THE RESTEK ADVANTAGE

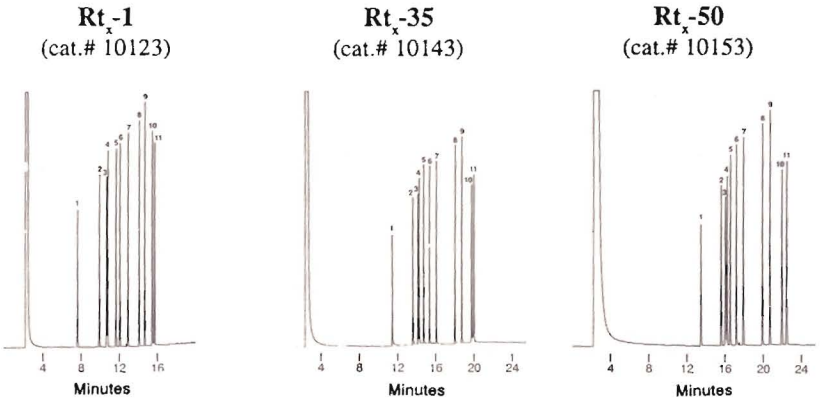
Drug Testing by Capillary GC

Improvements in fused silica capillary column technology have increased the utility of capillary gas chromatography as an accurate, rapid drug screening technique in analytical toxicology. High resolution capillary gas chromatography offers several distinct advantages in the area of drug analysis. The excellent inertness of the capillary column enables detection of both acidic, basic, and neutral drugs at low nanogram levels. High resolving capabilities allow the separation of several drug families and their metabolites on one phase. Capillary gas chromatography yields higher stability and allows more positive identification based on reproducible retention times.

Since a positive result can have potentially serious consequences, it is important that the confirmatory technique used is definitive. Capillary GC/FID is used for prescreening because it yields highly reproducible results and can be used for a wide range of analytes. The standard confirmatory technique for drug testing is GC/MS. A dual column confirmation system with two capillary columns of differing polarity can be used as an alternative to GC/MS confirmation. Confirmational analysis of drugs of abuse are shown on several different polarity columns.

in this issue...

Figure 1 - Common Barbiturates Baseline Resolved on an Rt_x-1, Rt_x-35, & Rt_x-50



1. Barbital
2. Aprobarbital
3. Butabarbital
4. Butalbital
5. Amobarbital
6. Pentobarbital
7. Secobarbital
8. Hexobarbital
9. Mephobarbital
10. Phenobarbital
11. Cyclobarbital.

30m, 0.25mm ID, 0.25µm
2.0µl split injection of 100µl/ml barbiturates

Oven temp.: 150°C (hold 5 min.) to 290°C @ 8°C/min. (hold 5 min.)

Inj. & det. temp.: 250°C / 300°C
Carrier gas: helium
Linear velocity: 20cm/sec.
FID sensitivity: 2×10^{-11} AFS
Split ratio: 10:1

Barbiturates

The widespread misuse of barbiturates has made it necessary for forensic laboratories to provide analytical services which specifically identify all common barbiturates. Because barbiturates have high polarity and low volatility, a GC column must possess thermal stability, high efficiency, good inertness, and selectivity. Barbiturates are typically chromatographed on a non-polar column (Rt_x-1) for primary screening and an intermediate polarity column (Rt_x-50) for confirmation.

Figure 1 shows the analysis of eleven common barbiturates on a 30m, 0.25mm ID, 0.25µm Rt_x-1, Rt_x-35, and Rt_x-50 run under identical conditions. The Rt_x-1, used as the primary column, provides baseline resolution of the barbiturates in twelve minutes. The Rt_x-35 or Rt_x-50, used as confirmation to the Rt_x-1, both provide baseline resolution in under

twenty-three minutes. The dual column systems offer baseline resolution, minimal analysis times, and immediate confirmation.

Opiates

The opiate drug class primarily refers to morphine and codeine, but is loosely used to describe a group of narcotic analgesics and their semi-synthetic derivatives. Opiate quantitation is typically performed on a non-polar column (Rt_x-1) or an intermediately polar column (Rt_x-50).

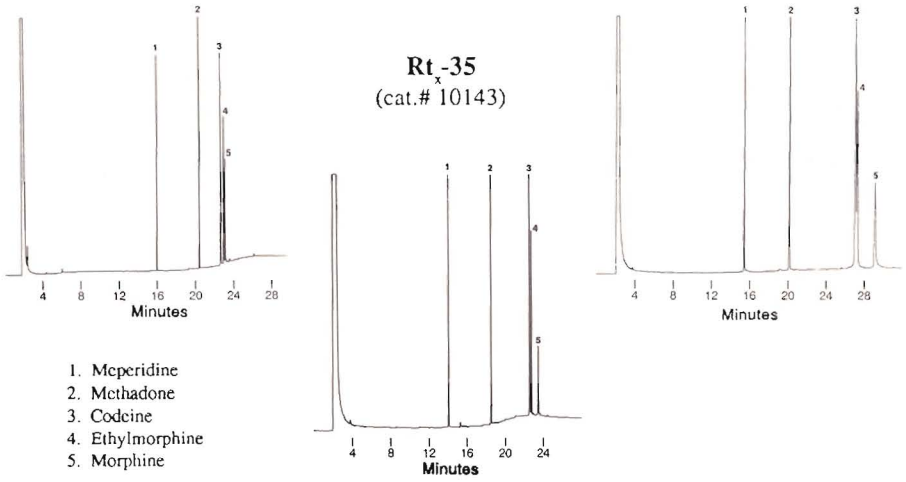
Figure 2 shows the simultaneous analysis of five opiates on a 30m, 0.25mm ID, 0.25µm Rt_x-1, Rt_x-35, and Rt_x-50. The Rt_x-1 and Rt_x-35 give baseline resolution between the critical components codeine, ethylmorphine, and morphine in under twenty-four minutes and show excellent peak symmetry. The Rt_x-50 can also be used as confirmation to the Rt_x-1, however, resolution between codeine and ethylmorphine is only seventy-five percent and the total analysis time is twenty-nine minutes.

Depressants

Depressants are a group of structurally similar compounds used as sedatives and abused for their euphoric properties. They are all weak bases and are typically analyzed on an intermediately polar column (Rt_x-50) by FID.

