel for nitrogen blowdown. Use two additional 1mL hexane rinses to ensure quantitative transfer of the tube elute.
7. Adjust the extract to the final volume (1 or 2mL) by using either nitrogen blowdown or a micro Snyder column. Measure the final volume with a syringe or by transferring the extract to a volumetric flask. The extract is ready for GC/ECD analysis.

Tips for Better Results

- 1g tubes will give the most consistent results regardless of final sample volume.
- Flow rate during elution should be either dropwise or gravity feed (no vacuum). This will reduce trichlorophenol breakthrough.

Product Listing

Rtx®-CLPesticides Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.25	-60 to 310/330°C	11120	11123
0.32mm	0.50	-60 to 310/330°C	11136	11139
0.53mm	0.50	-60 to 310/330°C	11137	11140

Rtx®-CLPesticides2 Column

ID	df (µm)	temp. limits	15-Meter	30-Meter
0.25mm	0.20	-60 to 310/330°C	11320	11323
0.32mm	0.25	-60 to 310/330°C	11321	11337
0.53mm	0.42	-60 to 310/330°C	11324	11340

Resprep™ 12- & 24-Port Tube Manifold

Complete manifolds include glass basin with built-in vacuum regulator, polypropylene top plate with 12 or 24 individual control valves, 12- or 24-position collection rack, and 12 or 24 Teflon® sample guides.

**Complete Resprep™
12-Port Manifold:**

cat.# 26077

**Complete Resprep™
24-Port Manifold:**

cat.# 26080

Resprep™ Florisil® SPE Cartridges

(All cartridges are polypropylene and have polyethylene frits unless otherwise noted)

3mL 500mg 24031/50-pk.

3mL† 500mg 24032*/50-pk.

6mL 1000mg 24034/30-pk.

6mL† 500mg 26086**/30-pk.

6mL† 1000mg 26085**/30-pk.

Notes:

†These cartridges are specified in the CLP Method

*Stainless steel frits

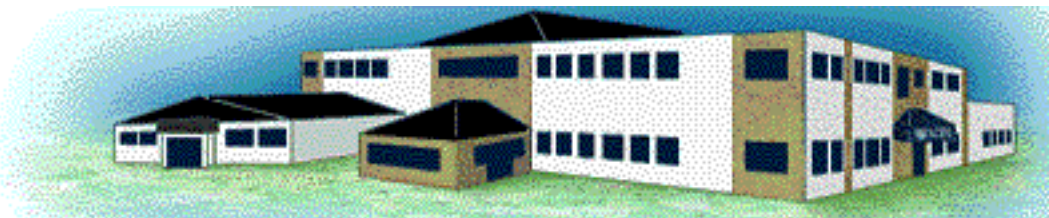
**Glass cartridges with Teflon® frits

Florisil® Cartridge Check Mix

	Each	5-pk.	10-pk.
	32017	32017-510	
w/data pack	32017-500	32017-520	32117

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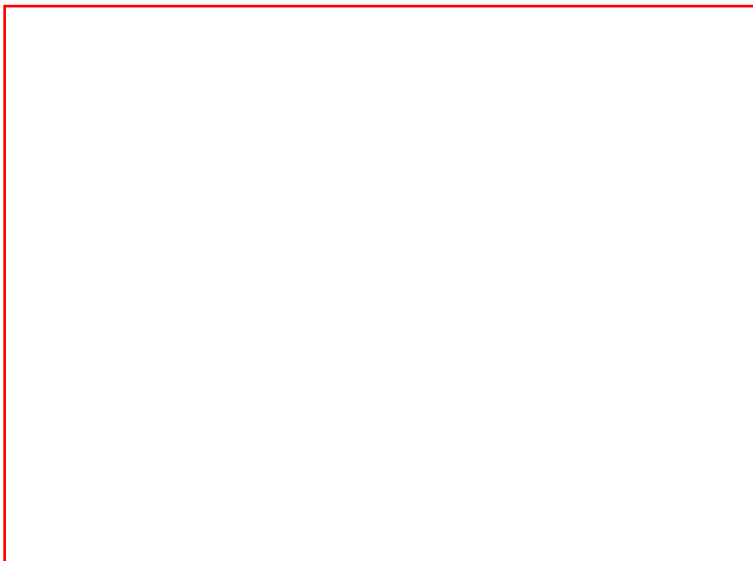
Environmental Applications Note #59586: Analysis of Triazine Herbicides

Triazine herbicides are a class of herbicides that have risen in popularity and usage over the last decade. Because of their low toxicity towards mammals, they are not considered to be a threat to the environment. However, triazine herbicides are relatively stable and can be detected in the environment for long periods of time following their application. Due to their long residence time, monitoring for the presence of triazine herbicides has become a concern. The Environmental Protection Agency (EPA) has addressed these issues by drafting a method for the determination of triazine herbicides in industrial and municipal wastewater.

EPA Method 619 is a gas chromatographic method designed for the analysis of triazine herbicides using packed columns. This method can be modified for use with capillary columns. Resolving the eleven analytes for this method, even with highly efficient capillary columns, can be very difficult. The structures for all of the triazine herbicides are very similar and most common stationary phases do not offer the polarity or selectivity needed to obtain efficient resolution of these compounds. By matching the chemical characteristics of these compounds to stationary phases with selective retention mechanisms, better peak shape and resolution can be achieved.

Triazine herbicides are referred to as s-triazines, meaning they are symmetrical in structure.

Triazines that have greater bio-activity generally contain halogenated (R1) and diamino (R2 and R3) functionalities (Figure 1). These herbicides are based on a six membered ring containing nitrogen constituents at the 1,3 and 5 positions. The electron density resulting from the inclusion of the nitrogen in the ring and the diamino substituted groups imparts significant polarity to these compounds. The degree of polarity will change depending on the functional groups present at either the R1, R2 or R3 substitution sites. Columns containing stationary phases with intermediate polarity are better suited for these compounds.



[Figure 2](#) shows the analysis on a Rtx®-50 column (50% methyl- 50% phenyl polysiloxane). The Rtx®-

50 is a common intermediate polarity stationary phase used for the analysis of pesticides and herbicides. It completely resolves five compounds and partially resolves the remaining six; thus, the Rtx®-50 can effectively be used for the primary analytical column. [Figure 3](#) shows the same compounds analyzed on an Rtx®-200 column. The Rtx®-200 is a trifluoropropylmethyl polysiloxane that has unique selectivity for compounds containing lone pair electrons. The Rtx®-200 completely resolves four compounds, partially resolves five, with two remaining unresolved. The elution pattern for the Rtx®-200 differs greatly from the Rtx®-50 making it an excellent choice as a confirmational column.

Used together, these columns combine to help the analyst positively identify and quantitate the compounds of interest. Since both columns are operated with the same temperature program conditions, simultaneous confirmation can be used. Several techniques for simultaneous dual column confirmation are available including a "Y" Press-Tight® Connector, a "Y" Vu-Union® Connector, a dual column direct injection tee, or a 2-hole ferrule.

Triazine herbicides are commonly applied to agricultural fields containing corn, apples, grapes, etc. Their stability increases their residence time in the environment, creating a general concern for their potential hazards. The Rtx®-50 and Rtx®-200 columns enhance the performance of triazine herbicide analyses. These columns offer a basis of quality that leads to accurate, reproducible results as required in EPA Method 619.

Product Listing

Columns (30m, 0.53mm ID, 0.50µm)

Rtx®-50	cat.# 10540
Rtx®-200	cat.# 15040

Dual Column Analysis

Dual Column Direct Injection Tee Kit

(includes all fittings/ferrules) cat.# 20412

Replacement tee: cat.# 20411

Replacement ferrules:

0.5mm ID graphite:
20201, 10-pk.
20228, 50-pk.

0.8mm ID graphite:
20202, 10-pk.
20224, 50-pk.

1/4" graphite:
20210, 10-pk.

Universal "Y" Press-Tight® Connectors

20405 (ea.)

20406 (3-pk.)

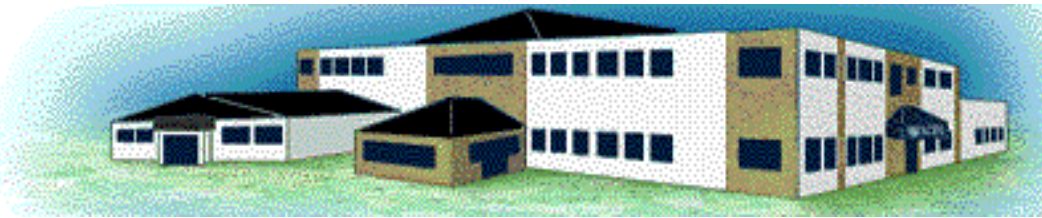
"Y" Vu-Union® Connectors	Replacement inserts:
cat.# 20432	20433 (ea.) 20434 (3 pk.)

Reference

1. Kearney, P.C. and Kaufman, D.D. Herbicides--Chemistry, Degradation, and Mode of Action. 2ed., Marcel Dekker, Inc., 1969.

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Rtx®-1: A New Bonded Packed Column for Simulated Distillation

- Rtx®-1 bonded packed column requires minimal conditioning ([fig. 1](#))
- Meets or exceeds all specifications of ASTM D2887-93 and D3710-93 ([fig. 2](#))
- Stable baseline to 350°C and repeatable RT's "right from the box" ([table 1](#))
- Deactivated Silcosteel® tubing and Silcoport™ packing for high inertness
- Column lifetime superior to existing Sim Dist columns ([fig. 4](#))

[Chromatograms & Tables](#)

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Simulated Distillation (Sim Dist), according to ASTM test methods D2887-93 or D3710-93 can be performed using either packed or capillary columns. Advantages of capillary columns are the columns are preconditioned so they can be used after only minimal conditioning, and the bonded stationary phases exhibit stable baselines and retention times. There are many laboratories currently using packed columns which would like to take advantage of bonded phases but do not have GC equipment that can be easily converted for use with capillary columns.

Restek's Rtx®-1 Sim Dist column is the first in a new generation of bonded packed columns having superior inertness and stability compared to conventional packed columns. These improvements are obtained by preparing the columns with Silcosteel® tubing and bonding the Rtx®-1 stationary phase to a highly deactivated Silcoport™ support. The column dimensions and packing (1/8" Silcosteel® with 10% Rtx®-1 on Silcoport™) are designed to exceed all requirements specified in ASTM Test Methods D2887-93 and D3710-93.

Bonded stationary phases require minimal conditioning and give stable baselines and [retention times](#) "right from the box."

Simulated distillation is a gas chromatographic procedure which differs from typical GC analysis

requiring peak resolution and integration. The sample is analyzed using a linear temperature program so that the retention time of the hydrocarbons are proportional to their boiling points. The sample boiling range distribution is calculated by comparing the sample area and its retention time with that of an alkane calibration standard. In order for the calibration to be valid for sample analysis, it is crucial that retention times be repeatable until the next calibration is performed. [Figure 1](#) is an example of the analysis of the Restek D2887 Calibration Mix (cat.# 31222) illustrating the typical pattern obtained for the alkanes under temperature programmed conditions. To demonstrate the stability of the Rtx®-1 column, a series of calibration standards were analyzed after only 30 minutes of conditioning at 350°C. [Table 1](#) shows the excellent retention time repeatability obtained with the column, indicating the column is suitable for sample analysis after minimal conditioning.

Column bleed is another important consideration for selecting a Sim Dist column. The baseline must be stable and free of any artifacts during the temperature program up to 350°C. Although baseline subtraction is permitted in the method, this compensation will produce errors if the baseline is not consistent. Conventional packed columns require up to 14 hours of conditioning and frequent updating of the baseline compensation run because the stationary phase is not bonded. Rtx®-1 columns, however, exhibit stable and reproducible baselines with just 30 minutes of conditioning. This results in fewer baseline blanks and less frequent calibration increasing laboratory productivity.

Rtx®-1 Sim Dist 2887 Packed Columns can also be used for gasoline range simulated distillation.

Simulated distillation of gasoline range hydrocarbons according to ASTM method D3710-93 can also be performed using the Rtx®-1 Sim Dist 2887 Packed Column. [Figure 2](#) shows the analysis of ASTM D3710 calibration mix with the addition of n-propane, 2-methyl propane, n-butane, n-hexadecane, and n-heptadecane. To achieve baseline separation of n-propane, 2-methyl propane, and n-butane, the GC oven was cooled to -30°C with liquid nitrogen. [Figure 3](#) shows the analysis of a composite gasoline sample under the same run conditions. Other volatile petroleum fractions such as kerosene and jet fuel can also be analyzed with this column.

Bonded stationary phases extend column lifetime.

