

# the Reporter

FOR EUROPE

MAGAZINE

Volume 13 October 2004 International issue

SUPELCO

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SIGMA-ALDRICH

## EDITORIAL

## LC-MS: Modern Purity Requirements for Solvents, Reagents and Standards

**Dear Chromatographer,**

Liquid chromatography-mass spectrometry (LC-MS) has become one of the most important tools in the analytical chemistry laboratory over the past decade. The structural information obtainable along with sensitive detection has made the technique indispensable in pharmaceutical, environmental and other scientific disciplines.

The sensitivity of LC-MS, however, is a double-edged sword. Along with the sensitive detection of the target analytes comes detection of trace amounts of impurities. These impurities may originate from solvents, reagents, HPLC columns or extraction materials such as solid phase extraction cartridges. For less sensitive detection techniques such as ultraviolet (UV) spectroscopy, impurities are tolerable or even go unnoticed. As we strive for lower and lower limits of detection with mass spectrometry, however, impurities often interfere with qualitative and quantitative measurements.

Trace impurities from chromatographic solvents are particularly troublesome for LC-MS practitioners. The constant influx of these impurities results in significantly increased background that can hinder the detection of trace target analytes. Small amounts of metal ion impurities often found in chromatographic solvents may also complicate spectral analysis through the promotion of multiple adduct ions. Our LC-MS ChromaSolv® line of chromatographic solvents are specially purified to minimize extractables and metal ion impurities. In addition to improving LC-MS detection, down time due to ion source contamination is minimized with the use of these solvents.

HPLC column bleed is another major source of background signal in LC-MS analyses. Supelco recently introduced a new line of LC-MS columns with highly stable bonding chemistry that promise low MS bleed and fast analysis times.

Sigma-Aldrich has recognized the unique needs of LC-MS practitioners. We offer a line of highly purified chromatography solvents, reagents and standards. Our solid phase extraction and chromatography media are prepared to minimize phase bleed. In addition, we offer stable isotopes, both off the shelf and customized for your application needs. For your LC-MS needs, visit the Sigma-Aldrich family of companies – the one that has it all.

Sincerely,



Dave Bell  
Applications Laboratory Supervisor

## MEET SUPELCO

## 35th International symposium on essential oils - ISEO 2004

September 29-October 2, 2004 - Giardini Naxos, Messina, Italy

**Symposium**

The 35th International Symposium on Essential Oils (ISEO 2004) will take place between September 29-October 2, 2004 in Giardini Naxos, Messina, Italy. The topics of the symposium will include all aspects of essential oils and related natural products ranging from analysis, biogenesis and chemistry to biological activity and utilization.

**Meet Supelco****HPLC 2004**

25th Int. Symposium on Chromatography	Paris, France	04.-08. Oct. 2004
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**Invitation**

Essential oils scientists and all other interested people are cordially invited to participate in the 35th International Symposium on Essential Oils (ISEO 2004) at Giardini Naxos, Messina, Italy.

For further information or registration (deadline June 15, 2004), please consult the website:

<http://pharma.unime.it/foodchem/iseo2004/> or contact [iseo2004@pharma.unime.it](mailto:iseo2004@pharma.unime.it)



## HPLC ARTICLE

## Rapid Analysis of Rifampicin Using Discovery LC-MS

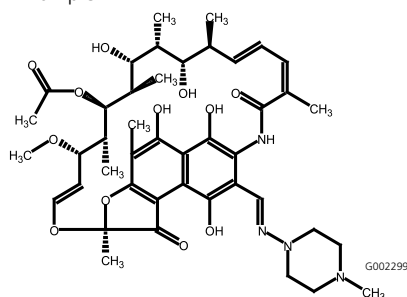
Stationary Phases David Bell dbell@sial.com**Abstract**

The desire for quality data in an increasingly timely manner places a significant burden on the modern analytical laboratory. In this article, the use of short LC-MS columns are shown to provide faster analysis times while retaining adequate resolution of the target analytes. The use of shorter dimension columns is often an effective choice where high throughput is required.

**Introduction**

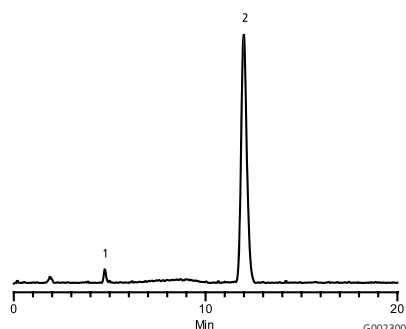
There exists a great interest in the modern analytical laboratory to reduce analysis time. One approach has been to use short HPLC columns to reduce chromatographic run times. The most common column length used in HPLC is 15cm. Although such lengths are sometimes necessary for adequate resolution, shorter columns can be substituted in situations where enough resolution is retained. Furthermore, with the growing use of LC-MS systems, qualitative and quantitative information is not as dependent on chromatographic resolution because the mass spectrometer is capable of providing additional selectivity based on mass resolution.

In this study the chromatographic run time for the analysis of the antibiotic rifampicin is contrasted between traditional column geometries and new, shorter columns. Adequate resolution is retained with a concomitant reduction of up to 10 minutes per chromatographic run.

**Figure A.** Rifampicin**Figure B.** Rifampicin on Conventional Discovery C18 Columns

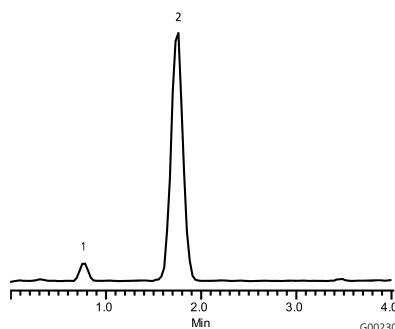
Column: Discovery C18, 15cm x 2.1mm ID, 5µm particles  
 Cat. No.: 50495521  
 Mobile Phase: 60:40, 33mM HCO<sub>2</sub>H : CH<sub>3</sub>CN  
 Flow Rate: 0.2ml/min  
 Temp.: 35°C  
 Det.: ESI (+), full scan  
 Inj.: 1.2µl  
 Sample: 10mg/l in 50% CH<sub>3</sub>OH

1. (M+H)<sup>+</sup> = 821.1 (likely oxidation product)
2. (M+H)<sup>+</sup> = 823.1 (rifampicin)

**Figure C.** Rifampicin on Short Discovery C18 Columns

Column: Discovery C18, 2cm x 2.1mm ID, 5µm particles  
 Cat. No.: 577507-U  
 Mobile Phase: 60:40, 33mM HCO<sub>2</sub>H : CH<sub>3</sub>CN  
 Flow Rate: 0.2ml/min  
 Temp.: 35°C  
 Det.: ESI (+), full scan  
 Inj.: 0.8µl  
 Sample: 10mg/l in 50% CH<sub>3</sub>OH

1. (M+H)<sup>+</sup> = 821.1 (likely oxidation product)
2. (M+H)<sup>+</sup> = 823.1 (rifampicin)

**Experimental**

Rifampicin (Cat. No. R3501) was run on Discovery C18 columns with internal diameters of 2.1mm and lengths of 2 and 15cm. See conditions in Figures B-C. The results were contrasted in terms of the retention of adequate resolution and the reduction in analysis time.

**Results**

The structure of rifampicin is provided in Figure A. Figure B shows a rifampicin analysis as acquired on a 15cm x 2.1mm Discovery C18 column. Two major responses are observed that are extensively resolved. Figure C depicts the same separation on a Discovery C18 2cm x 2.1mm column. As shown in this figure, the run time is reduced from approximately 12 minutes to just under 2 minutes while the resolution of the two responses is conserved. Adequate retention and resolution promises to provide a system capable of suitable qualitative and quantitative analysis. The reduced analysis time allows for a 6-fold increase in throughput.

**Conclusions**

The modern analytical laboratory is required to provide quality analytical data in a short amount of time. Faster analyses need not, however, necessitate a compromise in data integrity. Where standard analytical column dimensions (15cm, for example) provide significant resolution of target analytes, shorter columns may be an intelligent substitute. The shorter columns often provide a means of significantly reducing run times without loss of adequate resolution. Where mass spectrometry is utilized, loss of chromatographic resolution may be offset by the mass resolving power of the mass spectrometer.

Information Request.....1302

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

Your Problem Solving Partner in Chromatography

## Ordering information

Phase	ID (mm)	Length (cm)	Prod. No.
<b>Discovery LC-MS Columns</b>			
5µm Discovery C8 Columns	2.1	2	577501-U
	2.1	3	577502-U
	3.0	2	577503-U
	3.0	3	577504-U
	4.6	2	577505-U
5µm Discovery C18 Columns	4.6	3	577506-U
	2.1	2	577507-U
	2.1	3	577508-U
5µm Discovery Cyano Columns	3.0	2	577509-U
	3.0	3	577510-U
	4.6	2	577511-U
	4.6	3	577512-U
	2.1	2	577513-U
5µm Discovery Cyano Columns	2.1	3	577514-U
	3.0	2	577515-U
	3.0	3	577516-U
	4.6	2	577517-U
	4.6	3	577518-U

## Did you know...?

As column dimensions are decreased, the effect of extra column or system volume becomes more prominent in LC systems. This effect is usually observed as peak broadening when using columns of small inner diameter. Eliminating extra tubing, using small volume mixing devices, and lowering volume detector cells can greatly enhance the quality of the LC data when utilizing these small volume columns.

## HPLC Solvents

Riedel-de Haën has many years of experience with the production of high purity solvents. Production under clean room conditions, sophisticated distillation and extensive container cleaning processes ensure consistent quality, batch-to-batch. CHROMASOLV® grade solvents are notable for high transmittances in the UV spectrum and a low level of non-volatile impurities. From preparative HPLC grade P CHROMASOLV to the highest purity G CHROMASOLV (super gradient grade), Riedel-de Haën meets the quality requirements of modern HPLC.

**Related Products:** HPLC Grade Solvents Prod. No.

## CHROMASOLV Solvents for HPLC

The CHROMASOLV solvents are characterized by high UV-transmittance, consistent gradient testing for interfering peaks and base line drift, guaranteed suitability for fluorescence detection, low non-volatile components, free acid and free alkali and an exacting specification for low water content.

ACETONITRILE 34851

## G CHROMASOLV Solvents for HPLC Gradient Elution

The G CHROMASOLV grade was developed for sensitive gradient elutions at short wavelengths and with very high requirements with regard to UV-transmittance and fluorescence detection

ACETONITRILE 34998

## E CHROMASOLV Solvents for HPLC

This quality is suitable for measurements in the low wavelength range and has also been tested for suitability for gradient applications and for fluorescence detection.

ACETONITRILE 34888

## P CHROMASOLV Solvents for Preparative LC

The specifications of the P CHROMASOLV qualities are tailored fully to the requirements of preparative liquid chromatography. In particular this means low contents of non-volatile impurities and of free alkali.