Figure 3 shows the simultaneous analysis of five depressants on a 30m, 0.25mm ID, 0.25µm Rt_x-1 and Rt_x-35 at 260°C isothermal. Both columns give baseline resolution in 12.5 minutes and 30.5 minutes, respectively. Because the depressants are highly polar, they are retained longer on the Rt_x-35 than on the Rt_x-1. The Rt_x-50 can also be used as a confirmation column to the Rt_x-1, but not simultaneously. Total analysis time of the depressants on the Rt_x-50 at 260°C isothermal is forty-five minutes and peak shape is poor. If the depressants are run at 290°C, analysis time decreases to fifteen minutes and peak symmetry is restored. This occurs because the higher temperature decreases the k of the depressants and allows them to elute faster from the Rt_x-50.

Figure 2 - Confirmational Analysis of Opiates & Derivatives on an Rt_x-1, Rt_x-35, & Rt_x-50



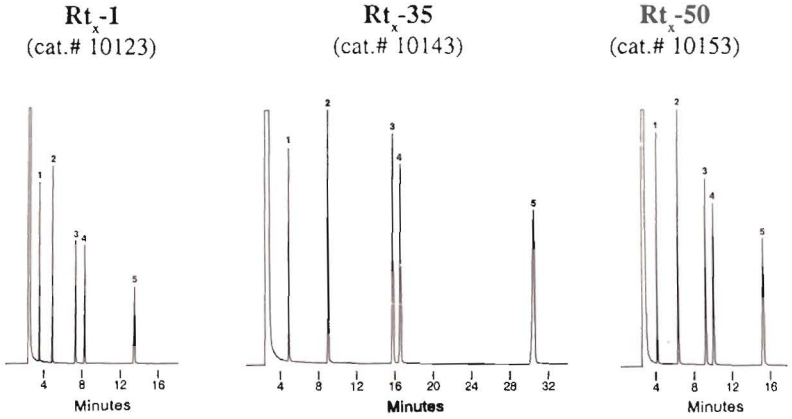
1. Meperidine
2. Methadone
3. Codeine
4. Ethylmorphine
5. Morphine

30m, 0.25mm ID, 0.25µm
2.0µl split injection of 0.1mg/ml opiate standard

Oven temp.: 100°C to 300°C @ 10°C/min. (hold 15 min.)

Inj. & det. temp.: 250°C / 300°C
Carrier gas: helium
Linear velocity: 20cm/sec.
FID sensitivity: 2 x 10^{11} AFS
Split ratio: 10:1

Figure 3 - Simultaneous Confirmational Analysis of Depressants on an Rt_x-1, Rt_x-35, & Rt_x-50



1. Glutethimide
2. Methaqualone
3. Diazepam
4. Chlorpromazine
5. Flurazepam

30m, 0.25mm ID, 0.25µm
2.0µl split injection of an 0.2mg/ml depressant standard

Oven temp.: 260°C isothermal (Rtx-1 & Rtx-35)
290°C isothermal (Rtx-50)

Inj. & det. temp.: 260°C / 300°C
Carrier gas: helium
Linear velocity: 20cm/sec.
FID sensitivity: 2 x 10^{11} AFS
Split ratio: 10:1

Base/Neutral and Acid/Amphetamine Drug Screen

Capillary GC gives forensic chemists the ability and confidence to perform drug analysis accurately. Capillary column inertness enables detection of acidic, basic, and neutral drugs at low nanogram levels and the high resolving capabilities allow the separation of several drug families and their metabolites on a single stationary phase.

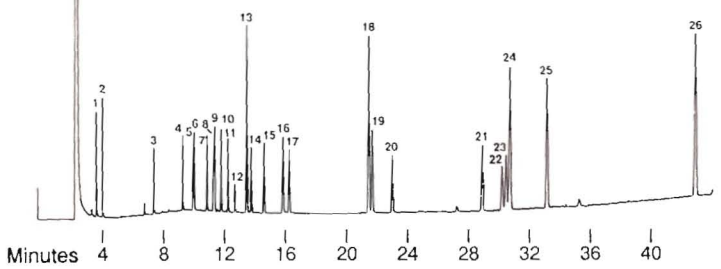
Figure 4 shows the analysis of the barbiturate, opiate, depressant, and stimulant mixes on the Rt_x-1 by GC/FID. The Rt_x-1 resolves all sample components in under forty-three minutes. Resolution between Methylphenidate and Pentobarbital is sixty percent. All other components are baseline resolved.

Another example of the excellent inertness and high resolving capabilities of capillary columns is shown in the analysis of a Tox-Clean drug standard mix. Figure 5 shows the analysis of barbiturates, depressants, tricyclic anti-depressants, opiates, and stimulants on the Rt_x-1. The Rt_x-1 yields resolution of a twenty component drug mix in under twenty-five minutes. Resolution between Imipramine and Oxycodone is seventy percent, all other drug components are baseline resolved. As seen from the chromatogram, all of the drug compounds have excellent peak symmetry on the Rt_x-1.

Detection and identification of unknown substances in biological materials is a challenging task for a forensic toxicologist. Forensic laboratories depend on methods that provide accurate drug identifications, yield rapid analysis times, and permit expanded detection limits of more drugs at lower concentrations. Capillary GC can provide accurate, rapid drug screening in analytical toxicology. Capillary columns have the necessary inertness to quantitate acidic, basic, and neutral drugs, have the separation efficiency to resolve several drug families in a single run, and have the high thermal stability to analyze highly polar, low volatility drugs and eliminate stationary phase bleed on sensitive detectors. ■

Figure 4 - Rt_x-1 Resolves Acidic, Basic, & Neutral Drugs

1. Amphetamine
2. Methamphetamine
3. Barbital
4. Aprobarbital
5. Butabarbital
6. Butalbital
7. Amobarbital
8. Methylphenidate
9. Pentobarbital
10. Meperidine
11. Secobarbital
12. Caffeine
13. Glutethimide
14. Hexobarbital
15. Mephobarbital
16. Phenobarbital
17. Cyclobarbital
18. Methaqualone
19. Methadone
20. Cocaine
21. Codeine
22. Ethyl morphine
23. Morphine
24. Diazepam
25. Chlorpromazine
26. Flurazepam