[Figure 4](#)

The Rtx®-1 stationary phase is bonded to the diatomite particles resulting in an immobilized coating which is resistant to solvents and lower in bleed than conventional packing. Since the packing is preconditioned, there is no need for extended conditioning in an oxygen-free system, otherwise high

bleed will result. Since GC systems often have leaks or carrier gas which contains oxygen, it is more likely that conventional columns will be damaged during the conditioning process. Figure 4a shows a conventional UCW-982 column after only 170 temperature cycles, demonstrating higher bleed and more tailing than the Rtx®-1 Sim Dist column (Figure 4b). Although actual column lifetimes depend upon the system and type of samples analyzed, the bonded stationary phase should result in longer lifetime than its non-bonded equivalent.

Rtx®-1 Sim Dist columns

have [equivalent polarity](#) to OV-101 and UCW-982.

In order for a stationary phase to be acceptable for ASTM methods, the column must not exhibit selective retention for aromatic hydrocarbons compared to aliphatic hydrocarbons. This is an important test because if the polarity of a column is different, the boiling point results will demonstrate a bias, especially for highly aromatic samples. The "polarity" of the bonded Rtx®-1 column was compared with OV-101 and UCW-982, two of the most common stationary phases currently used for simulated distillation. The results of the calculated boiling points for aromatics compared to the published boiling points appear in Table II. All three silicone columns tested are essentially identical in they elute aromatics at a slightly lower temperature than the alkanes. This confirms the polarity of the Rtx®-1 column is equivalent, and the boiling range values obtained will agree with OV-101 and UCW-982 columns.

Rtx®-1 is an excellent choice for Sim Dist using packed columns.

Simulated distillation is one of the most common GC analyses performed in the petroleum laboratory. ASTM test methods D2887 and D3710 can be performed with either packed or capillary columns, but until now the benefits of bonded phases were available only to capillary users. The Rtx®-1 packed column uses a bonded stationary phase which is immobilized on Silcoport™-a specially deactivated support. The columns are prepared using Silcosteel® tubing for inertness unavailable with conventional metal tubing. Rtx®-1 bonded packed columns require minimal conditioning and give stable baselines and retention even after only 30 minutes of operation at 350°C. If your laboratory has been looking for a better Sim Dist analysis, Restek's Rtx®-1 packed columns are the answer.

[Product List](#)

Chromatograms

[Figure 1: C5 to C44 calibration analysis after only 30 minutes conditioning.](#)

[Figure 2: Rtx®-1 SimDist 2887 packed columns can also be used for ASTM D-3710 analysis.](#)

[Figure 3: Perform simulated distillation of gasoline using the Rtx®-1 SimDist 2887 packed column.](#)

[Figure 4: Bonded packed columns exhibit lower bleed and longer lifetimes after 170 temperature cycles.](#)

Tables

[Table 1: Retention Time Repeatability](#)

[Table 2: No Polarity Differences](#)

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TABLE 1 - Retention Time Repeatability for Calibration after only 30 minutes conditioning.

Hydrocarbon	Min Rt	Max Rt	Avg RT	Stand. Dev.
C5	0.241	0.243	0.242	0.001
C6	0.493	0.497	0.495	0.002
C10	5.746	5.765	5.752	0.005
C20	18.482	18.491	18.486	0.004
C28	25.093	25.103	25.098	0.004
C40	32.160	32.171	32.166	0.004
C44	34.316	34.328	34.326	0.007

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TABLE 2 - Comparison of bonded and conventional packed columns indicates no polarity differences.

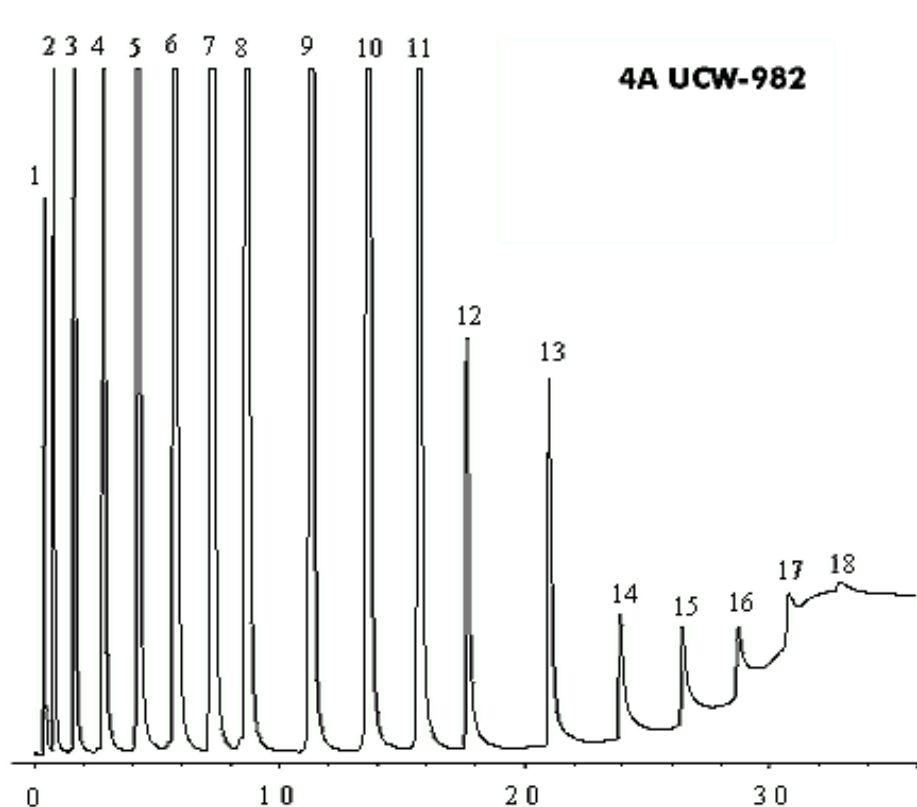
Aromatic Hydrocarbon	Published BP(°C)	Rtx®-1	UCW-982	OV-101
benzene	80	81.3	82	80.3
<i>p</i> -xylene	139	138.6	140.2	137.7
naphthalene	218	204.6	206.9	204.3
acenaphthylene	280	252.7	255.6	252.2
anthracene	342	304.1	307.2	303.4
chrysene	447	385.6	389.2	384.9
dibenzo(a,h)anthracene	524	452.3	455.7	450.4

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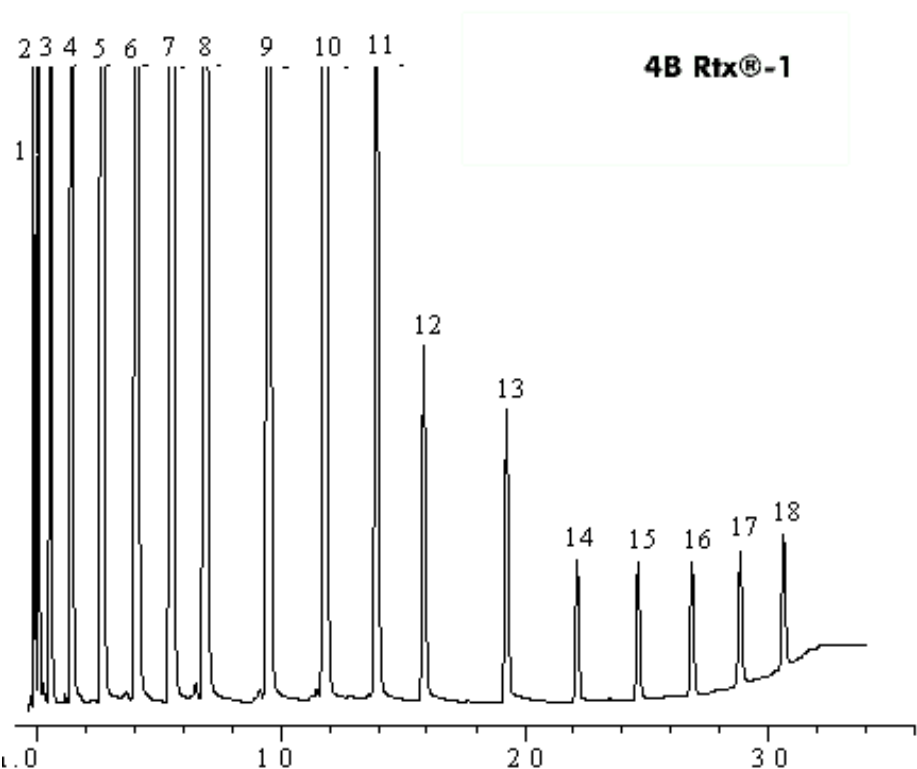
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Figure 4: Bonded packed columns exhibit lower bleed and longer lifetimes after 170 temperature cycles



Component list:

1. pentane
2. hexane
3. heptane
4. octane
5. nonane
6. decane
7. undecane
8. dodecane
9. tetradecane
10. hexadecane
11. octadecane
12. eicosane
13. tetracosane
14. octacosane
15. dotricontane
16. hexatricosane
17. tetracontane
18. tetratetracontane



Run Conditions:

25" x 1/8" Rtx®-1 Sim Dist 2887 Silcosteel® column
1.0µl direct injection of D2887 Calibration Mix

Oven temp: 35°C to 350°C @
10°C/min. (hold 5 min.)

**Injector &
Detector temp:** 350°C

Carrier gas: helium @ 25ml/min.

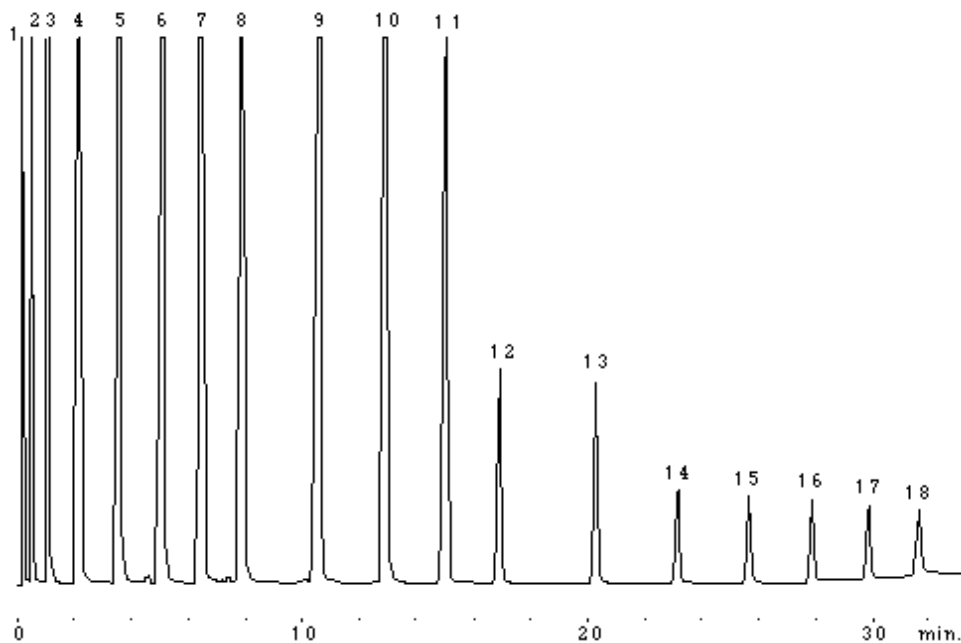
FID sensitivity: 256 x 10⁻¹¹ AFS

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Figure 1: C5 to C44 calibration analysis after only 30 minutes conditioning

**Component list:**

1. pentane
2. hexane
3. heptane
4. octane
5. nonane
6. decane
7. undecane
8. dodecane
9. tetradecane
10. hexadecane
11. octadecane
12. eicosane
13. tetracosane
14. octacosane
15. dotricontane
16. hexatricontane
17. tetracontane
18. tetratetracontane

Run Conditions:

25" x 1/8" Rtx®-1 Sim Dist 2887 Silcosteel® column
1.0µl direct injection of D2887 Calibration Mix

Oven temp: 35°C to 350°C @
10°C/min. (hold 5 min.)

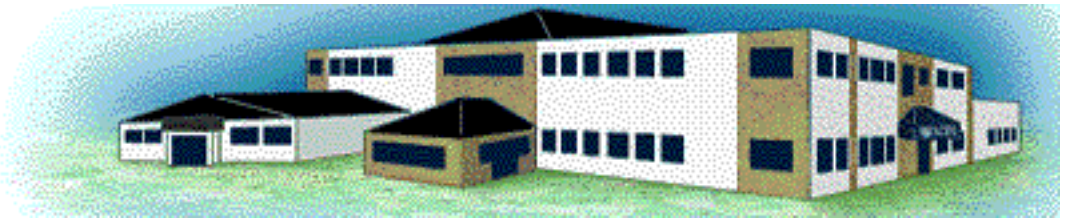
**Injector &
Detector temp:** 350°C

Carrier gas: helium @ 25ml/min.