ACETONITRILE 34989

## R CHROMASOLV Solvents for Routine Measurements in LC

This grade is suitable for isocratic analysis at low wavelengths, for example at 210nm or for gradients at high wavelengths, such as 254nm.

ACETONITRILE 34881

**i** Information Request.....1303

## LC/MS Nitrogen Generator N2-30

## Self-Contained Membrane Nitrogen Generator

## Gas Generator Benefits

The Parker Balston® model N2-30 is a self contained membrane nitrogen generator that produces 99% pure nitrogen with pressures up to 100 psig. It produces nitrogen by utilizing a combination of compressor, filtration, and membrane separation technologies. High and low pressure compressors filtered by high efficiency coalescing filters remove all contaminants down to 0.01 micron. Hollow fiber membranes subsequently separate the clean air into a concentrated nitrogen stream and oxygen enriched permeate stream, which is vented from the system.

The combination of these technologies produces a continuous on-demand supply of pure nitrogen. Typical applications include nebulizer gas, chemical and solvent evaporation, instrument purge and supply, evaporative light scattering detector use (HPLC), and sparging.



Analytical Gas Systems



## Principal Specifications

## Model No. N2-30

Maximum Outlet pressure	7 barg
Hydrocarbon Content	2ppm (excluding methane)
Atmospheric Dewpoint	50°C
Outlet Port Female	1/4" NPT
Min/Max Ambient Temperature	16°C/32°C
Electrical Requirements	230V/60Hz
Dimensions	41/BG barg (60/125 psig)
Shipping Weight	114kg
Nitrogen Flow Rate up to	271Lpm (Phthalate free)

## Ordering information

## Cat No.

SU861121 Parker LC/MS N2-30 Nitrogen Generator 220V

# HPLC ARTICLE

## Selection by Separation Problem, Part 3:

### PROBLEM 3: Too Much Resolution or Wasted Space in the Chromatogram

#### How does Discovery solve this problem?

The Discovery family of functionalized RP columns offers unique selectivity compared to C18. These chemistries provide different retention that can bring peaks closer together. Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

#### Demonstration 1: Too much resolution of alkaloids.

Alkaloids are naturally occurring bases with complex multicyclic ring structures. They are easily separated on the Discovery C18 column with good peak shape and adequate retention. However, there is excessive run time using C18, greater than 20 minutes. By changing to a more polar stationary phase such as the Discovery RP-AmideC16 as shown in Figure 1, a shorter analysis time is obtained with baseline resolution. If there is a requirement for shorter analysis time or you have too much resolution, consider going to a column that will provide different retention and offer unique selectivity such as the Discovery RP-AmideC16.

### Demonstration 2: High pH reduces excessive resolution of alkaloids.

There are usually multiple Discovery solutions to every HPLC separation problem. The mobile phase pH influences retention of ionic compounds. Excessive retention may be solved by running at high or low pH. Silica-based phases are not stable above pH 8. However, Discovery Zr particles are stable from pH 1 to 14 allowing the full range of pH to alter selectivity. Here, excessive resolution of the five alkaloids is solved by using a Discovery Zr-PBD column at pH 12.

Figure 2. pH Change Can Reduce Wasted Space in Chromatogram

- faster analysis from lower hydrophobicity
- better peak spacing (RP-AmideC16)
- better resolution of small impurities (RP-AmideC16)

Column: Discovery Zr-PBD 15cm x 4.6mm ID, 5µm particles (65718-U)  
 Mobile Phase: 90:10, 20 mM Potassium Phosphate (pH 8, 10 or 12):CH<sub>3</sub>CN  
 Flow Rate: 2.35ml/min  
 Temp.: 65°C  
 Det.: UV at 220nm  
 Inj.: 10µl, each compound 50µg/ml in mobile phase

1. Codeine
2. Strychnine
3. Papaverine
4. Quinine
5. Quinidine

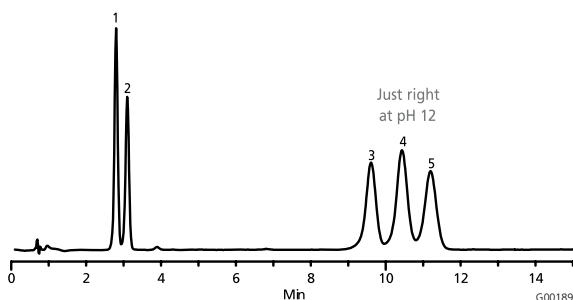
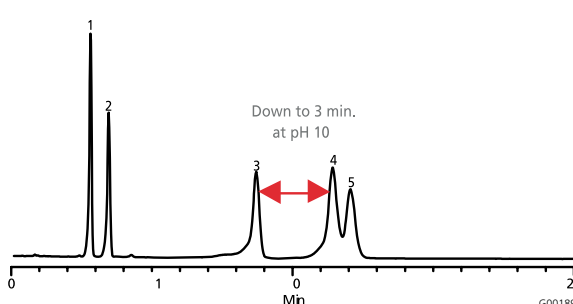
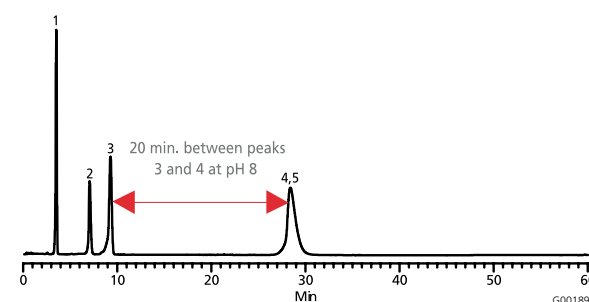
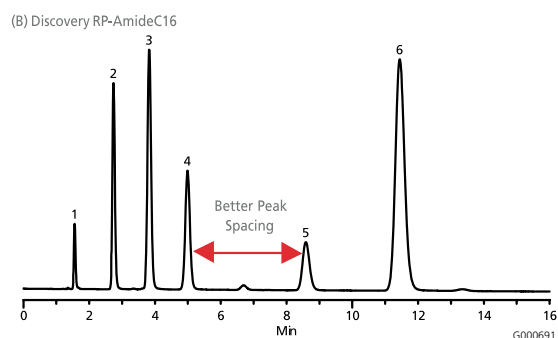
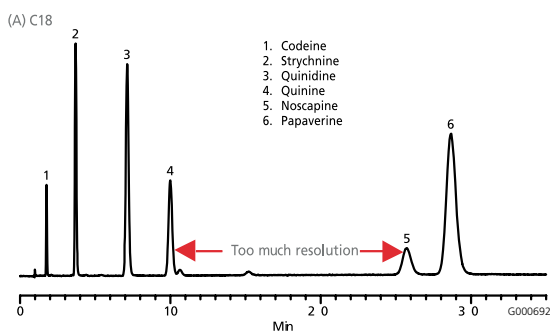


Figure 1. Discovery RP-AmideC16 Gives Better Resolution and Faster Analysis

- faster analysis from lower hydrophobicity
- better peak spacing (RP-AmideC16)
- better resolution of small impurities (RP-AmideC16)

Column: (A) C18 and (B) Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles  
 Mobile Phase: 80:20, 25mM Potassium Phosphate (pH 3.0):MeOH  
 Flow Rate: 2.0ml/min  
 Temp.: 35°C  
 Det.: UV at 254nm  
 Inj.: 10µl

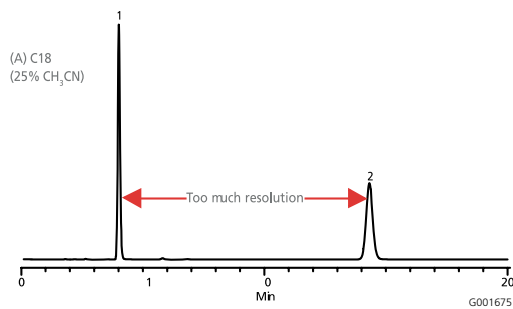


### Demonstration 3: Solving excessive retention of pharmaceutical compounds.

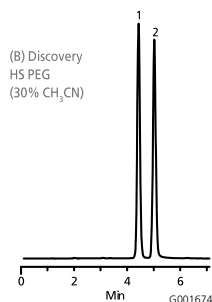
This example of excessive retention and resolution shows the skeletal muscle relaxant chlorzoxazone and its metabolite 6-hydroxychlorzoxazone. Analysis on a C18 column had excessive retention and resolution. The challenge was to reduce the retention of chlorzoxazone without losing retention of the more polar metabolite. By changing to a Discovery HS PEG column, run time and excessive resolution were decreased. Baseline separation was achieved in under six minutes. Many drug metabolites are more polar than the parent compound and subsequently elute before the parent compound. Discovery HS PEG is a good choice for looking at polar metabolites if there is a need for faster analysis while maintaining optimal resolution.

**Figure 3.** Chlorzoxazone - Excellent Separation on HS PEG; Excessive Retention and Resolution on C18

Column: Conventional C18, 15cm x 4.6mm ID, 5µm particles  
Mobile Phase: 20mM Acetic Acid in Water (pH 4.5 with Ammonium Hydroxide): CH<sub>3</sub>CN  
Flow Rate: 1.0ml/min  
Temp.: 30°C,  
Det.: UV at 285 nm  
Inj.: 10µl, each compound 100µg/ml  
1. 6-Hydroxychlorzoxazone  
2. Chlorzoxazone



Column: Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles (567416-U)  
Mobile Phase: 20mM Acetic Acid in Water (pH 4.5 with Ammonium Hydroxide): CH<sub>3</sub>CN  
Flow Rate: 1.0ml/min  
Temp.: 30°C,  
Det.: UV at 285 nm  
Inj.: 10µl  
1. 6-Hydroxychlorzoxazone  
2. Chlorzoxazone



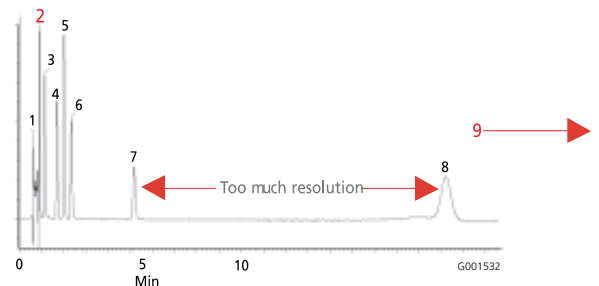
translates into greater LC-MS sensitivity. In this study, the unique selectivity of Discovery HS F5 toward basic solutes is exploited to provide retention under such conditions. The benefits for LC-MS experiments are discussed.

### Conclusion

These examples show that if there is a problem with excessive resolution or lengthy analysis time, the different selectivity or allowable pH range provided by Discovery functionalized reversed-phases may be the solution.

