THE RESTEK ADVANTAGE

NEW! Columns for Blood Alcohol Analysis

Faster Analysis Time and Baseline Resolution

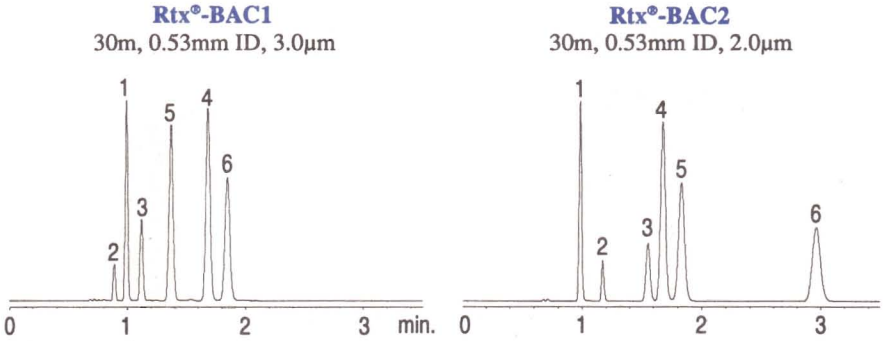
Testing for the presence and subsequent quantitation of ethanol in blood, breath and urine are the highest volume tests performed in forensic laboratories. In addition to ethanol, the detection of several other significant alcohols and their metabolites is necessary. Gas chromatographic assays provide the greatest amount of flexibility and specificity in analyzing for these volatile compounds. Headspace sampling is preferred over direct injection since it eliminates the build-up of non-volatile contamination at the head of the column.

Analysis time and resolution are two critical factors when developing a GC assay for ethanol. Analysis time for each sample should be as short as possible while maintaining baseline resolution for all analytes. Isothermal analysis is the method of choice because it eliminates the cooling down period between temperature programmed runs. Overall analysis time can be reduced in isothermal analyses by raising the temperature of the analysis or by increasing carrier gas flow rate. However, in attempting to shorten the analysis time, either by increasing the flow rate or by raising the temperature, many traditional capillary stationary phases fail to provide adequate resolution of all of the components commonly tested in blood alcohol analyses.

Figure 1 shows the analysis of low molecular weight alcohols and their metabolites on two new stationary phases developed specifically for blood alcohol analysis. These phases yield baseline resolution with very short analysis times for all of the compounds while providing elution order changes for four out of six compounds. These changes in elution order and retention

in this issue...

Figure 1 - Achieve baseline resolution of blood alcohols using dual columns in less than 3 minutes.



1.0ml headspace sample of Blood Alcohol Mix
Oven temp.: 40°C isothermal
Inj. & det. temp.: 200°C
Carrier gas: helium
Linear velocity: 80cm/sec. set @ 40°C
FID sensitivity: 1.28 x 10^{-10}AFS

1. acetaldehyde
2. methanol
3. ethanol
4. acetone
5. isopropanol
6. n-propanol

Chromatograms produced using a model 7000 static headspace autosampler on loan courtesy of The Tekmar Company.

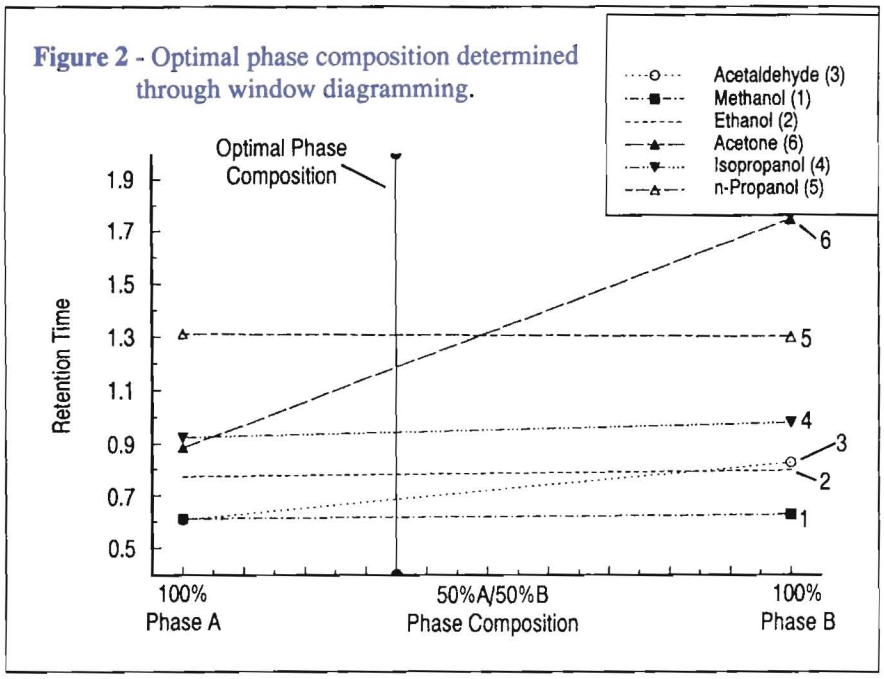


Figure 2 - Optimal phase composition determined through window diagramming.

time can be used as a confirmational tool in identifying the presence or absence of volatile compounds in forensic specimens. Dual column confirmational analyses significantly reduce the chance of false negative or false positive results.

See Hints for the Capillary Chromatographer on Performing Dual Column Analysis (pages 10 and 11)

Resolution between critical components and speed of analysis were finely tuned by altering the molar composition of certain functional groups in the stationary phase. By using the system of column development demonstrated by Mehran et al [1,2], two columns with dissimilar stationary phases were coupled together to evaluate changes in resolution for this group of six compounds. Window diagramming was then used to predict optimal stationary phase composition from a minimal number of experiments. In Figure 2, a plot of retention time versus stationary phase composition shows the phase compositions that should be able to perform the separation. In our experiments, four different functional groups were evaluated for their ability to alter specific separations. By combining two or more of these functional groups into a single, homogeneous stationary phase, complete resolution of all components can be achieved.

Optimal performance of these columns during headspace analysis depends on GC system set up. Band broadening can occur if there is excess dead volume in the sample flow path from the sample valve to the head of the column. Low volume sleeves or interfaces in the injection port significantly reduce the amount of excess volume at the exit end of the transfer line. Carrier gas flow rate through the sample loop and transfer line is also important in maintaining a narrow sample band width prior to the sample reaching the head of the column. Our experiments showed that a high flow rate of 30ml/min. was most effective in transferring the sample from the headspace unit to the column in a tight sample band. This flow was subsequently split between two columns by using a Universal "Y" Press-Tight® connector to provide simultaneous analyses from the same headspace sample.

The inclusion of multiple functional groups in stationary phases can significantly alter the retention characteristics for specific compounds. Restek's BAC columns incorporate functional groups into the stationary phase that have selective retention mechanisms for alcohols, ketones and aldehydes. Baseline resolution and elution order changes as well as short analysis time can be achieved for the analysis of blood alcohols.

References

1. M. F. Mehran, W. J. Cooper and W. Jennings, *Journal of High Resolution Chromatography and Chromatography Communications*, Volume 7 (1984) 215.

2. M. F. Mehran, W. J. Cooper, R. Lautamo, R. R. Freeman, and W. Jennings, *Journal of High Resolution Chromatography and Chromatography Communications*, Volume 8 (1985) 715.

Product Listing

Rtx®-BAC1
30m, 0.53mm ID, 3.0µm
cat.# 18001

Rtx®-BAC2
30m, 0.53mm ID, 2.0µm
cat.# 18000

Universal "Y" Press-Tight® Connector
cat.# 20405 each
cat.# 20406, 3-pack

Universal Angled "Y" Press-Tight® Connector
cat.# 20403 each
cat.# 20404, 3-pack

5m x 0.53mm ID Guard Column
cat.# 10045 each
cat.# 10045-600, 6-pk.

Ambient Air Analysis According to EPA Method TO-14

Since the Clean Air Act was introduced in 1990, much research has been done in developing methods for the analysis of Hazardous Air Pollutants (HAPs). One of the most common analytical methods for HAPs analysis is EPA compendium method TO-14. This method is designed for measuring relatively nonpolar volatile organic compounds. Method TO-14 involves sampling the air with a stainless steel SUMMA® canister followed by cryogenic sample preconcentration. The sample is then transferred to a gas chromatograph, analyzed by high resolution capillary gas chromatography and measured with a sensitive detector such as a mass spectrometer.[1]

There has been an increasing interest in analyzing polar volatile organics, in addition to the TO-14 compound list. Many of these polar compounds are chemically reactive and are commonly found at trace levels in air. Such compounds originate from a variety of industrial processes, automotive emissions, and consumer products, and some may be formed in the atmosphere by photochemical oxidation of hydrocarbons.[2] The measurement and detection of these compounds is very difficult and the analytical methods have not been completely developed.