FID sensitivity: 256 x 10⁻¹¹ AFS

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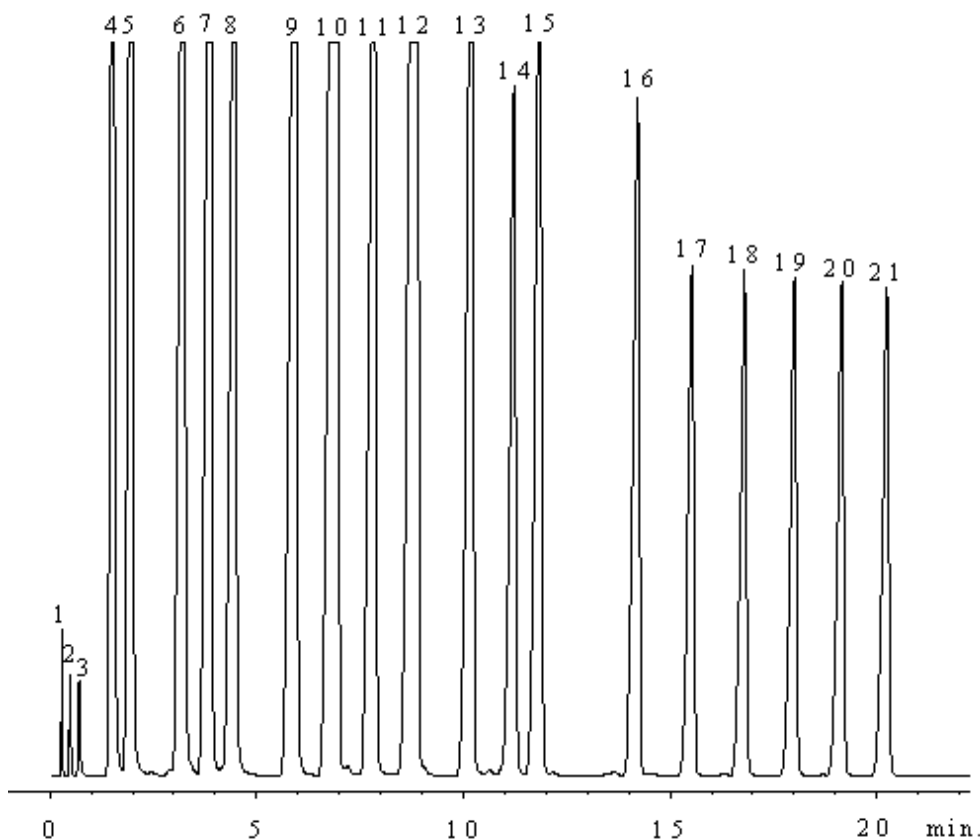
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Figure 2: Rtx®-1 SimDist 2887 packed columns can also be used for ASTM D-3710 analysis



Component list:

1. n-propane
2. 2-methyl propane
3. n-butane
4. 2-methylbutane
5. n-pentane
6. 2-methylpentane
7. n-hexane
8. 2,4-dimethylpentane
9. n-heptane
10. toluene
11. octane
12. p-xylene
13. n-propylbenzene
14. n-decane
15. n-butylbenzene
16. n-dodecane
17. n-tridecane
18. n-tetradecane
19. n-pentadecane
20. n-hexadecane
21. n-heptadecane

Run Conditions:

25" x 1/8" OD x 2mm ID Rtx®-1 Sim Dist 2887
D-3710 Calibration Mix + C3, C4, C16, & C17 added

Oven temp: -30°C to 250°C @
10°C/min.

**Injector &
Detector temp:** 250°C/300°C

Carrier gas: helium @ 25ml/min.

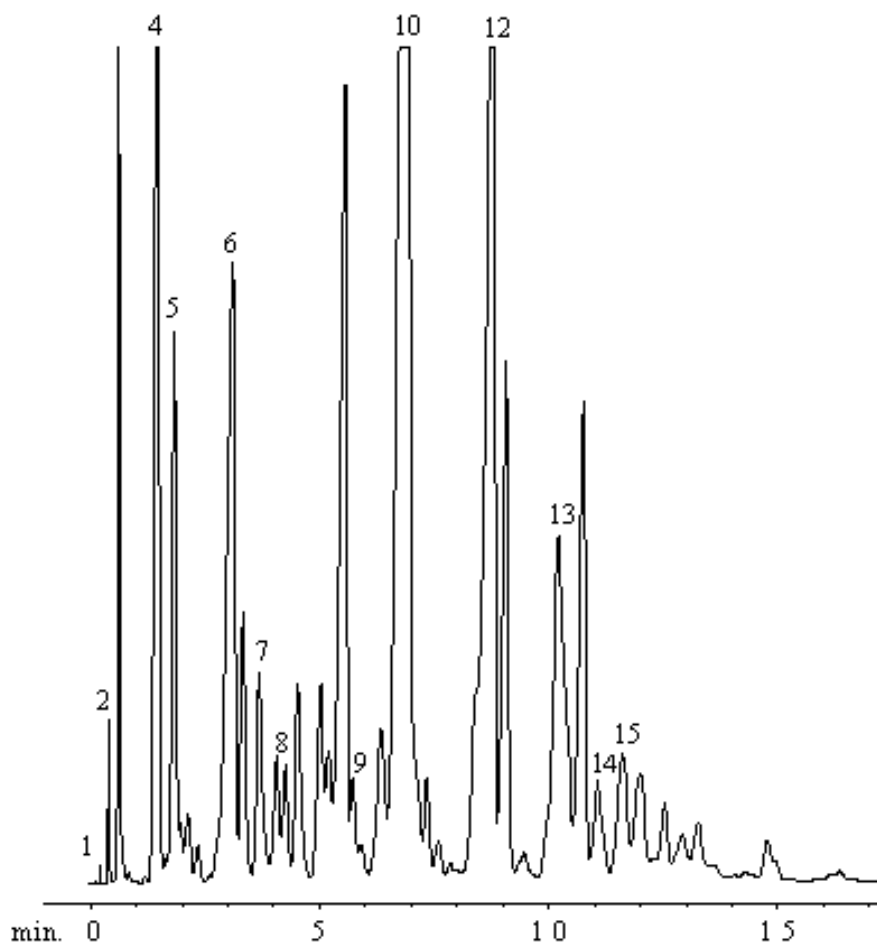
FID sensitivity: 256 x 10⁻¹¹ AFS

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Figure 3: Perform simulated distillation of gasoline using the Rtx®-1 SimDist 2887 packed column



Component list:

1. n-propane
2. 2-methyl propane
3. n-butane
4. 2-methylbutane
5. n-pentane
6. 2-methylpentane
7. n-hexane
8. 2,4-dimethylpentane
9. n-heptane
10. toluene
11. octane
12. p-xylene
13. n-propylbenzene
14. n-decane
15. n-butylbenzene
16. n-dodecane
17. n-tridecane
18. n-tetradecane
19. n-pentadecane
20. n-hexadecane
21. n-heptadecane

Run Conditions:

25" x 1/8" OD x 2mm ID Rtx®-1 Sim Dist 2887

1.0 µl direct injection of unleaded gasoline

Oven temp: -30°C to 250°C @
10°C/min.

**Injector &
Detector temp:** 250°C/300°C

Carrier gas: helium @ 25ml/min.

FID sensitivity: 256 x 10⁻¹¹ AFS

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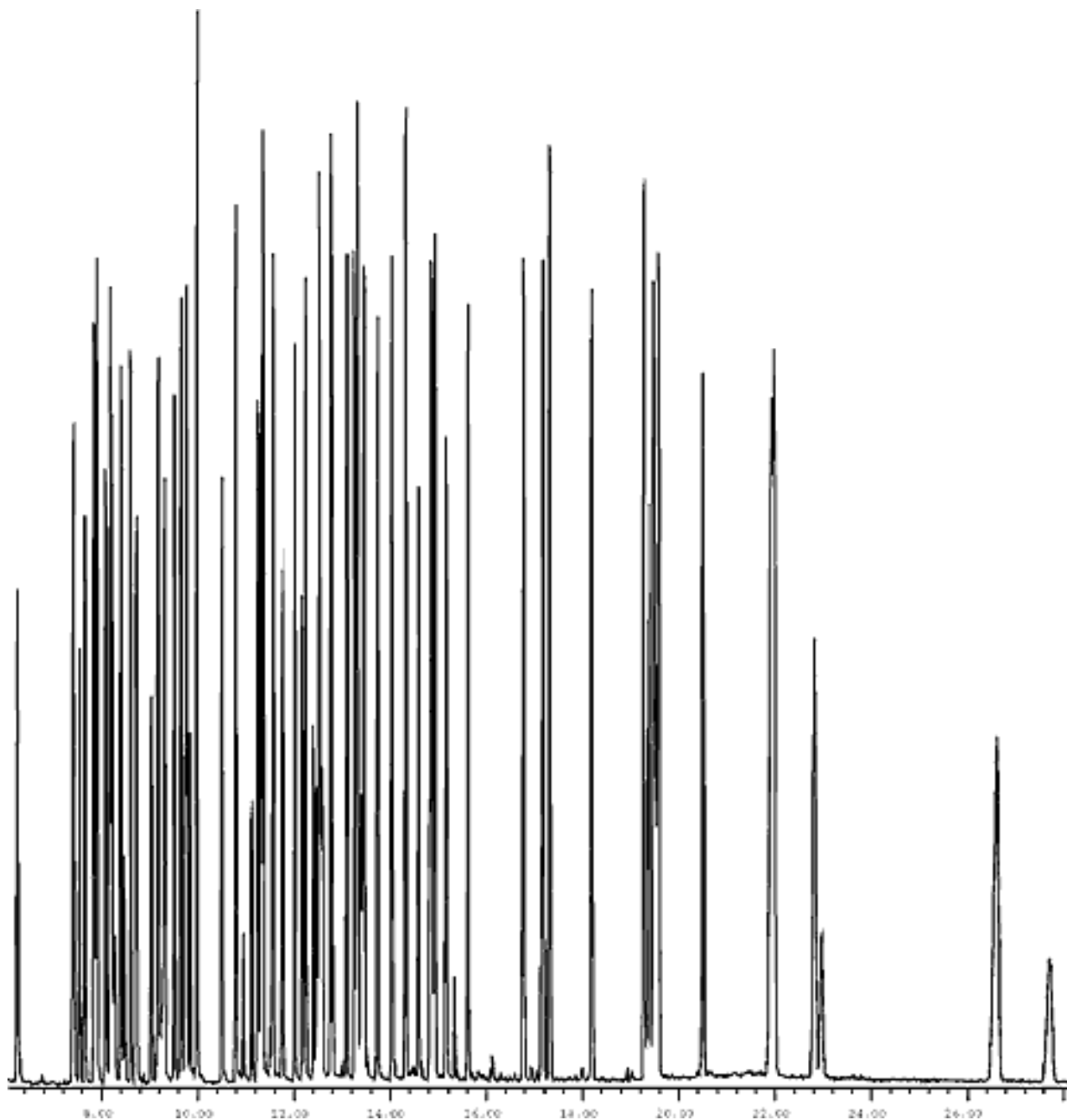
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Achieve low bleed and fast separation of US EPA Method 8270 semivolatile organics on a 0.28mm ID Rtx®-5MS column

[Analysis conditions](#)

[Retention Times](#)



Conditions

30m, 0.28mm ID, 0.50 μ m Rtx®-5MS (cat.# 12694)

160ng splitless injection of a Method 8270 calibration mixture (cat.# 31462)

Single gooseneck liner with fused silica wool (cat# 20798-200.1).

Oven temp.: 45°C (hold 3.5 min.) to 95°C @ 40°C/min, to 295°C @ 17°C/min. (hold 2 min.) then to 320°C @ 40°C/min. (hold 10 min.)

Inj./det. temp.: 300°C/310°C

Carrier gas: helium

Inj. Pressure Program: 30psi initial to 6.5psi @ 1 min. (-99psi/min.).
Split at 1 min.