**Figure 4.** Resolution of Phenolic Compounds on Discovery HS PEG Compared to Standard C18

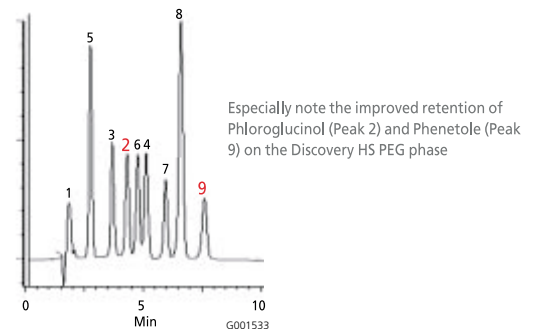
Columns: (A) Conventional C18 and (B) Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles  
Mobile Phase: 85:15, 10mM Ammonium Acetate (pH 6.8):MeCN  
Flow Rate: 1.0ml/min  
Temp: 20°C  
Det.: UV/Photodiode Array  
Inj.: 10µl (50µg/mL for each analyte)  
1. Uracil  
2. Phloroglucinol  
3. Pyrogallol  
4. Resorcinol  
5. Benzamide  
6. Catechol  
7. Phenol  
8. Nitrobenzene  
9. Phenetole



Column: Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles (567416-U)  
Mobile Phase: 20mM Acetic Acid in Water (pH 4.5 with Ammonium Hydroxide): CH<sub>3</sub>CN  
Flow Rate: 1.0ml/min  
Temp.: 30°C,  
Det.: UV at 285 nm  
Inj.: 10µl  
1. 6-Hydroxychlorzoxazone  
2. Chlorzoxazone

### (B) Discovery HS PEG

Note the selectivity differences of the phenolic compounds between the conventional C18 and the Discovery HS PEG phase.



### Demonstration 4: Solving excessive retention of hydroxylated compounds.

The last example in this section shows a set of phenolic compounds run under isocratic conditions on a C18. Note the excessive time between peaks 7 and 8 on the C18. By using the Discovery HS PEG phase, the excessive resolution is compressed to an ideal isocratic separation. Retention of analytes using mobile phases rich in organic modifier facilitates the desolvation process in electrospray ionization (ESI) sources. The increased ionization efficiency

**i** Information Request.....1301

# Discovery HS F5 Separates What C18 Cannot.



## Discovery HS F5 Delivers

- Increased Retention vs. C18
- Unique Selectivity vs. C18
- Improved Resolution vs. C18

**i** Information Request.....1301

# FREE

### USB Memory Stick

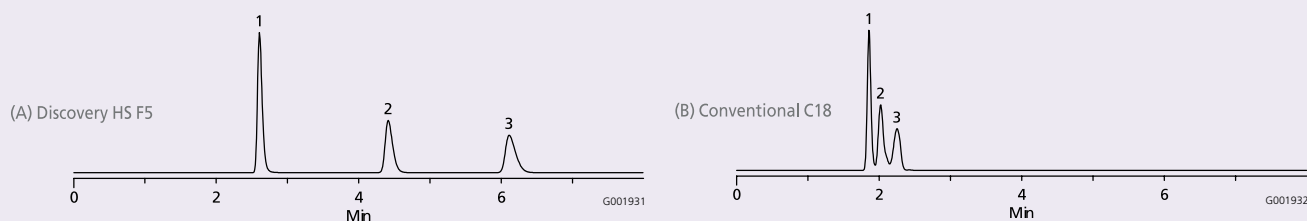
**Buy one column and get a free USB Memory Stick preloaded with our Method Development Brochure (Offer limited to 1 Stick per Customer)**

Quote Promotion Code F07 to qualify for this offer.  
Offer valid until 31st October 2004

## A Novel Fluorinated Reversed-Phase

Figure 2: Unique Selectivity of HS F5 Resolves Compounds Better than C18

Column: (A) Discovery HS F5 and (B) conventional C18, 15cm x 4.6mm ID, 5µm particles  
Mobile Phase: 10mM KH<sub>2</sub>PO<sub>4</sub>, pH 3.0 with H<sub>3</sub>PO<sub>4</sub>, (C18 separation has 5% CH<sub>3</sub>CN)  
Flow Rate: 1mL/min  
Temp.: 30°C  
Det.: UV at 280nm  
Inj: 10µL, each compound 100µg/mL  
1. Cytidine  
2. Cytosine  
3. 2'-Deoxycytidine



# Only the TRUE PEAKS are worth exploring- LC-MS CHROMASOLV® Solvents



## LC-MS CHROMASOLV® Solvents

- Accurately tested for LC-MS (analysis based on the reserpine test of many suppliers)
- Very low amount of metal ions, such as sodium and potassium
- High UV-transmittance for combination with UV and diode array detection
- Excellent gradient baseline particle tested
- Particle tested

## Specifications

Solvent content (GC)>99.9%(with the exception of ethylacetate:99.7%), LC gradient testing in UV and MS,metal impurities (Na,K,Mg,Ca:1ppm), UV-transmittance and fluorescence, particle tested.

## Ordering Information

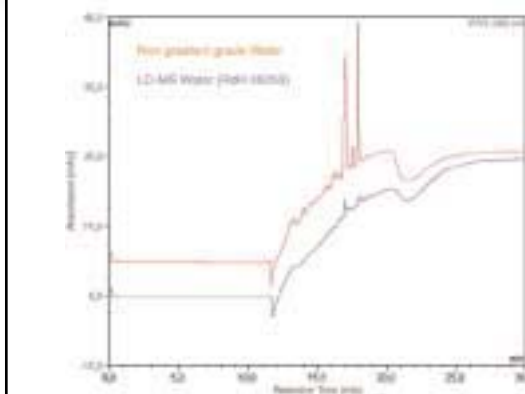
For product information and additional customized solvents, please feel free to contact us:

Frederik Pillong, Product Manager  
Phone ++41817552470, Fax ++41817552824  
Email:fpillong@sial.com

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



UV gradient at 205 nm, LC-MS Water (Cat. No. 39253) and non gradient grade water.



Product Range		
Prod. No.	Product Name	Pack Sizes
39253	CHROMASOLV® Water	1l
34967	CHROMASOLV® Acetonitrile	1l / 2.5l
34966	CHROMASOLV® Methanol	1l / 2.5l
34965	CHROMASOLV® 2-Propanol	1l / 2.5l
34972	CHROMASOLV® Ethylacetate	1l / 2.5l

**i** Information Request.....1304

## Did you know...?

Supelco offers a wide range of Hamilton HPLC syringes

### Ordering information Hamilton HPLC Syringes

Capacity	Model	Gauge	Length	Point	Prod No.	Qty.	Replacement Needles Prod No.
<b>700 Series Syringes For Rheodyne, Valco VISF-2, Altex and SSI Injection Valves</b>							
25µL	702SNR	22s	2"	#3	58381	-	--
50µL	705SNR	22s	2"	#3	58382	-	--
100µL	710SNR	22s	2"	#3	58383	-	--
250µL	725SNR	22	2"	#3	58384	-	--
500µL	750SNR	22	2"	#3	26222-U	-	--
<b>800 Series LC Syringes For Waters U6K Loop Injector</b>							
25µL	802RNW	25s	5cm	#3	58391	1	58398
50µL	805RNW	25s	5cm	#3	58392	1	58398
100µL	810RNW	25s	5cm	#3	58393	1	58398
250µL	825RNW	25s	5cm	#3	58394	1	58399
<b>1700 Series Gastight Syringes – For Rheodyne, Valco VISF-2, Altex and SSI Injection Valves</b>							
25µL	1702RNR	22s	2"	#3	20886	3	58649
50µL	1705RNR	22s	2"	#3	20887	3	58649
100µL	1710RNR	22s	2"	#3	20888	3	58649
250µL	1725RNR	22	2"	#3	20889	3	58650-U
500µL	1750RNR	22	2"	#3	20890-U	3	58650-U

# FREE

## Maglite Torch, when you buy any HPLC syringe

Promotional code: F99

(Product Number SU854200)  
For SGE see page 416 of the Supelco main catalogue. Offer valid until 31st October 2004



## HPLC Accessories

Rheodyne RheBuild Kits, Fittings, Tools

### RheBuild Kits

These kits include all of the tools and parts you need to repair your Rheodyne injector. The parts included are those most likely to be damaged, worn, or lost. Kits for front-loading injectors include: rotor seal, stator face assembly, isolation seal, needle guide, needle port cleaner, 2 hex keys, operating instructions, and a mini-manual. Parts may vary, depending on valve model.



Valve Model	Prod. No.
3725/3725i/3725-038/3725i-038	55043
7010	55044
7010 with stator	504602
7125/7126	55045
7125-081	55046
7410	55047
7520/7526	55048
7650/9650 TPMV	504629
7725/7725i/7726	55049
7750 TPMV Series	7750999
8125/8126	55050-U
9010/9040	55052
9125/9126	55051
9725/9725i/9726	55053
9750 TPMV Series	9750999
<b>KITS FOR SupelPRO/LabPRO UNITS</b>	
Injector with Purge <sup>1</sup>	54398-U
2-Channel Selector <sup>1</sup>	54394-U
3 Column Selector <sup>1</sup>	54388-U
11-Port/10-Position <sup>1</sup>	57443-U
2-Position, 6-Port <sup>1</sup>	54395-U
2-Position, 10-Port <sup>1</sup>	54396-U
Solvent Selector, 1/16"	54392-U
Solvent Selector, 1/8"	54393-U
Low Pressure Switching, 1/16"	54390-U

<sup>1</sup>Either stainless steel or PEEK unit.

For all Rheodyne products please refer to the Supelco Catalogue page 92.

# FREE

## RheoTool, when you buy any valve or rebuilt kit (Cat. No 55087-U)

Offer limited to 1 unit per customer

Promotional code: F02

Offer valid until 31st October 2004

Your Problem Solving Partner in Chromatography

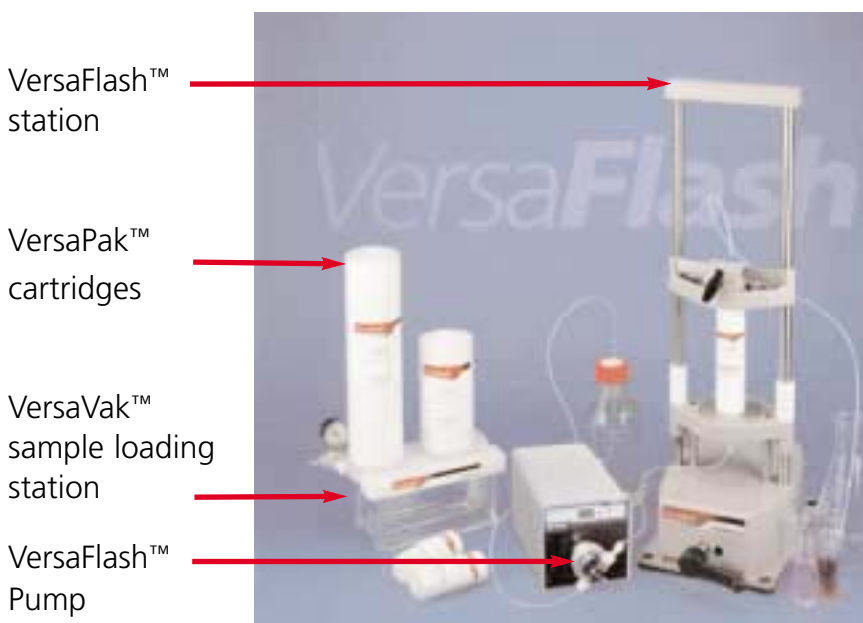
# VersaFlash™

## High Throughput Flash Purification

The VersaFlash high throughput flash purification system is a fast, easy and economical solution for the isolation and purification of natural products and target substances from reaction mixtures.

