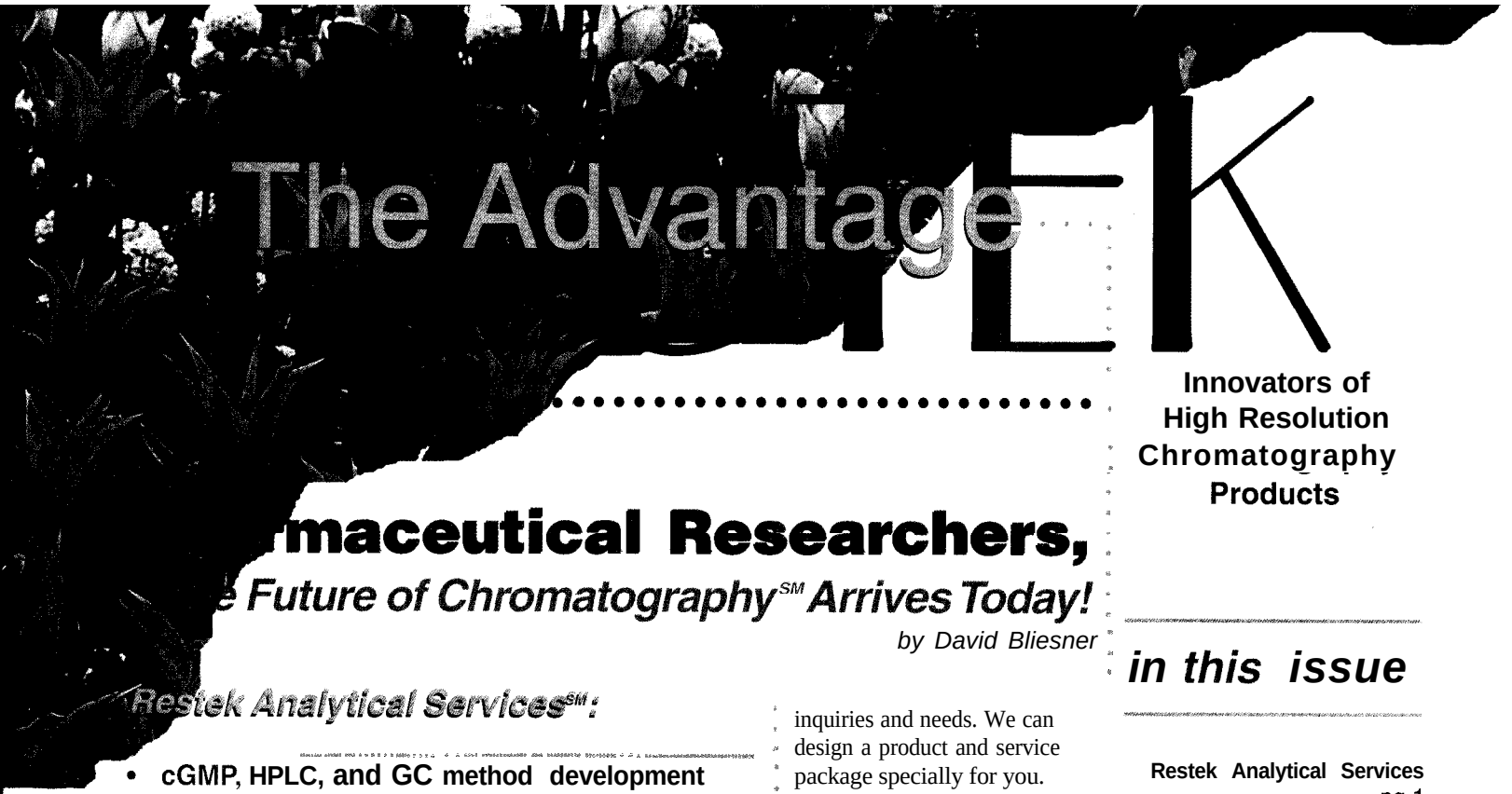

The Advantage RTK

Innovators of High Resolution Chromatography Products

rmaceutical Researchers, e Future of Chromatography℠ Arrives Today!

by David Bliesner

Restek Analytical Services℠:

- **cGMP, HPLC, and GC method development and validation**
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- **Full lines of HPLC and GC columns and accessories**
- **Innovative hardware and accessory designs**
- **Unsurpassed HPLC and GC technical support**
- **HPLC and GC educational services**

Restek Corporation is proud to announce the grand opening of Restek Analytical Services℠ (RAS). RAS is a unique and innovative, current Good Manufacturing Practices (cGMP)-compliant chromatography laboratory, designed to deliver quality products and services for the pharmaceutical market. No other column manufacturer can provide such complete services to support their products. Along with a full line of both HPLC and GC columns, RAS offers analytical method development and validation, HPLC/GC education and training, custom stationary phase design, and cGMP/regulatory services and support.