Figure 1 shows the analysis of a calibration mix of the polar and non-polar volatile organic compounds (VOCs) commonly found in air. The configuration used for this analysis involved sample preconcentration using a Tekmar AEROCAN™ 6000 and a glass bead trap cryogenically cooled to -165°C. The sample was desorbed and secondarily focused at -175°C on the head of a 60m, 0.32mm ID, 1.8µm Rtx®-502.2 capillary column using a cryofocusing module. Due to the volatility of these compounds a long length, thick film capillary column must be used. The Rtx®-502.2 stationary phase is specifically designed for the analysis of volatile organics in water, but is also an excellent choice for the analysis of VOCs in air.

Both polar and nonpolar volatiles can be effectively monitored using a 60m, 0.32mm ID, 1.8µm Rtx®-502.2 capillary column. This long length, thick film column provides the necessary resolution of VOCs in air as described in EPA compendium method TO-14.

References

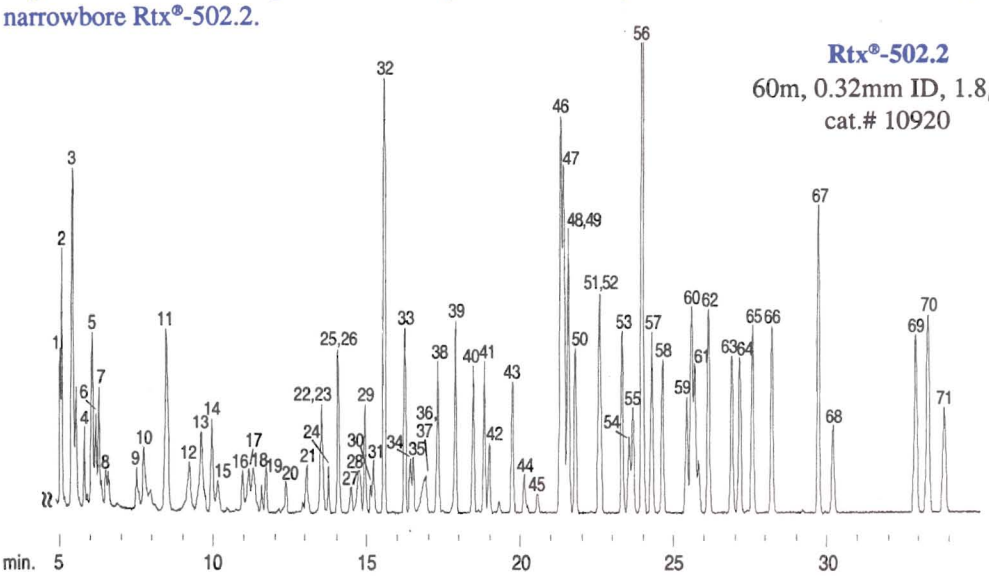
1. Winberry, W.T, Murphy, N.T, Riggan, R.M., *Methods for Determination of Toxic Organics Compounds in Air, EPA Methods, Compendium Method TO-14,* 1990

2. Kelly,T.J, Callahan, P.J., Pliel, J, Evans, G.F., *Method Development and Field Measurements for Polar Volatile Organic Compounds in Ambient Air,* Environ. Sci. Technol. 1993, 27, 1146-1153.

Peak List

1. chlorodifluoromethane
2. dichlorodifluoromethane
3. dichlorotetrafluoroethane
4. chloromethane
5. butane
6. vinyl chloride
7. 1,3-butadiene
8. acetaldehyde
9. bromomethane
10. chloroethane
11. trichlorofluoromethane
12. isopropanol
13. acetone
14. 1,1-dichloroethene
15. acetonitrile
16. dichloromethane
17. acrylonitrile
18. 1-propanol
19. trans-1,2-dichloroethene
20. 1,1-dichloroethane
21. methyl ethyl ketone
22. cis-1,2-dichloroethene
23. methacrylonitrile
24. chloroform
25. bromochloromethane
26. THF
27. 1,1,1-trichloroethane
28. n-butanol
29. heptane
30. 1,2-dichloroethane
31. benzene
32. 1,4-difluorobenzene
33. trichloroethene
34. ethyl methacrylate
35. 1,2-dichloropropane
36. 1,4-dioxane
37. bromodichloromethane
38. MIBK
39. octane
40. toluene
41. 2-hexanone
42. 1,1,2-trichloroethane
43. tetrachloroethene
44. dibromochloromethane
45. 1,2-dibromoethane
46. chlorobenzene-d5
47. chlorobenzene
48. m-xylene
49. p-xylene
50. 2-heptanone
51. styrene
52. o-xylene
53. isopropylbenzene
54. bromoform
55. 1,1,1,2-tetrachloroethane
56. 4-bromofluoromethane
57. n-propylbenzene
58. 1,3,5-trimethylbenzene
59. alpha-methyl styrene
60. t-butylbenzene
61. 1,2,4-trimethylbenzene
62. sec-butylbenzene
63. 1,3-dichlorobenzene
64. 1,4-dichlorobenzene
65. butylbenzene
66. 1,2-dichlorobenzene
67. dodecane
68. dibromochloropropane
69. 1,2,4-trichlorobenzene
70. hexachlorobutadiene
71. naphthalene

Figure 1 - Polar and nonpolar volatile organics commonly found in air can be successfully analyzed on a narrowbore Rtx®-502.2.



60m, 0.32mm ID, 1.8µm Rtx®-502.2 (cat.# 10920)
500ml of 10ppbv standard
Concentrated on an AEROCAN™ 6000 using a glass bead trap at -165°C then desorb at 200°C for 4 min. at 1 ml/min. and cryofocused using a cyrofocusing module at -175°C then desorb at 150°C.

Oven temp.:	35°C (hold 6 min.) to 120°C @ 15°C/min., then to 200°C@ 5°C/min., then to 220°C @ 25°C/min. (hold 10 min.)
Carrier gas:	helium at 1ml/min., linear velocity = 20cm/sec.
Det:	HP-5971A GC/MS
Det. temp:	280°C
Scan Range:	28-260
Solvent delay:	4 min.

Chromatogram courtesy of Allen Madden of The Tekmar Company

Now There are Two Vu-Union™ Designs

· Original design for standard and microbore capillary columns (graphite ferrules) ·
· New design for GC/MS and SFC systems (Vespel®/graphite ferrules) ·

Restek recently introduced the Vu-Union™ for connecting capillary columns. Although these connectors work well with graphite ferrules under normal GC conditions, an additional connector was needed for applications involving GC/MS and SFC applications that require Vespel® ferrules. Graphite ferrules provide excellent sealing properties under normal GC pressures, but are susceptible to oxygen permeation under vacuum and may leak when excessive pressure is used while Vepsel® maintains a leak-tight seal.

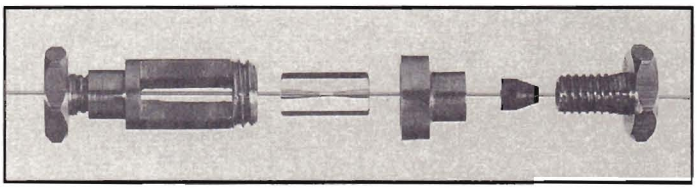
For vacuum and high pressure situations, Restek has developed a new Vu-Union™ for use with Vespel®/graphite ferrules for GC/MS or SFC. Figure 1 shows the disassembled version of the new Vu-Union™. A shortened version of the original Vu-Union™ glass insert allows more torque to be applied to the Vespel®/graphite ferrules without fear of cracking the insert. The addition of hex end nuts allows wrenches to be used for applying high torque to tighten the end fittings.

The new connector also maintains all the benefits of the original Vu-Union™: visual confirmation of the seal, deactivated glass inserts, and a stainless steel high-precision machined body. The new Vu-Union™ combines the benefits of a low dead volume Press-Tight® connector with the confidence of a ferrule seal for the vacuum and high-pressure conditions found in GC/MS or SFC.

The standard Capillary Vu-Union™ is an excellent choice for connections of guard columns to analytical columns, transfer lines at the inlet, or the repair of broken columns. The Capillary Vu-Union™ uses the original glass insert which accepts standard graphite ferrules. The ferrules fit into both ends of the insert, adding a positive seal to the end of the column and connector (Figure 2). The ferrule "seats" or deforms to the shape of the fitting, creating a secondary seal and preventing leaks.