The VersaFlash system includes:

### VersaFlash™ - The most versatile High Throughput Flash Purification System



### Easy Scale-up for Larger Samples:

- Remove the small used cartridge by turning the handle to idle position and pulling it out.
- Raise and adjust the upper platen to the proper height and re-tighten the toggle bolt.
- Secure the cartridge by turning the handle to the operate position. Ready to run.

Pure....

....& Simple

### Respond **NOW** for further information

or for technical questions, please contact the technical service department at your local Sigma-Aldrich Office.

For **online** information - please visit [sigma-aldrich.com/versaflash](http://sigma-aldrich.com/versaflash)

### Save Time:

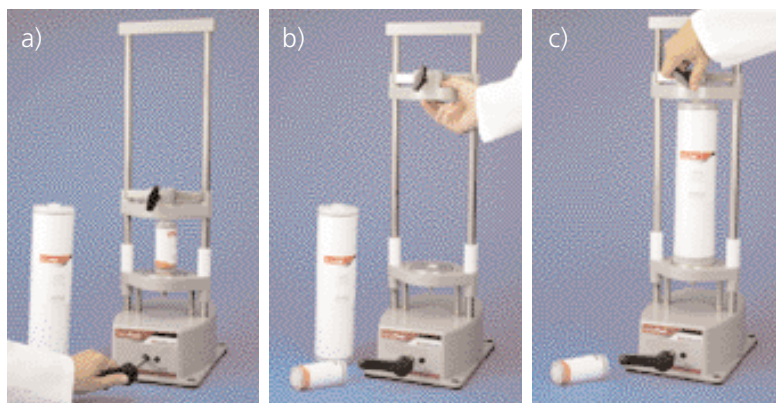
- Fast and easy cartridge change-over
- Fast elution of desired substances
- Speedy scale-up to larger sample loads
- One-step, bi-modal purifications
- Rapid loading and partial separation of multiple sample mixtures
- Instant mobile phase change-over

### Save Money:

- Pre-compressed cartridges eliminate the expense of additional compression barrels
- All VersaPak cartridges fit on the VersaFlash station
- Rev-Elution saves solvent costs
- Direct sample loading eliminates the cost of cartridge inserts

### Improve Performance:

- Spherical particles result in low band spreading
- Upward flow improves the separation
- Leak-resistant system design saves samples, solvents and cartridges
- Unobstructed cartridges allow monitoring of the separation



**i** Information Request.....1305

**S** SUPELCO

## SPME ARTICLE

# Improvements in SPME Reproducibility Through Enhancements in the Fiber Manufacturing Process

Robert Shirey [bshirey@sial.com](mailto:bshirey@sial.com)

Improvements have been made in the coating of the SPME fibers using an automated, continuous manufacturing process. This continuous process has resulted in significant performance advantages including:

- Bonding of the coating to the fiber core is improved because each individual layer is cured
- Coating thickness is more uniform throughout the 20m fiber strand compared to the uniformity of the coating thickness applied on multiple strands
- Application rate is motor controlled assuring that the adsorbent/binder ratio remains consistent over the entire fiber length
- Better coating application control within a lot assures better reproducibility between fiber lots

## Previous (batch) Coating Procedure

1. Applications of multiple coats were applied by pulling of individual fibers
2. Several layers applied before heat is applied for bonding
3. Fiber strand lengths limited to short lengths
4. 20-25 strands are coated per lot
5. Each strand is optically measured to obtain desired coating thickness range.

## Automated (continuous) Coating Procedure

1. Applications of multiple coats are applied with a motor controlled draw
2. Each layer is individually bonded by passing the fiber through a heating chamber
3. Fiber strand lengths are typically 20m in length consisting of one lot
4. The 20m fiber strand is measured optically at multiple sites throughout strand

Table 1 shows the precision of the PDMS-DVB fiber by making 6 extractions with 1 fiber of a mixture of solvents at various concentration levels in water containing 25% NaCl, pH 11 (0.05M Na<sub>3</sub>PO<sub>4</sub>). The fiber was immersed for 10 min with agitation and desorbed at 250°C and analyzed on a 30m x 0.32mm x 4.0µm SPB™-1 sulfur column.

The reproducibility of batch-coated and automated-coated (continuous coating fiber process) was evaluated and shown in Table 2 and 3 respectively.

The results from the 2 tables above show that the average variability of analyte response between fiber lots produced by the automated-coating process was much less than the average variability between lots of batch-coated fibers, 8.6% RSD and 21.1% RSD, respectively.

The coating thickness variation with the automated-coating process was also significantly less at 4.1% compared to the batch-coating process at 16% RSD. The results show that the coating thickness variation plays a major role in the overall variability for the PDMS-DVB fibers. This is verified in Figure A that compares coating thickness to analyte response.

**Table 1. Precision of Extractions with One Fiber**

Analytes	Concentration (ppm)	Average	% RSD
Methanol	75	263	1.3%
Dimethylamine	5	3874	3.5%
Ethanol	50	644	0.7%
Acetonitrile	25	1487	0.8%
Acetone	10	2575	0.7%
Isopropanol	10	1802	0.7%
n-Propanol	5	1719	0.6%
MTBE	2	1742	2.9%
1-Butanol	2	2550	0.3%
Trimethylamine	1	4574	4.5%
Dioxane	5	1993	0.7%
Pyridine	0.5	2175	1.4%
2-Hexanone	0.1	3539	1.6%
Hexanal	0.1	247	2.4%
Aniline	0.5	4058	1.4%
<b>Average</b>			<b>1.6%</b>

**Table 2. Analyte Response Variation Between Batch-Coated Fiber Lots**

Analytes	Average	% RSD
Methanol	217	24.3%
DMA	7223	43.4%
Ethanol	535	16.8%
Acetonitrile	1149	21.5%
Acetone	2052	23.2%
Isopropanol	1475	20.8%
n-Propanol	1412	21.2%
MTBE	1531	23.7%
1-Butanol	2155	22.8%
TEA	4529	8.0%
Dioxane	1694	23.3%
Pyridine	1772	20.4%
2-Hexanone	3227	16.9%
Hexanal	215	12.6%
Aniline	4234	22.2%
<b>Overall Average</b>		<b>21.1%</b>
<b>Coating Average, 59µm</b>		<b>16.0%</b>

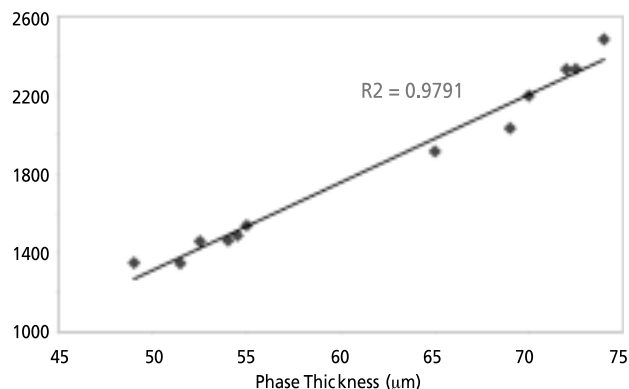
The variation between PDMS-DVB fibers is due primarily to the variability in coating thickness as shown in Figure A. We have shown that the automated fiber coating process improves reproducibility of fibers between and within lots from the standpoint of coating thickness and analyte response.

**Table 3. Analyte Response Variation Between Automated-Coated Fiber Lots**

Analytes	Average	% RSD
Methanol	291	7.1%
DMA	5069	19.1%
Ethanol	715	9.1%
Acetonitrile	1571	7.8%
Acetone	2873	6.9%
Isopropanol	2118	9.9%
n-Propanol	2001	9.8%
MTBE	2126	7.4%
1-Butanol	3119	9.6%
TEA	4747	8.5%
Dioxane	2405	8.6%
Pyridine	2599	8.5%
2-Hexanone	3939	3.3%
Hexanal	242	7.8%
Aniline	4745	5.5%
<b>Average</b>		<b>8.6%</b>
<b>Coating Thickness</b>	<b>71.7<math>\mu</math>m</b>	<b>4.1%</b>



**Figure A. Summed Area Counts vs. Coating Thickness**



**i** Information Request.....1306

# FREE

Start SPME now and get a **FREE** sampling stand. Buy a holder and a pack of fibers, and get a stand form.

Cat No. 57333-U (4mL vials) or 57357-U (15mL vials).

Stir plate not included. (Offer limited to 1 per customer)

Quote Promotion Code F12

Offer valid until 31 October 2004

## If you haven't yet received a copy of our 5th edition SPME CD - apply now

### Solid Phase Microextraction (SPME) Application Guide CD

The searchable CD format includes a big range of application references, which we organize by analyte and matrix for easy reference. There are new references added to this fifth edition along with several new bulletins. The guide helps the analytical chemist to choose the SPME fiber to use for their sample preparation problems. Most entries list the SPME conditions and instrumentation used for the application.

All the Supelco SPME literature is also found on the CD, including the Troubleshooting Guide and Guide to Quantitation with SPME.



**SPME Application CD**

**Update 5**

**i** Information Request.....1307

# 96-Well Starter Kit and PlatePrep Vacuum Manifold



The PlatePrep vacuum manifold consists of a clear acrylic top allowing for easier inspection of flow rates during SPE 96-well plate processing. The polypropylene base offers excellent chemical resistance while a single remote vacuum gauge/bleed valve controls flow through all the wells. Use this compact vacuum manifold in conjunction with a Discovery SPE 96-well plate to process up to 96 samples concurrently. The single valve control, parallel processing capabilities, and uniform flow dynamics allow for easier method development, reduces clutter, and allows for greater reproducibility. Unused wells can be covered and used at a later date.

### Starter Kit Includes:

- PlatePrep Vacuum Manifold
- 1 96 Sq. Well Collection Plate, 2ml, PP
- 2 Disposable Reservoir/Waste Trays, PVC
- 1 96 Sq. Well Piercable Cap Mat
- 5 Reagent Reservoirs
- 1 Cluster Tube Rack

### Ordering information

Description	Prod. No.
96-Well Starter Kit with PlatePrep Manifold	575650-U

## FREE

### FREE Mug

When you complete our online survey.

