THE RESIDEN

ADVANTAGE

Purge and Trap of Volatile Organics Using Narrowbore Capillary Columns

- Eliminates the need for a jet separator or secondary trapping.
- · Narrowbore columns result in shorter analysis times.
- · Meets MDLs for EPA methodology.
- · Reduces cost of analysis.

Volatile organic analyses (VOA) are commonly run using GC/MS systems and 0.53 mm ID columns. There is now increasing interest in utilizing narrowbore (0.18 - 0.25 mm) columns for VOA analysis because a simpler GC/MS system configura-

tion is used and shorter analysis times are obtained. Figure 1 shows an analysis of an EPA Method 524 calibration standard using a 0.18 mm ID column, split injection port and a direct connection to the GC/MS.

System Configuration

The narrowbore system utilizes a slightly different configura-

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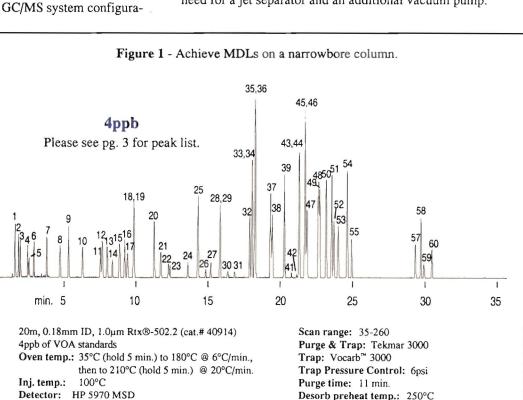
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tion than the more common systems used for 0.53mm ID columns. The injection port is a standard split/splitless inlet operated in the split mode. It is important to minimize dead volume between the transfer line from the purge and trap sampler and within the injection port, so Restek recommends connecting the purge and trap to the transfer line using a low dead volume union and a 1 mm ID injection port sleeve (See page 3). The exit end of the capillary column is directly interfaced into the mass spectrometer source, eliminating the need for a jet separator and an additional vacuum pump.



Linear velocity: 20cm/sec. set @ 35°C

Det. temp.: 280°C

Split ratio: 40:1

Desorb temp.: 260°C

Desorb flow rate: 20ml/min.

Chromatogram courtesy of Anne Williams, Tekmar Company

Desorb time: 2 min.

Optimum desorbtion flow rates for a purge and trap range between 20-30ml/min. When using a 0.53mm ID capillary column, the desorbtion flow rate is typically 10ml/min. This results in a wide sample band which usually requires refocusing to improve peak widths of early eluting components. When a short 0.53mm ID column is used, sub-ambient cooling is necessary. When using a long, thick film 0.53mm ID column, refocusing can be achieved at the head of the column. In the past, narrowbore columns were used but the purge and trap desorb flows were set at 1-2ml/min., which is the optimum flow for narrowbore columns. This low desorb flow results in unacceptable sample band widths and loss of resolution. One solution to this problem is to cold trap the sample band at the front of the narrowbore column. This technique requires an expensive cryofocusing unit and liquid nitrogen for operation.

A simpler, less expensive approach is to set a higher desorb flow of 20 to 30ml/min. and split the desorb flow using a split/splitless injection system. With a split ratio of 20:1 to 40:1 the column can be operated below 1ml/min., when it is close to the optimum flow rate. The faster desorb flow results in a very narrow sample band width. This low column flow rate is also compatible with a direct interface to all bench top GC/MS systems.

Meeting MDLs

Desorbing at high flow rates delivers a narrower band to the injector. However, splitting at the injection port not only reduces the amount of flow to the column, it also decreases the amount of the sample reaching the detector. To reach the required method detection limits it is recommended to purge a 25ml sample volume instead of 5ml. This will increase the amount of sample delivered to the system by 5 fold and compensate for some of the loss of sample caused by splitting.

Even when purging 25mls, there is a 4 or 5 times reduction in the amount of sample reaching the detector. However, the improved efficiency of the narrowbore column produces narrower sample band widths which result in taller peaks and better signal to noise ratio. If the same amount of sample is injected onto both a narrowbore and wide bore column, the smaller diameter column gives a narrower peak width, resulting in a stronger signal at the apex. By using the larger sample size and taking advantage of an increased signal to noise, the required MDLs can be achieved. Figure 1 shows a chromatogram of a 4ppb VOA standard analyzed on a 20m, 0.18mm ID, 1.0µm Rtx®-502.2, directly interfaced into the mass spectrometer source. This chromatogram was generated from a system that had a 20ml/min. desorb flow rate and a split ratio of 40:1.

In a system using 0.53mm ID columns and a jet separator, the noise level is higher compared to a narrowbore system. It is possible that this increase is due to a higher flow rate of helium carrier gas entering the MS source. A comparison of the noise levels of the 0.53mm ID system to the narrowbore system configurations is shown in Figure 2. The noise level is 4 times greater on the 0.53mm ID system compared to the narrowbore configuration.

Figure 3 shows a chromatogram of a 200ppb standard analyzed on a 40m, 0.18mm ID, 1.0µm Rtx®-502.2. This chromatogram was generated from a system that had a 30ml/min. desorb flow rate, a split ratio of 30:1, and direct interface into the mass spectrometer source. Although the 20 meter column reduces analysis time by about 5 minutes compared to the 40 meter column, the resolution of several components is lost. The improvement in peak shape for the early eluting gases is a result of the Trap Pressure Control available on the LSC 3000 system.

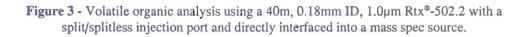
Figure 2 - Noise comparison of a 0.53 mm ID column vs. a narrowbore column directly interfaced into the mass spec source.

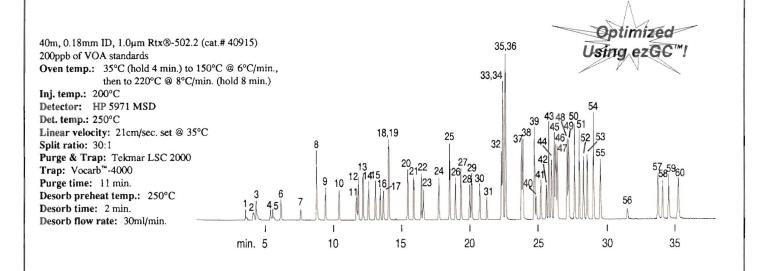
The noise level is 4 times less on the narrowbore setup.*

0.53 mm ID

p/p noise = 4.5e⁴ units

*The x and y scale are identical.