RAS has assembled seasoned researchers and analysts who have extensive experience in cGMP method development and validation. These experts are a perfect complement to our existing manufacturing staff. As a group, we understand the pressures and time constraints associated with pharmaceutical research and have practical experience analyzing many of the common drugs currently under development. The entire RAS staff is dedicated to helping you achieve your goals in a timely and compliant fashion.

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For further information, please request the RAS fulfillment folder (cat.# 59630). This folder includes details on quality assurance, research and development, analytical development and validation, laboratory and regulatory service, and HPLC columns and accessories.

Or, feel free to contact the director of the RAS laboratory, Dr. David Bliesner, to discuss your specific applications. He can be reached at 800-356-1688 or 814-353-1300, ext. 2193. Call us today and find out why RAS is truly the ***future of chromatography***.

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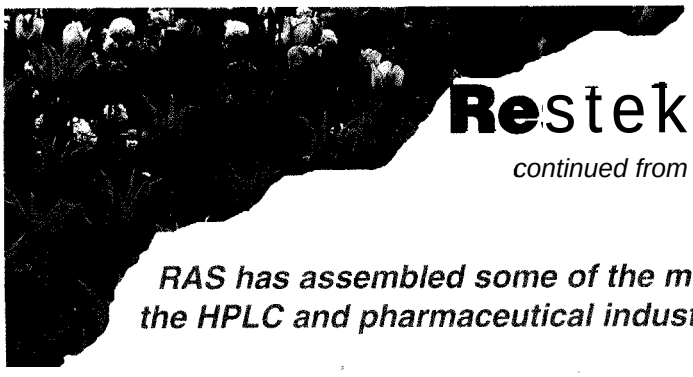

Restek Analytical Services℠

continued from page 1.

RAS has assembled some of the most talented, customer-oriented analysts in the HPLC and pharmaceutical industries. Meet the leaders of RAS:

Dr. David M. Bliesner, RAS Director

Expertise:
- HPLC method development and validation in a cGMP environment
- Qualification and operation of cGMP analytical laboratories
- Thin layer chromatography
- cGMP auditing and data review
- 15 years combined R&D and leadership experience

dbliesner@restekcorp.com

David S. Bell, RAS Senior Researcher

Expertise:
- HPLC method development and validation in a cGMP environment
- Thin layer chromatography
- 8 years of experience

dbell@restekcorp.com

Keith J. Duff, RAS R&D Group Leader

Expertise:
- HPLC bonded phase synthesis
- Synthetic chemistry
- HPLC method development
- 16 years of experience

keithd@restekcorp.com

Larry T. Peters, RAS Senior HPLC Technician

Expertise:
- HPLC column packing
- HPLC column bonding
- 16 years of experience

larryp@restekcorp.com

Randy L. Romesberg, RAS Production Group Leader

Expertise:
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"In the modern chromatography laboratory, you want one thing — reliable solutions to your chromatographic problems. Previously, when a method could not be reproduced, questions such as, 'Is it the column or did something change in the sample workup?' could not be answered by one source. By combining product development and method development under one umbrella, you can be assured that your problem will be solved with full accountability.