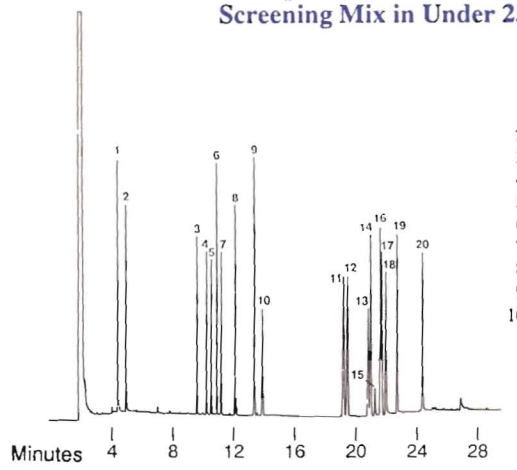


30m, 0.25mm ID, 0.25µm Rtx-1 (cat.# 10123)
2.0µl split injection of a 0.15mg/ml DOA standard

Oven temp..	150°C (hold 4 min.) to 200°C @ 10°C/min. (hold 2 min.) to 300°C @ 2°C/min. (hold 5 min.)
Inj. & det. temp..	250°C / 300°C
Carrier gas:	helium
Linear velocity:	20cm/sec.
FID sensitivity:	2 x 10^{-11} AFS
Split ratio:	10:1

Figure 5 - Rt_x-1 Resolves Twenty Component Drug Screening Mix in Under 25 Minutes

1. Amphetamine
2. Methamphetamine
3. Butabarbital
4. Amobarbital
5. Pentobarbital
6. Meperidine
7. Secobarbital
8. Glutethimide
9. Phencyclidine
10. Phenobarbital
11. Methaqualone
12. Methadone
13. Cocaine
14. Doxepin
15. Amitriptyline
16. Imipramine
17. Oxycodone
18. Desipramine
19. Pentazocine
20. Codeine



30m, 0.25mm ID, 0.25µm Rtx-1 (cat.# 10123)
2.0µl split injection of a 50µg/ml drug mix.

Oven temp.:	110°C (hold 2 min.) to 170°C @ 20°C/min. to 210°C @ 8°C/min. (hold 10 min.) to 260°C @ 15°C/min. (hold 10 min.)
Inj. & det. temp.:	250°C / 300°C
Carrier gas:	helium
Linear velocity:	20cm/sec.
FID sensitivity:	2 x 10^{-11} AFS
Split ratio:	10:1

Phase & Composition	length	ID	df	cat.#	price
Rtx-1	30m	0.25mm	0.25µm	10123	$370
Rtx-35	30m	0.25mm	0.25µm	10423	$370
Rtx-50	30m	0.25mm	0.25µm	10523	$370

Improving the Analysis of Residual Solvents in Pharmaceutical Products

The pharmaceutical industry employs a vast number of different solvents during its manufacturing process of drug products. Some of these solvents pose a potential health hazard if they remain in the finished product. To ensure the safety of the general public, methodology and guidelines for acceptable levels of these toxic solvents were established by the United States Pharmacopeia (USP).

The guidelines were first published in Chemical Tests and Assays, General Chapter ⟨467⟩ "Organic Volatile Impurities," pp. 2395-2397; Method I, from the third supplement to Volume XXII of the USP 1990. The six solvents specified in this method are listed in Table 1. The method outlines recommendations for sample preparation and final analysis. It also outlines several different sample preparation procedures to cover the wide variety of sample matrices. Most of these procedures involve the extraction or dissolution of the pharmaceutical product in water followed by capillary gas chromatography combined with Flame Ionization Detection (FID).

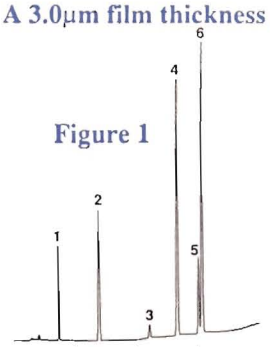
Table 1 - Six Solvents Found in Pharmaceutical Products

Compound	Concentration
Ethylene oxide	10ppm
Methylene chloride	100ppm
Chloroform	50ppm
Benzene	100ppm
Trichloroethylene	100ppm
1,4-dioxane	100ppm

The capillary column recommended in the USP method is a 30m, 0.53mm ID fused silica capillary column coated with a 5.0µm chemically cross-linked 5% phenylmethylsilicone stationary phase. Figure 1 shows the analysis of these six solvents on a 30m, 0.53mm ID, 5.0µm Rt_x-5 column. This column produces baseline resolution of all the components except for trichloroethylene and 1,4-dioxane which are approximately 95% resolved.

A 3.0µm film thickness promotes better resolution than a 5.0µm.

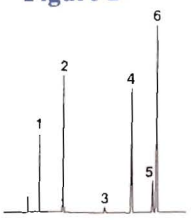
Figure 1



30m, 0.53mm ID, 5.0µm Rt_x-5 (cat.# 10279)

1. Ethylene oxide
2. Methylene chloride
3. Chloroform
4. Benzene
5. Trichloroethylene
6. 1,4-dioxane

Figure 2



30m, 0.53mm ID, 3.0µm Rt_x-5 (cat.# 10285)

1.0µl direct injection. Concentration 100ppm.
Oven temp.: 35°C (hold 5 min.) to 175°C @ 8°C/min. to 260°C @ 35°C/min. (hold 16 min.)
Inj. & det. temp.: 260°C
Carrier gas: helium
Linear velocity: 35cm/sec.
FID sensitivity: 2 x 10^{11} AFS

Understanding the various column parameters is important in selecting the proper column for a particular analysis. Column length effects both analysis time and separation of sample components. Generally, 30-meter columns are chosen as a compromise between fast analysis time and adequate resolution. Column diameter effects sample capacity and also dictates the type of instrumentation that is required. The 0.53mm ID columns exhibit excellent sample capacity and are easily adapted to packed or capillary column instruments. Film thickness also effects sample capacity as well as analysis time. In general, thin films are used when the majority of the sample contains high molecular weight components and thick films are used if the sample contains low molecular weight species.

Since this analysis is comprised primarily of six relatively low boiling solvents, a 30-meter column was an ideal choice. Because this method will be used in many different labs with different types of instrumentation, a 0.53mm ID column was chosen for its versatility. Very thick film columns, such as the 5.0µm column recommended in this method, are normally used for compounds that boil very close to room temperature. Several of these solvents in this method have boiling points above 80°C. These solvents are retained too long by the thick film resulting in longer analysis times and some loss in resolution. We have determined that reducing the film thickness to 3.0µm improves resolution of trichloroethylene and 1,4-dioxane and shortens the analysis time by approximately two minutes. Figure 2 shows the analysis of these six solvents on a 30m, 0.53mm ID, 3.0µm Rt_x-5 column.