<u>Compound</u>	<u>Retention Time</u>	<u>Compound</u>	<u>Retention Time</u>
2-Fluorophenol	6.25	3-Nitroaniline	12.41
Phenol-d5	7.41	2,4-Dinitrophenol	12.56
Phenol	7.43	Acenaphthene-d10	12.47
Bis(2-chloroethyl)ether	7.54	Acenaphthene	12.53
2-Chlorophenol	7.65	4-Nitrophenol	12.59
1,3-Dichlorobenzene	7.83	2,4-Dinitrotoluene	12.79
1,4-Dichlorobenzene	7.91	Dibenzofuran	12.77
1,2-Dichlorobenzene	8.17	Diethyl phthalate	13.10
1,4-Dichlorobenzene-d4	7.88	4-Nitroaniline	13.40
Benzyl alcohol	8.08	Fluorene	13.32
2-Methylphenol (o-cresol)	8.22	4-Chlorophenyl phenyl ether	13.25
2,2'-oxybis-(1-chloropropane)	8.27	4,6-Dinitro-2-methylphenol	13.44
N-Nitrosodi-n-propylamine	8.48	N-Nitrosodiphenylamine	13.45
4-Methylphenol (p-cresol)	8.42	2,4,6-Tribromophenol	13.74
Hexachloroethane	8.61	4-Bromophenyl phenyl ether	14.03
Nitrobenzene-d5	8.71	Hexachlorobenzene	14.32
Nitrobenzene	8.73	Pentachlorophenol	14.59
Isophorone	9.04	phenanthrene-d10	14.82
2-Nitrophenol	9.18	phenanthrene	14.85
2,4-Dimethylphenol	9.16	Anthracene	14.93
Bis(2-chloroethoxy)methane	9.31	carbazole	15.16
2,4-Dichlorophenol	9.51	Di-n-butyl phthalate	15.62
Benzoic acid	9.33	Fluoranthene	16.76
1,2,4-trichlorobenzene	9.65	Pyrene	17.16
Naphthalene	9.76	Terphenyl-d14	17.31
Naphthalene-d8	9.73	Butyl benzyl phthalate	18.19
4-Chloroaniline	9.84	3,3'-dichlorobenzidine	19.39
Hexachlorobutadiene	9.98	Benzo(a)anthracene	19.48
4-Chloro-3-methylphenol	10.50	Chrysene-d12	19.51
2-Methylnaphthalene	10.80	Chrysene	19.57
Hexachlorocyclopentadiene	11.13	Bis(2-ethylhexyl)phthalate	19.29
2,4,6-Trichlorophenol	11.25	Di-n-octyl phthalate	20.50
2,4,5-trichlorophenol	11.31	Benzo(b)fluoranthene	21.93
2-Fluorobiphenyl	11.37	Benzo(k)fluoranthene	21.98
1,1'-biphenyl	11.51	Benzo(a)pyrene	22.82
2-Chloronaphthalene	11.56	Perylene-d12	22.97
2-Nitroaniline	11.76	Dibenz(a,h)anthracene	26.60
Dimethyl phthalate	12.01	Indeno(1,2,3-cd)pyrene	26.63
2,6-Dinitrotoluene	12.17	Benzo(ghi)perylene	27.72
Acenaphthylene	12.24		

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HPLC Analysis of Vitamins

The market for nutritional and dietary supplements is growing tremendously. And, as these nutraceutical and dietary supplement markets grow, the need for simple, rugged, and accurate high performance liquid chromatography (HPLC) methods to analyze vitamins becomes increasingly important.

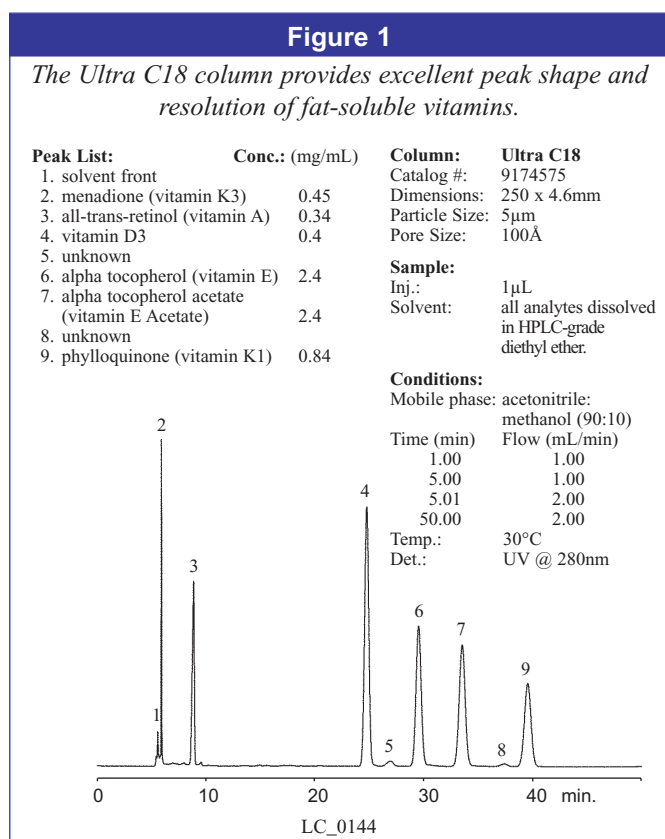
Vitamins encompass a wide range of organic compounds. They are required by the human body in only minute quantities. Yet, they have significant impact at the cellular level. Vitamins play a vital role in converting fat and carbohydrates to energy, regulating metabolism, and other bodily functions.

Vitamins can be broadly classified into two groups, water-soluble vitamins and fat-soluble vitamins. Fat-soluble vitamins include such constituents as Vitamin A (retinol) and Vitamin D3 (cholecalciferol). Vitamin A has been found to play an important role in proper growth and eye function, while Vitamin D is required for proper bone and tooth growth.

Fat-Soluble Vitamins

The Restek Ultra C18 column is an ideal first choice to separate a wide range of fat-soluble vitamins. Its very retentive, high-purity packing exhibits excellent peak shape. The silica has a carbon load of 20% and is fully end-capped, which eliminates any unwanted silanol interactions and improves column-to-column reproducibility.

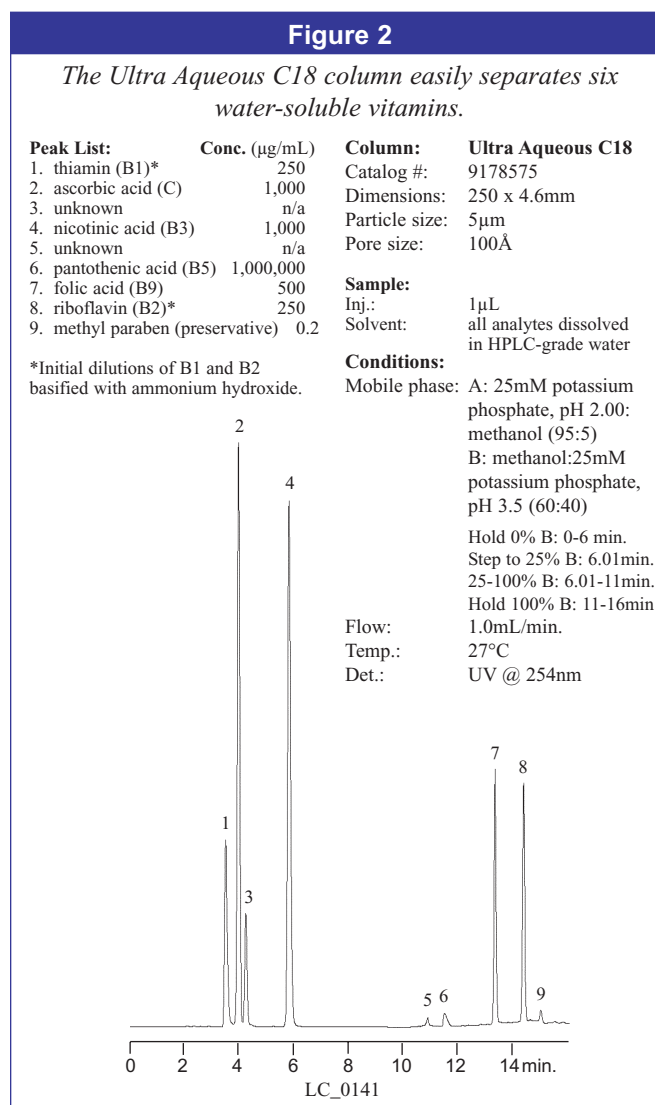
The fat-soluble vitamins are extremely hydrophobic and must be dissolved in an organic solvent. A mixture of six fat-soluble vitamins were separated using an Ultra C18 column (Figure 1).



The analytes were dissolved in diethyl ether. The sample solution was then analyzed using a simple isocratic mobile phase (acetonitrile:methanol [90:10]) with a stepped flow rate (1mL/min. from 1 minute to 5 minutes, then 2mL/min. from 5 minutes to 50 minutes). All six constituents are easily resolved and show excellent peak shape.

Water-Soluble Vitamins

The group of compounds known as water-soluble vitamins is very diverse chemically, including both basic and acidic molecules. Some of the water-soluble vitamins, such as thiamin and ascorbic acid, are very polar and thus difficult to retain by reversed phase HPLC. Many reversed phase methods for water-soluble vitamins require ion-pairing reagents in order to retain the more polar analytes. However, the Ultra Aqueous C18 column easily separates six water-soluble vitamins with a gradient elution and relatively simple mobile phases that contain no ion pairing reagents (Figure 2).



For simple, rugged, and accurate high performance liquid chromatography (HPLC) methods and columns to analyze fat- and water-soluble vitamins, turn to Restek for products and service.

■ *Ultra Aqueous C18, 5 μ m Columns*

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9178531	9178532	9178533	9178535
50mm	9178551	9178552	9178553	9178555
100mm	9178511	9178512	9178513	9178515
150mm	9178561	9178562	9178563	9178565
200mm	9178521	9178522	9178523	9178525
250mm	9178571	9178572	9178573	9178575

■ *Ultra Aqueous C18, 5 μ m Columns with Trident™ Inlet Fitting*

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9178532-700	9178533-700	9178535-700
50mm	—	9178552-700	9178553-700	9178555-700
100mm	—	9178512-700	9178513-700	9178515-700
150mm	—	9178562-700	9178563-700	9178565-700
200mm	—	9178522-700	9178523-700	9178525-700
250mm	—	9178572-700	9178573-700	9178575-700

■ *Ultra C18, 5 μ m Columns*

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9174531	9174532	9174533	9174535
50mm	9174551	9174552	9174553	9174555
100mm	9174511	9174512	9174513	9174515
150mm	9174561	9174562	9174563	9174565
200mm	9174521	9174522	9174523	9174525
250mm	9174571	9174572	9174573	9174575

■ *Ultra C18, 5 μ m Columns with Trident™ Inlet Fitting*

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9174532-700	9174533-700	9174535-700
50mm	—	9174552-700	9174553-700	9174555-700
100mm	—	9174512-700	9174513-700	9174515-700
150mm	—	9174562-700	9174563-700	9174565-700
200mm	—	9174522-700	9174523-700	9174525-700
250mm	—	9174572-700	9174573-700	9174575-700

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

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

Background

Penicillins and cephalosporins represent nearly sixty percent of antibiotics worldwide. These antibiotics possess a sulfur

atom within a five- or six-membered ring, attached to a four-member β -lactam ring. They are produced by fermentation processes using either selected fungi or species of *Streptomyces* bacteria. Derivatives are produced in two fashions:

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

Unfortunately, biosynthetic fermentation does not produce a "pure" antibiotic. Even after cleanup of the fermentation mash, some side reaction products will remain. Many of these side products are closely related to the primary analyte (Figure 1). Desired products, however, are created in the semi-synthetic process. Penicillin V is converted to amoxicillin through chemical intermediates and varies only slightly in structure (Figure 2). Similar reactions also occur during production of cephalosporin derivatives. The loss of

Figure 1

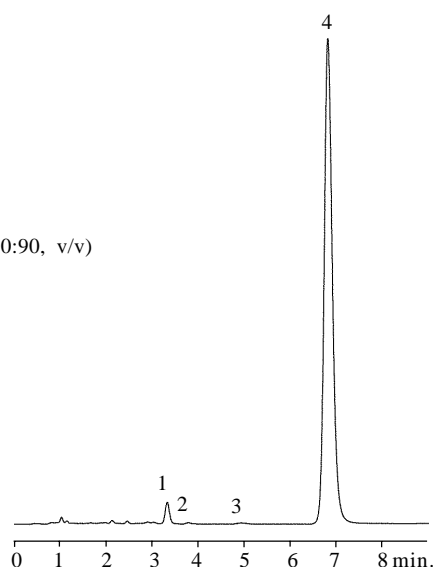
Ultra IBD separates penicillin V from other fermentation impurities.

Peak List:

1. unknown
2. unknown
3. unknown
4. penicillin V

Sample:

Inj.: 2.5 μ L
 Conc.: 1.2mg/mL
 Solvent: acetonitrile:water (10:90, v/v)



Column:

Ultra IBD
 Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5 μ m
 Pore Size: 100 \AA

Conditions:

Mobile Phase: 10mM ammonium formate, pH 2.5: acetonitrile (60:40, v/v)
 Flow: 1.2mL/min.
 Temp.: 30°C
 Det.: UV @ 270nm

LC_0096

a hydride ion to create a phenyl ring is the only structural difference between cephradine and its side product cephalixin (Figure 3). Semi-synthetic processes are used to create derivatives like cephaloridine.

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

pH near 7.4 may become important for stability and accurate quantitation.