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

Offer valid until 31 October 2004

## LpDNPH Cartridges

Use LpDNPH cartridges 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, where they are converted to the hydrazone derivatives. The derivatives are eluted from the cartridge in acetonitrile and are analyzed by HPLC. The S10x cartridge fits automated samplers (e.g., XonTech, Inc., ATEC Atmospheric Technology). Use in a XonTech unit requires a special, reusable adapter (Cat. No. 505307). The S10L cartridge is for analysts who prefer the shorter dimensions and do not need an adapter for sampling. The cartridge, reversible and eluted by connecting it to a syringe barrel<sup>1</sup>, acts as a reservoir for gravity-fed elution solvent. H10, H30, and H300 cartridges contain higher loadings of 2,4-DNPH, making them suitable for use in high concentration environments. The disposable ozone scrubber is similar to an S10L cartridge, containing 1.5g of high purity potassium iodide (ozone capacity at least 100,000ppb/hour).



### Ordering information

Prod. No.	Qty	Description
21026-U	10	S10
21024-U	10	S10 Starter Kit <sup>2</sup>
21014	50	S10
505293	10	S10x
505358	10	S10L
505315	10	H10
505323	10	H30
505331	10	H300
505285	10	Ozone Scrubber
<b>Accessories</b>		
21018-U	10	Cartridge Adapters for S10, H10, H30
57267	6	for H300
505307	10	XonTech Adapters for S10x Male Luer Fittings
21016	20	to 1/8" tubing
23364	20	to 3/16" tubing
24856	10	to 1/4" tubing
21017	20	Female Luer Fittings
21015	20	Female Luer Couplers
25064-U	20	Male Luer Couplers
21019-U	6	Lapel Clips
21012	100 nos	Bar Code Labels
57242	30	Polypropylene Tubes, 6ml <sup>3</sup>
21043-U	1	Universal Elution Rack

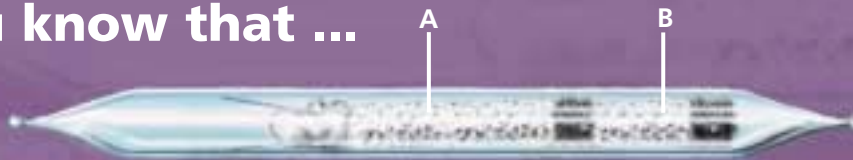
<sup>1</sup>We recommend a 6ml polypropylene tube, Cat. No. 57242.

<sup>2</sup>10 cartridges plus adapters and male luer fittings for various air sampling pumps.

<sup>3</sup>Reservoirs for S10L cartridges.

Your Problem Solving Partner in Chromatography

# Did you know that ...



A/B = adsorbent beds

## Supelco offers Solvent Desorption Tubes

### Ordering information

Orbo No.	Adsorbent	Bed Wt. (mg) A/B	OD x Length (mm)	SKC INC. Equivalent	Qty.	Prod. No.
<b>CHARCOAL</b>						
32 large	Activated coconut charcoal (20/40)	400/200	8 x 100	226-09	50	20228
32 small	Activated coconut charcoal (20/40)	100/50	6 x 75	226-01	50	20267-U
33	Activated petroleum charcoal (20/40)	700/390	8 x 150	226-36	50	20259
34	Activated coconut charcoal, specially treated	400/200	8 x 105	–	25	20211
301 <sup>1</sup>	Charcoal (20/40)	150	6 x 75	–	50	20039
303 <sup>1</sup>	Petroleum charcoal (20/40)	100/50	6 x 75	226-38	50	20040-U
304 <sup>1</sup>	Charcoal (low Ni) (20/40)	120/60	6 x 80	–	50	20041
306 <sup>1</sup>	Petroleum charcoal (20/40)	400/200	8 x 110	–	50	20073-U
351 <sup>1</sup>	4-tert-butyl catechol on charcoal	100/50	6 x 75	226-73	50	20042
353 <sup>1</sup>	HBr on petroleum charcoal	100/50	6 x 75	226-38-03	25	20044
354 <sup>1</sup>	Alkali-treated charcoal (AVL Barneby Cheney, 580-19)	100/50	6 x 75	226-67	50	20045
355 <sup>1</sup>	4-tert-butyl catechol on charcoal	110/55	6 x 75	–	50	20046
356 <sup>1</sup>	4-tert-butyl catechol on charcoal	400/200	8 x 110	–	50	20047
<b>CARBON</b>						
91	Carbosieve S-III carbon molecular sieve <sup>2</sup>	130/65	6 x 75	226-121	25	20360
90	Carboxen-564 3 carbon molecular sieve <sup>4</sup>	160/80	6 x 75	226-81	25	20358
92	Carboxen-564 carbon molecular sieve <sup>4</sup>	160/80	6 x 75	226-81	25	20362
78	HBr on Carboxen-564 carbon molecular sieve	400/200	6 x 110	–	25	20355
100	Carbotrap (20/40)	350/175	7 x 110	–	25	20255-U
101	Carbotrap (20/40)	100/50	6 x 75	–	25	20254-U
77 <sup>1</sup>	H <sub>2</sub> SO <sub>4</sub> -treated carbon bead (20/30)	500/250	8 x 150	226-29	50	20036
<b>SILICA GEL</b>						
52 small	Activated silica gel (20/40)	150/75	6 x 75	226-10	50	20229
52 large	Activated silica gel (42/60)	150/150	8 x 75	226-48	50	20263
507	Silica gel (20/40)	520/260	8 x 110	226-15	50	20870-U
53	Activated silica gel (20/40) with glass fiber filter	400/200	7 x 100	226-10-03	50	20265
502 <sup>1</sup>	Activated silica gel (20/45)	100/50	6 x 75	226-51	50	20030-U
504 <sup>1</sup>	Activated silica gel (45/60)	150/75	6 x 75	–	50	20031
506 <sup>1</sup>	Activated silica gel (45/60)	300/150	8 x 75	22226-10-04	50	20032
554 <sup>1</sup>	H <sub>2</sub> SO <sub>4</sub> -coated silica gel (20/40)	150/75	6 x 75	226-53	50	20033
<b>FLORISIL</b>						
60	Florisil (30/45)	100/50	6 x 75	226-39	50	20351
<b>POROUS POLYMERS</b>						
42 small	Supelpak 20E (20/40)	66/33	6 x 75	–	50	20262
42 large	Supelpak 20P (20/40)	100/50	10 x 100	–	50	20264-U
43	Supelpak 20U (20/40) <sup>4</sup>	100/50	8 x 100	226-30-04	50	20258
44	Supelpak 20E (20/40) <sup>4</sup>	100/50	8 x 100	226-30-04	50	20260-U
49P	Supelpak 20P (20/40) with glass fiber filter (OVS-2)	270/140	–	226-30-16	10	20350
23	2-(Hydroxymethyl)piperidine on Supelpak 20N (20/40)	120/60	6 x 85	226-118	25	20257-U
24	2-(Hydroxymethyl)piperidine on Supelpak 20N (20/40)	150/75	6 x 105	226-117	25	20231

For additional products not listed here, please see our Supelco Catalogue pp. 171

**i** Information Request.....1308

# FREE

## Tube cutter with your next order (one per customer).

Quote Promotion Code F11 to qualify for this offer. Offer valid until 31st October 2004



## AIR MONITORING

### ATIS Adsorbent Tube Injector System

#### ATIS™ Adsorbent Tube Injector System

The Supelco ATIS is a sample preparation device for adsorbent tubes. The Adsorbent Tube Injector System employs the technique of flash vaporization to vaporize the sample into a continuous flow of an inert gas, which carries the sample to the adsorbent tube. The sample pathway of the Adsorbent Tube Injector System is constructed of glass and stainless steel. The calibration standard is injected by a syringe through a replaceable septum in the center of the injection glassware, which is heated.

- Injecting calibration standards onto adsorbent tubes, to calibrate your analytical system
- Injecting surrogates and system monitoring compounds onto adsorbent tubes before or after sampling
- Removes moisture from tubes prior to analysis (Dry purging)
- Connect an air-sampling bag to the outlet of the ATIS to vaporize a calibration standards prior to assure complete vaporization.

The ATIS will accept either 1/4" or 6mm OD Thermal Desorption tubes. Included is a Luer/Hose Barb adapters to connect a variety of solvent desorption tubes.

The temperature range of the ATIS is ambient to 120°C. The flow range is 0 to 100mL/min.

The ATIS includes the injection glassware, a constant flow controller with a on/off valve, the heating source, spare parts along with all the necessary fittings and tubing. You simply plumb it to a regulated source of nitrogen or helium and plug it in to the appropriate electrical source.



P000849

#### Ordering information

Prod. No.	Description
28520-U	110VAC Model
28521-U	220VAC Model
28526-U	Replacement Injection Glassware

#### For Further Information

Refer to page 180 of the Supelco catalogue

“Have you seen our complete range of Air Monitoring products?”

See Supelco Catalogue p.170-183

## AIR MONITORING

### Air Sampling Pumps

#### Escort Elf Air Sampling Pump

An electronic laminar flow sensor in this easy-to-operate, state-of-the-art sampling pump provides constant flow control, unaffected by changes in battery voltage, temperature, sample load, or altitude. An internal secondary standard calibrates the pump continuously, requiring only monthly calibration with a primary standard. A built-in counter monitors total operating time, and reminds you when a primary calibration is required. The pump also features a low battery function with an indicator light, and blocked flow detection. An LED read-out alternately displays flow rate and elapsed sampling time. Order charger separately.



28160-U

#### Ordering information

Cat No.	Description
28160-U	Escort Elf Air Sampling Pump

#### Gemini Twin Port sampler

This pump attachment is designed for low flow industrial hygiene sampling, such as gas or vapor monitoring, using sorbent tubes. Two needle valves provide independent flow control for simultaneous collection on two tubes but, by closing the flow to one valve, a single tube can be used. The sampler is compatible with any personal sampling pump capable of a 1.5LPM flow rate at a load of 25 inches of water. Total flow cannot exceed 500mL/min. Each sampler comes with two tube protectors, one for small tubes (<2" long) and one for large tubes (<4 1/2" long), and tubing required to connect the sampler to the sampling pump.