Confirmational Analyses

It is not unusual, in many cases, for different sample components to have similar GC retention times. This can lead to misidentification and false positive results. In order to improve the accuracy of the analysis and avoid misidentifications, the use of a confirmational column is recommended. Several different columns were evaluated as potential confirmational columns. The two columns that exhibit the best potential as confirmational columns are either a 30m, 0.53mm ID, 3.0µm Rt_x-502.2 or a 30m, 0.53mm ID, 3.0µm Rt_x-1701. Both columns produce baseline separation of all

Standards Spotlight

Chemical Standards for 500 Series Methods

Restek is pleased to announce the continued expansion of our high quality environmental standards. These mixtures are manufactured in strict compliance with guidelines established by the EPA in their Contract Laboratory Program (CLP). Although these mixtures are NOT intended for use with the CLP program, the requirements specified in the 3/90 Statement of Work are the most stringent published by the EPA in any existing protocol.

501 Trihalomethane Mix
bromodichloromethane
dibromochloromethane
chloroform
bromoform
200µg/ml ea. in 1ml purge & trap grade methanol
Cat.# 30036 $25 ea.
30036-500 $40 ea. w/data pack
30136 $225 10pk. w/data pack

DW-VOC Mix #1
These compounds are currently REGULATED under the Safe Drinking Water Act. Although these materials are found in several additional EPA methods, laboratories may choose to monitor these compounds only.

Benzene	carbon tetrachloride
1,4-dichlorobenzene	1,2-dichloroethane
1,1-dichloroethene	1,1,1-trichloroethane
trichloroethene	vinyl chloride

200µg/ml ea. in 1ml purge & trap grade methanol
Cat.# 30037 $25ea.
30037-500 $45 ea. w/data pack
30137 $225 10pk. w/data pack

DW-VOC Mix #2
Regulations to monitor these compounds were promulgated by the EPA in the Federal Register, Volume 56, No. 20, January 30, 1991.

1,2-dichlorobenzene	cis-1,2-dichloroethene
trans-1,2-dichloroethene	1,2-dichloropropane
chlorobenzene	styrene
tetrachloroethene	toluene
ethylbenzene	o-Xylene
m-Xylene	p-Xylene

200µg/ml ea. in 1ml purge & trap grade methanol
Cat.# 30038 $25 ea.
30038-500 $55 ea. w/data pack
30138 $225 10pk. w/data pack

DW-VOC Kit
Contains 1ml each of the following regulated and promulgated volatile compound mixtures:
30036 501 Trihalomethane mix
30037 DW-VOC Mix #1
30038 DW-VOC Mix #2
Cat.# 30039 $60ea.
30039-500 $115ea. w/data pack

504 EDB/DBCP Mix
1,2-dibromoethane
1,2-dibromo-3-chloropropane
200µg/ml each in 1ml purge & trap grade methanol
Cat.# 30034 $25 ea.
30034-500 $40 ea. w/data pack
30134 $225 10pk. w/data pack

Additional Surrogates & Internal Standards for EPA Methods

α,α,α-trifluorotoluene
2000µg/ml in 1ml purge & trap grade methanol
Cat.# 30048 $25ea.
30048-500 $35 ea. w/data pack
30148 $225 10pk. w/data pack

1,2-dichlorobenzene-d4
2000µg/ml in 1ml purge & trap grade methanol
Cat.# 30049 $25 ea.
30049-500 $35 ea. w/data pack
30149 $225 10pk. w/data pack

Using Method 3500 listed in SW-846?

EPA Method 3500, listed in SW-846, requires the use of an acid matrix spike solution at a concentration of 200µg/ml. In the past, laboratories following this procedure could use our CLP acid matrix spike mix (cat.# 31005) to meet this requirement. In March, the EPA modified the 3/90 Superfund Statement of Work requiring acid matrix spikes at 150µg/ml. Restek changed the concentration of the CLP acid matrix spike mix (cat.# 31005) to 1500µg/ml to follow the new protocol. However, we are now offering an acid matrix spike mix prepared specifically for laboratories following the SW-846 procedures. This mixture is available from stock, complete with certificate of analysis and data packages.

Acid Matrix Spike Mix
Phenol
Pentachlorophenol
4-chloro-3-methylphenol
2-chlorophenol
4-nitrophenol
2000µg/ml ea. in methanol, 1ml per ampule
Cat.# 31014 $25 ea.
31014-500 $45 ea. w/data pack
31114 $225 10pk. w/data pack

Get Reliable GPC Calibrations!

Over the past several months we have had numerous customers request a CLP GPC Calibration Standard. In response to these requests, we are pleased to announce the availability of a GPC calibration standard for the CLP program. This mixture is offered as a qualitative standard that is used to verify resolution criteria of the GPC column and determine dump/collect times for sample clean-up. This mix is available in two convenient package sizes. Because this is a qualitative standard, a certificate of analysis and data packages are not available.

CLP GPC Calibration Mix
Dissolved in methylene chloride at the concentrations listed.

Corn oil	250mg/ml
Bis(2-ethylhexyl)phthalate	10mg/ml
Methoxychlor	2.0mg/ml
Perylene	0.2mg/ml
Sulfur	0.8mg/ml

1ml per ampule. Yields 10ml working solution.
Cat.# 32019 $15 ea.
32119 $135 10pk.
5ml per ampule. Yields 50ml working solution.
Cat.# 32023 $30 ea.
32123 $270 10pk.

Custom package sizes available upon request.

used to increase each column's resolving power and sample handling capacity. To minimize analysis time, high carrier gas flow rates using hydrogen as the carrier gas were employed. Hydrogen was chosen as the carrier gas to minimize the loss in column efficiency when run with high flow rates.* Results showed that all four columns have partial or complete co-elutions of some compounds. There are two co-elutions on the Rt_x-1 column (methyl silicone). Methanol and acetaldehyde are completely unresolved and acetone and isopropanol are only 40% resolved. The remaining compounds elute very close to one another because of the low polarity of the methyl silicone stationary phase. The Stabilwax column (polyethylene glycol) shows better selectivity than the Rt_x-1 column because of the increased stationary phase polarity, but ethanol and isopropanol co-elute with one another. The Rt_x-200 column (trifluoropropylmethyl silicone) had excellent resolution for all of the alcohols due to its selectivity for compounds with lone pair electrons. The only co-elution was with ethanol and acetaldehyde. When compared to the other phases, the Rt_x-1701 column (cyanopropylphenyl methyl silicone) had the best overall resolution. There were no complete co-elutions, with acetone and isopropanol being approximately 40% resolved.