Discussion of Analysis

The Restek Ultra IBD phase provides greater versatility for the LC/MS analysis of penicillins and cephalosporins compared to a C18 column. The Ultra IBD column is capable of providing retention for cephaloridine in reverse phase mode with up to 45% organic solvent in the mobile phase. A conventional C18 column loses all retention near 35% organic solvent. Unlike a C18 column, the IBD is capable of polar interactions in a normal phase mode with analytes that possess charged functional groups. The ability

Figure 2

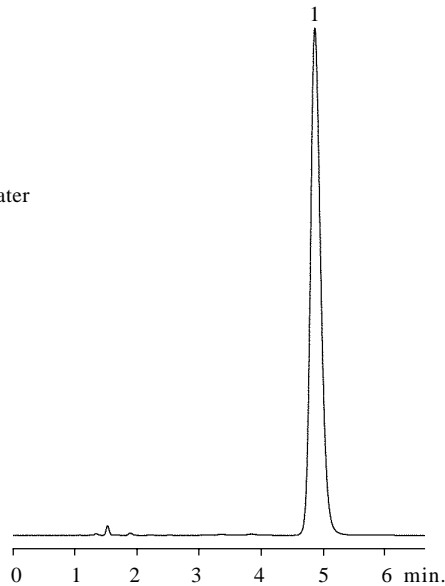
Ultra IBD shows excellent peak shape for amoxicillin.

Peak List:
1. amoxicillin

Sample:
Inj.: 5µL
Conc.: 1.5mg/mL
Solvent: acetonitrile:water
(10:90, v/v)

Column: Ultra IBD
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 10mM ammonium formate,
pH 2.5:acetonitrile (95:5, v/v)
Flow: 1.2mL/min.
Temp.: 30°C
Det.: UV @ 270nm



LC_0095

Figure 3

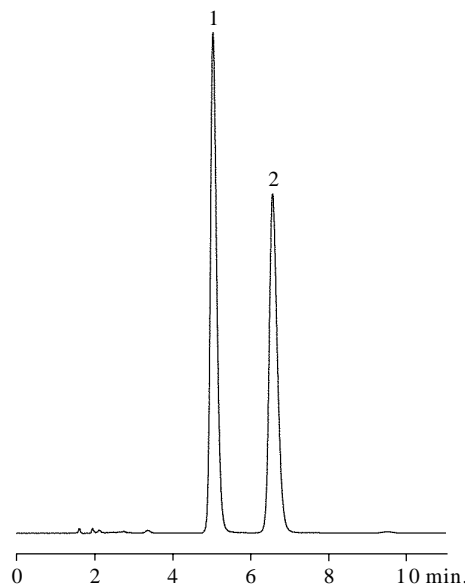
Ultra IBD shows great separation between cephalixin and cephradine, which differ only by a hydride ion.

Peak List:
1. cephalixin
2. cephradine

Sample:
Inj.: 10µL
Conc.: 500µg/mL
Solvent: acetonitrile:water
(10:90, v/v)

Column: Ultra IBD
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 10mM ammonium
formate, pH 2.5:
acetonitrile (90:10, v/v)
Flow: 1.2mL/min.
Temp.: 30°C
Det.: UV @ 270nm



LC_0094

to retain a compound such as cephaloridine in normal phase mode using levels of organic solvents above 50% in the mobile phase, will allow increased sensitivity by LC/MS (Figure 4).

The IBD column also provides other chromatographic benefits. The excellent peak shape for cephaloridine in both the reverse and normal phase modes (Figure 5) increases sensitivity and improves quantitation. Furthermore, the retention of cephalosporin and cephaloridine is essentially unaffected by the pH. This allows full control in the pH range of 2 to 8 for optimum stabilization of the cephalosporins and penicillins during analysis, provided hydrolysis is

not an issue. The IBD column has a unique blend of hydrophobic and polar character for better resolution of closely related compounds.

Conclusion

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

Figure 4

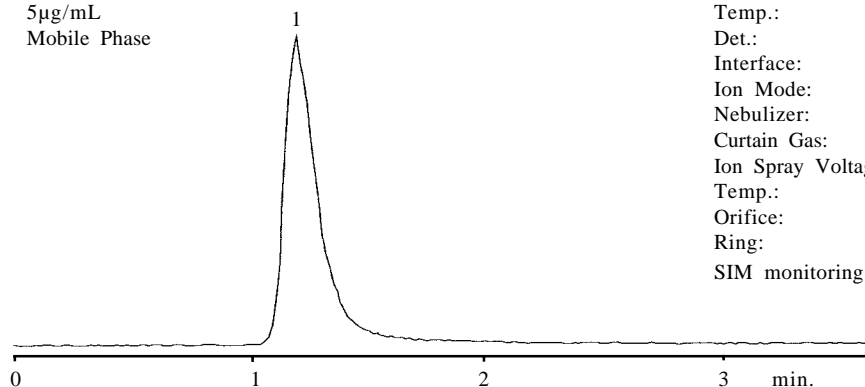
Ultra IBD allows increased LC/MS sensitivity of cephaloridine in normal phase mode.

Peak List:
1. cephaloridine

Sample:
Inj.: 5µL
Conc.: 5µg/mL
Solvent: Mobile Phase

Column: Ultra IBD
Catalog #: 9175552
Dimensions: 50 x 2.1mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 5mM ammonium acetate pH 7.4: acetonitrile (20:80)
Flow: 0.2mL/min.
Temp.: ambient
Det.: PE/Sciex API 150 EX
Interface: Turbo Ion Spray
Ion Mode: Positive
Nebulizer: 8L/hour
Curtain Gas: 12L/Hour
Ion Spray Voltage: 4700.0v
Temp.: 350°C
Orifice: + 10.0v
Ring: + 25.0v
SIM monitoring: 416 ± 3dal.



LC_0124

Figure 5

Ultra IBD shows excellent peak shape for cephaloridine in both normal and reverse phase modes.

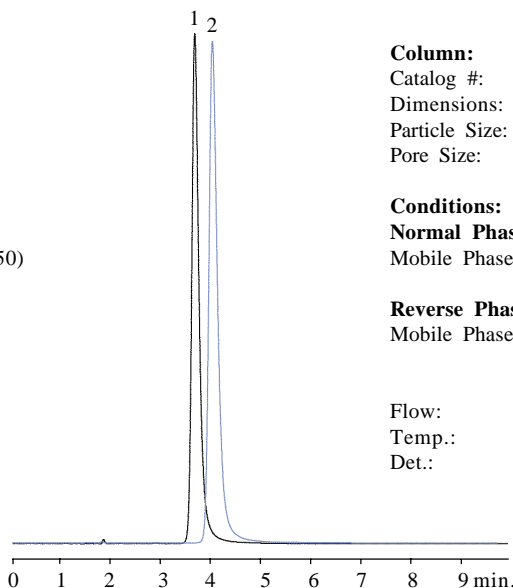
Peak List:
1. cephaloridine reverse phase
2. cephaloridine normal phase

Sample:
Inj.: 5µL
Conc.: 1mg/mL
Solvent: acetonitrile:water (50:50)

Column: Ultra IBD
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Normal Phase:
Mobile Phase: acetonitrile: pH 4.0 20mM ammonium phosphate (80:20, v/v)
Reverse Phase:
Mobile Phase: acetonitrile: pH 4.0 20mM ammonium phosphate (20:80, v/v)

Flow: 1.2mL/min.
Temp.: 27°C
Det.: UV @ 254nm



LC_0101&LC_0102

■ *Ultra IBD, 3µm Columns*

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365

■ *Ultra IBD, 3µm Columns with Trident™ Inlet*

Particle Size: 3µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175332-700	9175333-700	9175335-700
50mm length	9175352-700	9175353-700	9175355-700
100mm length	9175312-700	9175313-700	9175315-700
150mm length	9175362-700	9175363-700	9175365-700

■ *Ultra IBD, 5µm Columns*

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

■ *Ultra IBD, 5µm Columns with Trident™ Inlet*

Particle Size: 5µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175532-700	9175533-700	9175535-700
50mm length	9175552-700	9175553-700	9175555-700
100mm length	9175512-700	9175513-700	9175515-700
150mm length	9175562-700	9175563-700	9175565-700
200mm length	9175522-700	9175523-700	9175525-700
250mm length	9175572-700	9175573-700	9175575-700

■ *Ultra IBD Guard Cartridges*

Dimensions	cat.#	qty.
10 x 2.1mm	917550212	3
10 x 4.0mm	917550210	3
20 x 4.0mm	917550220	2

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Analyzing Cardiac Medications by HPLC

In the United States, cardiovascular disease is the leading cause of death. In an effort to reduce mortality from heart disease, several classes of medications are used to decrease high blood pressure, control arrhythmias (abnormal heart rhythms), and treat congestive heart failure. Many of these cardiac medications include beta antagonists, ACE inhibitors, diuretics, or calcium channel blockers.

High performance liquid chromatography (HPLC) is the preferred technique to analyze many of the compounds used in these medications. To maximize the effectiveness of the separation, a chromatographer should choose the column and conditions that best allow amplification of structural differences between matrix components, related compounds, and analytes. Proper HPLC column selection is dictated by the analyte and the sample matrix. In fact, selecting the appropriate analytical column is critical when analyzing cardiac medications because many of them contain basic

compounds, which tend to tail badly on poorly deactivated HPLC phases. Restek's fully end-capped Allure™ Basix, Ultra IBD (intrinsically base deactivated), and Ultra Cyano phases can use the basic nature of these compounds to achieve a separation that will not suffer from the problems normally resulting in peak tailing.

Angiotensin Converting Enzyme (ACE) Inhibitors

Ancient Egyptians used the ACE inhibitor, digoxin, as a poison. Ancient Romans used it as a wound dressing and heart stimulant. It is extracted primarily from the poisonous foxglove plant in a concentration of up to 0.4% by mass. A commercial digoxin standard claiming 100% purity is shown to be impure when the analysis is performed using the Ultra IBD column. The alternate selectivity of this phase to alkyl stationary phases results in the separation of two unknown impurity peaks in the digoxin standard (Figure 1).

Figure 1

Ultra IBD column provides alternate selectivity, which separates impurities in a digoxin standard.

Peak List:

1. unknown
2. unknown
3. digoxin

Sample:

Inj.: 10µL
 Conc.: 1000µg/mL
 Solvent: water:methanol (1:1, v/v)

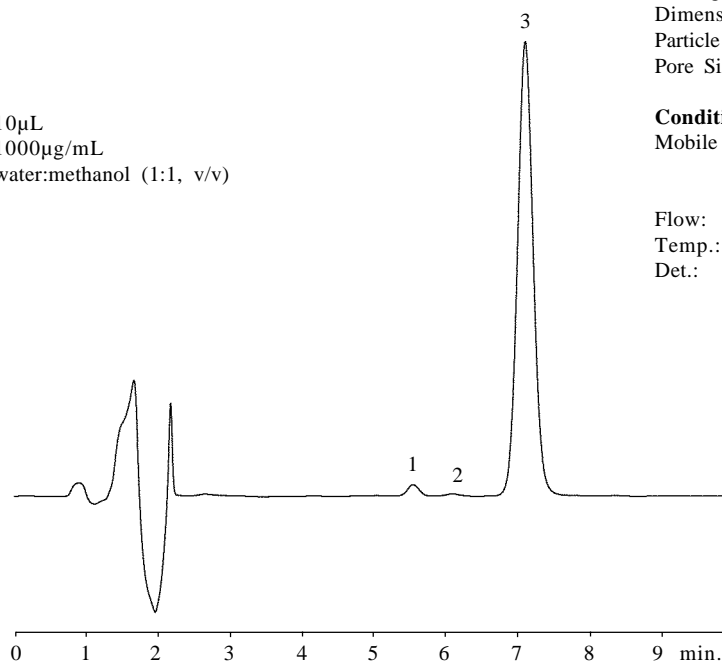
Column:

Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Ultra IBD

Conditions:

Mobile phase: water with 0.1% (v/v) acetic acid:acetonitrile (65:35, v/v)
 Flow: 1.0mL/min.
 Temp.: 27°C
 Det.: UV @ 220nm



LC_0068

Enalapril maleate, another common ACE inhibitor, can be separated by polar interaction using the Allure™ Basix phase. The Allure™ Basix column is able to interact with the basic amide and amine of enalapril to provide retention by a normal phase mechanism (Figure 2).

Calcium Channel Blockers

Verapamil, diltiazem, nifedipine, and nifedipine are a group of calcium channel blockers used to treat high blood

pressure, angina (chest pain), and/or some arrhythmias. These four compounds all contain a basic amine group. Additionally, nifedipine and nicardipine contain more basic nitrophenol and pyridine functional groups. Figure 3 demonstrates how basic functional groups can be used to affect retention and separation of these compounds using the Ultra Cyano column. Also, the Allure™ Basix Column, in the reverse phase mode, easily retains verapamil (Figure 4).

Figure 2

The Allure™ Basix column provides retention by a normal phase mechanism.