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David M. Bliesner, Ph.D.
Director, RAS℠

Restek Analytical Services-

The Advantage ... TEK

Innovators of High Resolution Chromatography Products

Restek's Silcosteel® HPLC Columns

...e strength of steel and inertness of glass

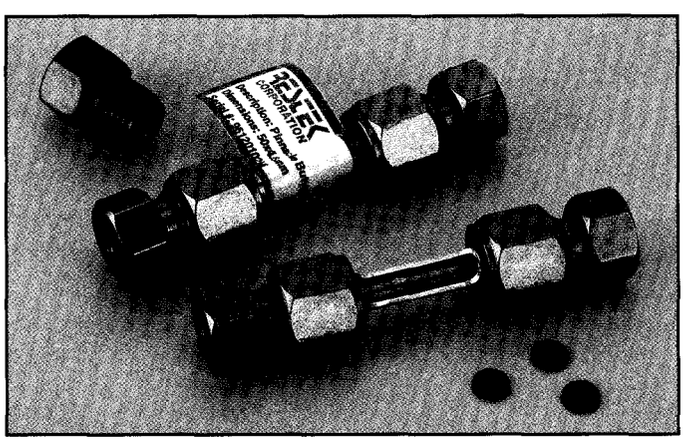
by Matt Piserchio

Combine the ruggedness and pressure limits of stainless steel with the inertness of PEEK or GLT™.

- **Provide a shield between the metal surface and active species.**
- **Applicable to all column dimensions.**

When analyzing active components, GC and HPLC sample pathways must be inert to prevent adsorption and poor chromatography. Unfortunately, GC sample pathways are constructed from metal to achieve and maintain the high temperatures needed for sample vaporization. In HPLC, columns and sample pathways require materials that can withstand high pressures. Contact of an active analyte with metal surfaces can result in adsorption, sample degradation, and inaccurate quantitation.

Silcosteel® is a proprietary process developed by Restek that applies a thin layer of material to the surface of metals. This coating acts as a passivation layer by providing a shield between the metal surface and the active species, eliminating detrimental interactions.



Silcosteel® GC columns and accessories have been proven effective by a significant number of satisfied customers. We have coated FID jets, FPD jets, entire injection ports, inlet seals, metal injection port liners, head space needles, nickel reaction tubes, and air sampling canisters. MXT® columns, rugged and inert metal GC capillary columns treated with Silcosteel®, are the column of choice in the process analyzer and portable GC markets. Now Silcosteel® technology is available for a full line of normal phase, reverse phase, and ion exchange HPLC columns.

Metal-free pathways are desired to reduce irreversible adsorption during HPLC analyses of proteins, peptides, and any other compounds that can undergo metal complexation.[1,2] Currently, columns constructed of PEEK (polyetheretherketone) or GLT™ (glass-lined stainless steel tubing) are utilized for such analyses. Although both materials are effective, each of these materials has definite limitations.

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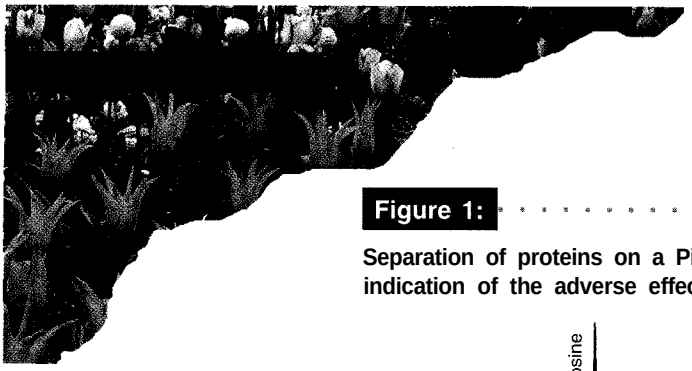