Restek's two Vu-Union™ designs give the chromatographer a number of choices for leak free connections for GC, GC/MS and SFC applications. Vu-Unions™ combine the benefits of a low dead volume connector with the confidence of a ferrule seal. The window allows visual confirmation of the seal between the column and connector. The Capillary and Microbore Vu-Unions™ are designed for graphite ferrules and low pressure conditions. The GC/MS & High Pressure Vu-Unions™ are designed for use with Vespel®/graphite ferrules under vacuum and high-pressure conditions. Deactivated glass inserts are available for all three systems to maintain system inertness.

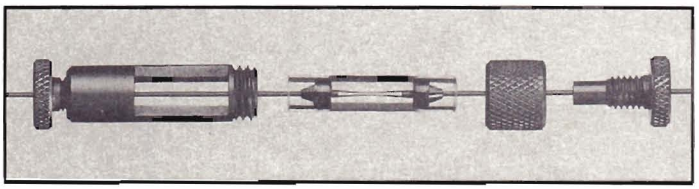
Figure 1 - A disassembled Vacuum/High Pressure Vu-Union™ shows the compact glass insert and hex end nuts.



Features of the Vacuum (GC/MS) and High Pressure (SFC) Vu-Union™:

- Maintains seal under high vacuum used with mass spectrometers.
- Seals under high pressures used in SFC.
- Low thermal mass.
- Uses tough Vespel®/graphite ferruels.
- End fittings incorporate wrench pads for maximum ferrule torque.
- Column seal visually confirmed.
- Universal, fits column IDs from 0.1 to 0.53mm ID.
- Connects columns without peak tailing or loss of efficiency.
- Combines the benefits of Press-Tight® and confidence of ferrule seals.
- Will not unpredictably disconnect.

Figure 2 - A disassembled Capillary/Microbore Vu-Union™ shows the primary and secondary sealing mechanisms.



Features of the Capillary and Microbore Vu-Union™:

- For use with pressures between 0 to 100psi.
- Seals easily without wrenches.
- Uses soft graphite ferrules that conforms easily to all tubing dimensions.
- Column seal visually confirmed.
- Universal, fits column IDs from 0.1 to 0.53mm ID.
- Connects columns without peak tailing or loss of efficiency.
- Combines the benefits of Press-Tight® and confidence of ferrule seals.
- Will not unpredictably disconnect.

Figure 3 - An assembled Capillary/Microbore Vu-Union™ shows the column connection.

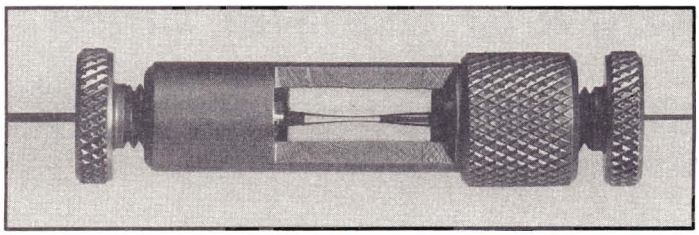
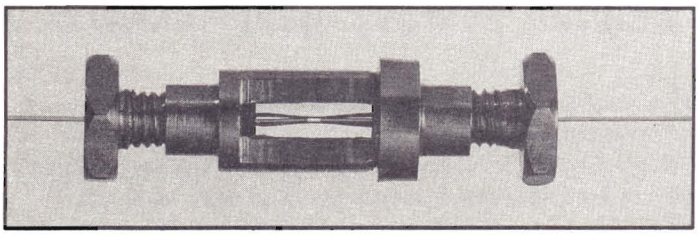


Figure 4 - An assembled Vacuum/High Pressure Vu-Union™ shows the column connection.



Capillary Vu-Union™

- Connect guard columns, transfer lines or repair broken columns.
- Seals easily with minimal torque.
- For use between 0 to 100psi.
- Deactivated, tapered glass insert for column ODs 0.35-0.74mm (IDs from 0.15mm* to 0.530mm).
- For use with standard graphite ferrules (ordered separately).

Capillary Vu-Union™, cat.# 20418 each
Replacement Inserts, cat.# 20419, 3-pk.

Microbore Vu-Union™

- Connect narrow bore tubing under operating conditions from 0-100psi.
- Deactivated, tapered glass insert for column ODs 0.15-0.45mm.
- For use with standard graphite ferrules (ordered separately).

Microbore Vu-Union™, cat.# 20416 each
Replacement Inserts, cat.# 20417, 3-pk.

Vu-Union™ Graphite Ferrules (for Capillary and Microbore Vu-Unions™)

- Easiest ferrules to use with the Vu-Union™.
- Universal, fits Vu-Union™ and connects capillary columns to most GC inlets.
- 450°C maximum operating temperature.

Ferrule ID	Fits Column ID	cat.# (10-pk.)
0.3mm	<0.20mm	20233
0.4mm	0.25mm	20200
0.5mm	0.32mm	20201
0.8mm	0.53mm	20202

Vacuum Vu-Union™

- Connect analytical columns to Mass Spec transfer lines.
- Use under vacuum conditions.
- Deactivated, tapered glass insert for column ODs 0.35-0.74mm (IDs from 0.15mm* to 0.530mm).
- Vespel Graphite ferrules only (ordered separately).

Vacuum Vu-Union™, cat.# 20427 each
Replacement Inserts, cat.# 20428, 3-pk.

High Pressure Vu-Union™

- Will not leak or crack under high-pressure SFC conditions.
- Deactivated, tapered glass insert for column ODs 0.15-0.45mm.
- Vespel®/graphite ferrules only (ordered separately).

High Pressure Vu-Union™, cat.# 20425 each
Replacement Inserts, cat.# 20426, 3-pk

High Pressure and Vacuum Vespel®/Graphite Vu-Union™ Ferrules

- Use only with the High Pressure and Vacuum Vu-Unions™.
- 60% Vespel®/40% graphite.
- 400°C maximum operating temperature.

Ferrule ID	Fits Column	cat.# (10-pk.)
0.3mm	<0.22mm ID - <0.4mm OD	20423
0.4mm	0.25mm ID - 0.4mm OD	20420
0.5mm	0.32mm ID - 0.5mm OD	20421
0.8mm	0.53mm ID - 0.8mm OD	20422

*seals with 0.15mm tubing with a 0.35mm OD

NEW! Hydroguard™
Water Resistant Guard Tubing and Transfer Lines

Dr. Konrad Grob recently published a challenge for chromatographers to develop water resistant guard/transfer line tubing[1]. He found the deactivation layer of a capillary column quickly degrades when it is partially filled with water and refluxed as a vapor (similar to steam cleaning). When transfer lines from purge & traps, air monitoring equipment, or other instruments carry condensed water vapor, the deactivated tubing quickly becomes active due to the creation of free silanol groups. These silanol groups subsequently cause adsorption of active oxygenated compounds such as alcohols and diols.

Restek's chemists investigated this phenomenon and found a solution, our Hydroguard™ deactivation process. By using a unique deactivation chemistry, a high density surface is created that is not readily attacked after an aggressive hydrolysis treatment. The high density surface coverage effectively prevents water vapor from reaching the fused silica surface beneath the Hydroguard™ deactivation layer.

The experiment was conducted by filling deactivated 30m, 0.25mm ID polyimide fused silica columns with 0.75ml of deionized water. One end of the column was flame-sealed, while the other end was evacuated under 0.8mm Hg of vacuum for 15 minutes, then flame-sealed. The columns were subsequently hydrothermally treated by heating at 120°C for one hour and dried with methanol before testing. An evaluation of surface changes was performed by connecting the 30m hydrothermal treated guard tubing to a 30m, 0.25mm ID, 0.25µm Rtx®-5 capillary column using a Vu-Union™ and testing with the Grob test mixture.