In choosing two columns for use in a dual column system for blood alcohol analysis, overall resolution and co-elutions with ethanol are the primary considerations. Although ethanol is completely resolved on the Rt_x-1 column, it was excluded because of the two co-eluting pairs and low resolving power. The Stabilwax column was dropped from consideration because ethanol co-eluted with isopropanol, a potential contaminant from skin preparation prior to collecting blood samples. Based upon the chromatography for the two remaining phases, the Rt_x-1701 column would be the choice for the screening column. It provides the best resolution of all compounds. The Rt_x-200 would then be the choice for the confirmational column. Because of the unique selectivity of the trifluoropropyl phase, the elution order for acetone and acetaldehyde relative to the other alcohols is dramatically altered. This shift in retention time and elution order provides the mechanism for confirmation of identity of any volatile intoxicants present. Acetone and isopropanol, which had been unresolved on the Rt_x-1701, are now completely resolved from one another. The co-elution of ethanol and acetaldehyde on the Rt_x-200 is a minor problem since clinically significant concentrations of acetaldehyde are rarely encountered.

". . . the Rt_x-1701 column would be the choice for the screening column. . . . The Rt_x-200 would then be the choice for the confirmational column."

The Rt_x-1701 and the Rt_x-200 were then installed into an instrument containing a packed column injection port and two FIDs. Installation of both columns into the injection port was achieved by using a "Y" Press-Tight®connector and a 10cm length of 0.53mm ID deactivated guard column tubing. A 1mm ID Uniliner®was used to adapt the packed column port for use with wide bore capillary columns. Because both columns were of the same length and internal diameter, carrier gas flow and samples to be analyzed were split evenly between the two columns. The detector ends of each column were then installed in separate FIDs. All other analytical parameters were the same. The chromatography achieved with this dual column system was identical to that obtained when using a single column.

Rt_x-1701 and Rt_x-200 columns can be used in a dual column configuration that provides rapid detection and quantitation for ethanol and associated volatile compounds in biological samples.

Product Listing

Phase & Composition	length	ID (mm)	df	cat.#	price
Rt_x-1701	30m	0.53	3.0	12085	$445
Rt_x-200	30m	0.53	3.0	15085	$475
Guard Column	5m	0.53	---	10045	$60
6-pack	5m	0.53	---	10045-600	$300
"Y" Press-Tight connector	---	---	---	20405	$55
3-pack	---	---	---	20406	$145

* longer analysis times and some loss of resolution may occur with helium as the carrier gas

Correction

In the last issue of The Restek Advantage (November 1991, Vol. 2 No. 5), there were some peak misidentifications in Figure 5 on page 4. Corrections are shown below:

As shown in newsletter		Correction
Peak#	Name	
3	Carbon tetrachloride	Tetrachloroethylene
6	n-Propyl nitrate	Bromoform
7	Methylene bromide	n-Propyl nitrate

Why Order Restek Standards?

- High concentration for maximum value
- Prepared for latest EPA protocols
- Complete data package available
- Packaged for laboratory convenience
- In stock for immediate delivery
- Bulk quantities available
- Custom orders welcomed
- Restek's 100% Satisfaction Guarantee

Restek manufactures its own line of high quality chemical standards for laboratories performing environmental analyses.

Most chemical standards are produced at concentrations from 1000µg/ml to 2000µg/ml to insure that an adequate volume of working solution can be prepared from a single ampule. Also, many individual mixtures can be combined to achieve the working calibration levels required by EPA protocols.

Our line of standards for the EPA Con-tract Laboratory Program (CLP) meet or exceed the quality specifications in the latest statement of work. The newest surrogates and target compounds that the EPA requires are included. Complete volatile, semi-volatile, and pesticide kits are available. A data pack that conforms to the EPA's documentation requirements for commercially obtained standards can be purchased with each mixture. These data packs have been accepted by EPA auditors in many regions as adequate documentation for commercially produced standards.

The difference between Restek chemical standards and those produced by other manufacturers

Restek provides all of the production and QA testing documentation for every lot of chemical standards purchased. Restek promotes a total quality program that begins before the raw materials arrive at our facility. Mixtures are designed for customer convenience and stability over long periods of time. Only the highest purity raw materials are purchased from reputable firms. These raw materials are extensively quality tested prior to their use in any mixture. Every compound is analyzed for purity and identity by melting point/refractive index, GC/FID, and GC/MS using high resolution fused silica capillary columns. Additionally, pesticides are analyzed by GC/ECD and the volatile gases are analyzed by GC/ELCD. Most compounds are 98% pure or greater. All raw materials and finished products are stored at reduced temperatures to increase shelf-life.

Our analytical balance calibrations are verified at six mass levels daily using ASTM class 1 weights. Two chemists independently prepare identical mixtures for each product. These mixtures are then analyzed in triplicate using high resolution fused silica capillary columns. The results from both lots are statistically compared to each other using a comparison of means and the Student's *t*-test. The criteria are established at the 95% confidence level with two degrees of freedom. A certificate of analysis is provided with each ampule. This certificate lists each component's exact gravimetric composition, and shows a typical chromatogram from that product.

Restek's Data Packs

The data packs provide customers with the necessary information for EPA audit documentation. The latest Superfund statement of work requires laboratories to have quality, detailed documentation on file for commercially purchased standards. These data packs include the following: melting point measurements for solids and refractive index for liquids, GC/FID purity analyses, GC/MS identity confirmation, copies of all lot sheets showing the exact gravimetric weights, copies of the final mixture's actual QA chromatograms, plus the statistical comparison data. For laboratories not performing Superfund analyses, the data pack can be used by their internal QA departments to review the quality of the standards used.