Peak List for Figures 1 and 3

- 1. dichlorodifluoromethane
- 2. chloromethane
- 3. vinyl chloride
- 4. bromomethane
- 5. chloroethane
- 6. trichlorofluoromethane
- 7. 1,1-dichloroethene
- 8. methylene chloride
- 9. trans-1,2-dichloroethene
- 10. 1,1-dichloroethane
- 11. 2,2-dichloropropane
- 12. cis-1,2-dichloroethene
- 13. bromochloromethane
- 14. chloroform
- 15. 1,1,1-trichloroethane
- 16. 1,1-dichloropropene
- 17. carbon tetrachloride
- 18. benzene
- 19. 1,2-dichloroethane
- 20. trichloroethene
- 21. 1,2-dichloropropane
- 22. bromodichloromethane
- 23. dibromomethane
- 24. cis-1,3-dichloropropene
- 25. toluene
- 26. trans-1,2-dichloropropene
- 27. 1,1,2-trichloroethane
- 28. 1,3-dichloropropane
- 29 tetrachloroethene
- 30. dibromochloromethane

- 31. ethylene dibromide
- 32. chlorobenzene
- 33. ethylbenzene
- 34. 1,1,1,2-tetrachloroethane
- 35. m-xylene
- 36. p-xylene
- 37. o-xylene
- 38. styrene
- 39. isopropylbenzene
- 40. bromobenzene
- 41. 1,1,2,2-tetrachloroethane
- 42. 1,2,3-trichloropropane
- 43. propylbenzene
- 44. bromobenzene
- 45. 1,3,5-trimethylbenzene
- 46. 2-chlorotoluene
- 47. 4-chlorotoluene
- 48. tert-butylbenzene
- 49. 1,2,4-trimethylbenzene
- 50. sec-butylbenzene
- 51. p-isopropyltoluene
- 52. 1,3-dichlorobenzene
- 53. 1,4-dichlorobenzene
- 54. n-butylbenzene
- 55. 1,2-dichlorobenzene
- 1,2-dibromo-3-chloropropane
- 57. 1,2,4-trichlorobenzene
- 58. hexachlorobutadiene
- 59. naphthalene
- 60. 1,2,3-trichlorobenzene

Conclusion

The use of narrow bore columns, in the split mode, can significantly reduce the costs associated with the GC/MS analysis of volatile organics. Instrument costs are reduced by eliminating the use of a jet separator. Column costs are reduced by using shorter length, narrow diameter columns. Reduction in analysis time further adds to cost savings. Required MDLs are easily achieved by using a 25ml sample volume and the improved signal to noise ratio produced by narrow bore columns.

Product Listing

cat.# 10969

Rtx®-502.2

20m, 0.18mm ID, 1.0μm	cat.# 40914
40m, 0.18mm ID, 1.0μm	cat.# 40915
30m, 0.25mm ID, 1.4μm	cat.# 10915
60m, 0.25mm ID, 1.4μm	cat.# 10916
Rtx®-624	
20m, 0.18mm ID, 1.0μm	cat.# 40924
40m, 0.18mm ID, 1.0μm	cat.# 40925
30m, 0.25mm ID, 1.4μm	cat.# 10968

60m, 0.25mm ID, 1.4µm **Injection Port Sleeves:**

1mm ID Split Sleeve for HP 5890 GCs

cat.# 20972 each cat.# 20973, 5-pk.

1mm ID Split Sleeve for Varian 1075/1077 GCs

cat.# 20970 each

cat.# 20971, 5-pk.

See Restek's 1993-1994 Chromatography Products Catalog for a complete listing of VOA standards.



Fire Debris Analysis

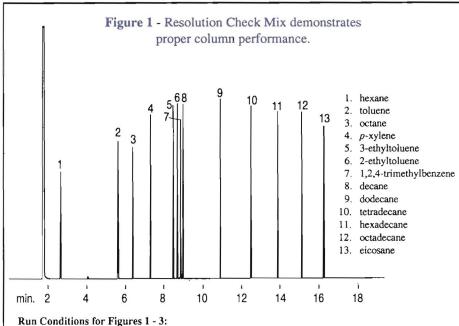
Capillary gas chromatography is the method of choice for analyzing suspected arson samples from fire debris Because of the complex composition of most accelerants used in arson cases, it is crucial that positive identification be made of the material used to start the fire. The American Society for Testing Materials (ASTM) has developed standard test procedures for analyzing these samples. The information presented in this article references ASTM E1387-90, "Standard Test Method for Flammable or Combustible Liquid Residues in Extracts from Samples of Fire Debris by Gas Chromatography".

As with all analytical procedures, sample collection, preservation, chain of custody, and sample preparation play a crucial role in the process. Samples may be extracted using several different techniques (1) for introduction into the gas chromatograph and are beyond the scope of this article.

Appropriate capillary column selection is the first decision the analyst must make. The ASTM standard allows the use of any

capillary column and conditions, provided that a Resolution Test Mix is completely resolved into the individual components. This resolution test mix consists of equal weights of the even numbered n-alkanes from C6 to C20, plus several aromatic compounds. The aromatics specified are: toluene, p-xylene, 2ethyltoluene, 3-ethyltoluene, and 1,2,4-trimethylbenzene.

Several different stationary phases and column configurations can provide the resolution needed. Typically, laboratories can use 30-meter columns coated with either Rtx®-1 (100% dimethyl polysiloxane) or Rtx®-5 (5% diphenyl 95% dimethyl polysiloxane). Film thicknesses can vary from 1.0 to 1.5 µm. Choice of column ID should depend upon sample capacity and detection system employed. The standard allows for the use of either FID, PID, or MS detectors. If MS detection is employed, use a 0.25mm ID column to minimize carrier gas flow. If FID or PID detection is employed, use a 0.53mm ID to maximize column capacity. By doing this, the analyst can minimize expensive duplicate analyses or dilutions if the concentration of accelerants are very high.



30m, 0.53mm ID, 1.5µm Rtx®-1 (cat.# 10170), 1.0µl split injection of E1387-90 Column Resolution Check Mix (cat.# 31224)

Oven temp.: 40°C (hold 3 min.) to 75°C @ 15°C/min., then to 275°C @ 20°C/min. (hold 5 min.) Inj./det. temp.: 250°C/285°C Carrier gas: hydrogen Linear velocity: 50cm/sec. @ 40°C FID sensitivity: 4.10 × 10-9AFS Split ratio:

> Figure 1 shows the complete resolution of all 13 components in the column resolution check mix on a 30m, 0.53mm ID, 1.5µm Rtx®-1 column with an FID detector. The linear velocity and temperature program chosen allow the entire analysis to be completed in about 16 minutes.

