

# the Reporter

EUROPE

Volume 20, March 2006 International, Issue

## Polar Embedded Group HPLC Phase Bioanalytics

**SUPELCO**

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Selectivity of Peptides on Amide Embedded Polar Group Phases Versus Alkyl Phases

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NEW Metal Fiber Assemblies for SPME Provide Higher Mechanical Stability

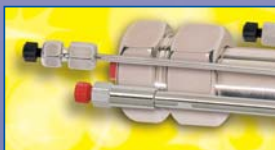
### GC

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**SIGMA-ALDRICH**

## EDITORIAL

## Reporter Forensic Editorial

Dear Reader,

### Is C18 always the best choice for HPLC separations?

Although by far the most frequently used phase, C18 may not always be the best choice. Because of its familiarity, it is often the first phase analysts go to when developing a new HPLC method. However, some separations may be faster and/or have better resolution on non-C18 phases that have different selectivity. In this Issue Number 20 of The Reporter, we discuss two Supelco HPLC phases that are viable alternatives to C18 for commonly encountered analytes.

The HPLC article that begins on page three presents the recently introduced embedded polar group (EPG) phase Ascentis™ RP-Amide. The alkyl-amide functionality of Ascentis RP-Amide gives it orthogonal selectivity to a C18. But unlike other EPG and amide-based HPLC phases, it is unique in its ultra-low bleed character and LC/MS stability. The authors compare retention of a variety of small molecule analytes on Ascentis RP-Amide and C18 phases and discuss some possible mechanisms behind the selectivity differences, seen primarily in H-bond donors.

Biomolecules also benefit from the selectivity differences of EPG phases, as the next HPLC article on page six describes. The authors show how merely substituting the single hydrogen of phenylalanine with the hydroxyl group of tyrosine results in enhanced retention of the corresponding peptide on the Ascentis RP-Amide column; consistent with the results from small molecule experiments.

EPG phases are alternatives to C18, but so are phases that simply have different alkyl chain length, especially when dealing with peptide and protein analytes. The HPLC article on page 6 reports subtle yet significant differences in elution pattern of tryptic peptides on Discovery® BIO Wide Pore C5, C8 and C18. Researchers who use peptide mass fingerprinting by LC/MS for protein identification can leverage those differences and choose best the chain length for their particular application.

### So, is C18 always the best choice for HPLC separations?

The answer is No. Although C18 is a workhorse phase, modern EPG phases and shorter chain length alkyl phases are viable alternatives for many separations. In fact, when working with polar analytes, and especially H-bond donors like hydroxyls or amines, consider using EPG phases, like Ascentis RP-Amide, as a first choice.

Best wishes for a successful analysis!

Sincerely,



Klaus Herick, Ph.D.  
Sales Development Manager HPLC Europe



## MEET SUPELCO - ANALYTICA 2006

25-28.April 2006, Munich

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## HPLC ARTICLE

# Selectivity of Peptides on Amide Embedded Polar Group Phases Versus Alkyl Phases

Hillel Brandes hbrandes@sial.com and Patrick Myers pmyers@sial.com

### Abstract

It has been known that small molecule analytes with hydrogen bond donor moieties display enhanced retention on embedded-polar group (EPG) phases (1, 2, 3). However, no such systematic study has been done with peptide analytes, until this report. Synthetic peptide sets, in which phenylalanyl residues were substituted with tyrosyl residues, were used to inspect relative differences in retention as a function of the degree of substitution. Indeed, substitution with the phenolic hydroxyl of tyrosine (versus a single hydrogen of phenylalanine) does confer enhanced retention of the peptide on the EPG amide phase. This is consistent with results using small molecules.

### Background

Ever since EPG phases were first reported (4), they have remained popular for chromatographing polar analytes. While these phases do retain compounds based on hydrophobicity, polar interactions can significantly contribute to retention as well. Deciphering the molecular interactions that confer particular retention on EPG phases has been of considerable interest. Various studies with small molecule analytes have indicated that amide EPG phases display particular retention for compounds that may function as hydrogen bond donors (1, 2, 3). While we have previously noted different selectivities of peptides on alkyl versus EPG phases, no systematic study has previously been performed to elucidate what molecular interactions may be responsible for the altered retention. In this article we report the first such investigation by inspecting the relative differences in retention of two sets of peptides, in which phenylalanyl residues of one set are substituted with tyrosyl residues in the other set. One of the more clear cases with small molecules, which show enhanced retention on amide phases, are phenols. Therefore, this comparison of phenylalanyl and tyrosyl residues was a logical first case to inspect possible molecular interactions that contribute to alternate selectivity of peptides on EPG phases.

### Methods and Results

Peptides obtained from Sigma-Genosys; chromatographic conditions indicated with figures.

Peptide set I (in order of elution):

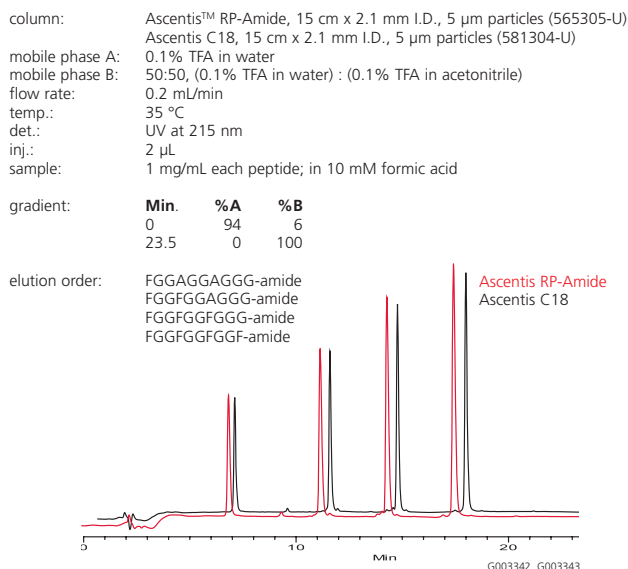
1. FGGAGGAGGG-amide
2. FGGFGGAGGG-amide
3. FGGFGGFGGG-amide
4. FGGFGGFGGF-amide

Peptide set II (in order of elution):

1. YGGAGGAGGG-amide
2. YGGYGGAGGG-amide
3. YGGYGGYGGG-amide
4. YGGYGGYGGY-amide

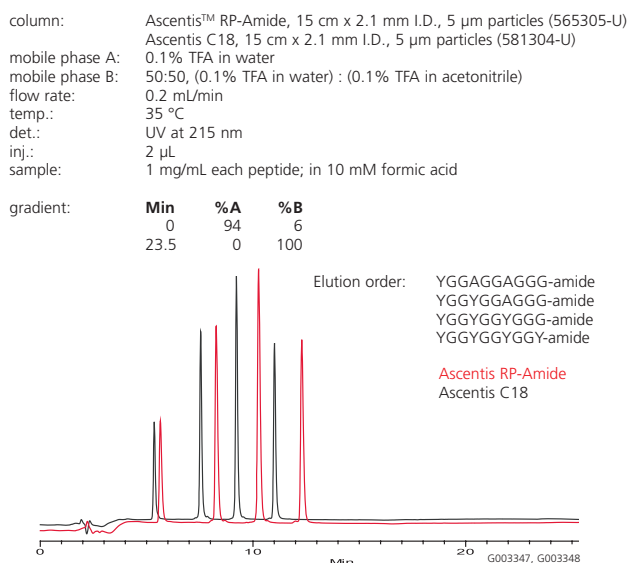
Figure 1 shows the retention of the peptide set I set on Ascentis C18 and Ascentis RP-Amide. For this peptide set, retention is conferred primarily by hydrophobicity. Thus the lower hydrophobic retention of the amide phase (as compared to C18) is demonstrated by the lower retention of the peptides. Figure 2 shows the retention of peptide set II on the two phases. Absolute retention differences of each peptide set on the two phases are not pertinent to this study but arise from the larger

**Figure 1.** Phenylalanyl Peptides (Peptide Set I) on Alkyl and Polar-Embedded Phases



hydrophobic retention index of phenylalanine versus tyrosine (5, 6). The clear difference in comparing the retention of each peptide set on the two phases, is that in the case of peptide set II, enhanced retention of the tyrosyl-substituted peptides occurs in an incremental manner with each additional substitution. That is, the difference in retention of each peptide (of set II) on the amide versus C18 increases as the number of tyrosyl residues increases. Thus, the phenolic hydroxyl of the tyrosyl side chain is conferring enhanced retention in a manner consistent with what has been previously observed with small molecules. If retention on the amide phase were strictly hydrophobic, the comparative elution patterns versus C18 for peptide set II would look like that of peptide set I (less retention of all peptides of set II on the amide phase versus the C18 phase).

**Figure 2.** Tyrosyl Peptides (Peptide Set II) on Alkyl and Polar-Embedded Phases



## Conclusions

We have shown conclusively for the first time, that hydrogen-bonding donor moieties of peptides confer enhanced retention on amide EPG phases in a manner consistent with what is observed with small molecules. This is likely to occur with other EPG phases that contain a carbonyl group.

We intend to expand this study with other possible hydrogenbond donors and acceptors of peptide side chains to further investigate which peptide functional groups may contribute to altered selectivity on EPG phases versus alkyl phases.

## References

1. Supelco poster. Separation Problem Solving Through Selectivity -Comparison of a New Stable Polar Embedded Phase with a New ODS Phase; T404130
2. Supelco poster. Designed HPLC Selectivity Enhancement; T405015
3. Supelco poster. Retention Mechanisms In Reversed-Phase Liquid Chromatography: Embedded Polar Group Stationary Phases. T405086
4. Ascah, T.L. & Feibush, B. 1990. J Chrom 506: 357-369
5. Guo *et al.* 1986. J Chrom. 359: 499-517
6. Sakamoto Y. *et al.* 1988. J Chrom 442: 69-79

## Did you know...?

Ascentis is the fourth generation of deactivated HPLC columns developed by the R&D team at Supelco. Ascentis is available in C18, C8 and the embedded-polar group RP-Amide phases.

## Ordering information

Prod. No.	Description	Dimensions
565305-U	Ascentis RP-Amide	15cm x 2.1mm, 5µm
581304-U	Ascentis C18	15cm x 2.1mm, 5µm

**i** Information Request .....2001, 2002

## HPLC ARTICLE

# Ascentis™ RP-Amide: A Universal, Ultra Low-Bleed Embedded Polar Group HPLC Phase

## Introduction

Although C18 is a workhorse phase and can achieve impressive separations, non-C18 phases can offer improvements in selectivity, retention and efficiency or peak symmetry, especially of polar compounds. However, stationary phase bleed can cause significant interference, which is often more pronounced when using non-C18 phases under LC/MS conditions. In this short communication we discuss the Ascentis RP-Amide, which has enhanced selectivity and polar retention compared to a C18, but does not exhibit phase bleed that can interfere with or reduce the MS signal.

## Polar-embedded phases in RP-HPLC

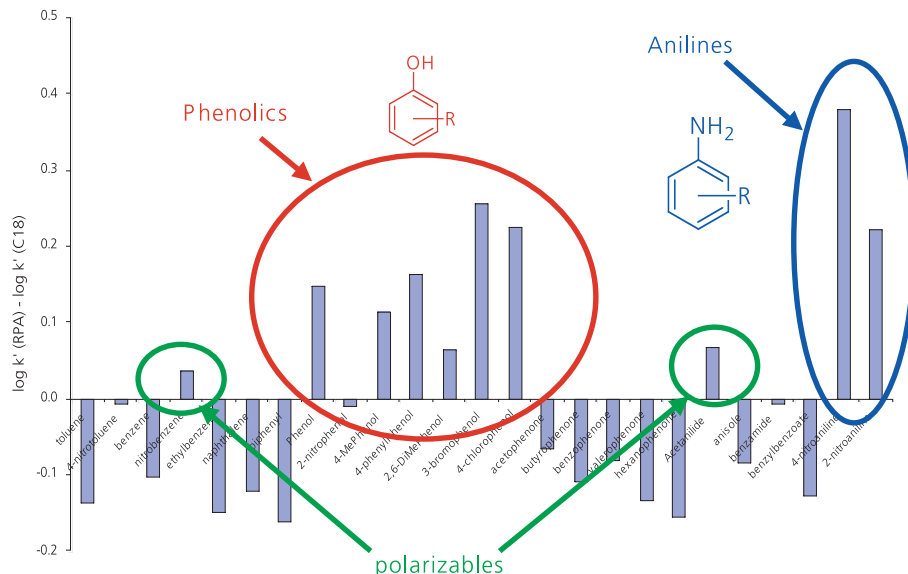
A group of stationary phases that is becoming a popular alternative to C18 is the so-called "embedded polar group (EPG)" phases. These stationary phases generally comprise a

hydrophobic region, which provides the reversed-phase retention, and a polar region or polar end-capping agent, which alters the selectivity of the phase. The precise description of the retention mechanism behind these phases and how they differ from C18 is complex and beyond the scope of this report. However, several recent publications have dealt with this subject in great detail (1-4).