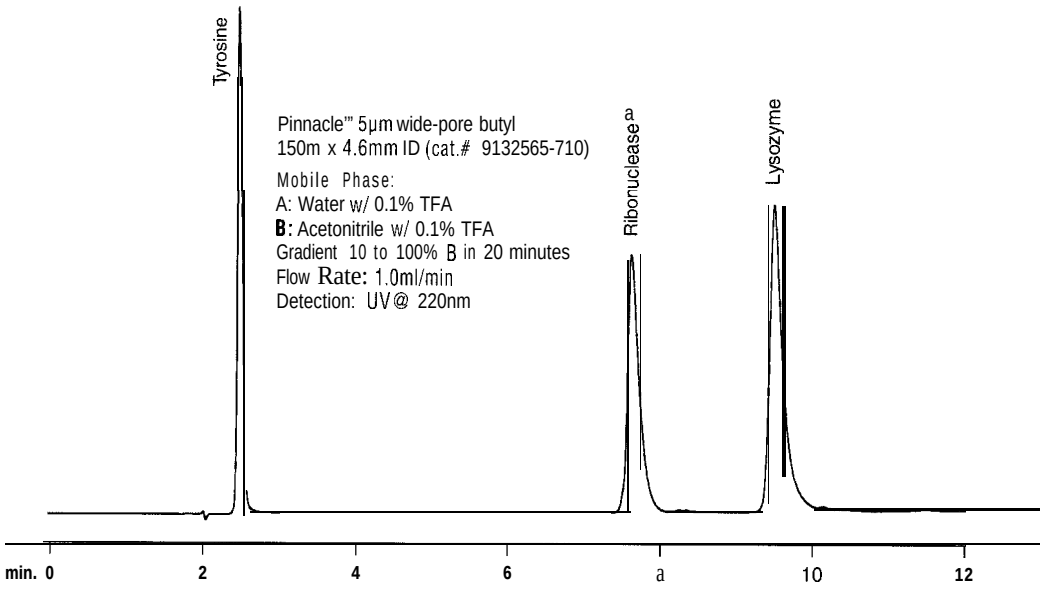
PEEK has a limited pressure threshold and can contain chemical impurities. GLT™ can crack, especially at the column inlet or outlet, causing a disturbance in the flow pattern resulting in peak distortion and adsorption. Finally, both PEEK and GLT'" are available in limited configurations. Silcosteel® combines the ruggedness, thermal stability, and pressure limits of stainless steel with the inertness of GLT" or PEEK. Silcosteel® can also be applied to any size HPLC column and virtually any other metal surface in the HPLC sample pathway.

The separation of proteins in **Figure 1** illustrates the inertness of the Silcosteel® layer. Ribonuclease A and Lysozyme elute with excellent symmetry using a standard gradient profile. Sharp, symmetrical peaks indicate the absence of the catalytic effect of metal surfaces that may cause protein adsorption. This will result in improved resolution and recovery when analyzing active species.

If your analyses demand a metal-free pathway, Restek has the Silcosteel®-treated HPLC column that is right for you. Our complete line of Pinnacle'" HPLC columns is now available in Silcosteel® hardware. ***Simply add a "-710" to your part number to add the benefits of Silcosteel® to your HPLC analysis.***

Figure 1:

Separation of proteins on a Pinnacle'" wide-pore butyl in Silcosteel® hardware shows no indication of the adverse effects of metal on protein adsorption.



For part numbers and prices, please call

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Add "-710" to your part number and get the benefits of Silcosteel® with your HPLC analysis!

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Pinnacle Octyl
Pinnacle Phenyl
Pinnacle Butyl
Pinnacle Methyl
Pinnacle Ultra C 18
Kromasil C 18
Kromasil C8
Kromasil C4
Nucleosil C 18
Nucleosil C8

Base Deactivated

Pinnacle ODS Amine
Pinnacle Octyl Amine
Pinnacle Phenyl Amine
Pinnacle Cyano Amine

Normal Phase

Pinnacle Silica
Pinnacle Amino
Pinnacle Cyan0
Nucleosil Cyano

ion Exchange

Pinnacle SAX
Nucleosil SCX

Specialty

Pinnacle TO-l 1
Pinnacle PAH
Pinnacle EcoSep
Pinnacle Wide-Pore Butyl

The FiestaTek

Innovators of High Resolution Chromatography Products

Fast GC Using Microbore Capillary Columns

by Kristi Sellers

Reducing instrument & operator time for gas chromatographic analyses has become an important consideration for many laboratories.

The use of microbore (O.lOmm ID) columns can significantly reduce analysis time without sacrificing resolution. The extremely high efficiency of microbore columns (-7000 plates/meter) can provide resolution of complex mixtures while using shorter lengths. Shorter columns are less expensive and reduce analysis times, resulting in a cost savings for the lab.

Some instrument companies have been promoting the benefits of fast screening columns, but the sacrifices required aren't always evident from their literature. The reduction of analysis time at the expense of resolution, sample capacity, and ease of use is not always an acceptable alternative. This article will discuss and demonstrate the benefits and limitations of O.lOmm ID columns.

Speed and Resolution

Table I compares the characteristics of microbore columns to conventional columns. This data holds the key to whether microbore columns are right for your analysis. The most striking difference of microbore columns is their high efficiency (plates/meter) compared to other diameters. **Table I** indicates that a O.lOmm ID column is 160% more efficient than a 0.25mm ID column. This high efficiency allows shorter columns to maintain excellent resolution and increase the speed of analysis. However, some of the other parameters in **Table I** illustrate limitations that may negate the usefulness of microbore columns in your laboratory. The effect of low flow rates, low sample capacity, and high operating pressures on your sample requirements will ultimately determine if microbore columns are an improvement for your laboratory.