> After establishing the correct conditions to obtain complete resolution of the test mix components, the analyst must then calibrate the instrument. In fire debris analysis, this involves purchase and preparation of common accelerants used to ignite fires and injection into the GC. Quantitation of unknown samples is not performed. The analyst must provide positive identification to the field investigators of any accelerants found in the samples collected. To do this, the analyst must be able to recognize typical chromatographic patterns of each accelerant. Figure 2 shows the chromatographic pattern obtained from an injection of an unleaded gasoline standard.

> To further complicate this analysis, many factors will change the chromatographic pattern obtained from fire debris. The first is weathering of the material from the heat of the fire along with dilution of water used to extinguish the blaze. This

Figure 2 - Unleaded Gasoline Standard (unweathered)

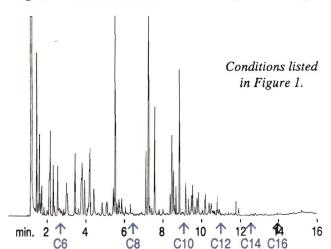
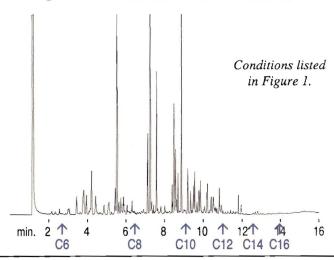


Figure 3 - Unleaded Gasoline (50% weathered)



weathering can dramatically change the chromatographic pattern of the material. Typically, lower boiling materials are lost by the heat, leaving the higher boiling compounds remaining. This type of weathering can be simulated in the laboratory by evaporating the material under controlled conditions. The advantage of performing the evaporation in the laboratory is that the exact amount of

Table I Typical Hydrocarbons Class # Range Examples Light Petroleum Pocket lighter fuel, petroleum ethers, C4-C8 some rubber cement solvents Distillates (LPD) C4-C12 Gasoline gasoline (ALL), some camping fuels Medium Petroleum C8-C12 mineral spirits, paint thinners, some Distillates (MPD) torch fuels, some charcoal fuels, some charcoal starters Kerosene C9-C16 Kerosene, No.1 Fuel Oil, Jet-A Fuel Oil, Jet-A Fuel, some charcoal starters, some torch fuels Heavy Petroleum C10-C23 No. 2 Fuel Oil Distillates (HPD) Diesel Fuel #2 0 Unclassified alcohols, acetone, toluene, some Variable lamp oils, camping fuels, lacquer thinners

identification for each type of accelerant, but in the end the experience of the analyst is crucial.

Adequate chromatographic resolution can be obtained on several different capillary columns. Typically, the best resolution can be obtained on a Rtx®-1 (100% dimethyl polysiloxane) stationary phase. Column configuration should be based upon the detection system being used and

sample capacity. Calibration with weathered petrochemical standards also plays an important part in identification of accelerants extracted from fire debris samples. ■

weight loss compared to the original starting material can be measured and controlled. Figure 3 shows the analysis of a 50% weathered unleaded gasoline. The gasoline has been weathered to a 50% weight loss and an exact concentration calibration standard was prepared with the remaining material. By analyzing known weathered products, the analyst can more readily recognize the type of original starting material.

There are five basic classes of complex petroleum distillates normally identifiable from arson samples. A sixth class of accelerants (Class 0) includes single compounds sometimes used and identified. Table I shows the complete list of classes, typical chromatographic range of each material (based upon hydrocarbon elution), and examples of each type.

There are additional variables which can make identification of the petroleum residue more difficult. Included would be coextracted volatiles and pyrolyzates from the fire debris. The extent of these co-extracted interferences would, in part, be dependant upon the sample preparation method used. The ASTM method does provide minimum requirements for class

References

ASTM Standard Practices for Fire Debris Extraction:
 ASTM E1412-91 Dynamic Headspace Concentration
 ASTM E1413-91 Passive Headspace Concentration
 ASTM E1385-90 Steam Distillation Concentration
 ASTM E1386-90 Solvent Extraction Concentration
 ASTM E1388-90 Sampling of Headspace Vapors
 ASTM E1389-90 Cleanup by Acid Stripping

Product Listing

Rtx®-1:

30m, 0.53mm ID, 1.5μm cat.# 10170

30m, 0.32mm ID, 1.0μm cat.# 10154

30m, 0.25mm ID, 1.0μm cat.# 10153



Standards Spotlight



Weathered Petrochemical Standards Now Available!

May be used for:

- · Underground Storage Tank Monitoring
- · ASTM fire debris analysis.

Laboratories monitoring underground storage tanks often find it difficult to determine the type of petrochemical detected during an analysis. The main reason for this is the petroleum product has weathered from exposure to air, water, sunlight, and microbial action. All of these factors can lead to misidentification of the original product.

A similar situation occurs for forensic analysts investigating potential arson cases. When arson is suspected, samples are taken from the site and analyzed using ASTM E1387-90

methods. In these methods, quantitation is not performed but identification of the accelerant (if any) is crucial. Again, weathering of the petrochemical can drastically change the chromatographic profile when compared to non-weathered material.

Restek is now offering as stock products, weathered petrochemical products to meet many of these difficult situations. All of these standards are prepared from commercially acquired materials. The material is then weathered in the laboratory based upon a specific weight loss from the original weight of starting material. While we cannot duplicate all environmental or arson factors, these standards may be useful in identification of the type of petrochemical detected.

Unleaded Gasoline (ASTM Class 2 Accelerant)

These standards are prepared from a single source (one refinery) product. Samples of regular and premium grade unleaded gasoline were collected, then blended in equal volumes. The weathered materials indicate the percent weight loss from original starting material. These standards are prepared at 5000µg/ml in purge & trap grade methanol, 1ml per ampule.

Unleaded Gasoline Standard (unweathered)

Cat.# 30096 each

> 30096-500 ea. w/data pack

30096-510 5pk.

30096-520 5pk. w/data pack

30196 10 pk. w/data pack

Unleaded Gas Standard - 25% Weathered

Cat.# 30097 each

ea. w/data pack 30097-500

30097-510 5pk.