## Benefits of polar-embedded phases over C18: polar retention, selectivity and aqueous stability

An example of the retention and selectivity differences between C18 and a polar-embedded alkyl-amide phase is shown in Figure 1. Under identical mobile phase conditions, the log  $k'$  of a series of low molecular weight compounds was measured on both Ascentis C18 and Ascentis RP-Amide columns. Besides

**Figure 1.** Depiction of selectivity difference for neutral, polar analytes between Ascentis RP-Amide and Ascentis C18 columns [ $\log k'(\text{RPA}) - \log k'(\text{C18})$ ]



the obvious difference in selectivity, the polar and polarisable compounds had longer retention on the Ascentis RP-Amide. Polar compounds also require highly aqueous mobile phases where C18 phases are not solvated. EPG phases, like the Ascentis RP-Amide, are highly solvated under aqueous conditions and do not exhibit retention anomalies and instability. An example of this benefit for the analysis of the polar compound glutathione was described in a previous Reporter article (5).

### LC/MS stability and column bleed

Column bleed can originate from elution of residual, un-bonded phase, acid hydrolysis of the bonded phase or dissolution of the silica substrate under basic conditions. EPG phases have been notorious in their tendency to exhibit LC/MS bleed. Although C18 phases also bleed, the C18 fragmentation patterns are generally simpler and do not interfere with positive ion mode MS as does bleed from nitrogenous polar-embedded phases.

### Ascentis RP-Amide overcomes bleed associated with polar-embedded phases

Common nitrogen-containing EPG stationary phases contain carbamate, urea or amide functional groups. Ascentis RP-Amide (Figure 2) is prepared using a proprietary silane bonded at high surface coverage and end-capped (6, 7). A comparison of the LC/MS bleed profile of Ascentis RP-Amide was made to other commercially-available nitrogen-containing polar-embedded phases. Mass range and elution time range were set to capture the majority of bleed ions. Two conditions in this study set the stage for bleed: low pH mobile phase can induce hydrolysis of the bonded phase and gradient elution will mobilise and elute any liberated phase material. Conditions and results appear in Figure 3.

The Ascentis RP-Amide (TIC "B" in Figure 3) exhibited the fewest number and lowest intensity of mass responses from column bleed. The urea phase (TIC "C") showed numerous intense mass responses in the investigated region. The carbamate phase (TIC "D") had lower intensity but a greater number of mass responses from phase bleed compared to the urea phase. A second commercially available amide phase (TIC "E") showed the same number of mass responses as the Ascentis RP-Amide, but at much higher intensity, proof that it is not only what is bonded, but how it is bonded that influences the performance and stability of the resulting phase. The Ascentis RP-Amide proprietary bonding and end-capping techniques ensure a low bleed, stable phase.

### Conclusion

Ascentis RP-Amide solves the dilemma of how to enhance selectivity and polar compound retention while maintaining LC/MS integrity. Compared to a C18, Ascentis RP-Amide has dramatically different elution pattern, enhanced polar compound retention and greater aqueous mobile phase stability. However, compared to competitive nitrogen-containing EPG columns, Ascentis RP-Amide exhibits fewer and less intense mass spectral bleed responses making it an ideal candidate for the most sensitive LC/MS applications.

### References

- (1) Euerby, M. R.; Petersson, P.; Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing polar embedded groups/amino endcappings using principal component analysis. *J. Chromatogr. A* 2005, 1088(1), 1-15.
- (2) Wilson, N. S.; Gilroy, J.; Dolan, J. W.; Snyder, L. R.; Column selectivity in reversed-phase liquid chromatography - VI. Columns with embedded or end-capping polar groups. *J. Chromatogr. A* 2004, 1026(1), 91-100.
- (3) Euerby, M. R.; Petersson, P.; Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns using principal component analysis. *J. Chromatogr. A* 2003, 994(1), 13-36.
- (4) Layne, J.; Characterization and comparison of the chromatographic performance of conventional, polar-embedded, and polar-endcapped reversed-phase liquid chromatography stationary phases. *J. Chromatogr. A* 2002, 957(2), 149-164.
- (5) Brandes, H.; Analysis of glutathione on Ascentis™ RP-Amide with MS detection. *The Reporter* 2005, 23.2, 1-2 ([www.sigmaaldrich.com/supelco/the\\_reporter/t205002.pdf](http://www.sigmaaldrich.com/supelco/the_reporter/t205002.pdf)).
- (6) Santasania, C. T.; Bell, D. S.; Mass spectral column bleed in nitrogen-containing, polar-embedded HPLC stationary phases. *The Reporter* 2005, 23.1, 1-2 ([www.sigmaaldrich.com/supelco/the\\_reporter/t205001.pdf](http://www.sigmaaldrich.com/supelco/the_reporter/t205001.pdf)).
- (7) Ascentis HPLC Column Brochure, Sigma-Aldrich/Supelco (T404114) ([www.sigmaaldrich.com/ascentis](http://www.sigmaaldrich.com/ascentis))

Figure 2. Structure of Ascentis RP-Amide stationary phase molecule

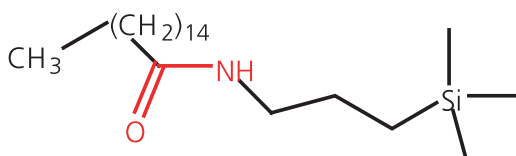
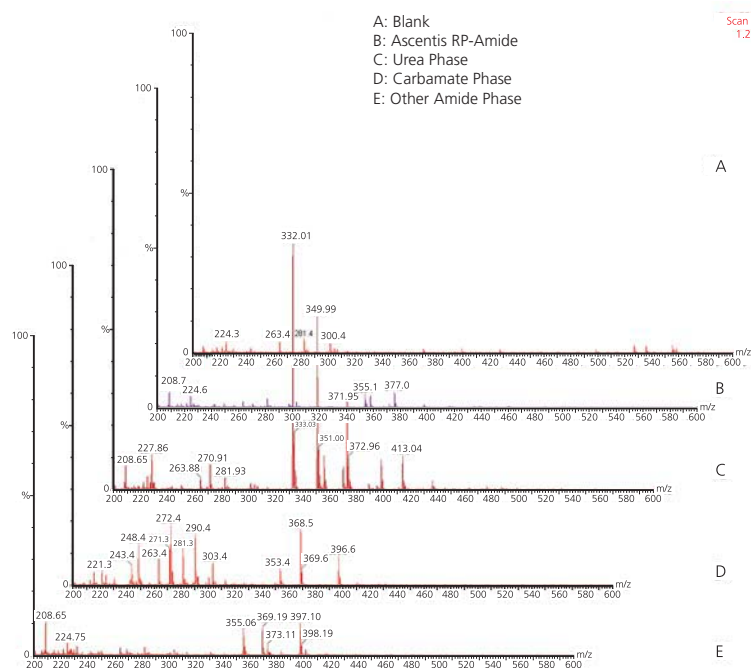


Figure 3. Low-bleed character of Ascentis RP-Amide vs. competitive polar-embedded phases

Bleed evaluation conditions: Each column was exhaustively washed with a 50% methanol solution prior to bleed evaluation. A blank run without a column in-line was acquired to establish responses due to system impurities. Each column was subjected to five gradient cycles of 5 – 100% acetonitrile in 0.1% formic acid. All solvents were of LC-MS CHROMASOLV® grade to ensure clean blank gradient baselines. Detection was by MS, ESI(+), full scan (m/z 50-1500). Bleed ions are typically observed in the collected mass range m/z 200 – 600 and elution time range of 14 – 16 minutes.



## Ordering information


Prod. No.	Particle Size (µm)	ID (mm)	Length (cm)
<b>Ascentis™ RP-Amide HPLC Columns</b>			
565300-U	3	2.1	5
565301-U	3	2.1	10
565302-U	3	2.1	15
565310-U	3	3.0	3
565311-U	3	3.0	5
565312-U	3	3.0	10
565320-U	3	4.6	5
565321-U	3	4.6	10
565322-U	3	4.6	15
565303-U	5	2.1	5
565304-U	5	2.1	10
565305-U	5	2.1	15
565306-U	5	2.1	25
565323-U	5	4.6	5
565324-U	5	4.6	15
565325-U	5	4.6	25

## Ordering information

Prod. No.	Solvent / Blend	Pack Size	Packaging
<b>LC-MS CHROMASOLV® Solvents and Blends</b>			
34967	Acetonitrile LC-MS CHROMASOLV®	1 L, 2.5 L	Amber bottle
39253	Water LC-MS CHROMASOLV®	1 L	Clear glass bottle
34966	Methanol LC-MS CHROMASOLV®	1 L, 2.5 L	Amber bottle
34673	Water with 0.1% formic acid LC-MS CHROMASOLV®	2.5 L	Amber bottle
34668	Acetonitrile with 0.1% formic acid LC-MS CHROMASOLV®	2.5 L	Amber bottle

Ascentis C18 and C8 phases and other dimensions of Ascentis RP-Amide are available. Please call or visit our website: [www.sigma-aldrich.com/ascentis](http://www.sigma-aldrich.com/ascentis)

Other solvents and package sizes are available. Please call or visit our website: [www.sigma-aldrich.com/lc-ms-solvents](http://www.sigma-aldrich.com/lc-ms-solvents)

 Information Request 2001, 2002, 2003

## HPLC ARTICLE

## Effect of Stationary Phase on Selectivity of Reversed-Phase HPLC Separations of Polypeptides

## Abstract

RP-HPLC separations of peptides and polypeptides are influenced by the chemistry of the bonded phase. The objective of most complex peptide separations is to obtain as much information about the sample as possible, especially when working with peptide maps. Therefore, it is to the researcher's advantage to run the sample on different stationary phases, like a C18, C8, and C5.

## What is Meant by Selectivity?

The resolution equation:  $R_s = (1/4) N^{1/2} \{(\alpha - 1)/\alpha\} \{k/(1 + k)\}$

tells us that retention ( $k$ ), efficiency ( $N$ ), and selectivity ( $\alpha$ ) each play a role in a chromatographic separation. There are few improvements that can be made to column efficiency if one is working with small particles and modern packing materials. Retention also gives limited options because of the need to keep analysis times as short as possible. However, selectivity has great power to increase resolution. Selectivity can be thought of as peak spacing. The further the peaks are spaced from one another, the better the selectivity. Selectivity, or separation factor, between peaks 1 and 2 is measured by the equation:

$$\alpha = k_2 / k_1$$

where  $k = (t_R - t_0) / t_0$

Of course, improvements in selectivity beyond allowing for complete baseline resolution of all sample components is of no additional benefit. Supelco Application Note 166 (T302166) showed that improvements in selectivity (and thus resolution) for a complex peptide sample can be achieved by altering the gradient slope and start conditions for the run. These are the most common strategies for optimizing selectivity with polypeptide samples, but there are other tools as well. In this short article, we will discuss the effect of stationary phase chemistry on the selectivity of peptide separations.

## How Does the Stationary Phase Effect Selectivity?

Unlike the partitioning mechanism exhibited by small molecules, retention of polypeptide analytes on a reversed-phase matrix is by differential adsorption to the stationary phase, primarily due to differences in their hydrophobicity. More hydrophobic peptides are retained longer by the bonded phase, and *vice versa*. By reducing the alkyl chain length of the bonded phase, not only is the hydrophobicity reduced, but also the total surface area that is in contact with the peptide analytes. For small molecule separations, a C18 and C8 will usually give the same selectivity, although different retention. However because of different retention mechanisms for peptide and polypeptide separations, the differences in selectivity between a C18, C8, and C5 can be dramatic. The same sample run on C18, C8, and C5 phases will yield different information about the sample, an important consideration for peptide mapping.

There are other factors that affect adsorption to the matrix, even when comparing only linear aliphatic alkyl bonded phases. These other factors involve polar or H-bonding interactions with the silica surface itself, or the indirect effects of the silica surface chemistry on the conformation of the bonded phase. Thus, not only differences in the hydrophobicity of the bonded phase can influence selectivity, but also secondary effects impacted by the bonding chemistry and surface silanols: bonding density, extent of endcapping of silanols, and type of bonding (mono-, di-, or trifunctional).