Peak List:

1. maleate salt
2. enalapril

Sample:

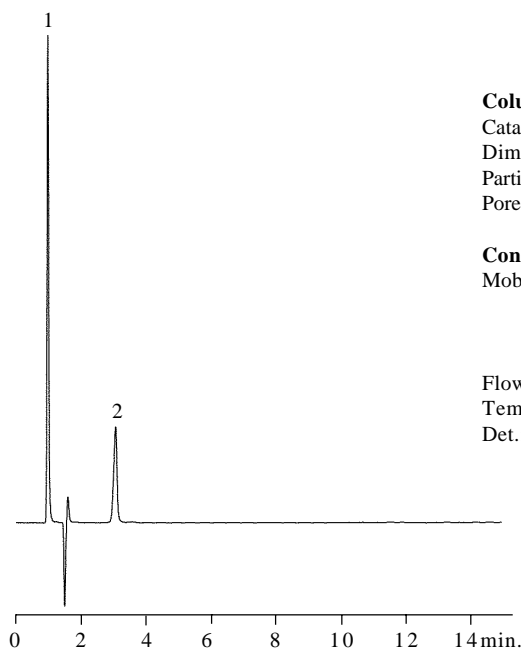
Inj.: 2.5µL
 Conc.: 1.1mg/mL
 Solvent: methanol:water
 (30:70)

Column: Allure™ Basix

Catalog #: 9161565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 60Å

Conditions:

Mobile phase: 10mM ammonium formate, pH 2.5: acetonitrile (20:80, v/v)
 Flow: 1.2mL/min.
 Temp.: 25°C
 Det.: UV @ 225nm



LC_0091

Figure 3

The Ultra Cyano column separates four of the most common calcium channel blockers.

Peak List:

1. diltiazem
2. nifedipine impurity
3. verapamil
4. nifedipine
5. nicardipine

Sample:

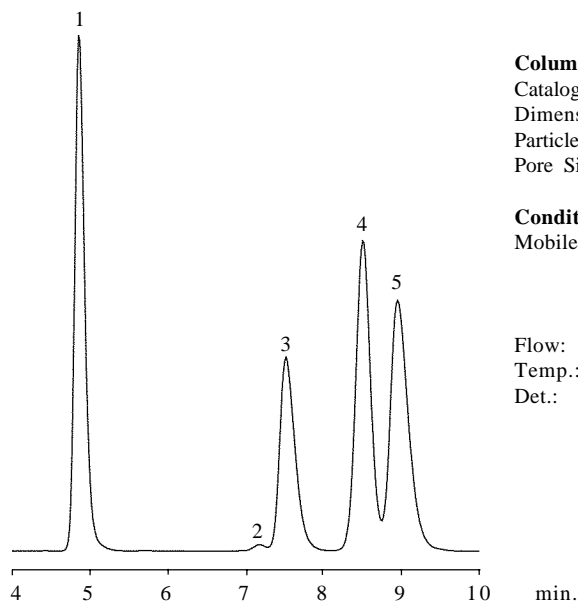
Inj.: 5µL
 Conc.: 100µg/mL
 Solvent: acetonitrile:water
 (1:1)

Column: Ultra Cyano

Catalog #: 9106565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:

Mobile phase: 20mM potassium phosphate monobasic, pH 3.0:acetonitrile (70:30, v/v)
 Flow: 1.2mL/min.
 Temp.: 30°C (± 1°C)
 Det.: 235nm



LC_0062

Beta Antagonists

Two of the more common beta antagonists are atenolol and metoprolol. The analytical techniques cited in some compendia methods for these compounds use a C18 phase with ion pairing agents. A simpler approach makes use of the nitrogen atom on these compounds as a key mechanism for separation. However, the basic amine groups allow analysis of these compounds using normal phase separation with the Allure™ Basix column.

Because metoprolol is more lipid-soluble than atenolol, it is more hydrophobic. Therefore, an increase in the organic composition of the mobile phase actually will enhance the retention of metoprolol with the Allure™ Basix phase. The Allure™ Basix column performs separation of these components, provides alternate selectivity to alkyl stationary phases, and reveals an impurity in the metoprolol (Figures 5 and 6).

Figure 4

The Allure™ Basix column offers alternative selectivity for verapamil in the reverse phase mode.

Peak List:

1. toluene (marker)
2. verapamil HCL

Sample:

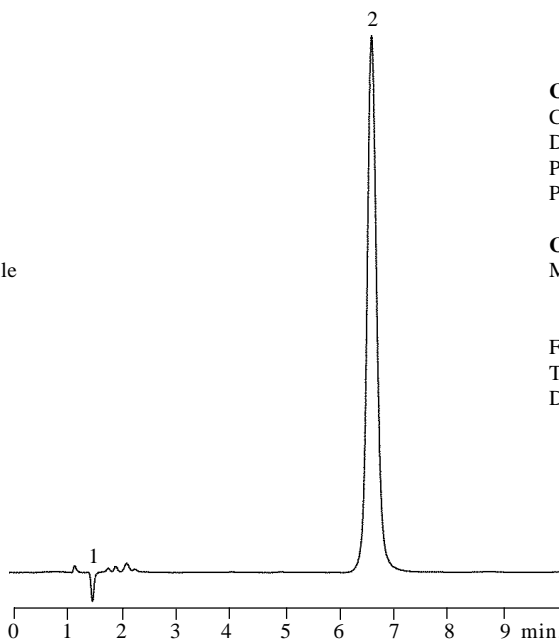
Inj.: 1 µL
Conc.: 1 mg/mL
Solvent: water:acetonitrile
(70:30, v/v)

Column: Allure™ Basix

Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5 µm
Pore Size: 60 Å

Conditions:

Mobile phase: 20mM ammonium acetate pH 4.5:
acetonitrile (65:35, v/v)
Flow: 1.2 mL/min.
Temp.: 25.0°C
Det.: UV @ 230nm



LC_0077

Figure 5

The Allure™ Basix column separates metoprolol and an impurity in 10 minutes.

Peak List:

1. unknown
2. metoprolol

Sample:

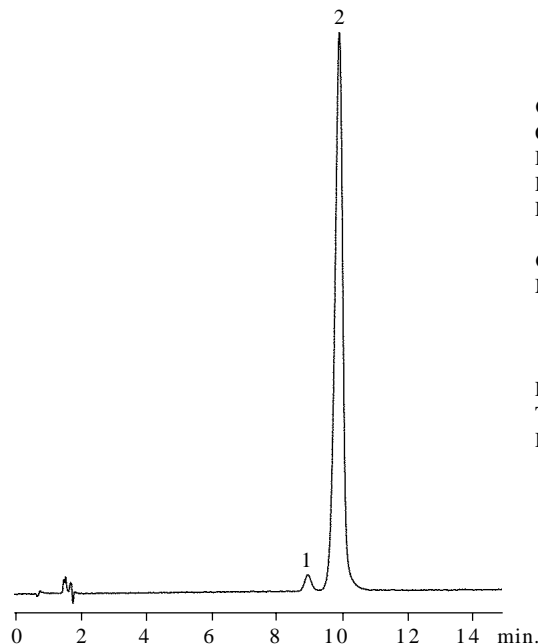
Inj.: 2.5 µL
Conc.: 1.5 mg/mL
Solvent: water:methanol
(70:30)

Column: Allure™ Basix

Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5 µm
Pore Size: 60 Å

Conditions:

Mobile Phase: 10mM ammonium formate, pH 2.5:
acetonitrile
(10:90, v/v)
Flow: 1.2 mL/min.
Temp.: 25°C
Det.: UV @ 225nm



LC_0090

Diuretics

Another important class of cardiac and high blood pressure medications are diuretics. These compounds rid the body of excess fluids and salt (sodium). Diuretics such as furosemide are used for the management of edema associated with chronic heart problems. The furosemide molecule contains carboxylic acid and basic sulfa-amine groups. The zwitterionic nature of furosemide makes it an ideal candidate for analysis using an Ultra IBD column (Figure 7).

Enlargement of the baseline reveals that the furosemide standard is not a pure substance (Figure 7, inset). The impurities may possibly be a reason why the therapeutic mechanism of furosemide is not completely understood.

The diuretic admixture of triamterene and hydrochlorothiazide (HCTZ) is used to remove excess fluid while attempting to limit the amount of potassium displaced from the body. The highly-charged HCTZ contains numerous amine and sulfonic groups. The high charge is the reason it elutes in

Figure 6

The Allure™ Basix column retains atenolol without ion pairing agents.

Peak List:

1. uracil (marker)
2. atenolol

Sample:

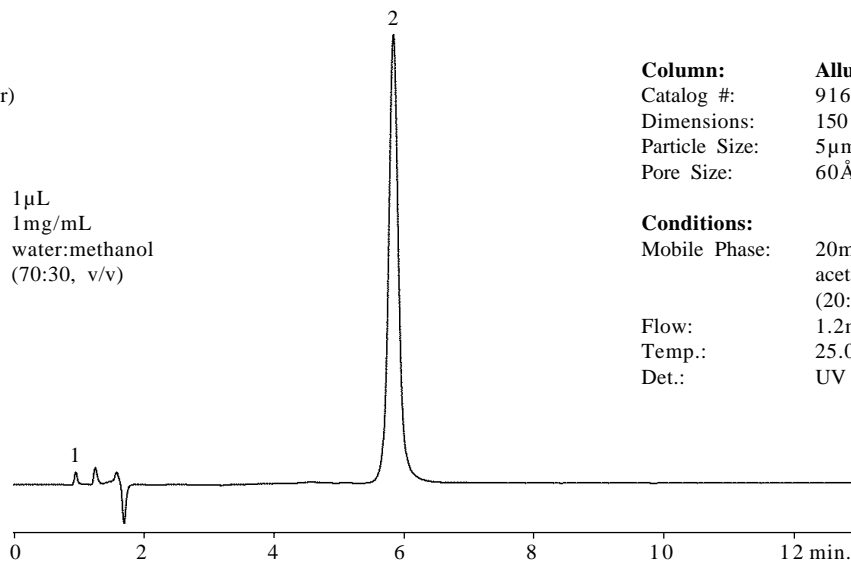
Inj.: 1 µL
Conc.: 1 mg/mL
Solvent: water:methanol
(70:30, v/v)

Column: Allure™ Basix

Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5 µm
Pore Size: 60 Å

Conditions:

Mobile Phase: 20mM ammonium acetate pH 4.5:acetonitrile (20:80, v/v)
Flow: 1.2mL/min.
Temp.: 25.0°C
Det.: UV @ 225nm



LC_0072

Figure 7

The Ultra IBD column separates impurities in furosemide.

Peak List:

1. uracil (marker)
2. furosemide

Sample:

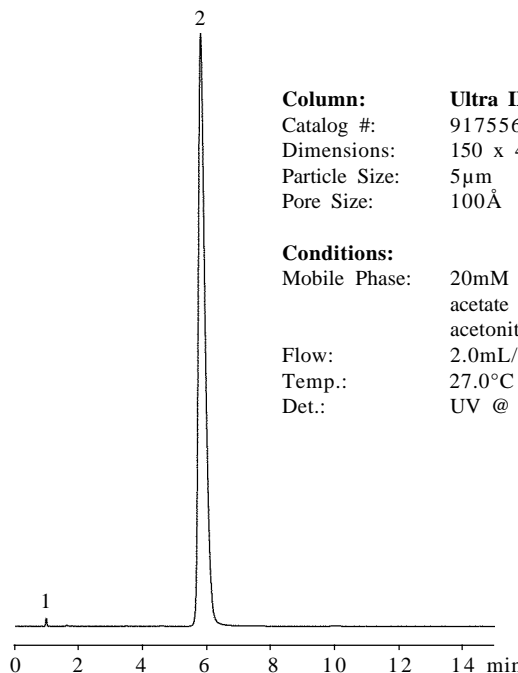
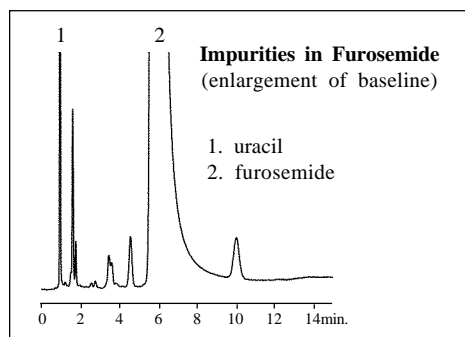
Inj.: 5 µL
Conc.: 1000 µg/mL
Solvent: acetonitrile:water
(60:40, v/v)

Column: Ultra IBD

Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5 µm
Pore Size: 100 Å

Conditions:

Mobile Phase: 20mM ammonium acetate pH 4.5: acetonitrile (70:30, v/v)
Flow: 2.0mL/min.
Temp.: 27.0°C
Det.: UV @ 280nm



LC_0071

Figure 8

The Allure™ Basix column easily resolves the diuretic admixture of triamterene and hydrochlorothiazide (HCTZ)

Peak List:

1. hydrochlorothiazide
2. triamterene

Sample:

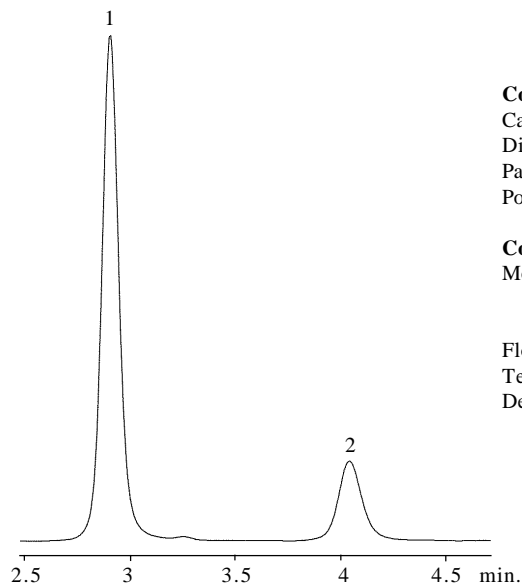
Inj.: 1 µL
Conc.: 500 µg/mL
Solvent: methanol:water:
tetrahydrofuran
(30:35:35)

Column: Allure™ Basix

Catalog #: 9161565
Dimensions: 150 x 4.6mm
Particle Size: 5 µm
Pore Size: 60 Å

Conditions:

Mobile Phase: 20mM ammonium
acetate, pH 4.5:
acetonitrile (65:35, v/v)
Flow: 1.2mL/min
Temp.: 25°C
Det.: UV @ 225nm



LC_0086

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Application Notes:

- (#59511) Improved HPLC Analysis of Analgesics
- (#59512) The Ultra IBD Column Allows HPLC Separation of Polar and Non-Polar Analytes from the Same Sample
- (#59510) HPLC Stationary Phase Selection for the Analysis of Steroids
- (#59118) Allure™ PFP Propyl HPLC Column Provides Improved LC/MS Analyses of Basic Compounds

Fast Facts:

- (#59728) HPLC Mobile Phase Accessories
- (#59896) Trident™ Integral HPLC Guard Column System
- (#59302) HPLC and LC/MS Column Kits
- (#59303) Allure™ Acidix HPLC Columns
- (#59314) Trident™ Direct Guard Column System
- (#59614A) Ultra IBD HPLC Columns

■ Allure™ Basix, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9161531	9161532	9161533	9161535
50mm length	9161551	9161552	9161553	9161555
100mm length	9161511	9161512	9161513	9161515
150mm length	9161561	9161562	9161563	9161565
200mm length	9161521	9161522	9161523	9161525
250mm length	9161571	9161572	9161573	9161575

■ Ultra Cyano, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9106331	9106332	9106333	9106335
50mm length	9106351	9106352	9106353	9106355
100mm length	9106311	9106312	9106313	9106315

■ Ultra Cyano, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9106531	9106532	9106533	9106535
50mm length	9106551	9106552	9106553	9106555
100mm length	9106511	9106512	9106513	9106515
150mm length	9106561	9106562	9106563	9106565
200mm length	9106521	9106522	9106523	9106525
250mm length	9106571	9106572	9106573	9106575

■ Ultra IBD, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365

■ Ultra IBD, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

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Analyze Nucleotides, Nucleosides, Purine, and Pyrimidine Bases Simultaneously with the Ultra IBD Column

Mixtures of nucleotides, nucleosides, and their respective purine or pyrimidine bases are difficult to analyze by reversed phase/high performance liquid chromatography (RP/HPLC). These compounds cover a wide range of polarities and functionalities, from the acidic nucleotides to the basic purines and pyrimidines, making it very difficult to retain and resolve all of them with conventional alkyl stationary phases. Traditional HPLC analysis of these compounds often uses a combination of reversed phase-ion pairing (RP-IP) and/or ion exchange (IEX) mode. Nucleotides often are analyzed by anion exchange, while nucleosides sometimes are analyzed by cation exchange. These methods are not compatible with all the solutes in the mixtures and they lack ruggedness.

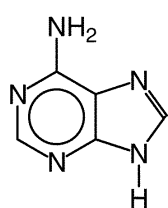
This Applications Note demonstrates that all three classes of compounds (nucleotides, nucleosides, and bases) can be analyzed by RP/HPLC using one column and the same,

simple isocratic mobile phase. This provides greater convenience, reproducibility, and ruggedness in developing methods for these mixtures. By using a unique, intrinsically base-deactivated stationary phase (i.e., the Ultra IBD column), simple RP/HPLC conditions were identified that can resolve any common purine or pyrimidine base from its related ribonucleoside and mono-, di-, and triphosphate nucleotides.

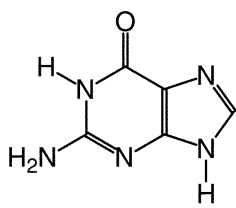
Nucleotides and nucleosides are derived from the nitrogenous bases shown in Figure 1. These nitrogenous bases are either purines (adenine and guanine) or pyrimidines (cytosine, uracil, and thymine). A nucleoside consists of a purine or pyrimidine base linked to a five-carbon sugar (pentose). A nucleotide is composed of a nucleoside plus one or more phosphate groups. Figure 2 shows the structures of a nucleoside and three nucleotides derived from adenine.

Figure 1

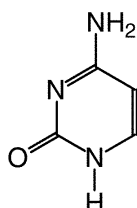
Nitrogenous purine and pyrimidine bases form nucleotides and nucleosides



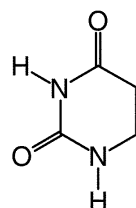
adenine



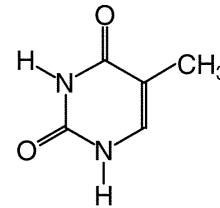
guanine



cytosine



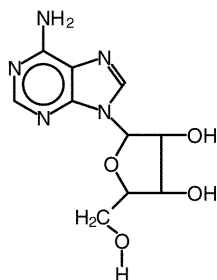
uracil



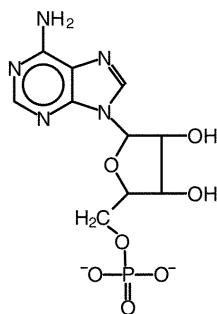
thymine

Figure 2

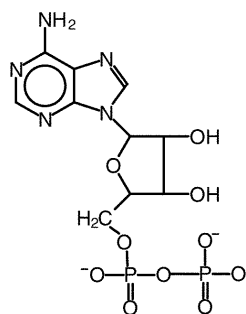
A nucleoside and three nucleotides derived from adenine



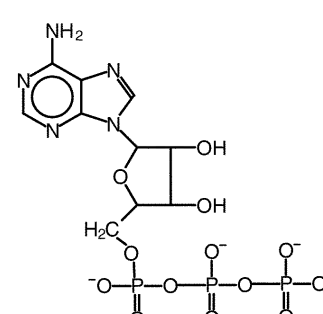
adenosine



5'-AMP



5'-ADP



5'-ATP

Adenosine is a ribonucleoside (adenine + ribose). ATP is a particularly important nucleotide, serving as a universal source of energy for biological processes.

The Ultra IBD column is particularly effective for retaining and resolving complex mixtures of nucleotides, nucleosides, and purine and pyrimidine bases. The unique Ultra IBD stationary phase is composed of a polar group within, or intrinsic to, an alkyl chain. The polar group gives extra

retention for many polar analytes as well as unique selectivity, a very high level of base deactivation, and compatibility with highly aqueous mobile phases. The Ultra IBD column is ideal for LC/MS because it often can resolve acidic, basic, zwitterionic and/or neutral compounds in a single analysis using simple mobile phases.

Figures 3 through 7 each show separations of one of the major purines or pyrimidines from its respective ribonucleo-

Figure 3

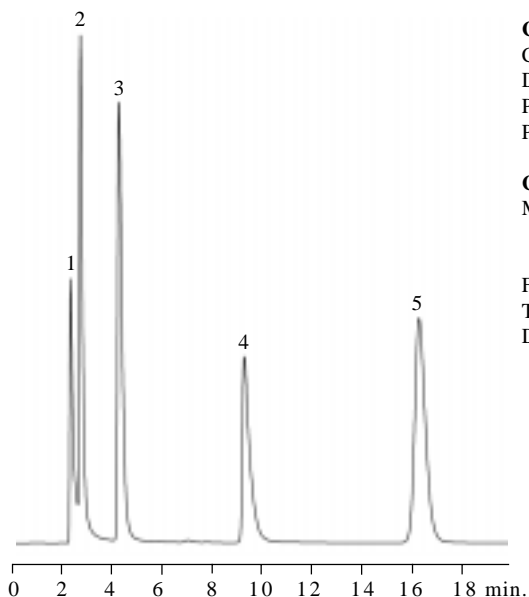
Ultra IBD separates adenine, adenosine, ATP, ADP, and AMP with simple isocratic reverse phase conditions.

Peak List:	Conc. (µg/mL):
1. ATP	258
2. ADP	320
3. AMP	274
4. adenine	84
5. adenosine	254

Sample:
 Inj.: 20µL
 Solvent: 20mM ammonium acetate, pH 5.8

Column: Ultra IBD
 Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:
 Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
 Flow: 1.0mL/min
 Temp.: 35°C
 Det.: UV @ 260nm



LC_0129

Figure 4

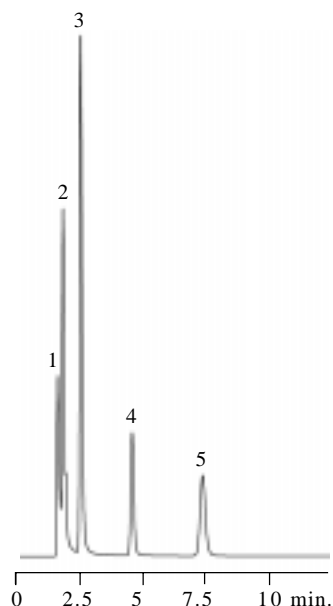
Ultra IBD separates guanine, guanosine, GTP, GDP, and GMP with simple isocratic reverse phase conditions.

Peak List:	Conc. (µg/mL):
1. GTP	212
2. GDP	260
3. GMP	362
4. guanine	80
5. guanosine	226

Sample:
 Inj.: 20µL
 Solvent: 20mM ammonium acetate, pH 5.8

Column: Ultra IBD
 Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:
 Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
 Flow: 1.0mL/min
 Temp.: 35°C
 Det.: UV @ 260nm



LC_0131

side and mono-, di-, and triphosphate nucleotide. Guanosine, uridine, cytidine, and thymidine are ribonucleosides derived from guanine, uracil, cytosine, and thymine, respectively. Note that each of these separations was achieved using the same conditions and that, in each case, the order of elution is the same: the triphosphate, the diphosphate, then the monophosphate nucleotide, followed by the base, and lastly the nucleoside. There are slight “shoulders” on the peaks for GDP (Figure 4), TTP (Figure 6), and UMP (Figure 7). These

compounds were present in the standards and were presumed to be impurities or degradation products.

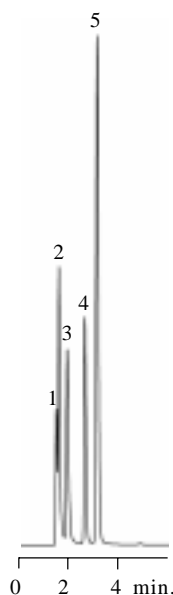
Table 1 lists the typical retention times obtained for all 25 of the compounds separated in Figures 3–7. While not all 25 compounds can be resolved in a single HPLC analysis, it is possible to analyze all of them using these chromatographic conditions and MS or MS/MS detection. Note that the mobile phase is compatible with MS detection, as all of its

Figure 5

Ultra IBD separates cytosine, cytidine, CTP, CDP, and CMP with simple isocratic reverse phase conditions.

Peak List:	Conc. (µg/mL):
1. CTP	248
2. CDP	322
3. CMP	248
4. cytosine	100
5. cytidine	340

Sample:
 Inj.: 20µL
 Solvent: 20mM ammonium acetate, pH 5.8



Column: Ultra IBD
 Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:
 Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
 Flow: 1.0mL/min
 Temp.: 35°C
 Det.: UV @ 260nm

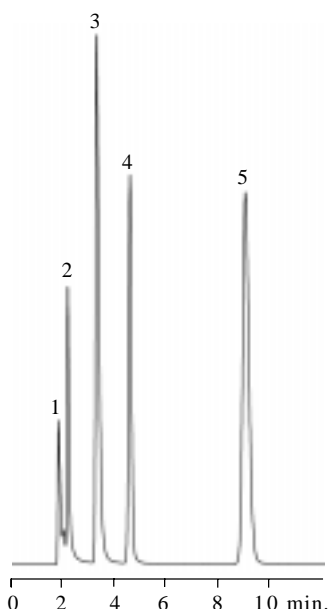
LC_0130

Figure 6

Ultra IBD separates thymine, thymidine, TTP, TDP, TMP with simple isocratic reverse phase conditions.

Peak List:	Conc. (µg/mL):
1. TTP	224
2. TDP	152
3. TMP	346
4. thymine	88
5. thymidine	318

Sample:
 Inj.: 20µL
 Solvent: 20mM ammonium acetate, pH 5.8



Column: Ultra IBD
 Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:
 Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
 Flow: 1.0mL/min
 Temp.: 35°C
 Det.: UV @ 260nm

LC_0132

components are volatile. Figure 8 shows that the Ultra IBD column can resolve a mixture of 11 various nucleotides, nucleosides, and bases using ultraviolet (UV) detection.