30097-520 5pk. w/data pack 30197 10 pk. w/data pack

Unleaded Gas Standard - 50% Weathered

Cat.# 30098 each

30098-500 ea. w/data pack

30098-510 5pk.

30098-520 5pk. w/data pack 30198 10 pk. w/data pack

Unleaded Gas Standard - 75% Weathered

Cat.# 30099 each

> 30099-500 ea. w/data pack

30099-510 5pk.

30099-520 5pk. w/data pack

30199 10 pk. w/data pack

Weathered Gasoline Kit

Contains one ampule (1ml) each of the following products:

Unleaded Gasoline Standard (cat.# 30096)

Unleaded Gas Standard - 25% Weathered (cat.# 30097)

Unleaded Gas Standard - 50% Weathered (cat.# 30098)

Unleaded Gas Standard - 75% Weathered (cat.# 30099)

Cat.# 30100 per kit

30100-500 w/data pack

Weathered Petrochemical Standards (cont.)

Mineral Spirits (ASTM Class 3 Accelerant)

There are four general types of material classed as mineral spirits, dependent upon boiling point range (BPR). Type I mineral spirits include stoddard solvent (BPR of 149-182°C), Type II mineral spirits have a high flash point (BPR of 177-196°C), Type III are odorless mineral spirits (BPR of 149-196°C) and Type IV are low dry point spirits (BPR of 149-174°C).

The standards listed below were prepared from an equal volume blend of Type I, II, and III mineral spirits. The weathered material indicated the percent weight loss from the original starting material. These standards are prepared at 5000µg/ml in methylene chloride, 1ml per ampule.

Mineral Spirits Standard (unweathered)

Cat.# 31225 each
31225-500 ea. w/data pack
31225-510 5pk.
31225-520 5pk. w/data pack
31325 10 pk. w/data pack

Mineral Spirits Standard - 25% Weathered

Cat.# 31226 each
31226-500 ea. w/data pack
31226-510 5pk.
31226-520 5pk. w/data pack
31326 10 pk. w/data pack

Mineral Spirits Standard - 50% Weathered

Cat.# 31227 each
31227-500 ea. w/data pack
31227-510 5pk.
31227-520 5pk. w/data pack
31327 10 pk. w/data pack

Mineral Spirits Standard - 75% Weathered

Cat.# 31228 each
31228-500 ea. w/data pack
31228-510 5pk.
31228-520 5pk. w/data pack
31328 10 pk. w/data pack

Weathered Mineral Spirits Kit

Contains one ampule (1ml) each of the following products: Mineral Spirits Standard (cat.# 31226)

Mineral Spirits Standard - 25% Weathered (cat.# 31227) Mineral Spirits Standard - 50% Weathered (cat.# 31228) Mineral Spirits Standard - 75% Weathered (cat.# 31229)

Cat.# 31237 per kit 31237-500 w/data pack

Kerosene (ASTM Class 4 Accelerant)

These standards are prepared from a single source (one refinery) product. The weathered materials indicate the percent weight loss from original starting material. These standards are prepared at 5000µg/ml in methylene chloride, 1ml per ampule.

Kerosene Standard (unweathered)

Cat.# 31229 each
31229-500 ea. w/data pack
31229-510 5pk.
31229-520 5pk. w/data pack
31329 10 pk. w/data pack

Kerosene Standard - 25% Weathered

Cat.# 31230 each
31230-500 ea. w/data pack
31230-510 5pk.
31230-520 5pk. w/data pack
31330 10 pk. w/data pack

Kerosene Standard - 50% Weathered

Cat.# 31231 ea. w/data pack 31231-510 5pk. 31231-520 5pk. w/data pack 31331 10 pk. w/data pack

Kerosene Standard - 75% Weathered

Cat.# 31232 each 31232-500 ea. w/data pack 31232-510 5pk. 31232-520 5pk. w/data pack 31332 10 pk. w/data pack

Weathered Kerosene Kit

Contains one ampule (1ml) each of the following products:

Kerosene Standard (cat.# 31229) Kerosene Standard - 25 % Weathered (cat.# 31230) Kerosene Standard - 50 % Weathered (cat.# 31231) Kerosene Standard - 75 % Weathered (cat.# 31232)

Cat.# 31238 per kit 31238-500 w/data pack



Weathered Petrochemical Standards (cont.)

Diesel Fuel #2 (ASTM Class 5 Accelerant)

These standards are prepared from a single source (one refinery) product. The weathered materials indicate the percent weight loss from original starting material. These standards are prepared at 5000µg/ml in methylene chloride, 1ml per ampule.

Diesel Fue	l #2 Standard	(unweath	nered)
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Cat.# 31233 each 31233-500 ea. w/data pack 31233-510 5pk. 31233-520 5pk. w/data pack 10 pk. w/data pack 31333

Diesel Fuel #2 Standard - 25% Weathered

each Cat.# 31234 31234-500 ea, w/data pack 31234-510 5pk. 31234-520 5pk. w/data pack 31334 10 pk. w/data pack

Diesel Fuel #2 Standard - 50% Weathered

Cat.# 31235 31235-500 ea. w/data pack 31235-510 5pk. 31235-520

each 5pk. w/data pack 31335 10 pk. w/data pack

Diesel Fuel #2 Standard - 75% Weathered

Cat.# 31236 each 31236-500 ea. w/data pack 31236-510 5pk. 31236-520 5pk. w/data pack 31336 10 pk. w/data pack

Weathered Diesel Fuel #2 Kit

Contains one ampule (1ml) each of the following products:

Diesel Fuel #2 Standard (cat.# 31233)

Diesel Fuel #2 Standard - 25% Weathered (cat.# 31234) Diesel Fuel #2 Standard - 50% Weathered (cat.# 31235) Diesel Fuel #2 Standard - 75% Weathered (cat.# 31236)

Cat.# 31239 per kit 31239-500 w/data pack

ASTM E1387-90 Fire Debris Analysis

Adequate column resolution is addressed in this protocol. Any capillary column can be used provided resolution of all analytes can be achieved. To demonstrate performance, a column resolution check mix must be analyzed prior to any sample analysis. Listed below is the required column performance mixture.

E1387-90 Column Resolution Check Mix

Contains the compounds listed at 2000µg/ml each in methylene chloride. Packaged 1ml per ampule.

hexane octane decane Cat.# 31224 each dodecane tetradecane hexadecane 31224-500 ea, w/data pack octadecane eicosane toluene 31224-510 5pk. 31224-520 5pk. w/data pack p-xylene 2-ethyltoluene 3-ethyltoluene 31324 10 pk. w/data pack 1,2,4-trimethylbenzene

Introducing ...