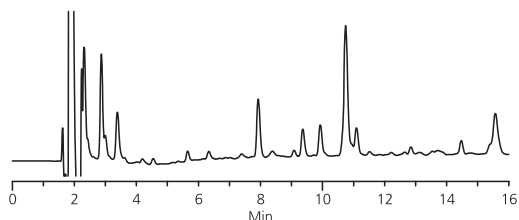
An example of selectivity differences conferred by bonded phase chemistry is shown in Figure 1 & 2. Here, a proteolytic digest of apohemoglobin is chromatographed on the three Discovery BIO Wide Pore reversed-phases C18, C8, and C5. The chromatograms displayed only represent a portion of the entire run to better illustrate the subtle, but significant, differences in selectivity conferred by each phase. Each of the phases displays better selectivity in different parts of the chromatogram. If the goal is purification of a specific peptide, then this has particular utility. If the goal is the best overall resolution of the entire sample, then a decision process should be applied, which evaluates the performance of each phase with its optimised method.

**Figure 1.** Proteolytic Digest Discovery BIO Wide Pore C18, C8 and C5 (0-16 minute window)

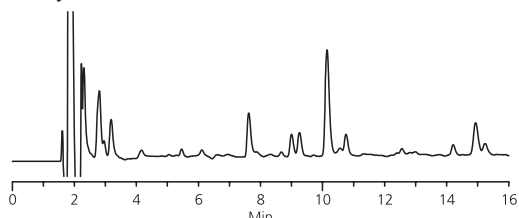
mobile phase A: 95:5, (0.1% TFA in water):(0.1% TFA in CH<sub>3</sub>CN)  
 mobile phase B: 50:50, (0.1% TFA in water):(0.1% TFA in CH<sub>3</sub>CN)  
 column: Discovery BIO Wide Pore, 15 cm x 4.6 mm, 5 μm  
 flow rate: 1.0 mL/min  
 temp: 30°C  
 det: UV, 215 nm  
 inj: 50 μL  
 sample: tryptic digest of carboxymethylated apohemoglobin

gradient:	Min	%A	%B
	0	100	0
	65	0	100

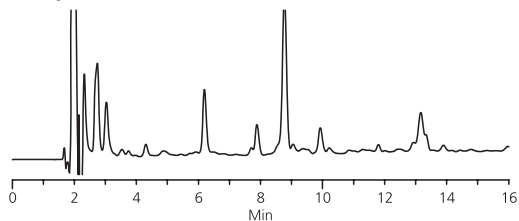
Discovery BIO Wide Pore C18



Discovery BIO Wide Pore C8



Discovery BIO Wide Pore C5



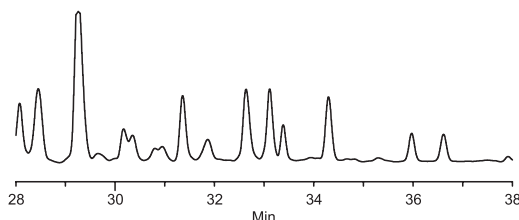
G001730,31,32

**Figure 2.** Proteolytic Digest Discovery BIO Wide Pore C18, C8, and C5 (28-38 minute window)

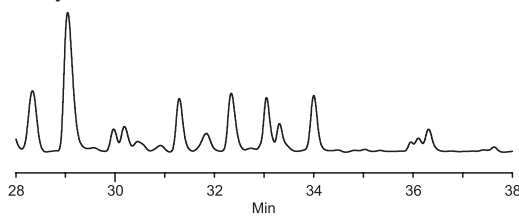
mobile phase A: 95:5, (0.1% TFA in water):(0.1% TFA in CH<sub>3</sub>CN)  
 mobile phase B: 50:50, (0.1% TFA in water):(0.1% TFA in CH<sub>3</sub>CN)  
 column: Discovery BIO Wide Pore, 15 cm x 4.6 mm, 5 μm  
 flow rate: 1.0 mL/min  
 temp: 30°C  
 det: UV, 215 nm  
 inj: 50 μL  
 sample: tryptic digest of carboxymethylated apohemoglobin

gradient:	Min	%A	%B
	0	100	0
	65	0	100

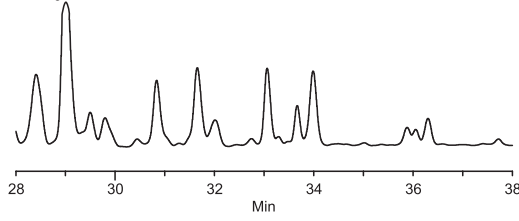
Discovery BIO Wide Pore C18



Discovery BIO Wide Pore C8



Discovery BIO Wide Pore C5



G001727,28,29

**Conclusion**

In conclusion, different bonded phase chemistries give subtle yet significant differences in selectivity toward peptides and polypeptides. Running the sample on each of the three Discovery BIO Wide Pore reversed-phase chemistries will yield different, useful information about the sample.

**Ordering information**

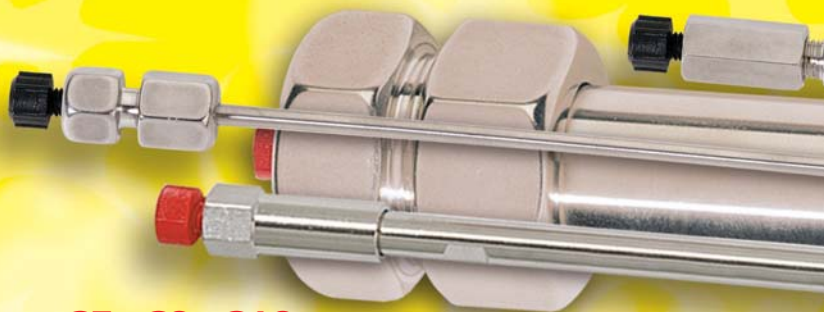
Prod. No.	Particle Size (μm)	ID (mm)	Length (cm)
<b>Discovery BIO Wide Pore C18</b>			
65603-U	3	0.18	5
65604-U	3	0.18	10
65526-U	3	0.32	5
65527-U	3	0.32	10
65517-U	3	0.5	5
65518-U	3	0.5	10
65606-U	5	0.18	5
65607-U	5	0.18	10
65608-U	5	0.18	15
65529-U	5	0.32	15
65519-U	5	0.5	15
568222-U	5	4.6	15

**Ordering information**

Prod. No.	Particle Size (μm)	ID (mm)	Length (cm)
<b>Discovery BIO Wide Pore C5</b>			
65609-U	3	0.18	5
65611-U	3	0.18	10
65531-U	3	0.32	5
65532-U	3	0.32	10
65520-U	3	0.5	5
65521-U	3	0.5	10
65612-U	5	0.18	5
65613-U	5	0.18	10
65614-U	5	0.18	15
65533-U	5	0.32	15
65522-U	5	0.5	15
568422-U	5	4.6	15
<b>Discovery BIO Wide Pore C8</b>			
567213-U	3	2.1	2.1
567214-U	3	2.1	2.1
567215-U	3	2.1	2.1
568300-U	5	2.1	2.1
568301-U	5	2.1	2.1
568302-U	5	2.1	2.1
568322-U	5	4.6	4.6

Other dimensions and guard columns available. Please visit [www.sigma-aldrich.com](http://www.sigma-aldrich.com).

Contact your local sales office. Website [sigma-aldrich.com/supelco](http://sigma-aldrich.com/supelco)



## Discovery BIO Wide Pore C5, C8, C18, PolyMA-SCX and PolyMA-WAX

### HPLC Columns and Capillaries - Solutions for Protein and Peptide Separation Challenges

#### Discovery BIO Wide Pore Reversed Phase Columns are ideal for:

- Proteomics and biotherapeutics: high efficiency protein and hydrophobic peptide separations
- LC/MS: no-bleed, capillary dimensions, and pH stability
- Protein purification: Scalable from analytical to preparative
- Rugged analyses: C5 has enhanced lifetime and pH stability over C4 phases

#### Reversed Phase Columns:

- C18: Reproducible separations batch-to-batch and column to column
- C8: Intermediate hydrophobicity makes it a good method development starting point
- C5: For proteins and hydrophobic peptides

#### PolyMA-SCX and -WAX Ion Exchange Columns:

- Excellent separation of protein isoforms
- High resolution at low sample load
- Quantitative recovery - a hydrophilic surface eliminates protein adsorption
- High efficiency
- Wide pH range

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**20% OFF** Promotion for all Discovery BIO wide pore HPLC columns!

Promotional code: T15  
Offer valid till May 31st 2006.

For a complete product listing, visit [http://www.sigmaaldrich.com/Brands/Supelco\\_Home/Spotlights/Discovery\\_HPLC.html](http://www.sigmaaldrich.com/Brands/Supelco_Home/Spotlights/Discovery_HPLC.html)

Information Request ..... 2004

If you haven't yet received a copy of our 5th edition SPME CD, request one today.

## Solid Phase Microextraction (SPME) Application Guide CD

The 5th edition of our SPME CD contains a comprehensive selection of SPME literature. It includes information on the basics of the technique, application-specific information, as well as bulletins about quantification and troubleshooting. The Included "SPME Applications Guide: Bulletin 925D" contains a bibliography of SPME citations that have been published in the scientific literature. For the 5th edition, this bulletin was updated to include 500 new entries bringing the total to nearly 1550 SPME references. For your convenience, we have sorted the bibliography by application to simplify browsing of the entries.



SPME Application CD

Update 5

Information Request ..... 2005

## TECHNICAL ARTICLE

# Control Your "T"s for better Quantitation with the Solid Phase Microextraction (SPME)

### Introduction

SPME is fiber coated with a liquid (polymer), a solid (sorbent) or a combination of both. The fiber coating removes the compounds from the sample by absorption in the case of liquid coatings or adsorption in the case of solid coatings.

Traditional sample preparation methods try to completely remove the analytes of interest from the sample. SPME does not work this way. With SPME, the amount of analyte removed by the fiber is proportional to the concentration of the compound in the sample. This is true when the fiber and the sample reach equilibrium or before equilibrium, as long as the sampling parameters are carefully controlled. The ability to use SPME quantitatively before equilibrium is reached permits much shorter sampling times producing a fast, economical, and versatile technique. We discuss in this article why controlling the sampling parameters of time, temperature, and technique are important to improve quantitation.

### Extraction Time

The extraction time is a critical parameter in the SPME sampling process. Figure 1 shows the typical relationship of extraction time to analyte absorbed on the fiber. Varying the amount of time that the fiber is exposed to the sample varies the analyte concentration on the fiber until equilibrium is reached. Once the analyte is at equilibrium between the fiber and the sample, its concentration will become constant. Consequently, controlling the extraction time is critical when working in the pre-equilibrium period. Use a stopwatch to time each extraction precisely. Figure 2 shows the poor reproducibility obtained for aromatic compounds in water when the sampling parameters are not well controlled or monitored.

### Temperature

Temperature also affects the equilibrium during extraction. If temperature is increased, the equilibrium distribution of analytes in the sample and the headspace is changed. During the sampling process, the fiber also establishes equilibrium with the sample and headspace. Variations in temperature will change the equilibrium and the resulting concentration of analyte on the fiber. The sample must be stabilized at the pre-determined optimal temperature before exposing it to the fiber. Be aware that variations in room temperature can cause nonreproducible results if sampling is performed at ambient temperature. Use a calibrated thermometer along side the sample to ensure a constant extraction temperature.

### Technique

The technique used in sampling will also influence the reproducibility. A reliable, reproducible technique is important whether using a headspace or direct immersion sampling approach. Be consistent with the fiber position and applying agitation, salting, or pH adjustments. Compared to Figure 2, Figure 3 shows how dramatically reproducibility is improved by paying attention to time, temperature, and technique throughout the sampling procedure. Unlike the previous set, for these extractions we were careful to add the same amount of salt to each sample vial and to stir the sample at the same speed during each extraction. We used a SPME sampling stand to consistently position the fiber just above the vortex of the stirring water. This maximised analyte absorption. We controlled

the sampling temperature and monitored it with a thermometer. Lastly, we timed each extraction precisely with a stopwatch.

### Conclusion

SPME is a fast, economical, and versatile technique to extract compounds from a wide variety of matrixes. By paying careful attention to the three "T"s: time, temperature, and technique during sampling, the reproducibility and accuracy of SPME can be greatly improved.