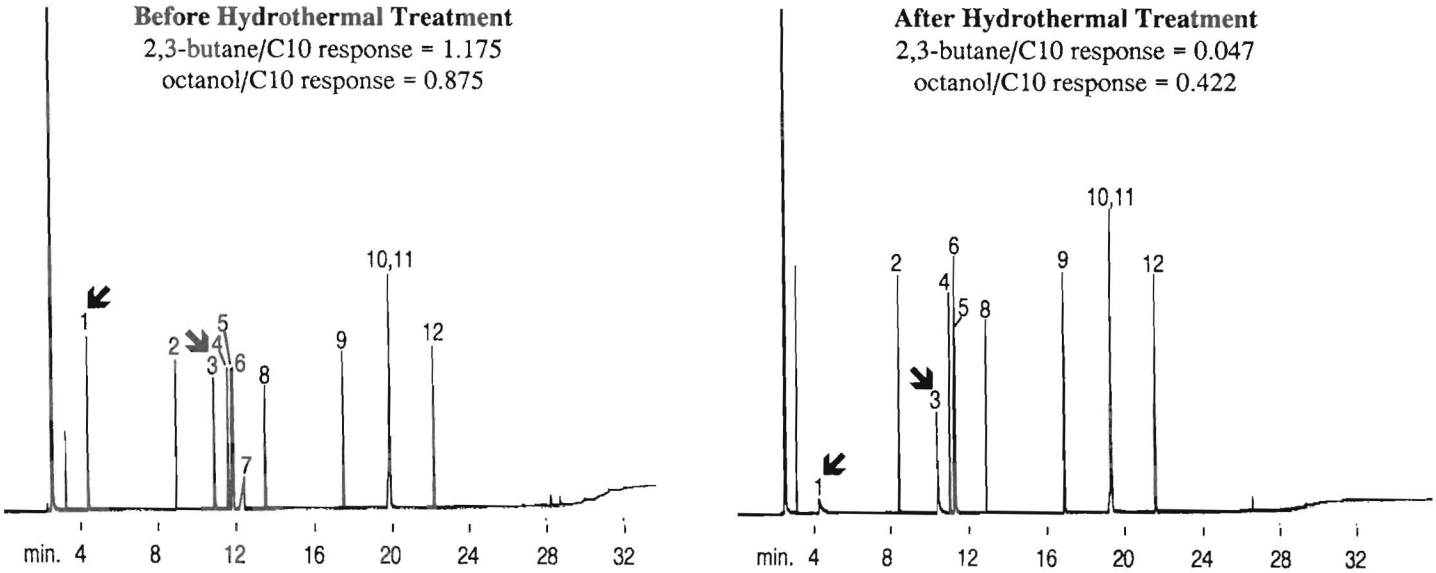
Figure 1 shows the standard Intermediate Polarity (IP) deactivated guard tubing before and after the hydrothermal treatment. Damage to the deactivation layer was indicated by the diminished response and tailing of 2,3-butanediol and octanol peaks. In contrast, the new Hydroguard™ deactivated surface was unaffected by the harsh hydrothermal treatment. Figure 2 shows the Hydroguard™ deactivated guard tubing before and after the hydrothermal treatment. The response of the 2,3-butanediol decreased slightly from 1.225 to 1.087 but the octanol response increased slightly from 0.857 to 0.913. This data shows the resistance of Hydroguard™ deactivated guard/transfer line tubing to aggressive water exposure.

Analysts using guard tubing or transfer line tubing that will be exposed to condensed water vapors should use Hydroguard™ guard tubing and transfer lines. For those analysts injecting organic solvents, Restek's standard IP deactivated guard tubing is preferred. Please call your local distributor if you are unsure which guard tubing to use.

References

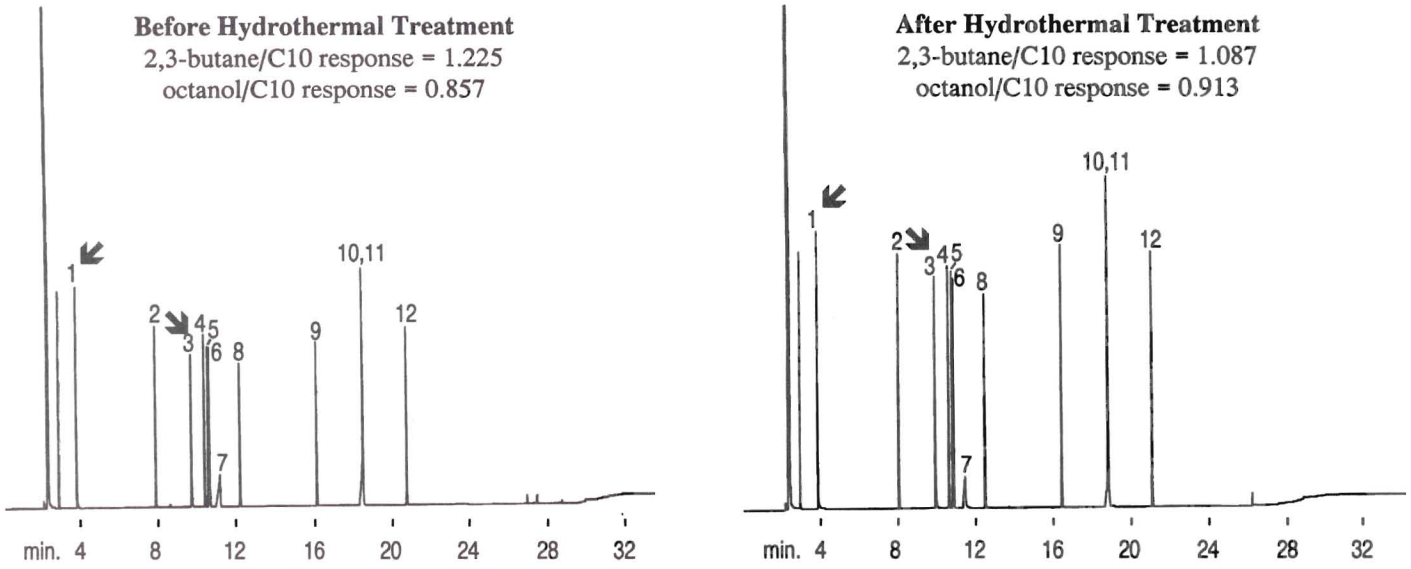
1. K. Grob and B. Schilling, "What Hinders the Further Development of Capillary GC?", *HRC & CC*, Vol 16, June 1993, pg 333-337.

Figure 1 - The Grob Test Mix shows that exposure to water at elevated temperatures causes a loss of tubing inertness with standard guard tubing. (30m, 0.25mm ID IP Guard Tubing)



Peak Identifications and Run Conditions are listed on page 7.

Figure 2 - Grob Test mix shows that the Hydroguard™ guard tubing resists damage from liquid or vaporized water. (30m, 0.25mm ID Hydroguard™ Tubing)



Peak List and Run Conditions for Figures 1 & 2

Grob Test Mix

1. 2,3-butanediol
2. decane
3. 1-octanol
4. undecane
5. nonanal
6. 2,6-dimethylphenol
7. 2-ethylhexanoic acid
8. 2,6-dimethylanaline
9. methyl decanoate
10. dicyclohexylamine
11. methyl undecanoate
12. methyl dodecanoate

30m, 0.25mm ID, 0.25µm Rtx®-5 column (cat.# 10223)
1µl split injection of Grob Test Mix (cat.# 35000)

Oven temp.: 40°C to 185°C @ 6°C/min., then to 325°C @ 15°C/min. (hold 10 min.)
Inj. & det. temp.: 325°C
Carrier gas: hydrogen
Linear velocity: 40cm/sec. set @ 40°C
FID sensitivity: 8 x 10^{-11} AFS
Split ratio: 40:1

Use Hydroguard™ Tubing for Connecting GCs to:

- Purge & Trap systems
- Headspace analyzers
- Summa canister sampling systems
- Air analysis equipment
- Other instruments that trap and release water vapors to GCs
- Any analytical instrument that needs an inert, water resistant pathway.

Benefits of Hydroguard™ Tubing:

- Resists degradation by water injections or condensation.
- Withstands harsh "steam cleaning" chromatography processes.
- Increases column lifetime.
- Reduces effects of dirty samples on column performance.
- Reduces downtime and maintenance.
- Protects expensive analytical columns.
- Prevents damage from harmful materials.

6-Packs of 5m Hydroguard™ Fused Silica Guard Columns & Transfer lines

Save money when you buy 6-packs!

Nominal ID	Nominal OD	Cat. #
0.25mm	0.40mm	10079-600
0.32mm	0.50mm	10080-600
0.53mm	0.75mm	10081-600

Hydroguard™ Fused Silica Guard Columns & Transfer Lines

- 5-meter lengths for convenient connections
- Copy of Grob test chromatogram included for each tubing lot

Nominal ID	Nominal OD	Cat. #
0.05mm*	0.35mm	10075
0.10mm*	0.35mm	10076
0.15mm	0.35mm	10077
0.18mm	0.40mm	10078
0.25mm	0.40mm	10079
0.32mm	0.50mm	10080
0.53mm	0.75mm	10081

Longer Length Hydroguard™ Fused Silica Guard Columns & Transfer lines

Nominal ID	10-meter cat.#	30-meter cat.#	60-meter** cat.#
0.25mm	10082	10085	10088
0.32mm	10083	10086	10089
0.53mm	10084	10087	10090

*not tested with Grob Mix due to a high pressure drop
** Restek recommends cutting 60m guard columns into shorter lengths. Using them full length may cause peak distortion.