Customer Choice Packaging

Since introducing chemical standards in 1990, we have offered customers a choice of purchasing either single ampules, single ampules with data packs, or economical 10 packs with data packs. Many laboratories may not be able to use 10 ampules of the same mixture within a reasonable period of time, but often purchase multiple single units.

To meet the needs of these customers, we are now offering environmental standards in 5-packs. The customer can choose a 5-pack at either a discount (compared to single ampule purchase) or a FREE data pack (for audit compliance).

For Restek environmental standards not listed here, use the five digit catalog number in our literature and add a three digit suffix for the product which best meets your needs. For example:

> cat.# 31006 SV Internal Standard Mix ADD "-510" for a 5-pack at a discount (31006-510) ADD "-520" for a 5-pack with FREE data pack (31006-520)



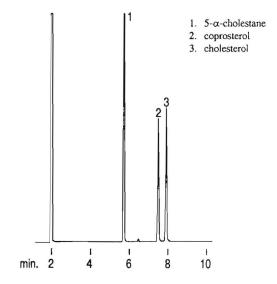
Analysis of Cholesterol and Other Dietary Sterols

The Importance of Cholesterol

The association of elevated levels of blood serum cholesterol with increased risk of heart disease has been widely publicized over the last decade. Since more people are monitoring their cholesterol intake, the demand for the qualitative and quantitative determination of cholesterol content in many foodstuffs such as butter, eggs, baked goods, etc., is rather significant. Capillary gas chromatography provides an efficient means of cholesterol analysis (AOAC methods 970.51, 976.26). (1) Figure 1 shows cholesterol and its metabolite coprosterol, along with the internal standard 5- α -cholestane, elute in less than 10 minutes on a 30m, 0.25mm ID, 0.50 μ m XTI $^{\Phi}$ -5. This column is an excellent choice for cholesterol because it is thermally stable to 330°C. It yields minimal stationary phase bleed and offers short analysis times.

The analysis of other sterols, such as plant sterols, are important as well. Plant sterols, or phytosterols, are constituents of food products such as vegetable oils. Brassicasterol, campesterol, stigmasterol, and β -sitosterol are commonly found in soybean, canola, olive and other vegetable oils that now replace cholesterol-rich sources of fat in the typical diet of the health-conscience consumer. Although similar in structure to

Figure 1 - Analysis of Cholesterol (AOAC 976.26) and Metabolite



30m, 0.25mm ID, 0.50 μ m XTI $^{\Phi}$ -5 (cat.# 12238) 1.0 μ l split injection of cholestane, coprosterol, cholesterol

on-column conc.: 250ng

Oven temp.: 330°C isothermal

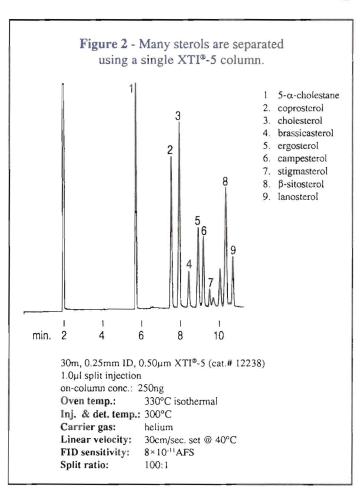
Inj. & det. temp.: 300°C Carrier gas: helium

Linear velocity: 30cm/sec. set @ 40°C

FID sensitivity: 8×10⁻¹¹AFS Split ratio: 100:1 cholesterol, plant sterols are not as readily absorbed as cholesterol, and decrease the artherogenic effects that cause heart disease in humans.

Some plant sterols, such as stigmasterol and ergosterol for example, are important due to their involvement in hormone production. Stigmasterol is used in the preparation of progesterone, and ergosterol is used to produce estradiol. (2)

All of the plant sterols mentioned above, combined with cholesterol, coprosterol, and lanosterol (a sterol found in wool wax) are well resolved on the XTI®-5 in under 12 minutes (Figure 2). Thus, qualitative and quantitative determination of many significant sterols can be performed using the XTI®-5 column at 330°C with 5-α-cholestane as an internal standard.



References

- 1) AOAC Official Methods of Analysis, 15th ed., 1990. pp. 976-7, 1103-1105
- 2) Cook, R.P., Cholesterol, 1958. pp. 4-5.

Extending Septa Life

Factors Affecting Puncturability and Fragmentation

A good septum must possess more than low bleed to meet today's laboratory requirements. Long life and low fragmentation are important to minimize instrument downtime. If a laboratory can make 500 injections instead of 50 injections before changing the septum and inlet sleeve, then more analyses can be performed and the cost per analysis can be reduced. The time savings is greater than initially apparent when you consider the time it takes the system to reequilibriate each time inlet maintenance is performed.

Thermolite® septa have been shown to exhibit the least amount of septum bleed and produce the least amount of artifacts or ghost peaks during a blank run. (1) But what about longevity and fragmentation? How many punctures can be made until the septum starts leaking and why? What causes septa fragments to be deposited in the injection port? These are questions our research group sought to answer in-order to provide the best, lowest maintenance septa possible.

Our experimental work focused on defining the variables that affect septa lifetime. The factors include:

- · septum torque or tightness
- · manual versus autosampler injections
- · septum nut design

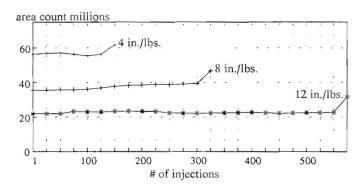
Most work in this study was performed using an HP 5890 GC with manual and autosampler injections. A PE Autosystem was used to double check the data. We expect Varian, Shimadzu, and other GCs to perform similarly but time did not permit verification prior to publication of this article. Testing was done by repeatedly injecting methanol and monitoring retention times and area counts. Septum leakage was monitored by sampling the air above the septum nut by using a thermal conductivity (TC) leak detector. Fragmentation was quantified by packing straight inlet sleeves with glass wool and measuring the amount of septa fragments deposited in the sleeves after a series of injections.

Interestingly, changes in peak area counts proved to be the best indicator of leaks. Since the HP 5890 GC is a back pressure controlled inlet, the component area count increased drastically as a septum leak developed. The increase in area count was directly related to the split ratio lowering as the back pressure regulator reduced flow to the split vent to compensate for the septum leak (2). Changes in peak areas did not occur with the head pressure controlled PE Autosystem. Septum leaks went undetected unless a TC leak detector was used to test septum integrity.