Figure 1. Absorption-Time Profile

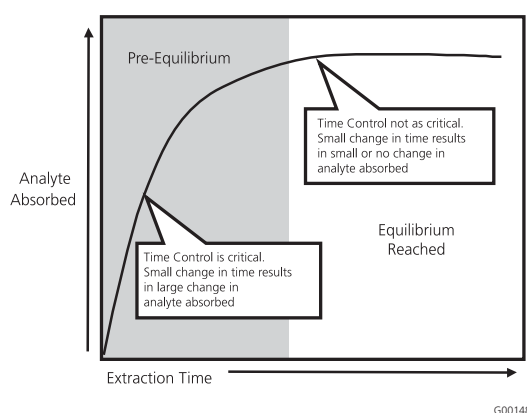


Figure 2. Results from Not Controlling the "T"s

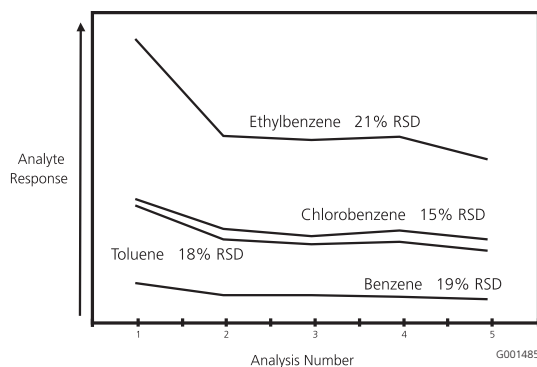
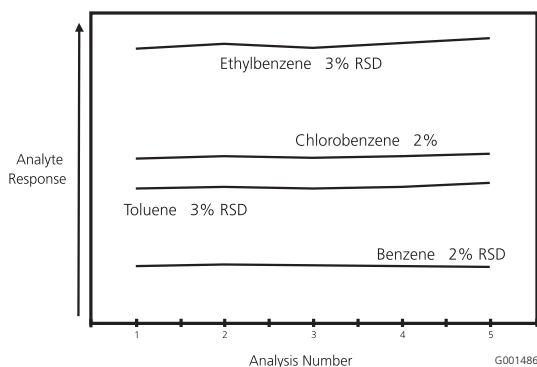


Figure 3. Results from Controlling the "T"s



## SPME ARTICLE Ultratrace Determination of Aroma-Active Thiols by Automated Headspace Solid-Phase Microextraction With In-Fiber Derivatization and GC-ECD and GC-MS-NCI

Laura Mateo-Vivaracho, Vicente Ferreira and Juan Cacho, Departamento de Química Analítica, Facultad de Ciencias, Universidad de Zaragoza, 50009 Zaragoza, España

Thiols very often have strong odors. Low molecular weight members are extremely unpleasant, but some higher molecular weight members at low concentrations have powerful and penetrating aromas responsible for the sensory characteristics of numerous products, such as mango, passion fruit, grapefruit, coffee and some wines. The analysis of these compounds is extremely difficult because of the low concentrations at which they must be determined ( $\text{ng L}^{-1}$  range), their reactivity and instability and their poor chromatographic and spectrometric properties. Present methods are long and tedious and require many steps.

In the present work, the applicability of a fast and automated headspace solid phase microextraction (HS-SPME) with in fiber derivatization with 2,3,4,5,6-pentafluorobenzyl bromide (PFBBBr) has been studied and applied to wine. Studied analytes were 2-methyl-3-furanthiol, 2-furanmethanethiol, 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexanol and 3-mercaptohexyl acetate. For this work poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB) SPME fibers at 65  $\mu\text{m}$  thickness were used. Method optimization has been carried out by using both GC-FID and GC-electron capture detection (ECD) and the final analytical determination is based on GC-MS in the Negative Chemical Ionization (NCI) mode.

One of the most critical steps of the method setup is to transfer an adequate and reproducible amount of derivatization reagent to the fiber. Different alternatives have been developed, making possible to transfer variable amounts ranging from 0.6 to 200 nmol of reagent to the fiber. An excess of reagent can cause serious contamination problems, while a deficit makes the response unstable. A value of 3 nmol has been selected

as compromise. This is achieved by exposing the fiber to the vapors of 10 mL of a 200 ppm solution (10 % acetone) of the derivatization reagent at 55°C for 5 minutes.

Different parameters that impact the process were investigated to optimize the analytical signal. Apart from the mass of reactive loaded, the stirrer speed, the time and temperature of the extraction-derivatization, the effect of salt, and the number of injection cycles that can be performed from one vial of the PFBBBr reagent have been investigated.

In general, it has been found that the critical parameter and the formation of derivatives, and therefore, high temperatures, times increase the analytical signal. Conditions of 55°C, 10 min and 250 rpm have been selected as the best compromise for getting good signals and not ruining the chromatographic performance. The method proposed is extremely sensitive, and it is possible to get clear signals for three analytes at concentrations below 0.1  $\text{ng L}^{-1}$  when using GC-MS in NCI mode. The method is also repeatable (rsd 12%), and the response is not matrix-sensitive because it does not differ between white and red wines. Other aspects considered in the method setup were the oxidation of analytes during the process.

### Acknowledgement.

This work has been funded by project AGL2004-06060/ALI. Lab. Análisis del Aroma y Enología. Dep. Analytical Chemistry, University of Zaragoza. Pza. San Francisco, s/n, 50009 Zaragoza, Spain.

\* Contact: Laura Mateo

E-mail: laura.mateo@unizar.es Tel.: 976761000 Ext. 3509

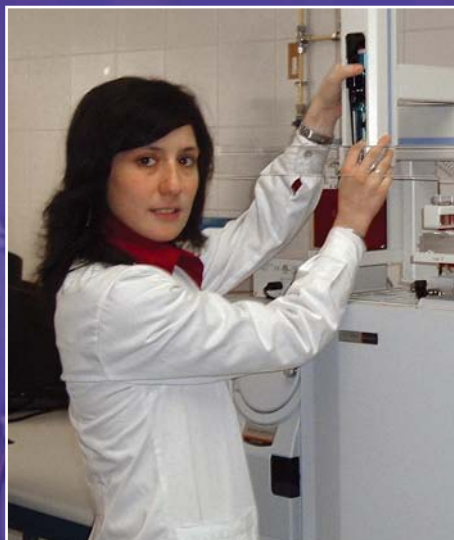
## Award Winner for:

### "Best Oral and Poster Communication on SPME"

11th Instrumental Analysis  
Conference (JAI), Barcelona  
15-17 November 2005

Dra. Laura Mateo Vivaracho  
Dr. V Ferreira  
Dr. Juan Cacho

Ultratrace determination of  
aroma-active thiols by automated  
headspace solid-phase microextraction  
with in-fiber derivatization  
and GC-ECD and GC-MS-NCI.



## SPME ARTICLE

# Metal Fiber Assemblies for SPME Provide Greater Mechanical Stability

Use of Solid Phase Microextraction (SPME) has grown significantly over the last ten years. The CTC CombiPAL™ autosampler is a valuable tool for high volume sample analysis using SPME. However the sample agitator on the CombiPAL™, which increases sample adsorption efficiency, can significantly stress the fiber leading to fiber damage and shorter fiber life.

The metal SPME fiber assemblies are manufactured with a flexible metal alloy used in the needle, plunger, and fiber core. The new design includes a thicker, flexible plunger that is much less likely to kink or break, and helps to reinforce the needle, especially when used in conjunction with the CombiPAL sample agitator. Since the new metal needle is more flexible and has a thinner wall than a standard stainless steel needle, it has been beveled to help it pierce septa materials more easily. As a result of this thinner needle wall and beveled tip, septa coring will occur requiring the use of the Merlin Microseal™ (the bevel tip of the fibers does not harm the Microseal)\* or similar septum-less sealing system for the GC instrument.

The alloy used in the metal fiber assemblies does not contain iron and is more inert than stainless steel. Improvements have been made in the coating of the fibers using an automated, continuous process. This process helps to bond the coating to the core and make a more consistent, reproducible fiber. Since the metal alloy core is slightly thicker, the actual coating diameter is slightly less than the stated value. However, the coatings placed on this inert, flexible metal core are similar in volume to those on the fused silica and StableFlex™ cores.

### Ordering information\*

Prod. No.	Description
<b>Fiber Coatings</b>	
57919-U	7 µm PDMS, Metal alloy, 1 cm length , Pk.1
57922-U	30 µm PDMS, Metal alloy, 1 cm length , Pk.1
57928-U	100 µm PDMS, Metal alloy, 1 cm length , Pk.1

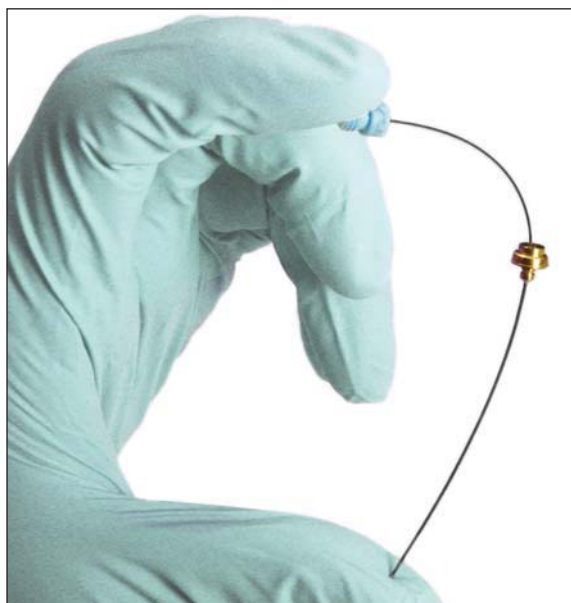
\* Additional fiber coatings for metal alloy fibers are currently under development

For more information request Literature T405058. (HXS)

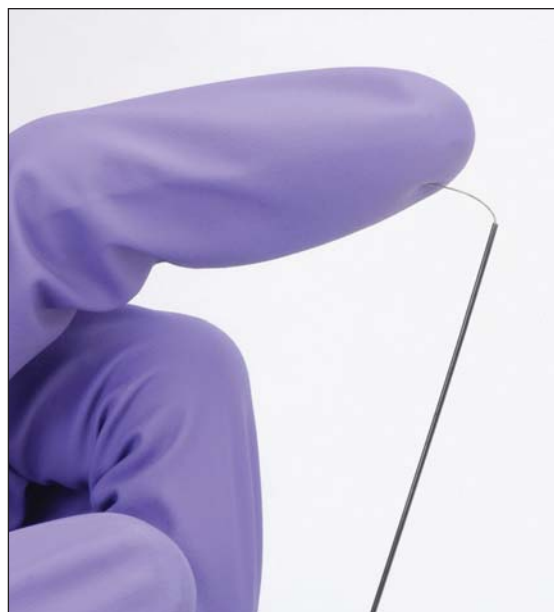
\*Merlin Microseal™ is applicable for Agilent, Varian, Thermo and Shimadzu instruments. Adapters maybe required.



The metal Fiber assemblies and fibers show high flexibility.



New flexible metal alloy SPME fiber



Conventional fused silica SPME fiber



## SPME ARTICLE

# Merlin Microseal™ Long Life Septum Replacement for GC Injector Ports are also Ideal for SPME

### Analytical chemistry in food science

The Merlin Microseal septum and nut are patented long-life replacements for the standard septum and septum nut in the capillary GC inlet system or the purged packed inlet system of Agilent, Varian, Thermo\* or Shimadzu\* gas chromatographs. The Microseal septum incorporates two sequential seals to provide a much longer run time between septum changes. It requires a 0.63 mm diameter blunt-tip syringe or a 23 gauge SPME fiber to seal properly. It is particularly useful for autosampler use, as it allows significant more samples to be run unattended while reducing the risk of lost or compromised data caused by septum leaks or by pieces of the septum falling into the injection port. In addition since there is not a fresh penetration surface the septum bleed is significantly reduced.

Because the syringe insertion force is much lower, the Merlin Microseal also allows for much easier manual injections. While actual life will vary depending on operating conditions, the Merlin Microseal septum will typically sustain over about 5,000 injections.

#### Ordering information

Prod. No.	Description
-----------	-------------

#### High pressure (up to 100 psi) Merlin Microseal

For Agilent 5800, 5900, and 6890 (Shimadzu with adapter installed)

24814-U	1 Nut and 2 Septa
24815-U	1 Nut and 1 Septum
24816-U	1 Septum

#### For Varian Models 3400,3800

24817-U	1 Nut, 1 Septum, 1 Inlet Adapter and O-Ring
24818-U	1 Septum

\*Please contact our technical service for information on other instrument manufacturers.