User Friendly Packaging

Restek's chemical standards are packaged with the laboratory's convenience in mind. First, every ampule is deactivated before being filled and sealed. There is no chance that active analytes can adsorb to the glass walls. We package every ampule in a flex square that contains THE AMPULE CRACKER™ to safely open the ampule, a deactivated vial to place unused portions of the mixture in, and an extra label. Several mixtures are offered in varying concentration and package sizes for different size laboratories.

In Stock for Immediate Delivery

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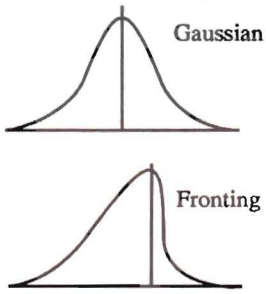
Hints for the Capillary Chromatographer

Sample Capacity and Column Overload

What is column overload?

Column overload occurs when the amount of sample injected exceeds the column's capacity for that component. Overload is normally observed as a fronting, non-gaussian peak shape (Figure 1). A column's capacity is a function of several parameters including the column's internal diameter (ID), its film thickness (df), the solubility of the compound in the column's stationary phase, and capacity factor (*k*).

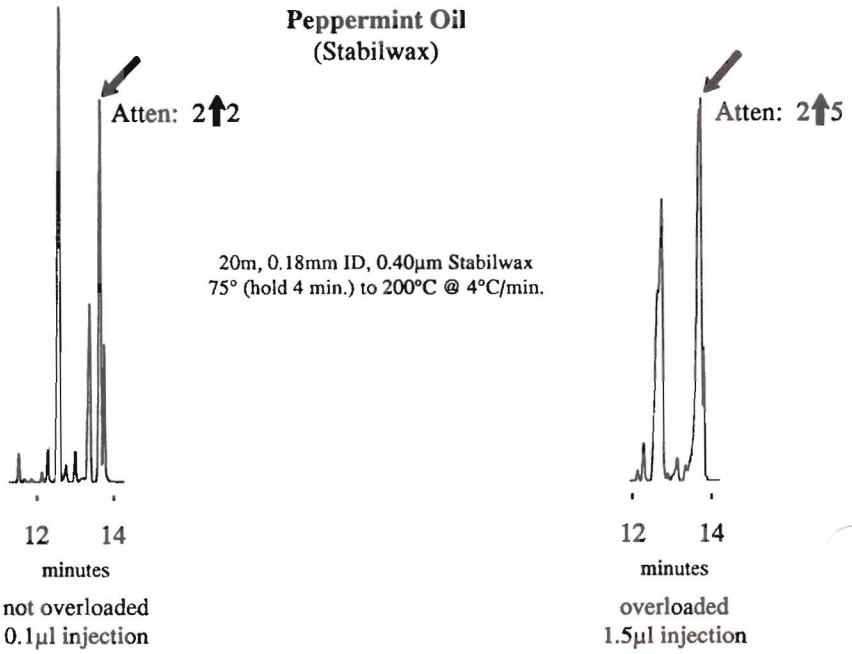
Figure 1 - Normal Gaussian vs. Overloaded Fronting Peak Shapes



Why is it important not to exceed a column's capacity?

Capillary columns have much lower sample capacities than packed columns, therefore, it is extremely important to optimize the amount of sample injected. When sample capacity is exceeded, peak symmetry is lost and resolution is affected. Because the peak shape will be much broader, resolution between two closely eluting peaks can be lost. Figure 2 shows the loss of peak symmetry and resolution in the analysis of peppermint oil on a Stabilwax column. In the first chromatogram, 0.1µl of neat peppermint oil was injected. At these low concentrations, very good resolution between the menthyl acetate, neo-menthol,

Figure 2 - Minimize the amount of sample injected to maximize resolution.



β-caryophyllene, and terpinene-4-ol is obtained. In the second chromatogram, 1.5µl of neat peppermint oil was injected. Because the sample concentration exceeded the column's capacity, a significant loss in resolution occurred.

How can overload be prevented?

Two choices are available to prevent overload:

- ▼ reduce the sample concentration reaching the column
- ▼ choose a column and run conditions that will allow greater sample capacity

To reduce the sample concentration reaching the column, the sample components can be diluted by increasing the split ratio, diluting with additional solvent, or by introducing a smaller amount.

How does column ID affect sample capacity?

As the column ID increases so does sample capacity. Table 1 shows typical column capacity for several different diameter columns. Figure 3 compares sample capacity on 0.25 and 0.53mm ID columns. Four hydrocarbons (heptane, octane, nonane, and decane) were analyzed at a concentration of 1000ng on

Table 1

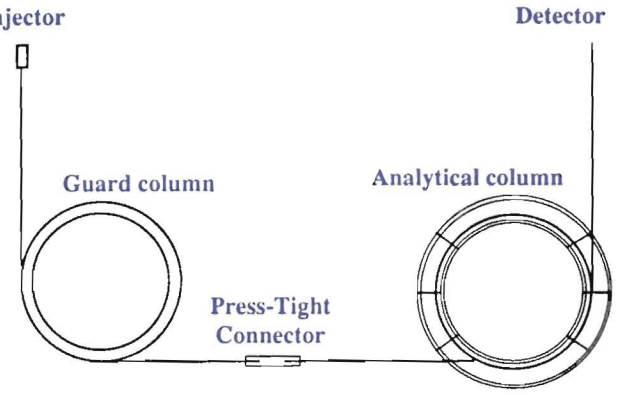
Column ID	0.18mm	0.25mm	0.32mm	0.53mm
Sample Capacity	<50ng	50-100ng	400-500ng	1000-2000ng

Hints for the Capillary Chromatographer

The Benefits of Guard Columns for Capillary Gas Chromatography

The use of guard columns has been commonplace in high performance liquid chromatography for many years. Their use as safeguards to protect the analytical column from highly retentive compounds and particles is well understood. It has only been in the past few years that the benefits of guard columns have been associated with capillary gas chromatography. Although guard columns prolong the life of capillary columns and protect them from sample contamination, they are not widely used in many laboratories. Understanding the basics of guard columns helps to dispel confusion and apprehension about their use.

Figure 1 - A Guard Column Connected to an Analytical Column.



What is a guard column?

A guard column for capillary chromatography is a short length of deactivated, uncoated fused silica tubing that is placed between the injection port and the analytical column. Figure 1 shows a diagram of a guard column connected to an analytical column.

What advantages do guard columns offer?

Prolong column lifetime.