Determining Organic Chemical Purity Using Differential Scanning Calorimetry

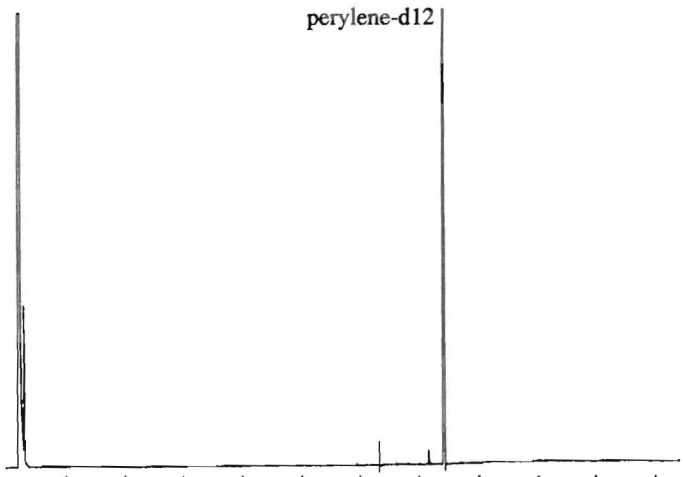
Analytical calibration standards must be made from correctly identified, high purity raw materials. Quantitative standards are prepared gravimetrically and their concentrations are typically assigned from this data. Gravimetric data alone can lead to erroneous results if the material contains varying waters of hydration, salts, or other inorganic impurities. Any impurity may affect the stability and concentration of the standard. Organic chemical purity is most frequently determined by GC/FID or GC/MS. These procedures alone may not yield complete information about the purity of a material. If the impurity is insoluble or does not respond to the detection system used it will go undetected. Since no one analytical technique can provide absolute chemical identification and purity determination, several complimentary techniques must be chosen to ensure the correct identification and purity determination of raw materials used for chemical standards.

Differential Scanning Calorimetry (DSC) has the potential to detect all of the impurities that cannot be identified by GC/FID and GC/MS purity assays including residual catalysts, solvents, water, and inorganic impurities. The extended temperature range of DSC from -170°C to over 600°C allows the determination of accurate melting points not achievable with most melting point equipment. These melting points can be used to help confirm the identity of a chemical.

DSC is an analytical technique where the enthalpy of a sample is measured as a function of its temperature. The instrument is calibrated for temperature, thermal lag, and enthalpy with high purity (99.999+%) metals of known properties. The purity assay is performed on 2 milligrams of the neat raw material, eliminating any possible solvent interferences. The sample is hermetically sealed inside an aluminum sample pan. The assay is run by cooling the sample to 25 degrees below its melting point, ramping the temperature to 25 degrees above the melting point and collecting data on the melting point transition. Any impurity that is soluble in the melt of the main component will cause a melting point depression, or a broadening of the melting transition. Water, residual catalysts, solvents, inorganic, and organic impurities can all be detected by DSC. If the amount of impurities is less than 3 mol/mol percent, the Van't Hoff equation will provide an accurate mol/mol purity determination for the raw material. The mol/mol percent purity is only equal to the weight percent purity when the molecular weights of the impurity and main component are the same. This DSC purity analysis is described in detail in ASTM Method E928-85.

In many cases the DSC data simply confirms the purity determination made by GC/FID. However, in some cases the GC/FID purity data can be misleading. Figure 1 shows a GC/FID analysis of a perylene-d12 sample. When the purity was determined to be 99.5%. However, Figure 2 shows a broad DSC melting point endotherm of this same sample, indicating a major impurity. The impurity was later identified as a residual inorganic catalyst that was not detected by GC/FID. While the exact purity could not be determined, the DSC indicated purity was much less than 97%.

Figure 1 - The GC/FID analysis of perylene-d12 gave a misleading purity value of 99.5 %.



30m, 0.32mm ID, 0.25µm Rtx®-5 (cat.# 10224)
1.0µl split injection of perylene- d12, concentration: 2mg/ml

Oven temp.:	50°C (hold 6 min.) to 300°C @ 10°C/min. (hold 15 min.)
Inj. & det. temp.:	300°C
Carrier gas:	hydrogen
Linear velocity:	40cm/sec. set @ 40°C
FID sensitivity:	1.28 x 10^{-10}AFS

Figure 2 - The DSC detected large amounts of impurities later identified as residual catalyst in the perylene-d12.

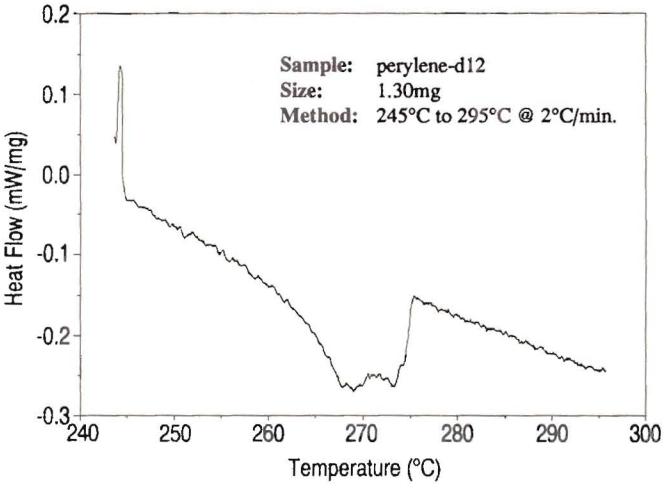
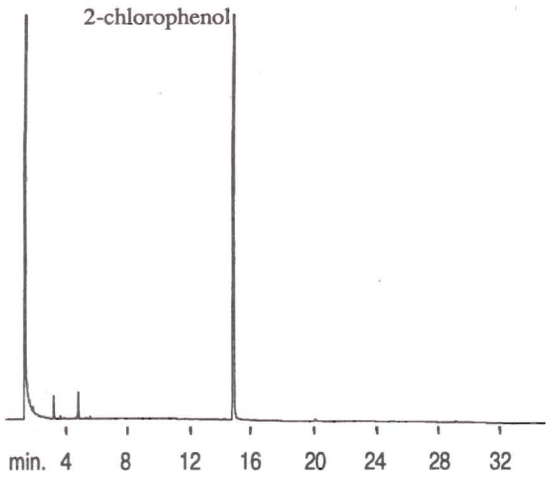


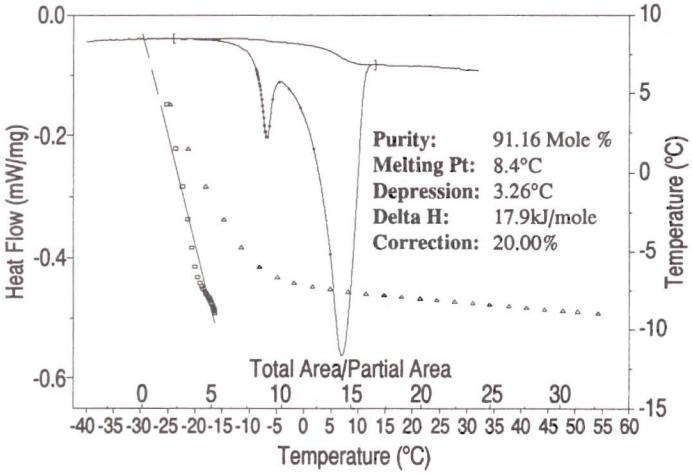
Figure 3 - GC/FID analysis of 2-chlorophenol did not indicate the presence of a major impurity.