### Vials and Septa for HS SPME with the CTC autosampler

To minimize the mechanical stress to the SPME fiber during injection into the sample vial the septum thickness should be around 1.5 mm. The most suitable septum material for SPME is PTFE/Silicon. The vials and caps below fulfill this requirements and are suitable for the CTC autosampler. The screw caps are easy to close and they are magnetic for suitability for the CTC autosampler:

#### Ordering information

Prod. No.	Description
-----------	-------------

#### Screw Cap Vials pk/100

SU860099	10 mL Clear glass, round Bottom 22.5 mm x 46 mm Height
SU860100	10 mL Amber, round Bottom 22.5 mm x 46 mm Height
SU860097	20 mL Clear glass, round Bottom 22.5 mm x 75.5 mm Height
SU860098	20 mL Amber, round Bottom 22.5 mm x 75.5 mm Height

#### Metal Screw Caps pk/100

SU860101	Aluminum screw cap PTFE faced/Silicon septum Thickness 1.3 mm
SU860103	Aluminum screw cap PTFE faced/Silicon septum Thickness 1.5 mm

#### Crimp seal vials & caps pk/100

SU860051	20 mL Headspace vial for SPME Pk.100
SU860052	Magnetic cap, 8mm hole, black Viton, 1.5 mm Pk.100
SU860053	Gold Seal magnetic seal closures PTFE/Silicon Thickness 1.5 mm

# 25% OFF

## Merlin Microseal offer

Promotional code: T18

Offer valid until 31st of May 2006



\* Additional Equipment necessary.  
Please contact the instrument manufacturer.



# 30% OFF

## 30% OFF Recommended HS Vials for SPME & CTC (Combi-PAL)

Promotional code: T20

Offer valid until 31st May 2006



SPE ARTICLE

# New Supelpak™-2SV Adsorbent Improves Environmental Sampling and Recovery of Difficult Semivolatile Compounds

Jim Walbridge jwalbridge@sial.com

Supelco is pleased to announce the availability of its newest adsorbent, Supelpak-2SV. This new adsorbent is optimized for the sampling and analysis of semivolatile pollutants, dioxins, furans, polynuclear aromatic hydrocarbons (PAHs) and chlorinated pesticides. Supelpak-2SV provides enhanced recovery of difficult compounds such as pentachlorophenol (PCP) and dinitrophenols and hence lower detection limits. Background levels from the adsorbent are low enough to eliminate the need for pre-cleaning, saving valuable time and money.

## Background

Environmental analysts analysing semivolatile pollutants according to various established methods (1) are required to collect samples using Amberlite XAD®-2 resin. These methods specify laborious, time-consuming cleaning procedures to reduce background contaminants on XAD-2 to acceptable levels. Analysts often find the need to process "precleaned" XAD-2 even further to reduce background levels so that lower detection limits can be obtained by GC-MS analysis. Additionally, difficult compounds such as PCP and dinitrophenols must show high recoveries following extraction by XAD-2.

## Improved Cleaning Process

For many years, Supelco has offered XAD-2 as well as Supelpak-2 and Supelpak-2B, pre-cleaned versions of XAD-2, meeting cleanliness requirements of specific environmental sampling methods (2). The Supelpak-2 series of resins has evolved to provide our customers with adsorbents that have lower background levels and increased recoveries for specific analytes. Our newest resin, Supelpak-2SV, is a dry and more highly purified version of XAD-2. Supelpak-2SV was developed specifically for the extraction and recovery of semi-volatile organic compounds from environmental samples. Produced using a proprietary cleaning process, Supelpak-2SV exhibits typical background levels of contaminants less than 1µg/g of resin measured as total chromatographable organics (TCO). Table 1 shows the low background levels extracted from Supelpak-2SV as analysed on an Equity™-5 capillary GC column.

## Improved Performance of Supelpak-2SV

Used directly from the bottle, Supelpak-2SV requires no costly in-house cleaning before use and is superior to other "precleaned" XAD-2 resins, even after additional cleaning.

Table 1. Typical Background Levels Extracted from Supelpak-2SV and Competitors Products

Adsorbent	TCO Background* µg/g
Supelpak-2SV lot 1	0.52
Supelpak-2SV lot 2	0.67
Supelpak-2SV lot 3	0.51
Manufacturer A	19.3
Manufacturer B	4743

\* Determined by GC/FID analysis. Sample prepared from 16-hour extraction of resin (40 grams) with dichloromethane

This results in savings in both lab time and hazardous solvent waste generation, disposal and associated costs. Characterisation by GC-MS has also shown Supelpak-2SV resin an excellent choice for semivolatile priority pollutants, PCBs, PAHs and dioxins/furans. A study of extraction efficiencies using a wide range of semivolatile compounds demonstrated recoveries >90% even for substituted phenols. Table 2 shows typical recoveries of substituted phenols from Supelpak-2SV compared to competitive products. Customers also appreciate other aspects of the product such as the availability of large single-lot batches of up to 10 kg. This significantly reduces a laboratory's labour for internal qualification for each lot of adsorbent.

## Conclusion

Produced using proprietary cleaning procedures, Supelpak-2SV, a dry and more highly purified version of XAD-2, offers environmental analysts significantly lower background levels of organic contaminants eliminating the need for further cleaning prior to use. Notoriously difficult compounds such as PCP and dinitrophenols show extraction recoveries typically greater than 90% on Supelpak-2SV, improving the accuracy and reliability of analytical results. Your laboratory will save considerable time and money by switching to Supelpak-2SV for your environmental sample collection and extraction needs. Supelpak-2SV can be ordered in three different package sizes to meet your usage requirements (see next page).

## References

1. US EPA SW-846, Method 0010, Method 8270C; California Air Resources Board (CARB) Methods 428 and 429, EPA Compendium Method TO-13A.
2. Supelpak-2 meets US EPA recommended criteria for purity in Level I Environmental Assessment Procedures Manual and as outlined in EPA SW-846, Method 10; Supelpak-2B meets EPA requirements for determining PCBs in water according to the Great Lakes National Program Office (GLNPO).

Table 2. Typical Recoveries of Phenols from Supelpak-2SV

Component	Recovery Lot #1910	Recovery Lot #1928	Recovery Lot #1932	Average Recovery 3 Lots	Competitor Data
Phenol	92	95	95	94	95
2-Chlorophenol	92	95	95	94	94
2-Methylphenol	92	95	95	94	92
2-Nitrophenol	93	95	96	95	95
2,4-Dimethylphenol	89	86	88	88	64
2,4-Dichlorophenol	89	90	93	91	100
2,4-Dinitrophenol	117	105	103	108	36
4-Nitrophenol	119	104	104	109	17
2,3,5,6-Tetrachlorophenol	113	104	106	108	112
2-Methyl-4,6-dinitrophenol	120	107	108	112	85
2,4,6-Tribromophenol	116	107	107	110	NA
Pentachlorophenol	123	109	108	113	85

## Ordering information

Prod. No.	Description	Pack Size
<b>Supelpak-2SV</b>		
13673-U	Supelpak-2SV	100 g
13682-U	Supelpak-2SV	250 g
13674-U	Supelpak-2SV	1000 g

For more information on XAD-2 and the Supelpak family of adsorbents, request the Supelpak-2 resins product information sheet, T405056 (HXM).

## Did you know...?

Supelpak-2SV can be obtained in single-lot batch sizes up to 10 kg. This will minimise the time that you spend on certifying different lots of adsorbent resin for your lab requirements. Specify your single lot needs at the time of order.

Supelco is one of the largest suppliers of small quantities of resins and media for research applications supplying research quantities of resins from Dow Chemical, Rohm & Haas and many other manufacturers. As these products demonstrate, we also routinely custom process resins to meet the exacting requirements of our customers through cleaning processes, repackaging or phase modification. For more information on our resin processing capabilities please contact Technical Service.

## Ordering information

Prod. No.	Description
<b>SLB-5ms Low-Bleed GC Columns</b>	
28471-U	30 m x 0.25 mm x 0.25 $\mu$ m
28473-U	30 m x 0.25 mm x 0.5 $\mu$ m

For a complete listing of all Supelco Standards, log on to our website: [sigma-aldrich.com](http://sigma-aldrich.com)

## FREE sample

## FREE sample of 100g Supelpak-2SV

Offer limited to 1 unit per customer

Promotional code: T21

Offer valid until 31st of May 2006

**i** Information Request ..... 2006

## SPE TUBE PRODUCT INFORMATION

### LpDNPH Air Monitoring Cartridges

## Introduction

The LpDNPH cartridge is an air sampling device designed for sampling carbonyls (e.g. formaldehyde) in ambient, indoor and industrial atmospheres. Carbonyls are trapped on a high purity silica adsorbent coated with 2,4-dinitrophenylhydrazine (2,4-DNPH), where they are converted to the hydrazone derivatives. The derivatives are eluted from the cartridge in acetonitrile and analyzed by HPLC.

## S10 Cartridge (Figure 1)

The unique solid phase extraction configuration makes the cartridge easy to use in the field as well as in the laboratory. Reusable adapters are available to connect the cartridge to the sampling pump. The built-in reservoir eliminates the need for attachment of a syringe for sample extraction/elution. It is recommended that sampling flow be in the opposite direction of elution flow.

## S10x Cartridge (Figure 2)

The LpDNPH S10x cartridge has a shorter tube length than the S10 cartridge, to fit automated samplers such as those manufactured by XonTech Inc. and ATEC Atmospheric Technology. Using a S10x cartridge, the air sampling flow will be in the direction opposite that for the S10 cartridge to accommodate the instrument's flow pattern.

Figure 1. LpDNPH S10 Cartridge

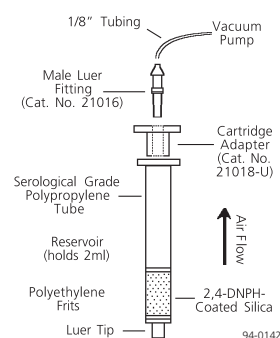


Figure 2. LpDNPH S10x Cartridge

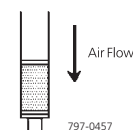
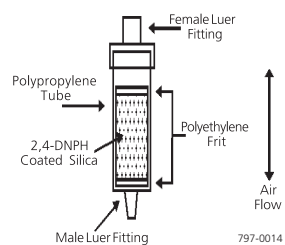


Figure 3. LpDNPH S10L Cartridge



## S10L Cartridge (Figure 3)

The LpDNPH S10L cartridge is configured for users who prefer the shorter dimensions without the need for an adapter for sampling. The cartridge is reversible and requires a syringe barrel for elution.

### Rezorian™ Cartridge (Figure 4)

Rezorian cartridges are polypropylene cartridges with luer lock syringe connections. These cartridges have polypropylene components and polyethylene frits making them solvent-compatible. Rezorian cartridges can be used inline with peristaltic pumps and low pressure HPLC systems, or with a syringe and a single sample processor. Rezorian cartridges can be used individually or connected in series.

Figure 4. Rezorian Cartridge

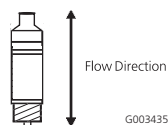


Figure 5. Universal Elution Rack



#### LpDNPH Cartridge Specifications:

adsorbent: chromatographic grade silica coated with 2,4-dinitrophenylhydrazine  
 particle size: 150-250 μm (60/100 mesh)  
 DNPH loading: 0.29% (1 mg/cartridge)  
 bed weight: approx. 350 mg  
 capacity: approx. 75 μg total carbonyls  
 cartridge length: S10 – 7.4 cm  
 S10x – 3.8 cm  
 S10L – 4.0 cm  
 Rezorian – 4.0 cm  
 background (per cartridge): <0.06 μg formaldehyde <0.15 μg acetaldehyde <0.38 μg acetone  
 pressure drop: <7kPa at 1.5 L/min (<28 inches water / <2.1 inches mercury)  
 storage: refrigerate (4 °C); protect from light  
 shelf life: 12 months

### LpDNPH cartridges feature:

- Low pressure drop, to enable use at high sampling rates (1.5-2 L/min.), even with personal sampling pumps
- High purity adsorbent, for better accuracy in trace analysis
- Bar code labels, for fast and accurate sample identification
- Individual packaging, to maximize sample integrity

### Method Applications:

US EPA TO11A — Method for the Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge Followed by High Performance Liquid Chromatography (HPLC)

US EPA IP-6A — Determination of Formaldehyde and Other Aldehydes in Indoor Air Using a Solid Adsorbent Cartridge

US EPA 600-R-98/161 (1998) — Technical Assistance Document for Sampling and Analysis of Ozone Precursors

US EPA 0100 — Sampling for Formaldehyde and Other Carbonyl Compounds in Indoor Air

NIOSH 2016 — Formaldehyde

ASTM D5197 — Standard Test Method for Determination of Formaldehyde and Other Carbonyl Compounds in Air

### Sample Collection (refer to specific methodology for greater detail)

As with any high purity-sampling device, care in handling the LpDNPH cartridges has to be exercised to minimize unintentional exposure. Ozone is a known negative interference when sampling for carbonyls. If you suspect the presence of ozone, use

an ozone denuder or scrubber as described in the methodology. The cartridges can be connected to the sampling pump using appropriate adapters, if necessary. The air sample is collected in the direction recommended for each cartridge configuration (see Figures 1-4), at flow rates up to 2 L/min. To avoid a sample breakthrough, the total amount of carbonyls collected should not exceed 75 μg. After sampling, both ends immediately need to be capped and the cartridges should be placed individually in the white foil bags provided. The bar code labels can be used to uniquely track each sample by placing one label on the bag and its duplicate. These then can be recorded in a sample log with sample identification. The sample loaded tubes should be stored at 4 °C and protected from light. One field blank and one lab blank should be included from the same lot with each batch of 10 samples.