Flow **Rates**

The low flow rates for microbore columns can be either an advantage or a limitation. Low flow rates are beneficial for GUMS users because the flow rates are well within the pumping capacity of most systems. In addition, the microbore prevents "pumping out the column" or operation below atmospheric pressure. This provides more efficiency

Table I:

Column Characteristics

Column ID	0.10mm	0.18mm	0.25mm	0.32mm	0.53mm
Theoretical plates/m	8,600	5,300	3,300	2,700	1,600
Effective plates/m	6,700	3,900	2,500	2,100	1,200
He flow @ 20cm/sec	0.1cc/min.	0.3cc/min.	0.7cc/min.	1.0cc/min.	2.6cc/min.
H_2 flow @ 40cm/sec	0.2cc/min.	0.6cc/min.	1.4cc/min.	2.0cc/min.	5.2cc/min.
Sample Capacity	5-10ng	IO-20ng	50-100ng	400-500ng	1000-2000ng
Operating Pressures	40.0psig	21.0psig	12.5psig	7.5psig	3.0psig

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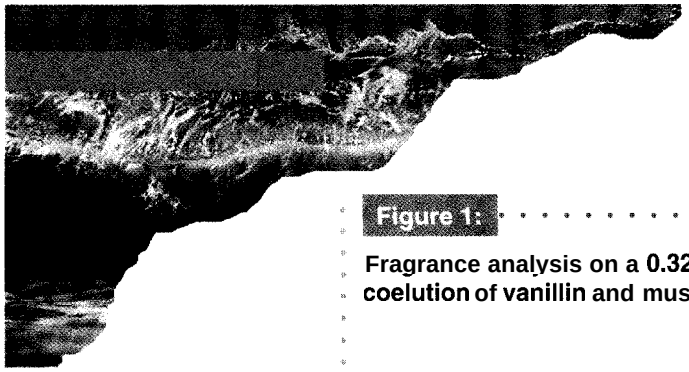


for the end user. However, low flow rates also translate into more flow path problems for the chromatographer. Unswept dead volume has disastrous consequences on the column performance.

Operating Pressures

Table I also shows that microbore columns require higher operating pressures which results in more ferrule leaks, septum leaks, and sample blow back through leaking syringe plungers. Connections need to be monitored for leaks more often. The pneumatic systems for older GCs are designed to operate at only 30psig and may need to be modified to handle higher pressures required for narrow bores. Operating microbore columns below optimum pressures will translate into poor resolution and poor performance.

Sample Capacity

A limiting factor of a microbore column is the amount of sample that can be injected onto the column. Table I indicates that the sample capacity of a microbore column is ten times less than a 0.25mm ID column. Therefore, the on-column injection should be at least ten times lower for a microbore column.

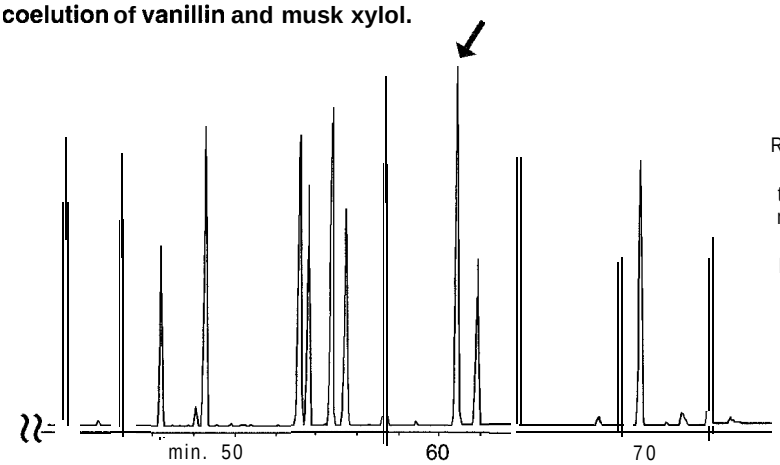
Sample cleanliness is another important factor to take into consideration when using microbore columns. Because the surface area of the O.lOmm ID columns is much lower than a conventional column, contamination will occur more rapidly when dirty samples are injected. This means that 0.25 or 0.32 mm ID columns will be more rugged and require less maintenance for dirty samples than microbore columns. Whenever possible, samples containing non-volatile residue should be avoided. If dirty samples are a must, extensive column and injection port maintenance is required. Otherwise, loss of resolution, ghost peaks, and a high background signal will result.