30m, 0.53mm ID, 3.0µm Rtx®-1 (cat.# 10185)
2.0µl split injection of 2-chlorophenol, concentration: 2mg/ml

Oven temp.:	40°C (hold 2 min.) to 200°C @ 5°C/min. (hold 30 min.)
Inj./det. temp.:	250°C/290°C
Carrier gas:	hydrogen
Linear velocity:	40cm/sec. set @ 40°C
FID sensitivity:	1.28 x 10^{-10}AFS

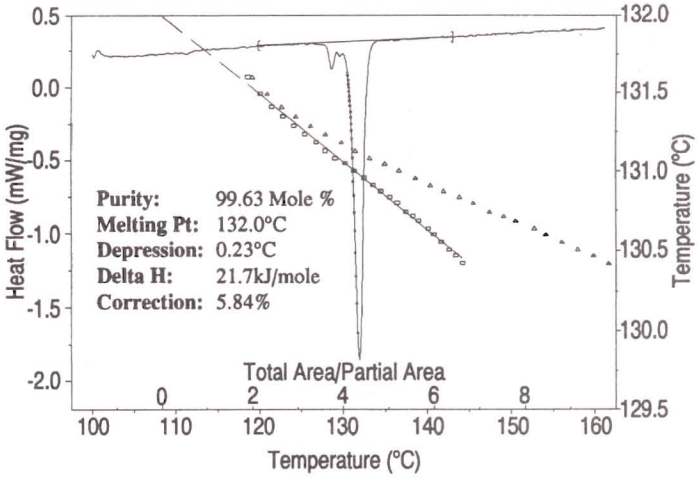
Figure 4 - The two DSC endotherms suggest a large impurity which was later identified as water.



A similar situation occurred with a sample of 2-chlorophenol. The GC/FID analysis indicated a purity of 99.0% (Figure 3). The DSC analysis showed two endotherms, again indicating a major impurity (Figure 4) which was undetected by GC. It was later determined that water, which does not respond on an FID, was the impurity.

All organic compounds are not suitable for purity analysis by DSC. The first constraint is that the raw material must be a single structural isomer with one discreet melting point between -150°C and 600°C. The material must be thermally

Figure 5 - The DSC run on pentobarbital indicated impurities that turned out to be other crystalline states of this material.



stable throughout the experiment while in contact with the aluminum pan and any residual room air inside the pan. Liquid raw materials must be crystallized inside the DSC pan before the experiment starts. Even though the DSC can cool a sample down to -170°C, crystallization is a thermodynamic and kinetic process, thus causing some materials to remain as super cooled liquids for long periods of time.

Figure 5 shows the DSC analysis of a pentobarbital sample. The results would indicate that two impurities existed in the sample. Further investigation revealed that pentobarbital contains three stabile crystalline phases and that the material was actually very pure.

Since no one analytical technique can give absolute chemical identification and purity determination for all organic compounds, multiple techniques must be chosen. DSC is an excellent technique to compliment chromatographic analysis. The combination of DSC, GC/FID, and GC/MS analysis can provide reliable chemical identification and purity determination for most organic compounds. Restek employs DSC in addition to GC-FID and GC-MS for the purity determination of raw materials that are used for our chemical standards.

Call your local distributor to request a copy of our new, 40-page *Chemical Standards Catalog*.

Hints for the Capillary Chromatographer

Techniques for Dual Capillary Column Confirmational Analysis

While capillary columns offer high resolution, they do not necessarily separate all components contained in complex mixtures. Coelutions can occur which decrease the quantitative and qualitative accuracy of an analysis. This is particularly a problem for ECDs, FIDs, NPDs and other detectors which do not give a positive identification for each peak. Even mass spectrometers cannot differentiate between structural isomers and must rely on the column for complete separation. Dual column confirmational analysis using two columns of different polarity can increase the reliability of GC data. If two peaks coelute on the first column, they can usually be separated on a second column of different polarity enhancing qualitative results. Quantitative results can be confirmed since the areas of the coeluting peaks on the first column should equal the combined areas of separated peaks on the second column.

There are three types of single inlet/dual column connection techniques commonly used. The technique chosen will depend on whether split/splitless or direct injections are performed. Only the "Y" Press-Tight® connector/guard column combination can be used with either split/splitless or direct injection techniques. The two-hole ferrule technique works best with split/splitless injections, whereas the direct injection tee is designed to function in a 1/4" packed column injection system operated in the direct injection mode. All three techniques will be described separately.

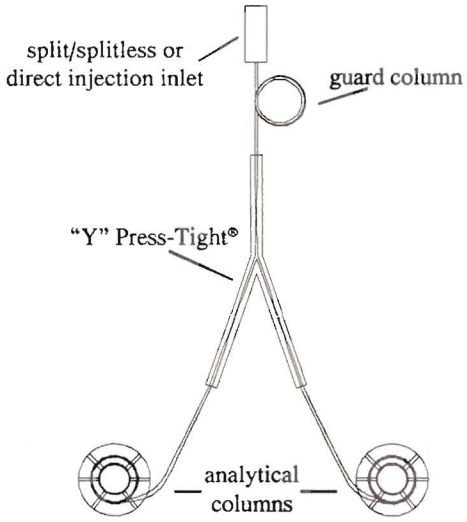
"Y" Press-Tight ® Connector with Guard Tubing

Figure 1 shows the "Y" Press-Tight® configuration for dual column confirmational analysis. A five-meter guard column is connected to the base of the "Y" Press-Tight® with the two analytical columns connected to each outlet leg of the connector. The guard column can be connected to either a split/splitless or direct injection inlet depending on the analyst's preference. The vaporized sample initially travels through the guard column until it reaches the "Y" Press-Tight® where the sample stream splits and a portion travels onto each column. The sample continues to travel through each analytical column until it reaches the detector and provides individual chromatograms.

Press-Tight® "Y"s connect fused silica tubing in the same fashion as a straight Press-Tight® connector. A square cut using a sapphire blade or ceramic scoring wafer is essential to forming a good seal. Examine the column end to make sure it is square and insert it into the Press-Tight® connector, pushing firmly until a uniform brown polyimide "ring" forms. In addition, a small amount of polyimide glue can be used to strengthen the connection.

Usually, the inside diameter of the guard tubing is chosen to match the analytical columns. However, 0.53mm ID guard tubing can be used with two 0.32mm ID analytical columns if the flow rate through the guard tubing is high enough to avoid band broadening. The combined flow rate through each analytical column should equal or exceed the carrier gas optimum flow rate through the larger bore guard tubing.

Figure 1 - The "Y" Press-Tight® configuration allows dual columns to be used in either a split/splitless or direct injection inlet.



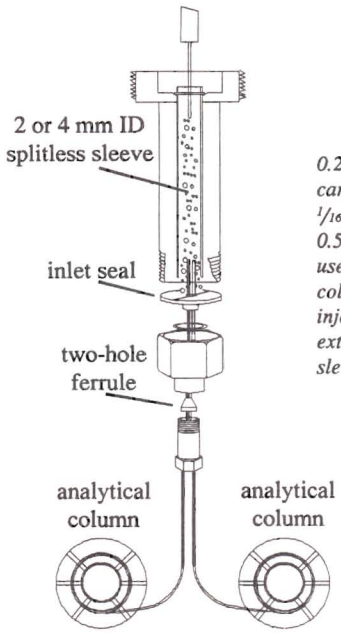
The "Y" Press-Tight® configuration offers versatility since it allows any diameter column or guard column to be connected to any inlet such as split/splitless or direct.

Two Hole Ferrule for Split/Splitless Injectors

Dual column confirmational analysis can also be performed by connecting two columns simultaneously to the same split/splitless inlet via a two-hole ferrule (Figure 2). Most 1/16" capillary inlet fittings will accommodate two 0.25 or 0.32mm ID capillary columns. However, two 0.53mm ID columns are too large to fit a standard 1/16" capillary inlet fitting and require a special 1/8" capillary inlet fitting with a 1/8" two-hole ferrule. Use a split or splitless liner with at least a 4mm ID to ensure that both column ends will fit into the sleeve. If 2mm ID inserts are used, the analyst runs the risk of the column end sitting too close to the sleeve wall which increases split/splitless mass discrimination effects. Standard gooseneck sleeves can not be used because the restriction is less than 1mm

and does not accommodate both columns side by side. Recently, extended goosenecks have become available which are designed with a 4mm internal base to accommodate even two 0.53mm ID columns simultaneously.

Figure 2 - Two hole ferrules can be used to allow dual column confirmational analysis in the same split/splitless inlet.



0.25 and 0.32mm ID column bores can be used with standard 1/16" inlet fittings. However, 0.53mm ID columns require the use of 1/8" fittings to allow both columns to fit side by side in the injector. Either straight or extended gooseneck split/splitless sleeves can be used.