### Sample Desorption

Analyse the sample within 30 days of sampling. Using gravity feed, elute the cartridge with 2 mL aliquots of acetonitrile and collect the eluate to the 3.0 mL mark on a volumetric flask. An elution rack (Fig. 5) or a vacuum manifold can be used to expedite multiple sample desorption. Ideally, desorption is in the direction opposite that of the air flow during sample collection, although this is not critical. Use only high purity acetonitrile that has been tested for carbonyl contaminants. Store the extract at 4 °C until analysis.

### HPLC Analysis

Analyse the extract by reversed phase HPLC, using a Discovery® HS C18 column with UV detection, as stated in methodology.

#### Ordering information

Prod. No.	Description	Pack Size
<b>LpDNPH Air Monitoring Cartridges</b>		
21026-U	LpDNPH S10	10
21014	LpDNPH S10	50
21024-U	LpDNPH S10	Starter Kit*
505293	LpDNPH S10x	10
505358	LpDNPH S10L	10
505361-U	LpDNPH S10L	50
54074-U	LpDNPH Rezorian	10
54075-U	LpDNPH Rezorian	50

\* Kit includes 10 cartridges plus adapters and male luer fittings for various air sampling pumps.

#### Accessories

Prod. No.	Description	Pack Size
<b>Connectors</b>		
21018-U	Cartridge Adapters	10
21016	Male Luer Fittings to 1/8" tubing	20
23364	Male Luer Fittings to 3/16" tubing	20
24856	Male Luer Fittings to 1/4" tubing	10
21017	Female Luer Fittings	20
21015	Female Luer Couplers	20
25064-U	Male Luer Couplers	20
<b>Plugs</b>		
504351	Male Luer Plugs	12
57098	Female Luer Caps	12
<b>Other</b>		
21019-U	Lapel Clips	6
505285	Ozone Scrubbers	10
57242	Syringe Barrels, 6mL <sup>1</sup>	30
21043-U	Universal Elution Rack	1

<sup>1</sup>Reservoirs for S10L cartridges.

# VersaFlash™

Flash Purification System

**VersaFlash -**  
the product family  
is growing!



## Versatility and simplicity that get results!

The VersaFlash Flash Purification system is a versatile tool that allows you to cover a broad spectrum of purification requirements and sample sizes without the need of additional equipment. The VersaPak cartridges are packed with spherical silica that provides higher capacities and efficiency compared to irregular silica. The latest addition to the VersaFlash line expands the versatility of the VersaFlash system by allowing the processing of more sample sizes on the same system.

New, 'small cartridges' for purification of small sample sizes:

- 23 x 55mm packed with 11g Silica or 15g C18 (particle size 20-45µm)
- 23x110mm packed with 23g Silica or 30g C18 (particle size 20-45µm)

The complete VersaFlash cartridge range covers a broad max. loading capacity range from 1.1g to 190g (see table).

**VersaPak Cartridge Characteristics and Sample Loading Guidelines**

Cartridge (ID x Length)	Particle Size (µm)	Packing Weight (g)	Column Volume (mL)	Maximum Capacity* (g)
<b>Silica</b>				
23 x 53 mm	20-45	11	15	1.1
23 x 110 mm	20-45	23	30	2.3
40 x 75 mm	45-75	51	60	5.0
40 x 150 mm	45-75	102	120	10.0
80 x 150 mm	45-75	410	480	34.0
80 x 300 mm	45-75	820	960	70.0
110 x 300 mm	45-75	1320	1850	130.0
<b>C18</b>				
23 x 53 mm	20-45	15	11	1.5
23 x 110 mm	20-45	30	20	3.0
40 x 75 mm	45-75	70	30	7.0
40 x 150 mm	45-75	140	60	14.0
80 x 150 mm	45-75	515	240	51.0
80 x 300 mm	45-75	1050	480	105.0
110 x 300 mm	45-75	1920	910	190.0

\* Several factors influence the maximum loading capacity of the column including similarity of compounds, sample matrix, concentration of reaction products and the elution solvent used.  
\* reusable peak adaptor needed. Please order separately.

**Please request our new VersaFlash Brochure (T403110B, FWL) for further details or contact your local Sigma-Aldrich office.**



**i** Information Request ..... 2007

**SUPELCO**

## GC ARTICLE

## A New Generation of GC Capillary Columns: SLB-5ms

Luigi Mondello and Rosaria Costa Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università degli Studi di Messina, Viale Annunziata, 98168 – Messina.

The heart of a gas chromatographic system, the capillary column, has been since its introduction the object of considerable improvement in terms of analytical performance. Particular attention has been devoted to the production of low bleed columns, resistant to very high temperatures and especially suited to gas chromatography-mass spectrometry (GC-MS). The wide employment of mass spectrometers as detection systems in GC applications has increased the necessity of low bleed columns in order to increase sensitivity and to obtain “cleaner” spectra, thus allowing easier peak identification. For this reason, in the last years there has been a high interest towards the so called “MS” columns specifically designed for GC-MS benchtop systems. MS columns are characterised by high inertness, low bleed and higher temperature operating conditions.

It must be added that the need for high thermal stability columns (capable of operating above 300°C) to analyse complex mixtures containing high boiling VOCs has become a common issue in the petrochemical industry. The methods that are exploited in order to increase the stability of the stationary phase are as follows:

- binding of the phase to the capillary surface (bonded phase)
- “cross-linking” the phase by the use of a radical initiator
- “Sol-gel” technology: the phase is encapsulated inside synthetic glass

Whatever technology is used, the final goal is always to reduce column bleed. The analytical sensitivity of a column is connected to the signal-to-noise ratio (S/N). Consequently, if the column bleed increases, the noise will increase as well.

Column bleed arises from the degradation of the stationary phase and is directly proportional either to the amount of stationary phase or to the column temperature. It is derived from the formation of cyclic (tri- and tetra-) polysiloxanes from thermal and oxidative degradation processes occurring in the stationary phase polymer. Due to a “backbiting” effect, these cyclic siloxanes are generated and, immediately after, separate from the stationary phase chain, thus producing noise in the detector signal.

Columns that exhibit low bleed have a longer lifetime and generally make quantitation and identification easier. It needs to be emphasised that a low level of bleed produces a better S/N ratio and avoids the formation of ion fragments unrelated to the matrix. Finally, a very low background greatly reduces the risk of contamination of the detector components, especially critical when using GC-MS.

Supelco has recently introduced a new low bleed GC capillary column, Supelco SLB-5ms, that has a significantly reduced bleed profile compared to conventional columns of the same 5% diphenyl/95% polydimethylsiloxane phase chemistry. SLB-5ms is the result of advances in the field of polymer chemistry and is characterized by improved cross-linking, a higher thermal stability and greater reproducibility.

Various parameters related to the column performance, such as resolution, analyte response, degree of bleeding and column life, have been accounted for during the development of this stationary phase. For the aforementioned reasons, SLB-5ms is particularly suited to GC-MS analysis. As can be seen in Figure 1, the SLB-5ms column produces an almost non-existent baseline rise, even at the final temperature of the oven program. SLB-5ms also passes the bleed measurements test. There are different methods that enable the evaluation of column bleeding. It may be measured in terms of detector response or, more effectively, in terms of ng bleed/sec. Another way to estimate this factor is through the calculation of the percentage rate of loss of the total volume of stationary phase.

Figure 1. Bleed arising from SLB-5ms compared to conventional 5-ms column

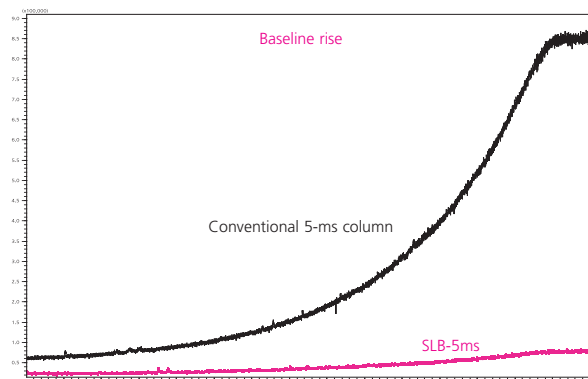


Figure 2 shows an example of absolute column bleed measurement related to a conventional 5-type column. A standard solution of octamethylcyclotetrasiloxane ( $C_8H_{24}O_4Si_4$ , known as D4) is injected in programmed temperature (50°C @ 7°C/min to 325°C, held 10 min). A slice of area under the baseline at the maximum temperature is measured and this value is divided by the D4 peak area. Dividing the result obtained by the time of the slice width will provide the corresponding measure of the absolute bleeding in ng bleed/sec. This method allows to define the exact degree of bleeding at the maximum operational temperature. The analytical performance of the SLB-5ms still shows to be excellent even in the case of complex matrices such as essential oils and perfumes (Figure 3).

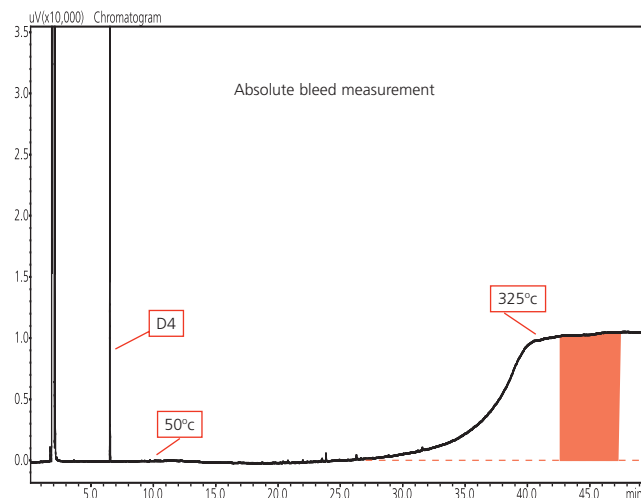
[www.sigma-aldrich.com/capillary-ms](http://www.sigma-aldrich.com/capillary-ms)



Supelco - Solutions in Chromatography

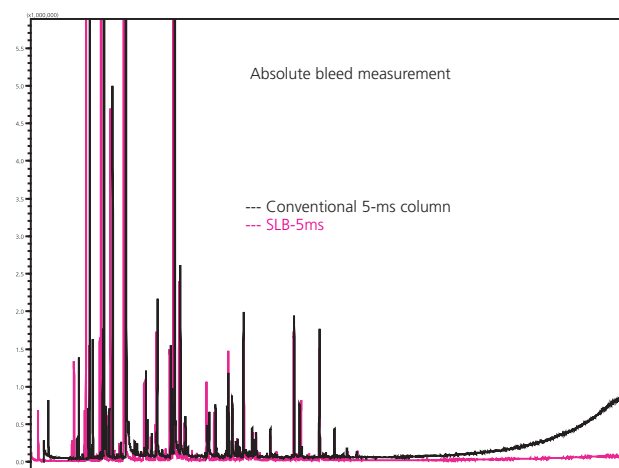
In order to test the reliability of the SLB-5ms columns, we compared the Linear Retention Indices obtained for SLB-5ms with those calculated for the same compounds identified in perfume into the conventional 5-type column. Finally, we made a comparison of these values with those reported in our GC-MS library (FFNSC) dedicated to flavour and fragrance compounds (Table 1).

**Figure 2.** Absolute bleed measurement on conventional 5-ms column



**i** Information Request .....2008

**Figure 3.** Essential oils on SLB-5ms vs. conventional 5-ms column



**Table 1: Comparison of flavour and fragrance compounds Linear Retention Indices (LRI)**

Compound name	LRI from FFNSC library	Conventional 5-ms column	SLB-5ms #1	SLB-5ms #2
Alpha-pinene	932	930	932	933
Limonene	1030	1028	1030	1030
Linalool	1101	1102	1101	1101
Linalyl acetate	1250	1250	1250	1250
Alpha-Isomethylionone	1473	1470	1473	1474
Methyl dihydrojasmonate	1649	1648	1649	1650
Alpha-hexyl cinnamaldehyde	1747	1743	1747	1746

## COLACRO XI - JUNE 26-30, 2006

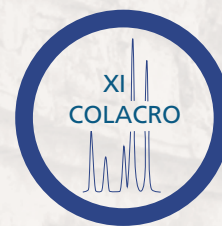
**Mérida, Yucatan, MEXICO Facultad de Química  
UNAM Depto. de Química Analítica**

Congreso Latinoamericano de Cromatografía y Ciencias Afines, is the most important International Latin-American event related to the divulgation of chromatographic science and related techniques. The Congress was set up in 1986 by prof. Fernando M. Lanças, who is teacher at the school of Chemistry of the São Paulo University, Brazil, with the aim of creating a debate about the most recent advances in Separation Science. COLACRO is open to all the people involved in such area. The intention is to allow the access to academic research, bringing prominent specialists of various parts of the world, and complimenting the scientific part at the same time, by presenting the advances in equipment and instrumentation thanks to the partnership made with the manufacturing companies.