Injector Considerations

Direct and on-column injection modes are not recommended due to the required low flow rates and small bore size of these columns. Therefore, trace analyses are difficult to perform with microbore columns. Split and splitless injections are the best alternatives. However, since microbore columns require low flow rates, speed of sample transfer through the liner to the column is a concern. Due to the high dead volume, poor peak shape, and response, loss of resolution will occur when 2 or 4mm ID liners are used in conjunction with microbore columns. Thus, lmm ID inlet liners are a must for sharp, well resolved, and recovered peaks. Not only is the inlet liner a consideration when

Figure 1:

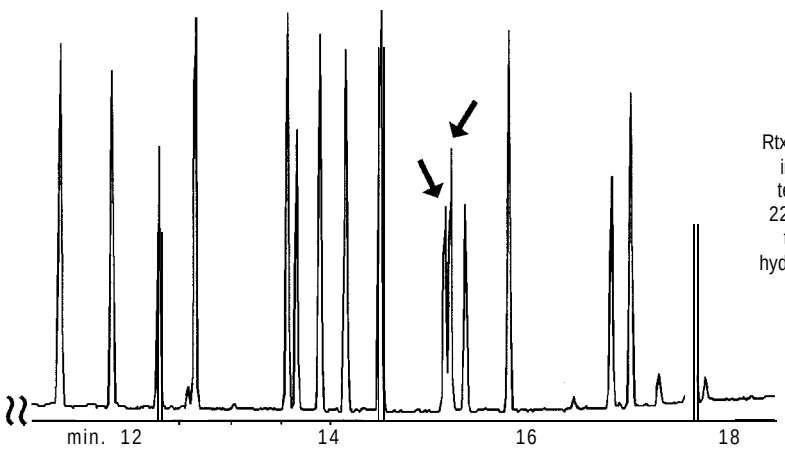
Fragrance analysis on a 0.32mm ID Rtx®-Wax column takes 75 minutes with complete coelution of vanillin and musk xylol.



30m, 0.32mm ID, 0.25µm Rtx-WAX (cat.# 12424). Splitless injection, 50mls/min. Oven temp.: 75°C to 225% @ 70°C/ min. (hold 15 min.). **Inj.&** det. temp.: 225°C. Carrier gas: helium, 30cm/sec. set @ 75%.

Figure 2:

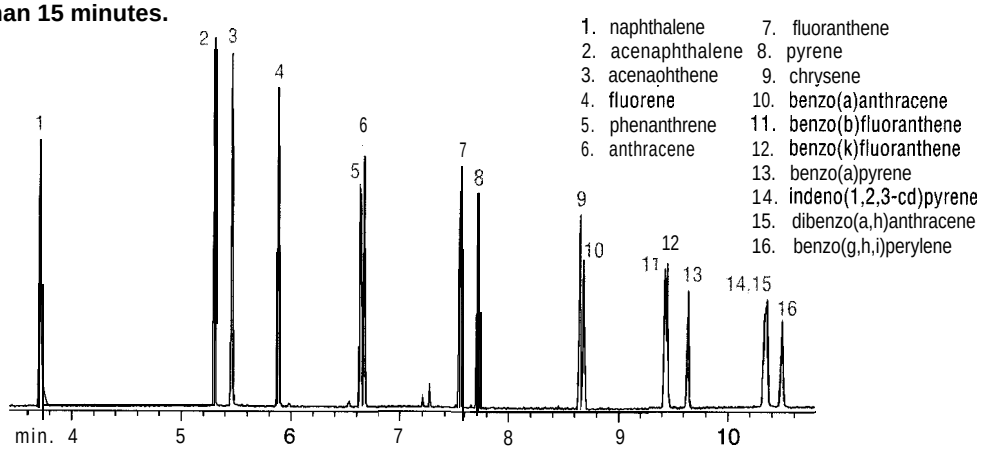
Fragrance analysis on the Rtx®-Wax microbore column reduces run times by 75% with increased resolution between vanillin and musk xylol.