Septum Nut Torque

Torque was the most significant factor that influenced septum lifetime. In general, the tighter a septum nut, the more injections could be obtained until leakage occurred. Figure 1 shows a graph that compares 4, 8, and 12 inch pounds of torque with 11mm septum in an HP autosampler. A septum leak is signified when the area counts deviate sharply from a straight line. The same study was repeated with a 10mm septum and showed similar results in the HP inlet. Surprisingly, even when the septum nut was tightened at 20 inch pounds of torque, the autosampler syringe easily pierced the septum without bending.

Figure 1 - Septa Life Increases as Torque Increases



20m, 0.18mm ID, 0.40μm Rtx®-1 (cat.# 40111)

1μl split injection of methanol

GC: HP 5890 Series II w/HP 7673 autosampler

Oven temp.: 100°C isothermal

Inj./Det. temp.: 260°C Detector: FID

Carrier gas: hydrogen

Manual vs. Autosampler Injections

Manual injections clearly caused more fragmentation and coring of the septum than injections made with an autosampler. Figure 2 shows a significant amount of septum particles deposited in an inlet sleeve after only 100 manual injections. Figure 2 also shows that fragmentation is almost non-existent even after 800 autosampler injections. This data indicates that using an autosampler will significantly minimize the need to perform inlet maintenance from septa fragmentation.

Figure 2 - Manual injections significantly increase fragmentation.



Septa fragments in Sleeve #1 Using Autosampler After 100 injections



Septa fragments in Sleeve #2 Using Manual Injection After 100 injections

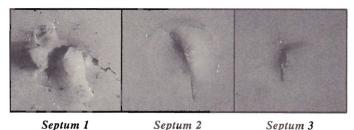


Septa fragments in Sleeve #3
Using Autosampler
After 800 injections

Septum Nut Design

Autosamplers produce little fragmentation because the syringe needle penetrates the septum in the same place each time creating only one small hole. In addition, the high speed injection made by the HP and PE autosampler further reduces damage to the septum. Manual injections allow a wider area of needle penetration and cause the septa to fragment and fall apart. The needle traps fragments upon insertion and deposits them into the inlet during sample injection. In order to reduce the area of penetration, we tested an HP septum nut with a small needle guide that closely matched the OD of a 26 gauge needle to direct the syringe through the same hole during each injection. Figure 3 shows that fragmentation and coring was drastically reduced with a 26 gauge needle guide was compared to the standard size needle guide. Figure 3 also shows that the

Figure 3 - Septum coring is significantly reduced when the needle guide directs the syringe through the same hole.



Septum 1 - coring from 100 manual injections with a standard HP septum nut.

Septum 2 - coring is reduced when a needle guide that closely matches the syringe OD is used.

Septum 3 - 800 injections with an autosampler still produces less coring than with a needle guide.

autosampler still produces less fragmentation and coring than the 26 gauge needle guide indicating that the speed of penetration also has a profound effect on coring.

The experimental results show that many factors affect Thermolite® septa lifetime with torque being the primary variable. In general, septa lifetime increased with the tightness of the septum nut. At a torque of 12 inch pounds, over 500 injections could be made without leakage. No difference was discerned between a 10 or 11mm septum for an HP split/splitless inlet. Injections made by an autosampler always resulted in longer septa lifetime and less fragmentation. The use of a special septum nut with a needle guide that closely fits the syringe OD significantly reduced coring and fragmentation for manual injections and approached the performance of an autosampler.

References

- Request Restek's Guide to Minimizing Septa Problems for data showing bleed comparisons with other manufacturer's septa.
- Request Restek's Operating Hints for Split/Splitless Injectors for more details on back pressure and head pressure designed inlets.

Product Listing

Needle Guide Septum Nut for HP 5890 GCs: cat.# 21309 each

(Please see pg. 14 for complete product description.)

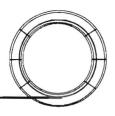
Thermolite® Septa

Septum Diameter	25-pack cat.#	50-pack cat.#	100-pack cat.#
5mm (3/16")	20351	20352	20353
6mm (1/4")	20355	20356	20357
7mm	20381	20382	20383
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

Instrument	Septum Size
Hewlett-Packard	
5890 series	10mm/11mm
5700, 5880 series	9.5mm/10mm
Varian	
packed column injector	9.5mm/10mm
split/splitless injector	IOmm/IImm
Perkin-Elmer	
Sigma series, 900, 990	11mm
8000 series	11mm
Tracor	
550, 560	9.5mm
220, 222	12.5mm
Gow-Mac (all models)	9.5mm
Fisons/Carlo-Erba	17mm
8000 series	
Pye/Unicam	7mm

Effective January 1, 1994, septa prices will increase - so stock up now!

Hints for the Capillary Chromatographer

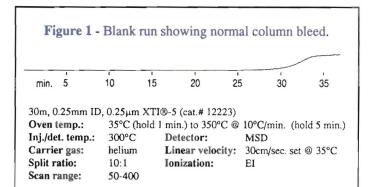


When is column bleed normal and when is it a problem?

What is column bleed?

Column bleed is the normal elution of stationary phase as the GC column is temperature programed. All columns will show a certain amount of bleed as the oven temperature increases. The question is whether the bleed is normal or excessive for your column and conditions. Column bleed only becomes a problem when it either interferes with quantitation or when it contaminates the detector.

A typical bleed profile for a temperature programmed run is shown in Figure 1. Column bleed is characterized by a gradual baseline rise which reaches a plateau at the final temperature of the program. This rise typically begins approximately 20 to 30°C below the maximum operating temperature of the column. Notice that there are no discrete peaks present in column bleed. The type of stationary phase as well as the dimensions of a capillary column will affect the amount of column bleed. For example, a polar phase usually exhibits more bleed than a non-polar phase. In general, the more stationary phase a column contains, the higher the column bleed. A long, wide bore, thick film column has more bleed associated with it than a short, narrow bore, thin film column. Operating at higher temperatures also increases bleed.



What are the most common causes of high column bleed?

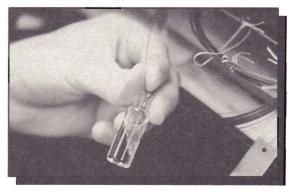
It is important to recognize that there are different causes of excessive column bleed. Studies have shown (1) that there are several common GC problems which can cause high bleed. Let's consider the most common causes for excessive column bleed and what steps can be taken to minimize it.