**Information and Registration: (52) (55) 56-22-37-86**

**Humberto Gómez Ruiz hgomez@servidor.unam.mx**

**Lucía Hernández Garcíadiago lhdez@servidor.unam.mx**



**XI CONGRESO  
LATINOAMERICANO DE  
CROMATOGRAFÍA  
Y CIENCIAS AFINES**

**JUNIO 26-30 2006  
MÉRIDA, YUCANTÁN  
MÉXICO**

INFORMES

**FACULTAD DE QUÍMICA UNAM  
DEPTO. DE QUÍMICA  
ANALÍTICA**  
(52) (55) 56-22-37-86

**Humberto Gómez Ruiz**  
hgomez@servidor.unam.mx  
**Lucía Hernández Garcíadiago**  
lhdez@servidor.unam.mx

**INCENTIVOS Y CONVENCIONES**  
**Carmen Junco Vallejo**  
cjunco@icsa.com.mx  
(55) 55-50-96-77 ext 113 o 114

## GC ARTICLE

# Protecting Capillary GC Columns

### SLB-5ms

Phase: Bonded and highly crosslinked; silphenylene polymer virtually equivalent in polarity to 5% phenyl polymethylsiloxane

Temp. Limits: 0.20 to 0.32 mm I.D. columns: -60 °C to 340 °C (isothermal) / 360 °C (programmable)

#### Ordering information

Prod. No.	Length (m)	D <sub>i</sub> (µm)	Beta
<b>0.20 mm ID Fused Silica</b>			
28513-U	30	0.20	250
<b>0.25 mm ID Fused Silica</b>			
28467-U	30	0.25	250
28469-U	15	0.25	250
28471-U	30	0.25	250
28472-U	60	0.25	250
28473-U	30	0.50	125
28476-U	30	1.0	63
<b>0.32 mm ID Fused Silica</b>			
28557-U	15	0.25	320
28482-U	30	0.25	320
28532-U	30	0.32	250
28484-U	30	0.50	160
28487-U	30	1.0	80

### Extend Column Life With Guard Columns

A decrease in peak shape quality in a capillary GC system can typically be traced to the inlet end of the column. Over time, the inlet end of the column becomes contaminated from an accumulation of non-volatile material. The phase can also be damaged from the continuous condensation and vaporisation of solvent and analytes. Inevitably, active analytes will adsorb to the contaminated / damaged section, leading to peak tailing, loss in resolution, and reduced response. When chromatography degrades to an unacceptable level, performance is restored by clipping the contaminated / damaged section off the inlet end of the column.

To extend the lifetime of capillary GC columns, Supelco recommends using a 3 m long guard column. A guard column is a short piece of uncoated deactivated fused silica tubing which is placed in-line between the GC injection port and the analytical column. The guard column will take the brunt of the contamination / damage. By clipping the guard column periodically to restore performance instead of the analytical column, the analytical column remains unaltered. Therefore, chromatography (retention times and resolution) is not affected.

### Fused Silica Guard Columns

For use as guard columns to protect your analytical column from damaging sample components. Match the deactivation of the tubing with the polarity of the injection solvent.

Deactivation	Injection Solvents	Max. Temp.
Non-Polar	Alkanes, Carbon disulfide, Ethers	360 °C
Intermediate Polarity	Acetone, Methylene chloride, Toluene	360 °C
Polar	Acetonitrile, Methanol, Water	250 °C

### Capillary Column Butt Connector

This device consists of a double-tapered ferrule and a stainless steel compression housing with a threaded cap. Small and light (2.3 cm x 0.6 cm, 4.4 g with ferrule), it provides a gas tight seal. This unit maintains inertness with no change in column efficiency.

#### Ordering information

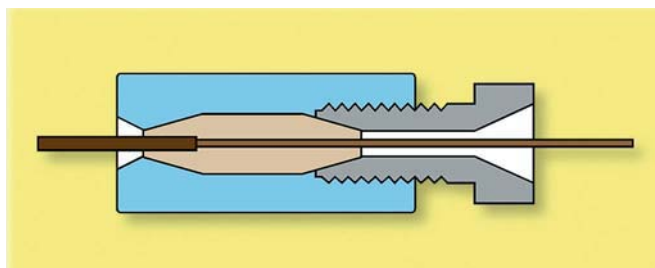
Prod. No.	Length (m)	ID (mm)
<b>Non-Polar Deactivation</b>		
25722	3	0.25
25742	5	0.25
25723	3	0.32
25743	5	0.32
<b>Intermediate Polarity Deactivation</b>		
25727	3	0.25
25747	5	0.25
25728	3	0.32
25748-U	5	0.32
<b>Polar Deactivation</b>		
25752-U	5	0.32

#### Prod. No. Description

23804	Capillary Column Butt Connector, body only
<b>Supeltext M-2B Ferrules, pack of 2</b>	
22453	To connect 0.10/0.25 mm ID to 0.10/0.25 mm ID
22454	To connect 0.32 mm ID to 0.32 mm ID

[www.sigma-aldrich.com/capillary-ms](http://www.sigma-aldrich.com/capillary-ms)

Capillary Column Butt Connector



# FREE

**Buy any SLB-5ms column and get a Capillary Cleaning Tool (Cat. No 23740-U) for FREE**

Offer limited to 1 unit per customer

Promotional code: F69

Offer valid until 31th of May 2006

## GC ARTICLE

## Solutions for Analyses of Fatty Acids: New Regulations Require Trans Fat Content to be Reported on Food Labels

By Michael D. Buchanan: mbuchanan@sial.com Roberto Ferrari: rferrari@europe.sial.com

On July 9, 2003, the United States Food and Drug Administration (FDA) issued a regulation requiring manufacturers to list trans fatty acids (FA), or trans fat, on the Nutrition Facts panel of foods and some dietary supplements. In addition, over 100 publications appeared evaluating the effects of dietary fats or fatty acid (FA) supplementation on various plasma parameters, including lipids / lipoproteins.

Also In Europe the interest of pattern of FA, polyunsaturated fatty acids (PUFA) and trans fatty acids is under investigation by several research groups. With this rule, consumers will have more information to make healthier food choices that could lower their consumption of trans fat as part of a heart-healthy diet. Scientific reports have confirmed the relationship between trans fat and an increased risk of coronary heart disease (CHD). Food manufacturers have until January 1, 2006, to list trans fat on the nutrition label. FDA estimates that by 2009, trans fat labeling will have prevented from 600 to 1,200 cases of coronary heart disease and 250 to 500 deaths each year. (1) There is also a push for Nutrient Level Claims for eicosapentaenoic acid (C20:5n3, EPA), docosahexaenoic acid (C22:6n3, DHA) and alpha linolenic acid (C18:3n3, ALA). All of these claims result from scientific studies related to coronary artery disease and the effect of the omega 3 fatty acids in hormone synthesis.

Food analysts currently use the 100 m SP-2560 column for detailed analyses of fatty acid isomers. Unfortunately, this procedure may take 38 minutes or more to perform. Due to the issuing of the new regulation, more analyses need to be performed now than in the past. Therefore, analysts need a faster method than the current 100 m SP-2560 provides.

**New SP2560 improved Column For Fast GC**

Supelco has recently developed a 75 m Fast GC (0.18mm ID) version of the SP-2560 that allows the same detailed FAME analysis to be completed in just 21 minutes. Using hydrogen carrier gas, a 45% reduction in analysis time over the traditional 100 m SP-2560 column is realized. This shorter analysis time will allow analysts to keep up with the demand for higher throughput caused by this new trans fat labeling regulation.

**FAME Standards**

There are numerous individual and standard solutions of FAMES ranging in carbon number from C4:0 to C24:1 including saturates, monounsaturated (cis and trans) and polyenoic FAMES. Fifty-eight individual fatty acids are noted in the method. Seventeen are saturated-predominately even chain but does include odd carbon chains. Twenty are monenes and the remaining twenty one individual FAMES are polyenoic containing from two to six double bonds. A few conjugated C18:2 FAME compounds are also included in the polyenes. All of the individual standards and mixes are designed to provide representation of the major fatty acids contained in the major dietary sources of fat from food. These are grain or seed oils, land animal, dairy and marine fish. These standards are available in our Supelco Catalogue and on our website:

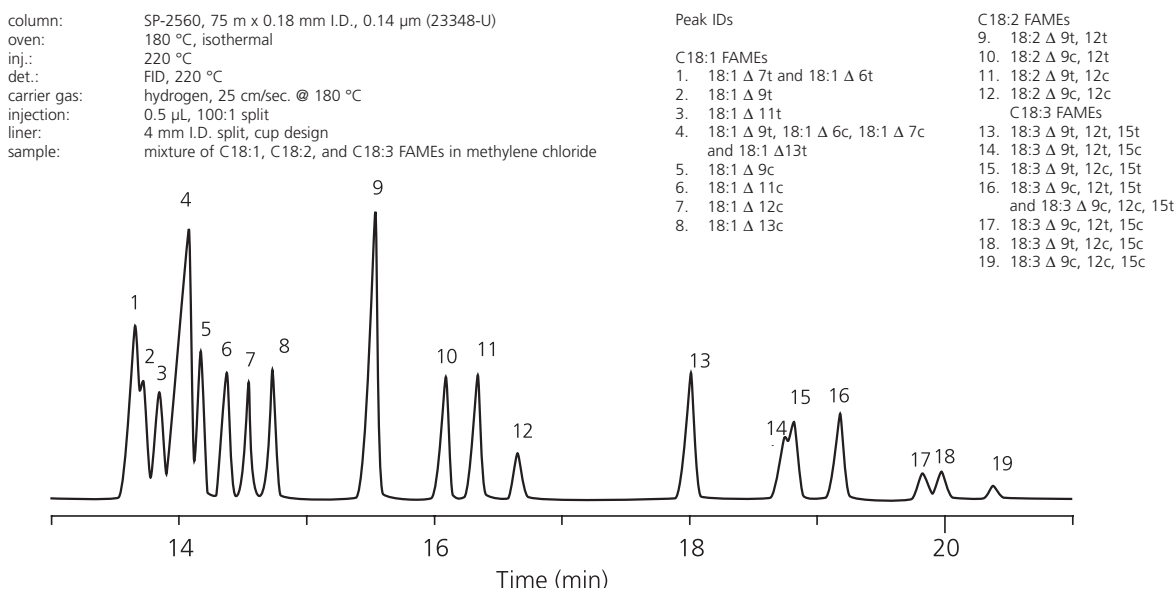
<http://www.sigma-aldrich.com/fame>

1. Information from the US FDA available at: [www.fda.gov/oc/initiatives/transfat/](http://www.fda.gov/oc/initiatives/transfat/).

**Ordering information**

Prod. No.	Description
23348-U	SP-250 75m x 0.18mm, 0.14µm

**Figure 1.** Fast Analysis of cis and trans FAME isomers on 75 m SP-2560



## STANDARDS & REAGENTS ARTICLE

### Air Monitoring Standards

The Supelco Air Monitoring Standards are suitable for use with a wide range of chromatographic sample determinations. They have been developed under different official norms. The standards are quantitative formulations for use as chromatographic calibration or spiking solutions. Products include a Certificate of Analysis

describing lot-specific production and analytical information. Free data packets are available for most of these products. Data packets contain data on raw materials and final production. Request the data packet when ordering the standard; the order number is the same as that for the standard, preceded by the letters DP.