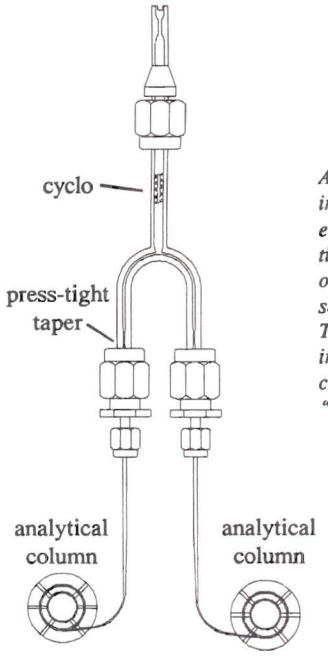
Direct Injection Tee

Many analysts prefer to perform dual column confirmational analysis using direct injection into a 1/4" packed column injection port. Special glass inlet "T"s are available to allow direct connections into two 0.32 or 0.53mm ID columns (Figure 3). The connection from the column inlet to the "T" is made via a Press-Tight® taper as the primary sealing mechanism and a 1/4" to 1/16" reducing fitting as the secondary sealing mechanism. A proper Press-Tight® seal between the column and glass inlet Direct Injection "T" is essential to prevent peak tailing and can be visually observed in Restek's Dual Direct Injection Tee. For the direct injection "T" to function properly, the sample must be thoroughly vaporized prior to the "T" splitting point. Glass wool can be used but may detract from the inertness of the system. Devices such as inverted cups or glass screws (cyclos) can also be incorporated into the inlet leg to ensure complete sample vaporization. These devices also ensure a high degree of inertness since they can be deactivated as a complete unit.

Uniform Sample Splitting

Regardless of which type of dual column system you choose, both column diameters and lengths should be the same. This will ensure that the same amount of sample reaches each column. Slight differences in flow rates between each analytical column are acceptable. However, large flow differences cause an excessive amount of sample to be delivered preferentially onto one column resulting in lower sensitivity for the other column.

Figure 3 - A dual column direct injection "T" allows two 0.32 or 0.53mm ID columns to be securely connected to one 1/4" packed column inlet.



A dual column direct injection "T" incorporates a glass screw to ensure complete sample vaporization prior to splitting the sample onto two columns. The dual sealing mechanisms of a Press-Tight® taper and reducing fitting increase the ease of use and confidence over the Press-Tight® "Y" configuration.

Simultaneous dual column confirmational analysis increases qualitative and quantitative reliability without increasing analysis time. The "Y" Press-Tight® can be used with any injection mode. The two column ferrule technique can only be used for split/splitless injectors, whereas the dual column direct injection "T" must be used in 1/4" packed column injection ports. No conclusive evidence exists that favors one technique over the other when analyzing adsorptive compounds. However, the two-hole ferrule technique used in the splitless injection mode exhibited the highest amount of molecular weight discrimination. Direct Injection is preferred over splitless injection when analyzing high molecular weight compounds, because it minimizes molecular weight discrimination. (For more information on molecular weight discrimination, request Restek's *Guide to Direct/On-column Flash Vaporization Injection.*) Therefore the direct injection tee or the "Y" Press-Tight® in the direct injection mode is recommended over the two-hole ferrule when analyzing high molecular weight compounds or samples with a wide boiling point range. Otherwise, the choice depends on the analyst's personal preference and inlet limitations.

See Restek's *Chromatography Products Catalog* under Dual Column Analysis or call your local distributor for more information. ■

Analyzing Neutral Sterols on an Rtx®-225 for Colon Cancer Research

A special thanks to Dr. Lynne Ausman and Ni Rong of the School of Nutrition, Tufts University, Medford, MA 02155; USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111, for providing the information for the following article.

Neutral sterols are a class of compounds which include cholesterol and its main degradation products coprostanol and coprostanone. These compounds are formed in the colon with the action of microbial enzymes. Studies of humans that have cancer or exhibit a high risk of colon cancer show that these individuals may have a different amount of cholesterol breakdown than individuals at a lower risk of the disease.

In 1983, Dr. Lynne Ausman of the School of Nutrition at Tufts University, studied a colony of cotton-top tamarins, a new world monkey, exhibiting a high incidence of spontaneous colitis and colon cancer in captivity.[1] In working with these animals, she studied their normal lipid and bile acid metabolism using packed column gas chromatography. The results indicated that these monkeys have a low rate of microbial conversion of cholesterol to secondary products.[1]

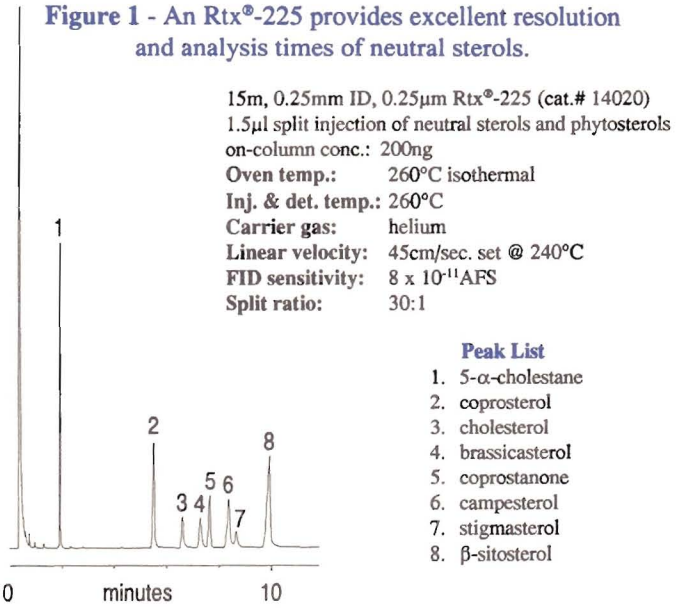
In an effort to determine how diet could slow down or even prevent the disease in humans, Dr. Ausman expanded her research by investigating the fecal sterol output in relation to the type of vegetable oil consumed in the diet. Several vegetable oils show a hypolipidemic response (lower lipid levels) when fed in place of the saturated fats in the "average American" diet. However, some vegetable oils work better than others. These vegetable oils contain plant sterols, termed phytosterols, which humans obtain also through the consumption of fruits, vegetables, grains and grain oils in their diet.

Capillary Gas Chromatography in Fecal Sterol Research

Capillary gas chromatography was used in this research to monitor fecal sterols. The method required separation of the neutral sterols: cholesterol, coprosterol and coprostanone, as well as plant sterols: β-sitosterol, campesterol, brassicasterol, and stigmasterol that were present in the specimens. Although non-polar stationary phases such as the Rtx®-1 (100% dimethyl polysiloxane) and Rtx®-5 (5% diphenyl-95% dimethyl polysiloxane) are suitable for analysis of cholesterol and other sterols, a more polar stationary phase was required for resolution of coprostanone and cholesterol. All analytes, including the cholesterol and coprostanone, are completely resolved on the Rtx®-225 (50% cyanopropylmethyl-50% phenylmethyl polysiloxane), an intermediately polar stationary phase.

Figure 1 illustrates the analysis of these neutral sterols and phytosterols, along with 5-α-cholestane as the internal standard. All analytes are well resolved in 10 minutes and show good peak shape on the 15m, 0.25mm ID, 0.25µm Rtx®-225.

Figure 1 - An Rtx®-225 provides excellent resolution and analysis times of neutral sterols.



There have been studies on humans that suggest those with colon cancer or those at high risk may not metabolize cholesterol to the extent of those who are at a lower risk of the disease. Dr. Ausman and her colleagues are studying consequences on cholesterol metabolism by replacing saturated fats with vegetable oils, which appear to have a hypolipidemic and/or anticholestermic effect. This is monitored by analyzing fecal sterol profiles including neutral sterols and phytosterols by capillary gas chromatography. The neutral sterols cholesterol and coprostanone coelute on non-polar stationary phases commonly used in sterol analysis. The Rtx®-225, however, is an excellent choice for the analysis of the neutral sterols: cholesterol, coprosterol, and coprostanone; and the phytosterols: β-sitosterol, campesterol, brassicasterol, and stigmasterol. All components are well resolved, illustrate good peak symmetry, and are quickly analyzed on this stationary phase.

Dr. Ausman's research also involves analysis of fecal bile acid profiles by capillary gas chromatography. This will be described in a future issue of *The Restek Advantage*.

Reference

1. Ausman, Lynne M., Julia A. Johnson, Catherine Guidry, and Padmanabhan P. Nair, Comp. Biochem Physiol. Vol. 105B, Nos. 3/4, pp. 655-663, 1993.

Product Listing

Rtx®-225
15m, 0.25mm ID, 0.25µm
cat.# 14020

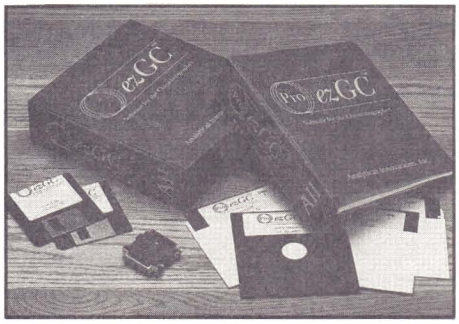
Pro ezGC™
Method Development Software Updates

Pro ezGC™ has become more powerful with new Version 1.5!