#### Ordering information

Prod. No.	Description
28118-U	Gemini Twin Port Sampler <sup>1</sup>

- CE approved.
- 1 Replacement parts available.
- 2 Use to charge battery pack from automobile dashboard (most), or run with charged
- 3 Approvals for PAS 500: ETL — Class I Groups A, B, C, D; Class II Groups E, F, G;



28118-U

Your Problem Solving Partner in Chromatography

# Fast Analysis of Fish Oils and Animal Lipids on the SUPELLOWAX 10 Column

Katherine Stenerson kstenerson@sial.com

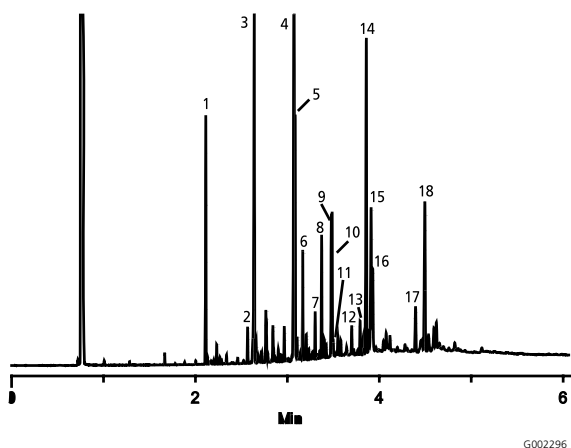
Polyunsaturated fatty acids (PUFAs) are found in both fish and land animal food sources. Recent research findings suggest that there are potential health benefits associated with the consumption of certain PUFAs. Specifically, these studies indicate that the omega-3 PUFAs found in fish may lower a healthy individual's risk of developing cardiovascular disease (1). Capillary GC is the analytical method of choice for fatty acids due to its ability to provide high selectivity and resolving power for complex mixtures. Current methodologies for the analysis of fatty acids in food require saponification, and esterification of samples prior to the analysis of methyl esters using a standard polyethylene glycol (PEG) capillary column. The Omegawax™ 250, 320, and SUPELLOWAX™ 10 capillary columns are often used for the analysis of omega-3 and omega-6 PUFAs from fish and animal sources. Polyethylene glycol phase columns such as Omegawax and SUPELLOWAX provide minimal overlap in fatty acid methyl esters (FAMES) of different carbon chain lengths, and elute FAMES according to their degree of unsaturation. This provides for good separation of the predominate omega-3 PUFAs C22:6n3 (DHA) and C20:5n3 (EPA), and the predominate omega-6 PUFA C20:4n6 (2).

**Figure A.** Analysis of FAMES in Cod Liver Oil on the SUPELLOWAX 10, 15m x 0.10mm ID, 0.10µm

#### Identical conditions for Figures A, B and C

Column: SUPELLOWAX 10, 15m x 0.10mm ID, 0.10µm  
 Cat. No.: 24343  
 Oven: 140°C, 40°C/min to 280°C (2 min)  
 Inj.: 250°C  
 Det.: FID, 260°C  
 Carrier gas: Hydrogen, 50cm/sec, constant  
 Injection: 0.2µL, 200:1 split  
 Liner: Split, cup design  
 Samples: 50mg/mL in methylene chloride  
 PUFA-1, Marine Source (Cat. No. 47033)

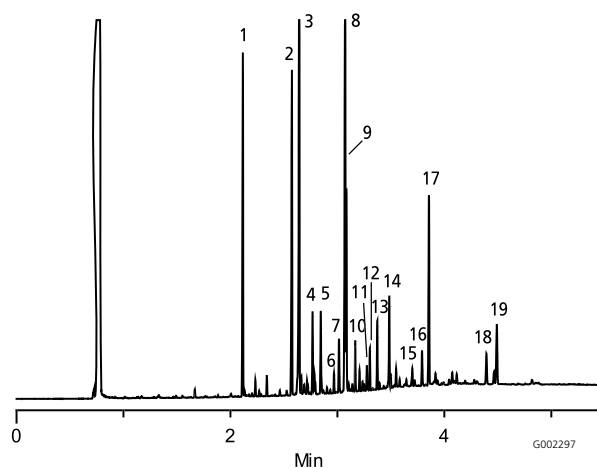
1. C14:0	5. C18:1n7	9. C20:1n11	13. C20:4n3
2. C16:0	6. C18:2n6	10. C20:1n9	14. C20:5n3
3. C16:1n7	7. C18:3n3	11. C20:1n7	15. C22:1n11
4. C18:1n9	8. C18:4n3	12. C20:4n6	16. C22:1n9
			17. C22:5n3
			18. C22:6n3



**Figure B.** Analysis of FAMES in Menhaden Oil on the SUPELLOWAX 10, 15m x 0.10mm ID, 0.10µm

Sample: 50mg/mL in methylene chloride  
 PUFA-3, from Menhaden Oil (Cat. No. 47085-U)

1. C14:0	6. C16:4n1	11. C18:3n4	16. C20:4n3
2. C16:0	7. C18:0	12. C18:3n3	17. C20:5n3
3. C16:1n7	8. C18:1n9	13. C18:4n3	18. C22:5n3
4. C16:2n4	9. C18:1n7	14. C20:1n9	19. C22:6n3
5. C16:3n4	10. C18:2n6	15. C20:4n6	



An example of a popular fast GC method is shown in Figure A. An analysis of various natural mixtures containing FAMES on a 0.10mm ID SUPELLOWAX 10 column provides an excellent example of the selectivity of the column. A shorter column with a narrow ID was used in combination with a fast oven ramp rate and hydrogen carrier gas to reduce the average analysis time of 40-45 minutes to just under 5 minutes. The analyses of methylated samples of cod liver and menhaden oil are presented in Figures A and B. The 15m x 0.10mm ID column had resolution comparable to the Omegawax 250 and 320. C20:5n3 (EPA) and C22:6n3 (DHA) were resolved from other sample components, and the analysis demonstrated the high levels of these PUFAs present in marine samples. The analysis also re-vealed the relative differences in the fatty acid compositions between the two types of fish. A FAME sample derived from an animal source is presented in Figure C. This sample shows significantly lower levels of EPA and DHA than the two fish samples, with the omega-6 fatty acid C20:4n6 as the predominate PUFA.

Fast GC is becoming increasingly popular due to its ability to increase productivity by reducing analysis time. The 15m x 0.10mm ID x 0.10µm SUPELLOWAX 10 can be used for the fast analysis of FAMES. By employing the principles of fast GC, one can realize a reduction in analysis time as much as 10-fold.

**References**

1. Kris-Etherton, P.M., Harris, W.S., Appel, L.J., *Circulation*, 106, 2747-2757 (2002.)
2. Supelco Application Note T394034 (1994.)

For more information request Reproducible Analyses of Omega-3 and Omega-6 Fatty Acid Methyl Esters by Capillary GC, T394034 (APO), and Analyzing Fatty Acids by Capillary Gas Chromatography,

**Trademarks**

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- Chromosorb - Manville Corp.
- Finnigan - Thermo Finnigan, LLC Corp.
- OV - Ohio Valley Specialty Chemical Co.
- Teflon - E.I. duPont de Nemours

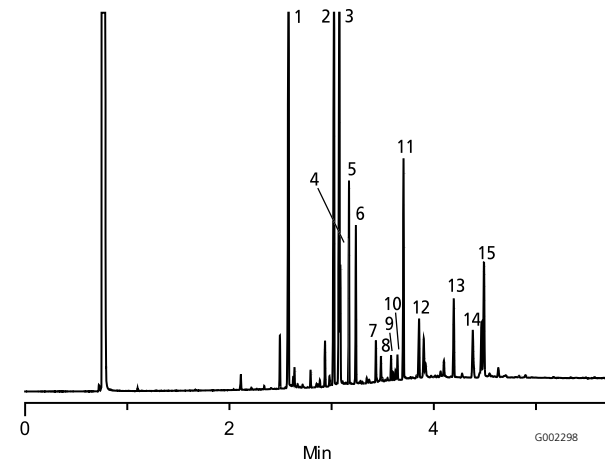
**Patents**

SPME - Technology licensed exclusively to Supelco. US patent #5,691,206; European patent #523,092

**Figure C.** Analysis of FAMES in Menhaden Oil on the SUPELCOWAX 10, 15m x 0.10mm ID, 0.10µm

Sample: 50mg/mL in methylene chloride  
PUFA-3, from Menhaden Oil (Cat. No. 47085-U)

- |            |             |             |
|------------|-------------|-------------|
| 1. C16:0   | 6. C18:3n6  | 11. C20:4n6 |
| 2. C18:0   | 7. C20:0    | 12. C20:5n3 |
| 3. C18:1n9 | 8. C20:1n9  | 13. C22:5n6 |
| 4. C18:1n7 | 9. C20:2n9  | 14. C22:5n3 |
| 5. C18:2n6 | 10. C20:3n6 | 15. C22:6n3 |



**i** Information Request.....1309

**OFFER**

Please see our offer on the back page.

**Did you know...?**

The presence of moisture during a derivatization reaction will reduce the effectiveness of the reagent and may affect the derivative yield. Prevent moisture related problems by always purchasing reagents packaged under nitrogen, and by selecting the smallest practical size. Tightly seal opened reagents during storage. Use a microliter syringe when working with small reagent quantities to reduce exposure to atmospheric moisture. Add anhydrous sodium sulfate to the reaction mixture to trap water that may be present in the sample.

**GC ACCESSORIES**

**Supelcarb Split Vent Trap**

**Adsorb toxic compounds longer than with conventional traps.**

Typical split vent flow rates of 10-100ml/min carry most of the injected sample into the laboratory atmosphere. Many GC manufacturers recommend venting the split injection systems into a fume hood, but this is often impossible due to space constrictions. Another approach is to connect an adsorbent trap to the split vent outlet.

A Supelcarb Split Vent Trap traps a broad range of organic compounds. The high-capacity carbonaceous adsorbent has been specifically prepared for split vent applications – a narrow particle size distribution and spherical shape allow tight packing and less gas channeling than the irregular shape of activated charcoal particles.

Data show that breakthrough on the Supelcarb trap occurs after approximately 2 weeks at 65ml/min. Breakthrough on other traps often occurs before the manufacturer's recommended replacement time. We recommend using Supelcarb traps for longer effective trapping, and replacing the trap every two weeks.



For Further Information

please see Supelco Catalogue, page 271

**BREAKTHROUGH ON SPLIT VENT TRAPS**

	TYPE A	TYPE B	TYPE C	SUPELCARB
Breakthrough Time (Hours)	22.5	49.5	249.0	374.0
Breakthrough Volume (Liters)	88	193	971	1458
No. of Injections Before Breakthrough	45	99	498	748
Manufacturer's Recommended Replacement Time	168 hr	300 inj.	720 hr	336 hr

**Ordering information**

Prod. No.	Description
<b>SUPELCARB SPLIT VENT TRAP STARTER KIT</b>	
22536	(one trap and an attachment kit)
<b>REPLACEMENT SUPELCARB TRAPS</b>	
2253502	Two Traps
2253505	Five Traps

**TRIAL OFFER 35% OFF**

**for one cartridge**

Quote Promotion Code X58 to qualify for this offer.  
Offer limited to one discounted cartridge per customer  
Offer valid until 31st October 2004

Your Problem Solving Partner in Chromatography

# Packed Columns

## Agilent Technologies™



Supelco the **authorized supplier** of packed GC columns for **Agilent Technologies and Agilent Technologies customers.**

Agilent Technologies has made a decision to exit the packed GC column business.

In an effort to maintain a continuous supply of product to their customers, **Agilent Technologies has named Supelco the authorized supplier of packed GC columns for Agilent Technologies and Agilent Technologies customers.**

For your convenience, you may reference an Agilent Technologies part number with your first order. In most cases, your Sigma-Aldrich/Supelco representative will provide you with a corresponding Supelco part number for future orders.

- All glass columns will fit Agilent/HP 5880, 5890, 5987 and 6890 GCs of configuration A, on-column injection, all detectors except TCD.
- All stainless steel columns are general configuration. You can carefully bend to fit most GCs.