#### Air Monitoring Standards

##### Air Monitoring Standards, General

##### Chemical Standards for American Society for Testing and Materials (ASTM) Methods

ASTM D4861	PCBs In Air
ASTM 4947	Chlordane & Heptachlor
ASTM 5197	Aldehydes
ASTM 5578	Ethylene Oxide
ASTM D5836	2,4 and 2,6- Toluene Diisocyanate (TDI)

##### Chemical Standards for NIOSH and OSHA Methods for Workplace Atmospheres

NIOSH 2001/OSHA 32	Cresol in Indoor Air
NIOSH 2005	Nitrobenzenes in Indoor Air
NIOSH 2501/OSHA 52	Acrolein in Indoor Air
NIOSH 2541/OSHA 52	Formaldehyde in Indoor Air
NIOSH 5503	PCBs in Indoor Air
NIOSH 55061,5515	Polynuclear aromatic hydrocarbons (PAH) in Indoor Air
NIOSH 5519	Endrin in Indoor Air
OSHA 32	Phenol in Indoor Air
OSHA 42, 47	Isocyanates in Indoor Air

##### Chemical Standards: Air Pollutants in Indoor Air (IP)

IP1	Volatile Organics (BP 80-200°C) in Indoor Air by GC/MS
IP6A/TO11	Aldehydes & Ketones in Indoor Air by HPLC/UV
IP7/EPA610	Polynuclear aromatic hydrocarbons (PAH) in Indoor Air by HPLC/UV
IP8	Organochlorine Pesticides in Indoor Air by GC/ECD

##### Chemical Standards: Carbonyls in Ambient Air

CARB	Carbonyl DNPH Mix 1 (Varied concentration)
CARB1004	Carbonyl-DNPH Mix1 (Same concentration)
CARB1004	Carbonyl-DNPH Mix2 (Same concentration)

##### Single Component Carbonyl-DNPH Solutions

These solutions of DNPH derivatives are designed as quantitative calibration mixtures for use where a multicomponent solution is not suitable. At concentration indicated in 1 mL acetonitrile, in an amber glass ampul.

##### Chemical Standards: Toxic Organic Compounds in Air (TO)

TO1-1/TO-2	Volatile Organic Compounds
TO-4/TO-10	Organochlorine Pesticides in Indoor Air by GC/ECD
TO-5/TO-11	Aldehydes & Ketones in Indoor Air by HPLC/UV
TO-7	N-Nitrosodimethylamine by Capillary GC-MS
TO-8	Cresol & Phenols by HPLC/UV
TO-13	Polynuclear aromatic hydrocarbons (PAH) by GC/FID & HPLC/UV

##### Custom Chemical Standards

You can choose from more than 2000 raw materials to define the composition, concentration and formulation you need. We can:

- Quote on most formulation requests.
- Manufacture most custom orders.
- Provide quantitative and qualitative product testing.
- Provide custom packaging.

You can find these standards divided into classes in our [Supelco catalogue\\*](#) or on our [web page](#) [www.sigma-aldrich.com](http://www.sigma-aldrich.com)

\*Please refer to the table of content page of the Chemical Standards chapter in the [Supelco catalogue](#).

## STANDARDS &amp; REAGENTS

How to Select the Right Ion Pair Chromatography Reagent By Rainer Walz rwalz@sial.com

**The key to resolving complex mixtures of polar and ionic molecules is to select the right Ion Pair Chromatography (IPC) reagent.**

The key to resolving complex mixtures of polar and ionic molecules is to select the right Ion Pair Chromatography (IPC) reagent. Alkyl sulfonates are a good first choice for basic solutes, whereas quaternary amines are useful for the acidic ones. Once this is done, the method can be further optimised by adjusting the pH and the concentration. Tables 1 and 2 give you an overview of IPC reagents for the separation of cations and Table 3 shows IPC reagents suitable for the separation of anions.

For a complete list of products, please visit [www.sigma-aldrich.com/ipc](http://www.sigma-aldrich.com/ipc)

**Table 1. Selection of solid IPC reagents suitable for cation separation sorted by carbon chain length.**

Prod No.	Brand Compound (solid)	Carbon Length
52862	Fluka1-Hexanesulfonic acid sodium salt monohydrate	C6
51832	Fluka1-Heptanesulfonic acid sodium salt monohydrate	C7
74882	Fluka 1-Octanesulfonic acid sodium salt monohydrate	C8

**Table 2. Selection of IPC reagent concentrates suitable for cation separation sorted by carbon chain length. Concentrates are available in packages with 6 ampoules. Dilute to 1-liter with HPLC grade water (Cat. No. 95304) to obtain a 0.005M eluent solution.**

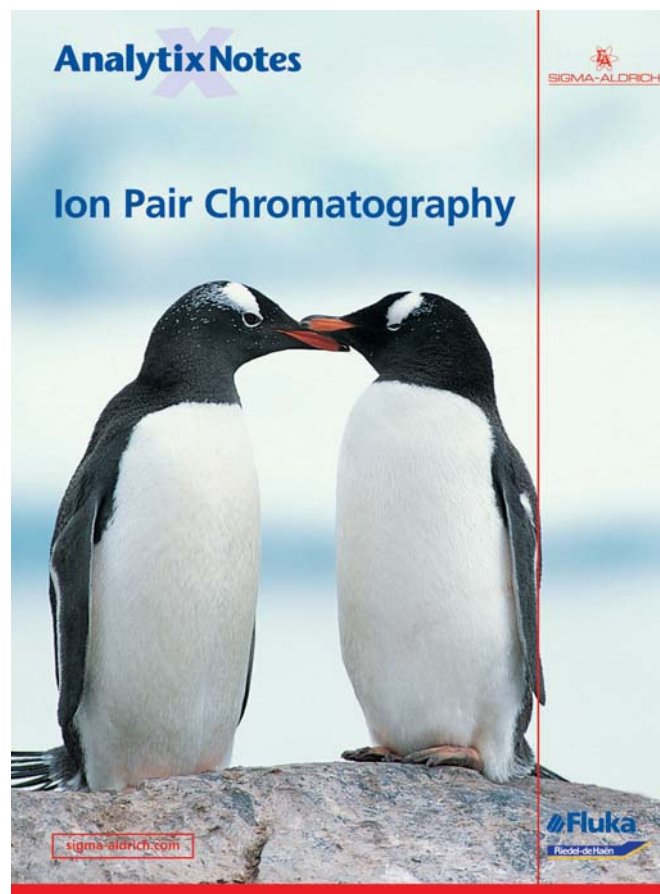
Prod No.	Brand Compound (concentrate)	Carbon Length
52864	Fluka1-Hexanesulfonic acid sodium salt concentrate (~0.33M)	C6
51834	Fluka1-Heptanesulfonic acid sodium salt concentrate (~0.33M)C7	C7

**Table 3. Overview of solid IPC reagents suitable for anionic separation sorted by carbon chain length.**

Prod No.	Brand Compound (solid)	Carbon Length (longest chain)
88106	FlukaTetrapropylammonium hydrogensulfate	C3
86847	FlukaTetrabutylammonium hydrogensulfate concentrate	C4
86853	FlukaTetrabutylammonium hydrogensulfate	C4
87299	FlukaTetrahexylammonium hydrogen sulfateC6	C6

One of the biggest challenges facing scientists working in pharmaceutical research and drug discovery is the separation and identification of biological substances. Since most of these compounds are ionic or polar, the use of reversed phase-high performance liquid chromatography (RP-HPLC) is somewhat restricted.

In the past, the approach used to separate charged analytes was ionic suppression. This technique is based on the pH adjustment of the mobile phase to result in a non-ionised analyte. However, this requires extensive method development and is only suitable for single compounds or simple mixtures where the pKa's of the analytes lie close together. Furthermore, the silica supported on bonded columns is only stable within a pH range of 2-8.

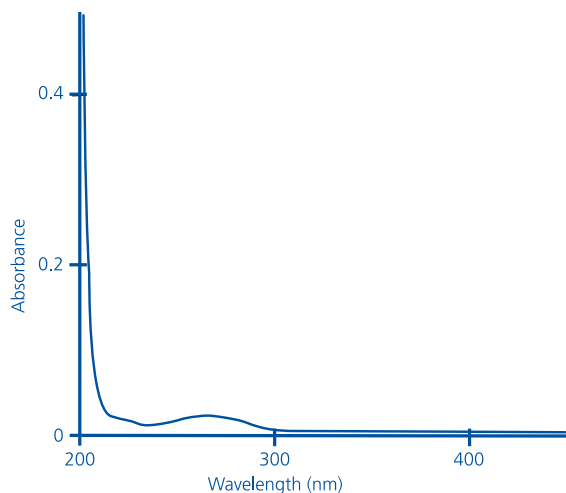


Do you want to know more about IPC? Please ask us for the Analytix Notes on Ion Pair Chromatography (FZI). Send back the enclosed reply card or download it from [www.sigma-aldrich.com/analytix](http://www.sigma-aldrich.com/analytix)

The limitations of ionic suppression led to the development of Ion Pair Chromatography (IPC). IPC is a more general and applicable approach that allows the separation of complex mixtures of polar and ionic molecules. The selectivity is determined by the mobile phase: the organic eluent is supplemented with a specific ion-pairing reagent. The IPC reagents are large ionic molecules having a charge opposite to the analyte of interest, as well as a hydrophobic region to interact with the stationary phase. The counter-ion combines with the ions of the eluent, becoming ion pairs in the stationary phase. This results in different retention, thus facilitating separation of analytes. IPC is now an established and reliable technique which provides:

- Reduced separation times
- Highly reproducible results
- Sharper peak shapes
- Simultaneous separation of ionised and non-ionised analytes in one run
- Wide choice of additives to improve separation.

**Figure 1.** UV absorption performed on tetrabutylammonium bisulfate (Cat. No 86853). Concentration: 10% in water, measured against water. Cell: quartz (1cm).



Fluka IPC reagents are of the highest purity and exhibit minimal extinction in the low UV (Figure 1). They have excellent transparency down to 200 nm, even at high concentrations. In addition, they are tested for the absence of insoluble matter (Figure 2). Non-absorbing impurities such as redox traces, which may interfere with the sample, are also checked. Finally, the suitability test is performed using a very steep gradient (Figure 3).

**Figure 2.** Filter test performed on tetrabutylammonium bisulfate (Cat. No 86853).

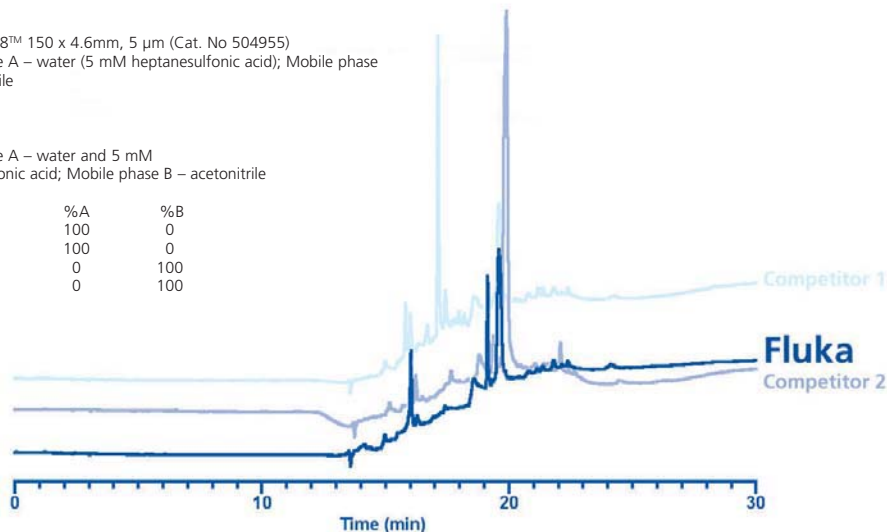
20 ml of the reference solution is filtered through a Millipore® 0.45 µm Membrane filter. After air drying it is compared with a blank. The filter shows no insoluble matter.



**Figure 3.** Gradient test for Sodium 1-heptanesulfonate monohydrate (Cat. No 51832)

column: Discovery C18™ 150 x 4.6mm, 5 µm (Cat. No 504955)  
 mobile phase: Mobile phase A – water (5 mM heptanesulfonic acid); Mobile phase B – acetonitrile  
 flow rate: 1.0 mL/min  
 temperature: 35°C  
 detection: UV, 205nm  
 gradient: Mobile phase A – water and 5 mM heptane-sulfonic acid; Mobile phase B – acetonitrile

Time (min)	%A	%B
0	100	0
10	100	0
20	0	100
30	0	100



**i** Information Request .....2011, 2012

# LPLC Resins and Media



Riedel-de Haën

## Your source for media samples for laboratory research and pilot-scale development efforts.

We offer a full line of off-the-shelf resins and media from all of the leading manufacturers including Dow Chemical, Rohm and Haas, Mitsubishi Chemical, Amersham-Biosciences, Sigma-Aldrich and Tosoh Biosep. In addition to the standard products, Supelco offers custom packaged quantities and media processing meet customer requirements. Just a few of the Supelco media processing capabilities include:

- Cleaning
- Pre-wetting
- Blending
- Drying
- Ion-exchange
- Sanitising and sterilising
- QC testing including Certificate of Analysis
- Custom Packaging (including clean room)



A media selection guide is given in the Supelco catalog in the beginning of the Resins & Media chapter. For more information on the custom capabilities please contact our Technical Service.

[sigma-aldrich.com](http://sigma-aldrich.com)


Contact your local sales office. Website [sigma-aldrich.com/supelco](http://sigma-aldrich.com/supelco)



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