A guard column protects and prolongs the lifetime of an analytical column in several ways. It traps non-volatile residues and prevents them from collecting at the head of the analytical column. These non-volatile residues may be very high molecular weight organic compounds, inorganic salts, or particulate materials. If these contaminants enter the analytical column, they can cause adsorption of active compounds and loss of resolution. When this contamination begins to affect sample analysis, a small section of the analytical column must be removed to restore proper performance. However, each time a section of the column is removed, retention times change, some resolution is lost, and column length is decreased, eventually resulting in a useless analytical column. By trapping this contamination in the guard column, the analytical column remains the same length and stays cleaner longer.

Decrease maintenance requirements.

Since there is no stationary phase on the guard column, the amount of time the sample spends in it is minimized. This reduces the interaction between sample components and the contamination from non-volatile residue. Therefore, guard columns allow more injections to be made before residue interferes with analytical results.

Improve resolution.

Many analysts are reluctant to use guard columns because they believe that they will lose resolution. In fact, guard columns aid in focussing the components by decreasing aerosol formation and actually increasing separation efficiency. The guard column acts as a retention gap to help focus the sample at the head of the column. When a sample is injected, it first exists as vapor and aerosol. Without a guard column the vapor begins to partition in and out of the column's stationary phase. The aerosol portion of the sample does not partition in the phase and moves out ahead of the vaporized sample. This results in broader, less efficient peaks and, in extreme cases, can cause split peaks. Since a guard column is not coated with stationary phase, there is no interaction with the vaporized sample or the aerosol. They move along together in a tighter band. The aerosol vaporizes in the guard column so that when the sample reaches the coated column it is completely vaporized. This produces sharper, more efficient peaks, as shown in Figure 2. Table 1 shows the results of analyzing 2,6-dimethylphenol on a 30 meter, 0.53mm ID, 1.0µm Stabilwax®column with and without a guard column. The efficiency of the 2,6-dimethylphenol peak was measured in each case and the results show a 3.1% increase in efficiency with the guard column.

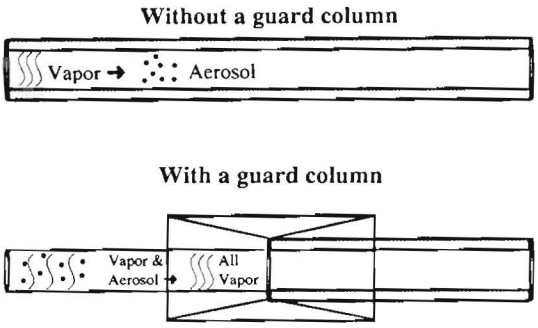
Table 1 - Column Efficiency Data
(1µl split injection of 2,6-dimethylphenol)

30m, 0.53mm ID, 1.0µm Stabilwax

Without guard column	With guard column
Total plates = 51500	Total plates = 53100
Plates/meter = 1716	Plates/meter = 1770

3.1% increase in plates

Figure 2 - Guard columns increase separation efficiency because the aerosol completely vaporizes before it reaches the coated column.



How is the guard column connected to the analytical column?

A Press-Tight®Connector is the simplest and most common way to connect a guard column to the analytical column. These connectors do not require ferrules and work on the principle of radial compression. Once heated, the polyimide on the outside of the tubing bonds to the inside of the connector, making a permanent, leak-free seal. There are several key steps to ensure a leak-free seal. First, cut the column ends squarely with a device designed for cutting fused silica tubing, such as a sapphire scribe or a ceramic scoring wafer. (Pointed scoring devices are not recommended.) Second, clean and lubricate the tubing by wiping the ends with a tissue moistened with methanol or deionized water. Next, firmly insert the tubing into the connector taper and check for leaks. If no leaks are found, bond the tubing to the connector by heating it to 200°C.

When should a guard column be replaced?

As the guard column becomes contaminated with non-volatile residue, the performance of the entire chromatographic system will begin to deteriorate. This is normally exhibited as a drastic decrease in the response of active compounds. Figure 3 shows the analysis of phenols on an Rt_x-5 with a guard column. The response of all of the phenols is excellent. Figure 3 also shows the same analysis after repeated injections of a sample containing significant quantities of non-volatile residue. The reduction in the response of 2,4-dinitrophenol and pentachlorophenol indicate that the guard column is contaminated and must be replaced.

How often must a guard column be replaced?

The life expectancy of a guard column depends on the length of the guard column, the amount of non-volatile residue in the samples, and the number of samples run. When analyzing dirty samples, the guard column becomes contaminated quickly. Normally, contamination deposits in the first .5 to .8 meters of the guard column. If a short guard column (1-meter or less) is used, it must be completely replaced when it becomes overly contaminated. If a longer guard column (5-meters) is used, the contaminated section can be removed without re-connecting the analytical column.

How long should a guard column be?

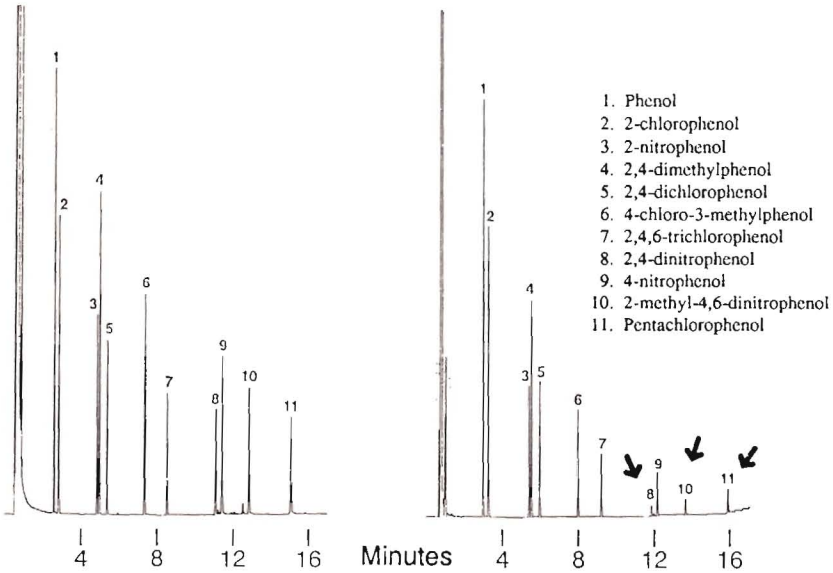
A guard column should be long enough to keep non-volatile residue from entering the column, but short enough so that the analysis time is not significantly increased. Five meter guard columns are more cost effective, reduce the frustrations of making the connection between the guard column and analytical column, and are preferred by most analysts over 1-meter guard columns. If a very long guard column (>10-meters) is used, the residence time of the sample components increases, resulting in longer analysis times.