See a short list of our Products list. For the complete list please take a look to **Your Supelco Catalogue pp.236.**

## For 'All' your packed column needs.

### Ordering information

Agilent Cross-Reference	Description	Type	Length	OD	ID	Prod. No.
19001A-101	80/100 Chromosorb 101	SS	6 ft	1/8"	2.1mm	12712
19001A-102	80/100 Chromosorb 102	SS	6 ft	1/8"	2.1mm	13794
19001A-103	80/100 Chromosorb 103	SS	6 ft	1/8"	2.1mm	13104-U
19001A-A01	80/100 HayeSep A	SS	6 ft	1/8"	2.1mm	13105-U
19001A-A11	10% OV-1 on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13106-U
19001A-A52	5% OV-1 on 100/120 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13107-U
19001A-B11	10% OV-17 on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13109-U
19001A-B51	5% OV-17 on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13114-U
19001A-D11	10% OV-101 on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13115-U
19001A-D12	10% OV-101 on 100/120 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13116-U
19001A-F12	10% OV-225 on 100/120 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13119-U
19001A-G11	10% Silar 5 CP on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13121-U
19001A-J11	10% SE-30 on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13122-U
19001A-J51	5% SE-30 on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13124-U
19001A-K11	10% Silar 10 CP on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13125-U
19001A-M11	10% Carbowax 20M on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13126-U
19001A-M12	10% Carbowax 20M on 100/120 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13127-U
19001A-M51	5% Carbowax 20M on 80/100 Chromosorb W HP	SS	6 ft	1/8"	2.1mm	13128-U
19001A-MA1	45/60 Molecular Sieve 5A	SS	6 ft	1/8"	2.1mm	13130-U
19001A-MA2	60/80 Molecular Sieve 5A	SS	6 ft	1/8"	2.1mm	13133-U
19001A-MX1	45/60 Molecular Sieve 13X	SS	6 ft	1/8"	2.1mm	13134-U
19001A-MX2	60/80 Molecular Sieve 13X	SS	6 ft	1/8"	2.1mm	13136-U
19001A-N00	80/100 Porapak N	SS	6 ft	1/8"	2.1mm	13141-U
19001A-N01	80/100 HayeSep N	SS	6 ft	1/8"	2.1mm	13144-U
19001A-P00	80/100 Porapak P	SS	6 ft	1/8"	2.1mm	13146-U
19001A-Q00	80/100 Porapak Q	SS	6 ft	1/8"	2.1mm	12437
19001A-Q01	80/100 HayeSep Q	SS	6 ft	1/8"	2.1mm	13801
19001A-QS0	80/100 Porapak QS	SS	6 ft	1/8"	2.1mm	13787
19001A-R00	80/100 Porapak R	SS	6 ft	1/8"	2.1mm	13156-U
19001A-S00	80/100 Porapak S	SS	6 ft	1/8"	2.1mm	13161-U
19001A-T00	80/100 Porapak T	SS	6 ft	1/8"	2.1mm	13163-U

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



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

# GC Accessories for Agilent Technology Instruments

Reduce your Risk, Prevent Problems,  
Save Time and Money

*Teaming Supelco's capillary GC columns with our high performance accessories will reduce the risk of chromatographic problems and instrument downtime.*

Accessory items are for Agilent Technologies instrument models: 4890, 5880, 5890, 6890.

## Ordering information

1. INLET LINERS	Agilent X-Ref	Pk/5	Pk/25
<b>Split Injection</b>			
78.5x6.3mm, 4mm ID, wool packed	19251-60540	20486,05	20486,25
78.5x6.3mm, cup design	18740-80190	20510,05	20510,25
78.5x6.3mm, cup design, wool packed	18740-80190	20482,05	20482,25
<b>Splitless</b>			
78.5x6.5mm, tapered	5781-3316	20466,05	20466,25
78.5x6.5mm, tapered, wool packed	5062-3587	20478,05	20478,25
78.5x6.5mm, 2mm ID	5181-8818	20513,05	20513,25
78.5x6.5mm, dual tapered	5181-3315	20485,05	20485,25
<b>Direct/Wide-Bore</b>			
78.5x6.5mm, 1.5mm ID	18740-80200	20517,05	20517,25
78.5x6.3mm, 0.75mm ID for SPME*		26375,05	26375,25
<b>2. SEPTA</b>		<b>Pk/50</b>	
Thermogreen LB-2 , 11.0mm (7/16")	5183-4757	20654	
Thermogreen LB-2 , 9.5mm (3/8")	5181-1283	20652	
<b>3. FERRULES</b>		<b>Pk/10</b>	<b>Pk/50</b>
<b>GC Inlet - Supeltex M-4 Graphite</b>			
0.20-0.25mm Column ID	5080-8853	24811-U	24819-U
0.32mm Column ID	5080-8853	24809-U	24813-U
0.53mm Column ID	5080-8773	24808-U	24812-U
<b>GC Inlet - Supeltex M-2A Vespel-Graphite</b>			
0.20-0.25mm Column ID	5181-3323	24803-U	24807-U
0.32mm Column ID	5062-3514	24802-U	24806-U
0.53mm Column ID	5062-3512	24801-U	24804-U
<b>GC/MS Interface - Supeltex M-2A Vespel-Graphite</b>			
0.20-0.25mm Column ID	5062-3508	24826-U	28022-U
0.32mm Column ID	5062-3506	24824-U	28023-U
0.53mm Column ID	5062-3538	24823-U	28024-U
<b>4. INLET SEALS</b>		<b>Pk/2</b>	<b>Pk/10</b>
Gold-Plated	18740-20885	23318-U	23319-U
Stainless-Steel	18740-80220	23316-U	23317-U
<b>5. O-RING SEALS</b>		<b>Pk/10</b>	<b>Pk/25</b>
Therm-O-Ring	5180-4182	21004-U	21004-U
<b>6. COLUMN NUTS</b>		<b>Pk/2</b>	
Column Nut for 1/16" Ferrules	5181-8830	24833-U	
Brass Nut for GC/MS Interface		28034-U	
<b>7. MSD NUTS</b>		<b>Pk/2</b>	
MSD Source Nuts for Agilent Technologies		22517-U	
<b>CAP KIT CASE</b>			
GC Accessory Case for Agilent Technologies	28035-U		

Trademarks, Agilent Technologies - Agilent Technologies, VESPEL - E. I. du Pont de Nemours & Co., Inc. CapSeal Bullet, Supeltex, Thermogreen, Therm-O-Ring - Sigma-Aldrich Co. \* SPME - Solid Phase Micro Extraction, Mfr.# - Agilent Technologies Part number . Pack sizes might be different.

# GC PRODUCT INFORMATION

## Gas Delivery, Gas Purifiers

### OMI Indicating Purifiers

- Simultaneously remove O<sub>2</sub>, water vapor, CO, CO<sub>2</sub>, most sulfur compounds, most halogen compounds, alcohols, phenols to less than 10ppb
- Purify helium, hydrogen, nitrogen, argon-methane
- Color change indicates purifier exhaustion
- Glass body does not diffuse air or off-gas
- Ideal for Hall, ECD, GC/MS detection systems
- OMI-4 purifier protects multiple instruments (three times the capacity of OMI-2 tubes)

### Irreversibly remove contaminants from carrier gas.

Install an OMI purifier downstream from your primary gas purifying device, and tell at a glance whether or not oxygen and water vapor are being effectively eliminated from your system. The OMI purifier will provide point-of-use gas polishing and final visual assurance of gas quality before the gas enters the GC. OMI purifier tubes contain Nanochem resin, developed for the demanding gas purity needs of the semiconductor manufacturing industry. As little as 1ppm of oxygen or water will change the indicating resin from black to brown.

Spent tubes are easily replaced. Simply unscrew the end assembly from the tube holder and replace the tube. The design prevents air from entering the new tube during installation.

Note: For optimum performance, we do not recommend storing OMI tubes for longer than 6 months.

### Protect Your Column from Many Carrier Gas Contaminants

CONTAMINANT	OMI PURIFIER	INDICATING DEVICES	NON-INDICATING OXYGEN TRAPS
Oxygen	Yes	Yes <sup>1</sup>	Yes <sup>1</sup>
Water	Yes	No	Maybe
Carbon monoxide	Yes	No	Maybe
Carbon dioxide	Yes	No	No
Alcohols/Phenols	Yes	No	No
Sulfur-containing compounds	Yes	No	No <sup>2</sup>
Halogen-containing compounds	Yes	No	No <sup>2</sup>

<sup>1</sup>If incoming oxygen level does not exceed 10ppm.

<sup>2</sup>Corrosive compounds may poison some of these devices.

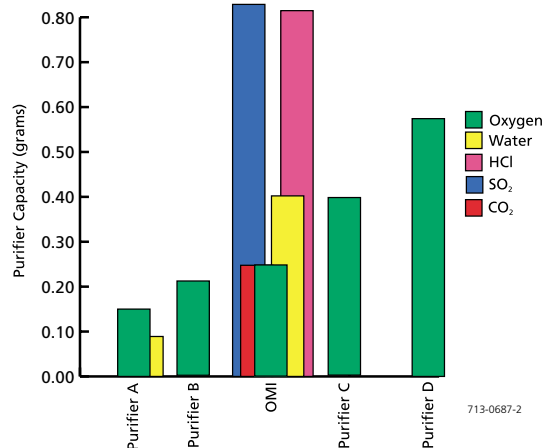
### Ordering information

Prod. No.	Description
23906	OMI-2 Purifier Tube <sup>3</sup>
23921	OMI-2 Tube Holder, 1/8" fittings <sup>3</sup>
23917	Seal Kit for OMI-2 Tube Holder (includes 2 Teflon seals and tool)
23909	OMI-4 Purifier Tube <sup>3</sup>
23926	OMI-4 Tube Holder, 1/8" fittings <sup>3</sup>
23900-U	OMI-1 Replacement Tube <sup>4</sup> (includes 2 ferrules)
22311	3/8" Ferrules (pk. of 10)
21517	1/4" to 1/8" Swagelok SS Reducer

<sup>3</sup>First time users must order both purifier tube and corresponding holder. Holder is reusable.

<sup>4</sup>Will not fit OMI-2 tube holder – use with OMI-1 installation kit only (kit no longer available).

### OMI Indicating Purifier Removes as Much Oxygen as Most Nonindicating Purifiers



### DIMENSIONS OF OMI PURIFIERS

#### OMI-2

Tube: 6"/15.2cm x 5/8"/1.6cm OD

Tube Holder: 10"/25.4cm x 1 1/2"/3.8cm OD

Endfittings: 2 1/2"/6.4cm

#### OMI-4

Tube: 12"/30.5cm x 1 1/2"/3.8cm OD

Tube Holder: 16"/40.6cm x 1 1/2"/3.8cm OD

Endfittings: 2 1/2"/6.4cm

Please refer to the Supelco Catalogue, page 303

## GC ARTICLE

# Customer Application Note on Fast GC

P.Delconte(\*) and R.Ferrari(\*\*) (\*) Stazione Sperimentale Combustibili S.Donato Milanese (MI) Italy (\*\*) Sigma Aldrich srl Milan Italy

The TCEP Capillary column with TCEP phase are commonly used for fuel analysis, because of their slightly different polarities the Benzene and other bigger aromatic compounds elute after the n-Decane.