- Increase your labs' competitive advantage!
- Optimize temperature and pressure programming parameters to decrease analysis times and increase sample throughput.
- Improve resolution to meet or exceed method protocols.
- Optimize column length, diameter and film thickness ***before*** purchasing the column.
- Import data from ASCII or AIA(ANDI) formats to reduce data entry time.
- Calculate Kovats and Linear Temperature Program Indices, as well as Equivalent Chain Length (ECL) values, for qualitative analysis.

The addition of several new features allows simultaneous optimization of column length, internal diameter, and film thickness, as well as pressure programming. These features are added to the temperature program optimization features already in place. By using *Pro ezGC™*, you can improve the resolution of your analysis, shorten analysis times, increase sample throughput, and save money.

Pro ezGC™ uses thermodynamic retention indices (TRIs) to calculate retention times and elution characteristics for a set of components on a given stationary phase. By entering a column dead time and two temperature programmed runs of experimental data, the user can calculate TRIs. TRIs are then used to predict the performance of these components when any of the column parameters (length, ID, film thickness, carrier gas, or flow control) are changed. By using component libraries and TRIs generated by Restek, you can pick the best column and run conditions without ever installing a column. GC method development and analysis optimization couldn't be easier.



Version 1.5 now allows simultaneous optimization of temperature programs, column length, ID, film thickness and flow or pressure parameters. New component libraries include FAMEs, Pesticides, and PCBs. Call your local distributor to request a complete listing of all the component libraries.

***Pro ezGC™* Software ver. 1.5:**
cat# 21481, $1495

***Pro ezGC™* ver. 1.0 to *Pro ezGC™* ver. 1.5:**
cat.# 21485, $595

***ezGC™* ver. 1.0 or 1.5 to *Pro ezGC™* ver. 1.5:**
cat.# 21482, $1095

Three New Retention Index Libraries Available

Fatty Acid Methyl Ester (FAME)
cat.# 21455

FAME thermodynamic retention index libraries are now available for 70 compounds on the Rtx®-2330 and Stabilwax® stationary phases. All straight chain saturates from methyl butanoate(C4:0) to methyl tetradocosanoate(C24:0) are included, along with unsaturates ranging from monounsaturate methyl undecenoate (C11:1) to the polyunsaturate methyl docosahexanoate(C22:6).

Environmental - Pesticides/Herbicides (Part 1)
cat.# 21456

A collection of 62 chlorinated pesticides from EPA methods 505, 507, 508, 608.1, 608.2, 1618, and CLP Pesticides, as well as 19 derivatized phenoxy-acids found in EPA methods 515.1, 8150B, and 615 are included in this library. Thermodynamic retention indices are provided on the Rtx®-5, Rtx®-35, and Rtx®-1701 stationary phases.

Environmental - PCBs
cat# 21454

A complete collection of retention indices for the 209 polychlorinated biphenyls (PCBs) on the Rtx®-5 stationary phase are included in this library.

Other Retention Index Libraries Available:

Food and Flavor Volatiles (cat.# 21451)
Drugs & Pharmaceuticals (cat.# 21453)
Environmental - Volatiles (cat.# 21452)
Solvents & Chemicals - Part 1 (cat.# 21450)

Please call your local distributor for additional information.

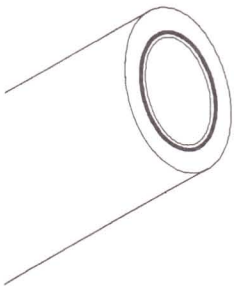
Stabilwax® and MXT®-WAX

The Longest Lasting, Most Inert, Bonded Carbowax® Capillary Columns Available

- Compatible with all solvents including water.
- Resists oxidative degradation.
- Available in polyimide coated fused silica (Stabilwax®) or fused silica lined stainless steel (MXT®-WAX).
- Polymer stable to 250°C.
- Available in a wide variety of IDs, lengths, and film thicknesses.

Stabilwax® (Fused Silica)		(Crossbond® Carbowax® - provides oxidation resistance) Polymer stable to 250°C **Applications:** FAMEs, flavors, acids, essential oils, amines, solvents, xylene isomers, BTEX, EPA Method 603.			
	df (µm)	**temp. range**	**15-Meter**	**30-Meter**	**60-Meter**
0.25mm ID	0.10	40 250°C	10605	10608	10611
	0.25	40 250°C	10620	10623	10626
	0.50	40 250°C	10635	10638	10641
0.32mm ID	0.10	40 250°C	10606	10609	10612
	0.25	40 250°C	10621	10624	10627
	0.50	40 250°C	10636	10639	10642
	1.00	40 240°C	10651	10654	10657
0.53mm ID	0.10	40 250°C	10607	10610	10613
	0.25	40 250°C	10622	10625	10628
	0.50	40 250°C	10637	10640	10643
	1.00	40 240°C	10652	10655	10658
	1.50	50 230°C	10666	10669	10672
	2.00	50 230°C	10667	10670	-----

MXT®-WAX (Silcosteel®)		(Crossbond® Carbowax® - provides oxidation resistance) Polymer stable to 250°C **Applications:** FAMEs, flavors, acids, essential oils, amines, solvents, xylene isomers, BTEX, EPA Method 603.			
	df (µm)	**temp. range**	**15-Meter**	**30-Meter**	**60-Meter**
0.28mm ID	0.25	40 250°C	70621	70624	70627
	0.50	40 250°C	70636	70639	70642
	1.00	40 240°C	70651	70654	70657
0.53mm ID	0.25	40 250°C	70622	70625	70628
	0.50	40 250°C	70637	70640	70643
	1.00	40 240°C	70652	70655	70658
	1.50	50 230°C	70666	70669	70672
	2.00	50 230°C	70667	70670	-----



NEW! Pre-Cleaned Copper Tubing

- Use for plumbing GC systems.
- Specially cleaned to be chromatographically free of background contamination.
- Adheres to ASTM B-280.

cat.#	OD	wall	ID	length
21590	1/8″	0.030″	0.065″	50′
21592	1/4″	0.030″	0.190″	50′

Chemical Standards - New USP 467 Calibration Mixture Available

In the November-December 1993 edition of Pharmocopeial Forum (Volume 19, Number 6) additional modifications have been proposed to USP Method 467. This most recent modification has been introduced to address requirements of the European Pharmacopeia Commission and the Japanese Pharmaceutical Manufacturers Association.

The proposal is to add three additional analytes to the method. These compounds are acetonitrile, pyridine, and 1,2-dichloroethane. Restek has been closely monitoring all proposed modifications, and has available from stock the required calibration mixture. This mixture is shipped complete with an MSDS and a certificate of analysis.

International USP 467 Calibration Mixture

benzene	100µg/ml
chloroform	50
1,4-dioxane	100
methylene chloride	500
trichloroethene	100
acetonitrile	50
pyridine	100
1,2-dichloroethane	100

Prepared in methanol, 1ml/ampul.
Cat.# 36003 each
36103 per pack of 10 ampuls

Other USP 467 Calibration Mixtures Available from Restek:

Revised USP 467 Mixture

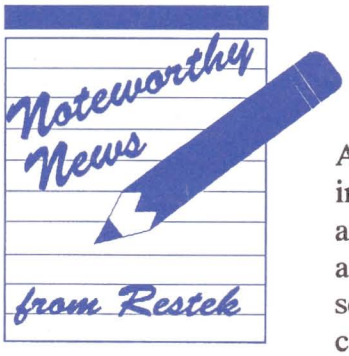
benzene	100µg/ml
chloroform	50
1,4-dioxane	100
methylene chloride	100
trichloroethene	100

Prepared in dimethyl sulfoxide, 1ml/ampul.
Cat.# 36001 each
36101 per pack of 10 ampuls

Proposed USP 467 Mixture

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

Prepared in methanol, 1ml/ampul.
Cat.# 36002 each
36102 per pack of 10 ampuls



Restek Achieves ISO 9001 Certification

We are proud to announce that Restek's quality system has been officially granted ISO 9001 registration by the AT&T Quality Registrar. ISO 9001 is the most encompassing ISO standard, which not only includes a quality assurance system for manufacturing, but also includes quality assurance systems for product design, development, and service. We are very proud of this accomplishment and will continue to improve our quality systems to bring you the best products and services.

The Restek Advantage is printed on recycled paper.