Guard columns help prolong the life expectancy of capillary columns and are an excellent and economical alternative to column replacement. Analysts working with dirty samples find that the use of guard columns significantly reduces column replacement costs and time lost in troubleshooting column contamination problems. ■

Figure 3

Phenols show excellent response on an Rt_x-5 with a newly installed guard column.

Reduced response factors show guard column contamination.

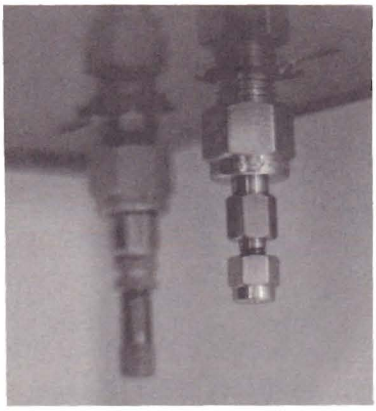


15m, 0.32mm ID, 1.0µm Rtx-5 (cat.# 10251)
0.2µl split injection of phenols.

Oven temp.: 80°C to 290°C @ 8°C/min.
Inj. & det. temp.: 310°C

Carrier gas: hydrogen
Linear velocity: 45cm/sec.
FID sensitivity: 8×10^{-11} AFS
Split ratio: 9:1

HP FID/NPD Detector Adaptor Fitting



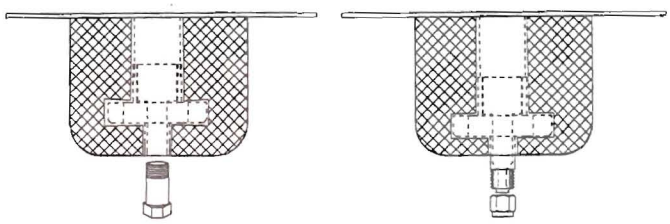
After wrestling with HP's detector adaptor fitting, Restek's chemists decided to engineer an improved version. First we replaced the awkward column fitting with a 1/16" Swagelok® type nut that uses standard graphite or Vespel®/graphite ferrules. Then we shortened the fitting to make it more compact and improved the wrench pad to prevent it from turning when installing a capillary column. The end result? An easy-to-use, sturdy stainless steel fitting that makes capillary installation easy. All the parts needed for installation (1/4"SS nut, 1/4" Vespel ferrule, 1/16"SS nut, and 0.4mm ID graphite ferrule) are included. Replaces HP Part numbers 19244-80610 and 5921-21170.

cat.# 20884, $55 per kit

HP Capillary Inlet Adaptor Fitting

HP fitting is hard to see when inserting capillary column

Restek's fitting makes column insertion easy



HP 5890 capillary inlet fittings are frustrating because it is difficult to see where the column is inserted. Restek's chemists engineered a simplified HP 5890 capillary inlet fitting that uses 1/16" Swagelok®-type nuts and standard graphite or Vespel®/graphite ferrules. We carefully machined the threads and matched the stainless steel types to eliminate seizing onto the injector body. We also paid careful attention to the fitting depth to keep inlet insertion distances exactly the same and designed it to use the same type of inlet disc. The end result? An easy-to-use, sturdy stainless steel fitting that makes capillary column installation easy. All the parts needed for installation (the inlet disc, washer, 1/16" SS nut, and 0.4mm ID graphite ferrule) are included. Replaces HP part numbers 18740-20800, 05921-21170, and 18740-20880.

cat.# 20633, $60 per kit

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- Produces square, even cuts
- Eliminates distortion of the tubing
- Replaceable cutting wheel

This lightweight tool is designed to cut 1/16" tubing squarely, is easy to operate, and prevents distortion.

estek 1/16" Tubing Cutter: cat.# 20192, $75 each
Replacement Wheels: cat.# 20185, $10/3-pack

Standard Capillary Ferrules

Restek has graphite and 60% Vespel®/40% graphite ferrules to use with capillary columns. The graphite ferrules are made from highly-compressed ribbon that will not crack or split under torque. The 60% Vespel/40% graphite ferrules are designed to seal easily with minimal torque and are re-useable. Both are stable to 400°C.

Ferrule ID	Fits Column ID	Graphite			Vespel/graphite		
		cat.#	price	quan.	cat.#	price	quan.
0.4mm	0.25	20200	$25	10-pk	20211	$30	10-pk
0.5mm	0.32	20201	$25	10-pk	20212	$30	10-pk
0.8mm	0.53	20202	$25	10-pk	20213	$30	10-pk
0.4mm	0.25	20227	$100	50-pk	20229	$120	50-pk
0.5mm	0.32	---	---	---	20231	$120	50-pk
0.8mm	0.53	20224	$100	50-pk	20230	$120	50-pk

News from Restek

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Don't miss the Restek Wizards at Pittsburgh Conference 1992 in New Orleans. We will be presenting nine technical papers and four posters at this year's show (please check insert for a complete listing of times and topics).

We are anticipating a terrific conference and are looking forward to seeing everyone there. Please visit our booth (#2612) and bring along the coupon insert to receive a free wizard fanny pack. Eighteen of our technical support chemists will be there to serve your chromatographic needs.

Starting Mid-February,

Restek will be open on Saturdays (10am-2pm EST) for orders, shipping (Federal Express only), and technical service.

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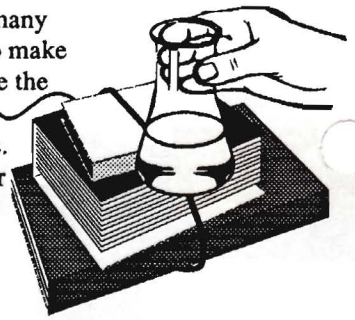
With shrinking budgets and research funds drying up, many colleges and universities have found it more difficult to make ends meet. This often means not being able to purchase the equipment necessary to educate the chromatographers of tomorrow or to complete important research projects. Restek is working to stretch your budgets a little further with the development of a new Educational Discount Program. This program is available to any accredited college or university. For more information contact Restek's Marketing Department at 800-356-1688.



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