The stationary phases used in capillary columns are susceptible to oxidation. If the column exhibits a high baseline rise, the column may have been subjected to oxygen at a relatively high temperature. This can be from a leak either in the injection port area or in the gas lines immediately preceding the injector. Oxygen can also be present in the carrier gas as a contaminant from the gas cylinder. It is important to prevent oxidation by

using oxygen traps on the carrier gas lines and by carefully leak checking the flow system and inlet after column installation.

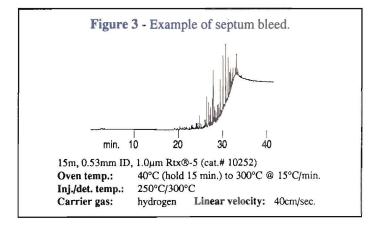
Exposing the column to high temperature without flow or operating the column at temperatures above the manufacturer's recommended maximum will also result in stationary phase damage. This most commonly occurs when a column is conditioned without confirming carrier gas flow, or when a cylinder of carrier gas empties during temperature programming. Restek recommends that flow through the column be verified, before conditioning, by either detecting a non-retained peak or by submerging the detector end of the column in a small vial containing methanol and observing the bubbles (Figure 2).

Figure 2 - Confirm column flow by submerging the column outlet in a vial of methanol.



If the column has been exposed to high molecular weight sample residue, the baseline may exhibit a rise similar to column bleed. When a column has been contaminated, discreet peaks are usually observed in the chromatogram at elevated temperatures. Frequently, solvent rinsing can rejuvenate the column by extracting the contamination. Phase degradation can also result from injecting samples containing strong acids or bases or excess derivatizing reagents.

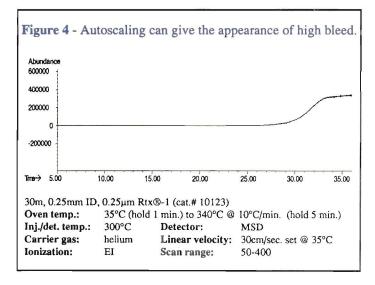
Sometimes septum bleed is confused with column bleed because the electron impact spectra obtained with mass spectrometry are similar for both. Septa bleed is easily recognized as a distinct pattern of discrete peaks in a chromatogram, whereas column bleed normally does not result in individual peaks. In Figure 3, notice the pattern of multiple peaks just before the baseline begins to rise from the normal column bleed. The best techniques for minimizing septum bleed are using low bleed septa, frequently replacing used septa, using a septum purge, and completing a blank run when the column has been at temperatures below 100°C for several hours.



How can column bleed be accurately measured?

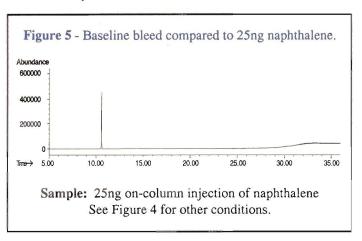
To determine how much column bleed is acceptable for an analysis, you must have an understanding of the necessary detection limits, the type of column being used, and the detector and signal sensitivity during operation. When analyzing trace components with very sensitive detectors, even a small amount of column bleed can interfere with the analysis. If using long length, thick film columns, more bleed will be experienced than with short length, thin film columns. The combination of stationary phase type and detection system used can have a profound effect on how much column bleed is exhibited. For example, nitrogen sensitive detectors (TSDs or NPDs) would exhibit a higher baseline signal from a cyanopropylphenyl stationary phase than Flame Ionization Detectors (FIDs).

It is important to be careful when interpreting the chromatogram obtained with a blank run. Some data systems use an autoscale feature which normalizes the intensity axis to the largest peak in the chromatogram. If there are no peaks, then the chromatogram is drawn with the baseline at full scale, giving the illusion that the column has high bleed (Figure 4).



A simple way of accurately measuring column bleed is to inject a known concentration (i.e. 25ng on-column) of a component that shows good response on the detector being used and temperature program the column to its maximum temperature. Measure the peak height of the component and compare it to

the baseline offset from the bleed. Although the relative intensities of these two values depend upon several factors, these values can serve as a reference point to compare with other columns and systems. Figure 5 shows the bleed level on the same column shown in Figure 4, however, a 25ng injection of naphthalene was included as part of the blank run. Notice that Figures 4 and 5 have the same absolute amount of bleed (500,000 counts), but the bleed level in Figure 5 appears much lower because the plot is scaled relative to the 25ng naphthalene peak. Without the naphthalene injection, an analyst can be fooled into believing that the bleed level is much higher than it actually is.



How can column bleed be minimized?

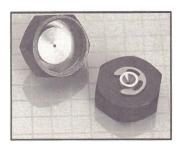
To minimize column bleed, there are several precautionary measures. All systems should be installed with oxygen and moisture traps on the carrier lines. When installing a column it is important to check the entire system for leaks. This includes any column connections, injection port fittings, and carrier lines. All columns should be conditioned following the manufacturer's recommendation. Additional routine conditioning may be required to remove high molecular weight residue, depending on the type of samples you are running. If the column becomes extremely contaminated from dirty samples, rinsing the column may be necessary in order to rejuvenate it. Routine replacement of the septum will eliminate leaks resulting from coring and/or cracking. On GC/MS systems, it is very easy to monitor for air and water leaks. Acceptable levels of air and water vary from system to system, so check with the manufacturer for the recommended limits.

Once there is a leak free system and the column is conditioned, make an injection of a standard sample and program the column to its maximum temperature. The relative height of the peak to the height of the maximum baseline will give a fair assessment of the column bleed.

 M.A. Hayes, J.J. Harland, H.D. Rood and K.T. Klatt, "Proceedings of the Tenth Int. Symp. on Cap. Chrom.", May (1989).

Peak Performers

Needle Guide Septum Nut for HP 5890 GCs



Increase septa lifetime and decrease maintenance requirements with Restek's new septum nut for 26 gauge needles. This new septum nut directs the needle through the same hole, minimizing coring and leakage. For additional details on how our Needle

Guide Septum Nut can extend septa lifetime, see the article, "Extending Septa Life", on pages 10-11. (Similar to HP part number 18740-60835 except with 26 gauge hole).

Needle Guide Septum Nut for HP 5890 GC: cat.# 21309 each

0.3mm ID Ferrules for the Capillary Vu-Union™

Due to numerous customer requests, we have introduced 0.3mm ID graphite and Vespel®/graphite ferrules for the Vu-Union™. The 0.3mm ID ferrule fits 0.18 or 0.22mm ID tubing and is also recommended for some manufacturers 0.25mm ID tubing with ODs close to 0.3mm.