10m, O.lOmm ID, 0.20µm Rtx-WAX (cat.# 41603). Splitless injection, 250mls/min. **Oven** temp.: 55°C (hold 1 min.) to 225°C @10°C/min. **Inj.&** det. temp.: 225°C. Carrier gas: hydrogen, 7Ocmlsec. set @ 55°C.

Figure 3:

Polynuclear aromatic hydrocarbons on an Rtx®-5 microbore column are analyzed in less than 15 minutes.



10m, 0.10mm ID, 0.10µm Rtx-5 (cat.# 41201). 0.5µL splitless injection. 41psi initial pressure, hold 2 min. 8 psi/min. to 99psi (hold 1.87 min.). 275°C, vent open @ 1 min. **Oven temp.:** 40°C (hold 0.5 min.) to 90°C @ 70°C/min. then to 100°C @ 5°C/min then to 310°C @ 30°C/min. (hold 2 min.).

using microbore columns for split or splitless injections, but other parameters specific to the type of injection method must also be optimized.

In a split injection, the choice of inlet liner and initial temperature will affect peak shape, response, and resolution the most. **Figure 1** shows part of a typical fragrance analysis on a conventional column (0.32mm ID). Under optimal conditions (4mm ID inlet liner and initial temperature of 75°C), the analysis time is more than 70 minutes and the separation of vanillin and musk xylol could not be achieved. By switching to a microbore column and optimizing run conditions (lmm ID inlet liner and initial temperature of 55°C), we were able to reduce the analysis time to 18 minutes and attain 80% resolution of the vanillin and musk xylol as shown in **Figure 2.** The 1 mm ID inlet liner improved the recovery and peak shape of the early eluting compounds.

Figure 3 illustrates a splitless PAH analysis on a 10m, O.lOmm ID, 0.10µm Rtx®-5 using an optimized inlet liner and inlet pressure. When a 2mm ID inlet liner was used, high molecular weight discrimination occurred. By changing to a lmm ID inlet liner, high molecular weight discrimination was eliminated. However, this change caused peak splitting of the early eluting compounds. The peak splitting was eliminated completely when pressure programming was applied in place of constant pressure.

Detector Considerations

Detector design and flows must be optimized when using microbore columns. Make up gas flows may need to be increased to minimize detector dead volume and compensate for the lower column flow rates. Since peak widths are approximately half compared to conventional columns (< 1 second), fast integrator and detector electrometers must be used. Integrator sampling rates must be increased over rates used for 0.25mm ID columns since the peaks are much narrower with microbores. If the sampling rate is too slow, then poor integration and non-reproducible peak areas will result. Check with your instrument company and data system manufacturer to be sure your system is capable of handling microbore sampling rates.

Microbore columns can produce shorter analysis times, equivalent resolution, and provide cost savings. But remember, converting your conventional system to a microbore system isn't as easy as changing columns. Column capacity, sample purity, and injector and detector conditions must be considered and optimized for a successful analysis. Keep in mind that when switching from conventional capillaries to microbore columns, there may be the need to optimize inlet temperatures, liners, and GC run conditions.

Product Listing:

Microbore Capillary Columns			
0.10mm ID, 0.10µm			
Column	**temp. limits**	**lo-meter**	**20-meter**
Rtx®-1	-60 to 330/350°C	41101	41102
Rtx®-5	-60 to 330/350°C	41201	41202
Rtx®-Wax	20 to 250°C	41601	41602
0.10mm ID, 0.20µm			
Column	**temp. limits**	**lo-meter**	**20-meter**
Rtx®-Wax	20 to 2401250°C	41603	41604
0.10mm ID, 0.40µm			
Column	**temp. limits**	**10-meter**	**20-meter**
Rtx®-1	-60 to 320/340°C	41103	41104
Rtx®-5	-60 to 320/340°C	41203	41204

Contact Restek's GC experts to discuss the suitability of Microbore or other GC columns for your specific application.