The unique stationary phase of the Ultra IBD column can retain and resolve mixtures of nucleotides, nucleosides, and purine and pyrimidine bases by RP/HPLC, using isocratic

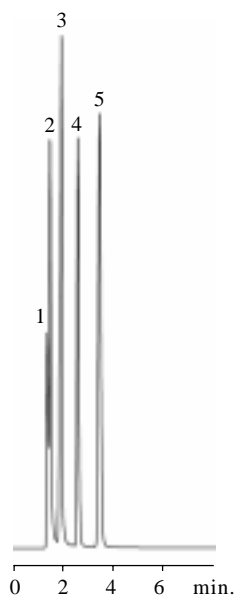
elution with a simple, volatile mobile phase. A single set of chromatographic conditions can resolve any of the common purine or pyrimidine bases from its respective ribonucleoside and mono-, di-, and triphosphate nucleotides.

Figure 7

Ultra IBD separates uracil, uridine, UTP, UDP, UMP with simple isocratic reverse phase conditions.

Peak List:	Conc. (µg/mL):
1. UTP	360
2. UDP	360
3. UMP	284
4. uracil	90
5. uridine	230

Sample:
 Inj.: 20µL
 Solvent: 20mM ammonium acetate, pH 5.8



Column: Ultra IBD
 Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:
 Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
 Flow: 1.0mL/min
 Temp.: 35°C
 Det.: UV @ 260nm

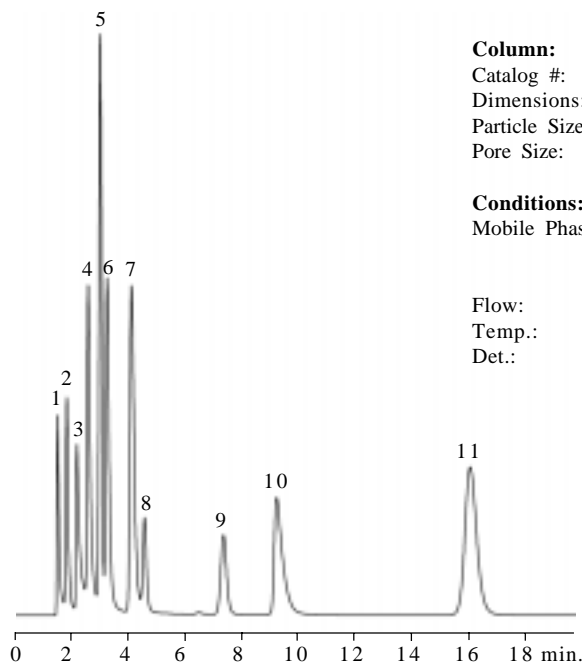
LC_0133

Figure 8

Ultra IBD separates an 11-component mixture of nucleotides, nucleosides, and nitrogenous bases.

Peak List:	Conc. (µg/mL):
1. CDP	146
2. CMP	113
3. ATP	117
4. ADP	145
5. cytidine	155
6. TMP	157
7. AMP	125
8. guanine	36
9. guanosine	103
10. adenine	38
11. adenosine	115

Sample:
 Inj.: 20µL
 Solvent: 20mM ammonium acetate, pH 5.8



Column: Ultra IBD
 Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:
 Mobile Phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
 Flow: 1.0mL/min
 Temp.: 35°C
 Det.: UV @ 260nm

LC_0128

Table 1

Typical retention times for common nucleotides, nucleosides, and purine and pyrimidine bases.

Compound	Retention Time ¹ (min.)
CTP	1.5
UTP	1.5
CDP	1.6
UDP	1.6
GTP	1.7
TTP	1.8
CMP	1.9
GDP	1.9
UMP	2.0
TDP	2.2
ATP	2.3
GMP	2.6
Cytosine	2.6
ADP	2.7
Uracil	2.7
Cytidine	3.1
TMP	3.3
Uridine	3.6
AMP	4.2
Guanine	4.6
Thymine	4.6
Guanosine	7.4
Thymidine	9.0
Adenine	9.3
Adenosine	16.2

1. Retention times are for 150x4.6mm column; Flow rate: 1.0mL/min; Mobile phase: 20mM ammonium acetate, pH 5.8; Methanol. (97.5:2.5, v/v).

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Application Notes:

(#59511) Improved HPLC Analysis of Analgesics

(#59512) The Ultra IBD Column Allows HPLC Separation of Polar and Non-Polar Analytes from the Same Sample

(#59510) HPLC Stationary Phase Selection for the Analysis of Steroids

(#59118) Allure™ PFP Propyl HPLC Column Provides Improved LC/MS Analyses of Basic Compounds

Fast Facts

(#59728) HPLC Mobile Phase Accessories

(#59896) Trident™ Integral HPLC Guard Column System

(#59302) HPLC and LC/MS Column Kits

(#59303) Allure™ Acidix HPLC Columns

(#59314) Trident™ Direct Guard Column System

(#59614A) Ultra IBD HPLC Columns

■ *Ultra IBD, 3µm Columns*

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365

■ *Ultra IBD, 3µm Columns with Trident™ Inlet*

Particle Size: 3µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175332-700	9175333-700	9175335-700
50mm length	9175352-700	9175353-700	9175355-700
100mm length	9175312-700	9175313-700	9175315-700
150mm length	9175362-700	9175363-700	9175365-700

■ *Ultra IBD, 5µm Columns*

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

■ *Ultra IBD, 5µm Columns with Trident™ Inlet*

Particle Size: 5µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175532-700	9175533-700	9175535-700
50mm length	9175552-700	9175553-700	9175555-700
100mm length	9175512-700	9175513-700	9175515-700
150mm length	9175562-700	9175563-700	9175565-700
200mm length	9175522-700	9175523-700	9175525-700
250mm length	9175572-700	9175573-700	9175575-700

■ *Ultra IBD Guard Cartridges*

Dimensions	cat.#	qty.
10 x 2.1mm	917550212	3
10 x 4.0mm	917550210	3
20 x 4.0mm	917550220	2

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HPLC Analysis of Basic Pharmaceutical Compounds on an Ultra Cyano Phase

Pharmaceuticals designed for use as antihypertensive agents are challenging samples for high performance liquid chromatography (HPLC) methods development. The challenge arises when the basic functional group in the sample interacts with a residual silanol group in the column packing, causing peak tailing and difficult quantitation.

When compared to C18 phases, a bonded base-deactivated cyanopropyl phase exhibits the best peak shape for basic pharmaceutical compounds. However, cyano phases on traditional Type A silica can be unstable under certain pH conditions (Scheme 1). In order to combat these problems, we've developed new deactivation and bonding chemistries for the Ultra Cyano HPLC column, which increase phase stability and eliminate potential hydrolysis when incorpo-

rated with a highly pure, Type B silica support. The high bonding density (8% carbon) increases the retention/capacity factor for analytes, resulting in better resolution of complex mixtures.

Figure 1 illustrates the separation of calcium channel blockers, a common analysis containing compounds with these basic functional groups, using an Ultra Cyano HPLC column. The 150 x 4.6mm column produces good resolution in less than ten minutes and excellent peak symmetry without tailing. Through changes to the deactivation, bonding chemistry, and bonding density, Ultra Cyano HPLC columns provide a superior chromatographic solution to difficult pharmaceutical analyses.

Figure 1

HPLC Separation of Popular Calcium Channel Blockers Shows Good Peak Shape and Resolution in 10 minutes

Peak List:

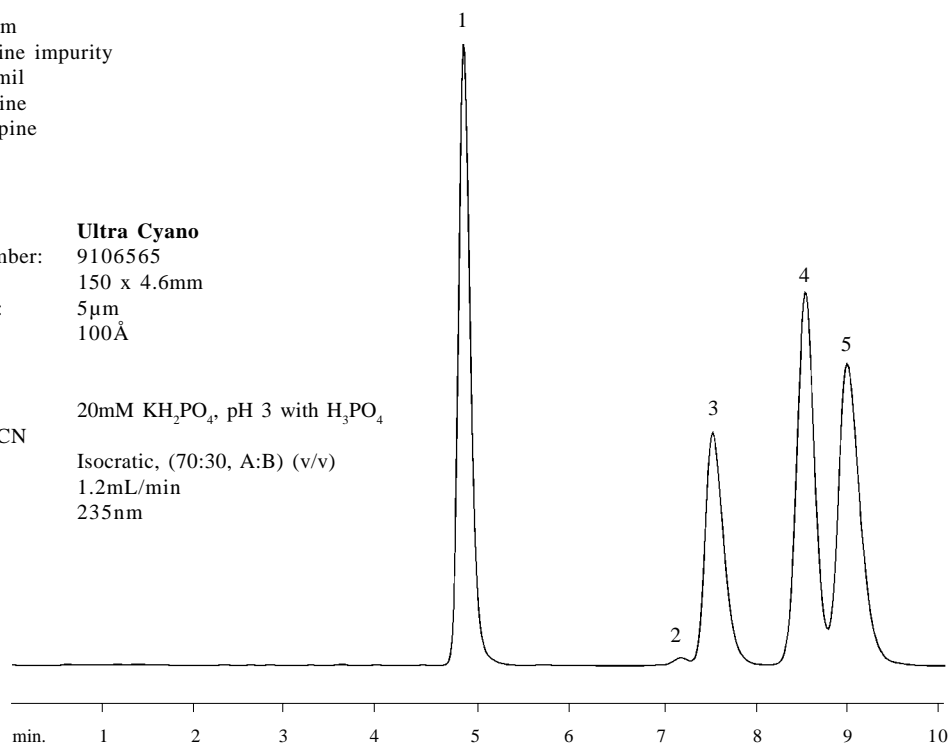
1. diltiazem
2. nifedipine impurity
3. verapamil
4. nifedipine
5. nicardipine

Column: Ultra Cyano

Catalog Number: 9106565
 Dimensions: 150 x 4.6mm
 Particle Size: 5µm
 Pore Size: 100Å

Conditions:

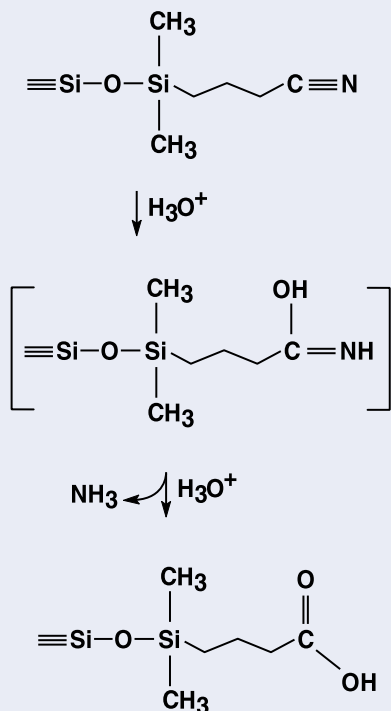
Solvent A: 20mM KH₂PO₄, pH 3 with H₃PO₄
 Solvent B: ACN
 Mode: Isocratic, (70:30, A:B) (v/v)
 Flow Rate: 1.2mL/min
 Wavelength: 235nm



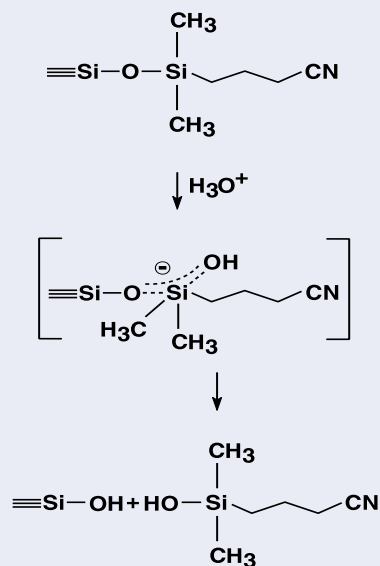
Scheme 1

Hydrolysis of Cyano Bonded HPLC Phase Causes Instability

Hypothetical



Probable



Ultra Cyano, 5µm Particle size

Length (mm)	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30	9106531	9106532	9106533	9106535
50	9106551	9106552	9106553	9106555
100	9106511	9106512	9106513	9106515
150	9106561	9106562	9106563	9106565
200	9106521	9106522	9106523	9106525
250	9106571	9106572	9106573	9106575

Ultra Cyano, 5µm, w/ Trident Guard Inlet Fitting

Length (mm)	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30	9106532-700	9106533-700	9106535-700
50	9106552-700	9106553-700	9106555-700
100	9106512-700	9106513-700	9106515-700
150	9106562-700	9106563-700	9106565-700
200	9106522-700	9106523-700	9106525-700
250	9106572-700	9106573-700	9106575-700

Ultra Cyano Guard Cartridges

Length (mm)	cat.#	Qty.
10 x 2.1	910650212	3
10 x 4.0	910650210	3
20 x 4.0	910650220	2

Also available in 3µm particles.

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