0.3mm ID graphite ferrules: cat.# 20233, 10pk.
0.3mm ID Vespel®/graphite ferrules: cat.# 20423, 10pk.

ezGC™ and Pro ezGC™ Method Development Software

 $ezGC^{\text{TM}}$ and $Pro\ ezGC^{\text{TM}}$ method development software calculates the peak widths and retention times for a given set of chromatographic conditions and then displays the resulting chromatogram. In addition, the software predicts the optimum temperature program for a given analysis that provides baseline resolution in the shortest time. The advanced features of $Pro\ ezGC^{\text{TM}}$ make GC computer modeling even more powerful through accessing libraries generated in your lab or purchased through Restek.

ezGC™ Software: cat.# 21480

Pro ezGC™ Software: cat.# 21481

ezGC™ to Pro ezGC™ Upgrade: cat.# 21482

All software includes both $5^{1}/4^{11}$ and $3^{1}/2^{11}$ disks.

Retention Index Libraries:

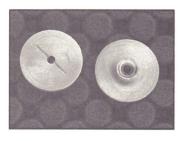
Food & Flavor Volatiles: cat# 21451 Drugs & Pharmaceuticals: cat.# 21453 Environmental - Volatiles: cat.# 21452 Solvents & Chemicals (Part 1): cat.# 21450

Receive a FREE Retention Index Library!

Order $Pro\ ezGC^{m}$ now and receive a free Retention Index Library. This offer ends December 31, 1993. Use the part numbers below when order your free library.

Pro ezGC[™] w/Food & Flavor Volatiles, cat.# 21481-515 Pro ezGC[™] w/Drugs & Pharmaceuticals, cat.# 21481-516 Pro ezGC[™] w/Environmental - Volatiles, cat.# 21481-517 Pro ezGC[™] w/Solvents & Chemicals, cat.# 21481-518

Improved Inlet Seals for HP 5890 GCs



The metal inlet seal at the base of an HP 5890 GC capillary injection port comes into contact with the sample when it vaporizes. This can cause decomposition of active components such as alcohols, pesticides, and drugs. To reduce breakdown and

adsorption of active compounds, Restek offers gold plated and Silcosteel® treated inlet seals. The gold surface offers better

inertness than standard stainless steel. Restek's unique Silcosteel® process places micron thin layers of fused silica and a deactivation layer over the stainless steel to provide inertness similar to a fused silica capillary column.

Gold Plated Inlet Seals (similar to HP part# 18740-20885):

cat.# 21305, 2-pk. cat.# 21306, 10-pk.

Silcosteel® Treated Inlet Seals:

cat.# 21307, 2-pk. cat.# 21308, 10-pk.

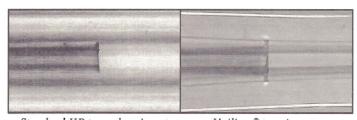
Direct Injection into HP 5890 Packed Column GCs

Restek has optimized the design of the direct injection inlet system for HP 5890 GC packed injection ports. The first area addressed was the tolerances on the disposable glass inserts. Chromatographers have expressed a desire to change the glass inserts by pulling them through the septum nut weldment at the top of the inlet. The current tolerances used by HP cause a large proportion of liners to get stuck at the top of the inlet, requiring complete removal of the direct injection metal sleeve adapter. Removal of the sleeve adapter increases maintenance time and forces the analyst to re-condition the column to stabilize the system. Restek closely monitors every glass insert to make sure it can be changed by simply removing the septum nut weldment and pulling it out with a needle file. In addition, each glass insert is deactivated with our high temperature silanization procedure to maintain the integrity of an inert fused silica capillary system.

Restek also developed a special glass insert (Uniliner®) that incorporates a press-tight taper at the base. The press-tight taper seals tightly onto the end of the fused silica capillary column reducing any chance of dead volume. Figure 1 shows the excessive dead volume between the column and standard HP type glass insert. The Uniliner® design seals tightly against the outside of a fused silica capillary, significantly reducing dead volume and peak tailing. Uniliner® type glass inserts are deactivated and designed to seal with 0.25, 0.32, and 0.53mm ID capillary columns.

The final area we addressed was to re-engineer the metal direct injection sleeve adapter. HP's sleeve adapter for packed purged ports requires a special male capillary nut that uses only short, non-standard ferrules. Restek's design uses a standard 1/16" Swagelok®-type male fitting that uses standard nuts and

Figure 1 - The standard HP type glass insert allows dead volume, whereas the Uniliner® type insert seals tightly onto the column end.



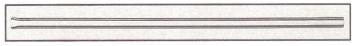
Standard HP type glass insert

Uniliner® type insert

ferrules. We also strengthened the wrench pad at the base of the metal inlet sleeve to make it easier to tighten the column nut. Both the standard glass inserts and the Uniliner® type inserts work with either Restek's metal direct injection sleeve or HP's sleeve adapter for packed purged injection ports.

DI Glass Inserts for an HP 5890 Packed Column GC

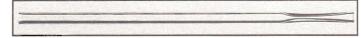
- · Tolerances closely controlled.
- · Can be removed from the septum nut weldment.
- · Deactivated and fully inert to active compounds.
- Similar to HP part# 5181-3382 or #5080-8732.



cat.# 20967, 5-pk. cat.# 20968, 25-pk. cat.# 20969, 50-pk.

DI Uniliners® for an HP 5890 Packed Column GC

- Press-tight taper forms dead volume free connection to column.
- · Minimizes solvent and peak tailing.
- Fits 0.25, 0.32, and 0.53mm ID MXT® or fused silica capillary columns.
- · Can be removed from the septum nut weldment.
- · Deactivated and fully inert to active compounds.
- Fits same sleeve adapter as HP part# 5181-3382 or #5080-8732.



cat.# 20964 each cat.# 20965, 5-pk. cat.# 20966, 25-pk.

DI Sleeve Adapter for an HP 5890 Packed Column GC

- · Uses a standard 1/16" capillary nut and ferrules.
- · Convenient wrench pad at base.
- · Includes 1/4" graphite ferrule and SS nut.
- Works with HP or Restek's DI glass inserts or Restek's DI Uniliners® for an HP 5890 packed column GC.
- · Similar to HP part # 19244-80540.



cat.# 21303, each

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