The Advantage

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High Resolution
Chromatography
Products

Restek's Silcosteel® HPLC Columns

...e strength of steel and inertness of glass

by Matt Piserchio

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- **Provide a shield between the metal surface and active species.**
- **Applicable to all column dimensions.**

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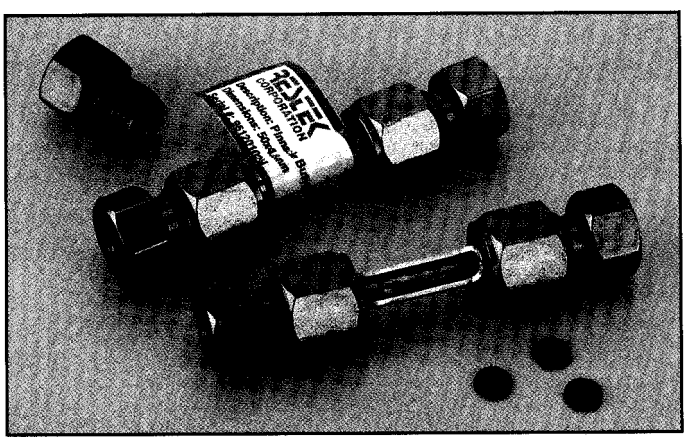

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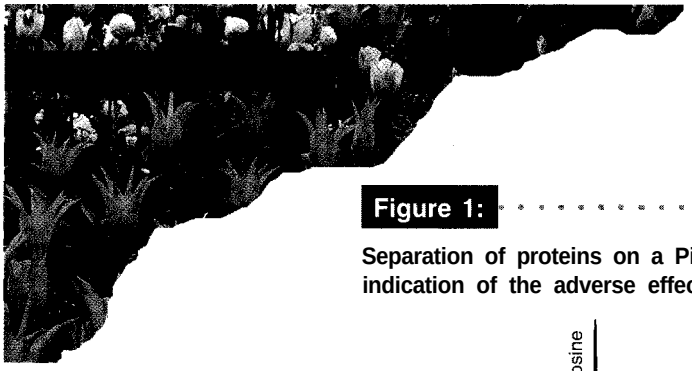

Figure 1:

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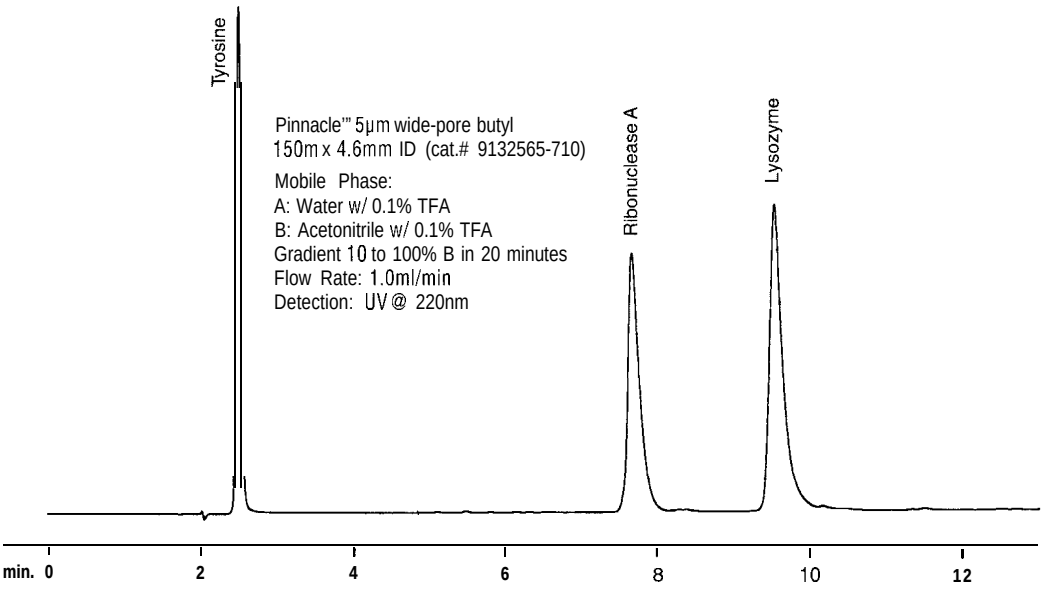

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Pinnacle ODS
Pinnacle Octyl
Pinnacle Phenyl
Pinnacle Butyl
Pinnacle Methyl
Pinnacle Ultra Cl8
Kromasil C 18
Kromasil C8
Kromasil C4
Nucleosil C 18
Nucleosil C8

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