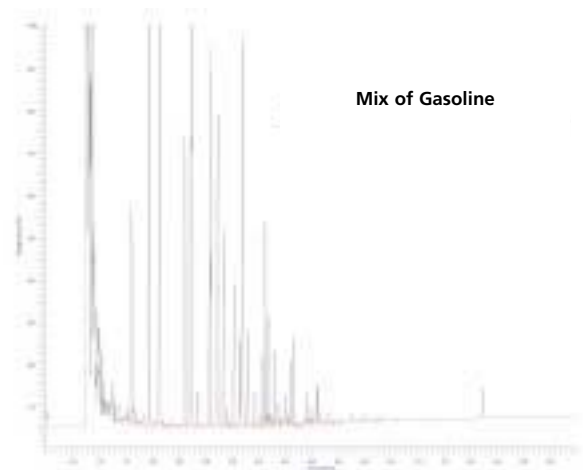
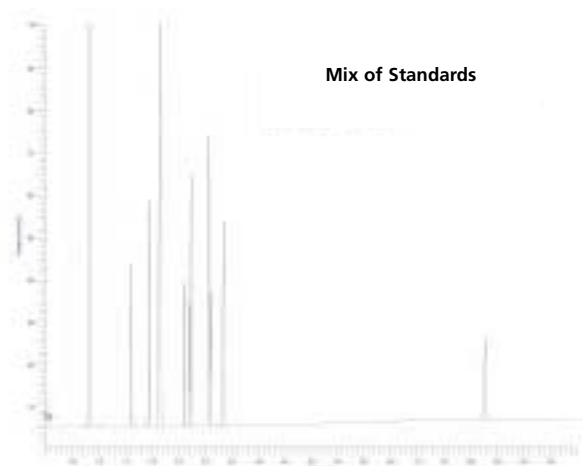
In this experiment two TCEP capillary columns, one traditional narrow bore column and the other being the corresponding Fast version have been evaluated.

From the following chromatograms, the analysis time has been reduced by a factor of 5 from 45 minutes to less than 9 minutes. Resolution has been maintained and peak shape improved. It is clearly shown within this application that we are able to reduce runtime significantly with Supelcos' Fast GC column.

### Supelco Fast GC Column

Capillary Column: TCEP 15 m x 0.10mm ID, 0.18 µm film,  
Carrier Gas: Helium  
Injection system: split 1:400  
Injector temp: 220°C  
Linear velocity: 46 cm/sec constant flow

Column Temp: 40°C  $\xrightarrow{25^\circ\text{C}/\text{min}}$  130°C final time 8 min.  
Detector : FID, 200°C



Description	
<b>TCEP fast capillary column.</b>	<b>Prod. No.</b>
<b>15m x 0.10mm ID x 0.18 mm film</b>	
Tube Holder: 16"/40.6cm x 1	28348-U.
1/2"/3.8cm OD	

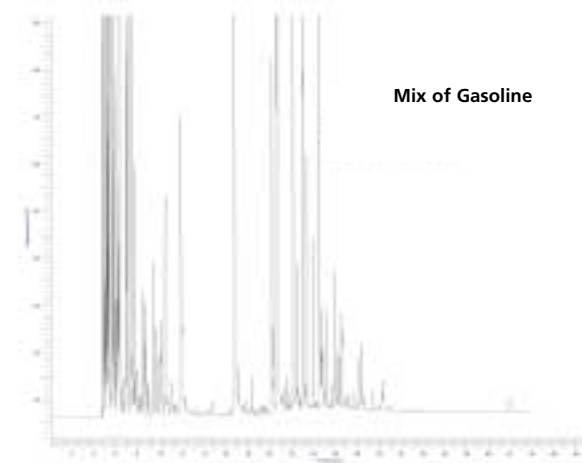
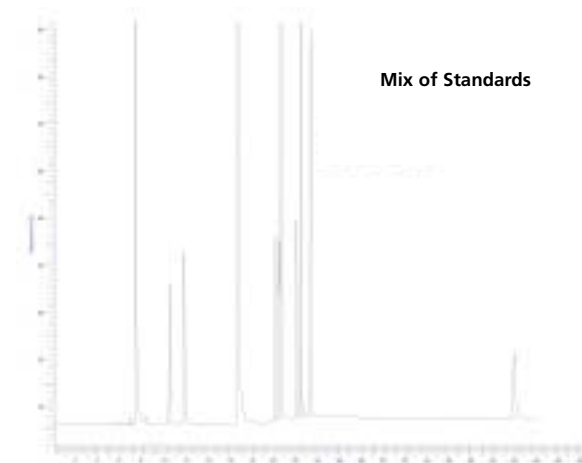
Compound list of Std mix used:		
Name	Rt 0.25mmm. ID	Rt 0.10mmm. ID
Isocane	6.70	0.82
Benzene	10.45	1.62
MEK	11.83	1.95
Toluene	16.75	2.18
Ethylbenzene	20.18	2.62
m.Xylene	20.60	2.71
p.Xylene	20.66	2.74
n.Propilbenzene	22.11	3.09
o.Xylene	22.48	3.11
1,3,5 Trimethylbenzene	22.70	3.35
Naphtalene	42.00	8.28

### Ref. Columns (Competitor)

Identical conditions for Figures A, B and C

Capillary Column: TCEP 50 m x 0.25mm ID, 0.40 µm film,  
Carrier Gas: Helium  
Injection system: split 1: 250  
Injector temp:: 220 °C  
Linear velocity: 22 cm/sec constant flow

Column Temp: 40°C  $\xrightarrow{10^\circ\text{C}/\text{min}}$  130°C final time 16.5 min.  
Detector : FID, 220°C



Your Problem Solving Partner in Chromatography

## STANDARDS &amp; REAGENTS ARTICLE

## A Fluorescence Derivatization Reagent for the Liquid Chromatographic Determination of Airborne Isocyanates

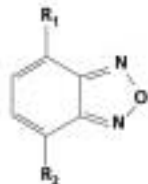
Hartmut Henneken, Uwe Karst and Martin Vogel [m.vogel@utwente.nl](mailto:m.vogel@utwente.nl) ..... Faculty of Science and Technology and MESA+ Institute for Nanotechnology, University of Twente, Enschede, The Netherlands.

Currently, mono- and diisocyanates are widely applied for the industrial production of pharmaceuticals, pesticides or polyurethanes (PURs). Owing to their high reactivity, isocyanates are toxic. Isocyanates in general have strong sensitising properties and are supposed to be most common inducers of occupational asthma. Furthermore, exposure to mono- or diisocyanates is prone to cause irritations of the respiratory system, which may lead to acute pulmonary oedema. Therefore, characterisation and sensitive quantification of this class of analytes is important in the fields of workplace analysis.

Today, modern analytical approaches for the determination of isocyanates base on the derivatization with nucleophilic reagents such as alcohols or secondary amines. The derivatization is followed by a chromatographic separation with mostly photometric or fluorimetric detection. Currently, a number of different derivatizing agents are available. However, only few of them offer sufficient sensitivity, for example, 9-(N-methylaminomethyl)-anthracene (MAMA) [1] or dibutylamine (DBA) [2]. Although for MAMA, HPLC detection can be done both photometrically or fluorimetrically, absorption maxima in the short-wavelength range of the electromagnetic spectrum may lead to interferences from matrix constituents. With respect to DBA, the application is restricted to mass spectrometric detection as the reagent is lacking any chromophoric or fluorophoric group.

In analytical chemistry, derivatizing agents based on the 2,1,3-benzoxadiazole backbone (Figure 1) have found increasing application mainly for bioanalytical or environmental purposes. Although all benzoxadiazoles are likely to be excellent chromophores with absorption maxima in a range > 350 nm accompanied by high molar absorptivities, fluorescence characteristics are restricted to a limited number of benzoxadiazole compounds.

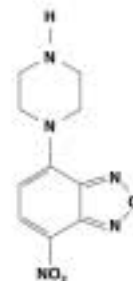
**Figure 1.** Structure of the benzoxadiazole (benzofurazan) backbone



Earlier, 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBDPZ) (Figure 2) had been applied to the determination of carboxylic acids [3]. Making use of NBDPZ for isocyanates, however, both mono- and diisocyanates can be determined by means of reversed-phase (RP) liquid chromatography with subsequent photometric, fluorimetric [4] or even mass spectrometric detection [5, 6].

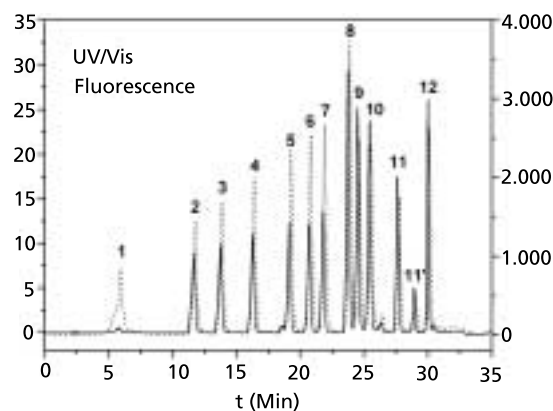
Isocyanates readily react with NBDPZ, thus yielding the respective urea compounds. NBDPZ urea derivatives possess UV-Vis absorption maxima in the range of 480 nm, high molar

**Figure 2.** Structure of NBDPZ



absorptivities (~25,000 L·mol<sup>-1</sup>·cm<sup>-1</sup> per NBDPZ moiety), and additionally, they show good fluorescence characteristics. Excitation wavelengths are at ~ 470 nm, while emission is observed at ~ 535 nm. Thus, compared to all established amine derivatizing agents, NBDPZ provides for the most red-shifted absorption maxima. Using a binary gradient consisting of an aqueous buffer and acetonitrile, reversed-phase C18 LC columns show good results for the separation of NBDPZ and its corresponding isocyanate derivatives. However, to overcome a co-elution problem of the derivatives of 2,6-toluene diisocyanate (2,6-TDI) and 1,6-hexamethylene diisocyanate (HDI or HMDI), a phenyl-modified reversed-phase column shows best chromatographic resolution [4].

**Figure 3.** Liquid chromatographic separation of NBDPZ (1) and the derivatives of methyl isocyanate (2), ethyl isocyanate (3), propyl isocyanate (4), butyl isocyanate (5), pentyl isocyanate (6), phenyl isocyanate (7), 2,6-toluene diisocyanate (8), 1,6-hexamethylene diisocyanate (9), 2,4-toluene diisocyanate (10), isophorone diisocyanate (11/11'), methylene bis(phenyl isocyanate) (MDI) (12) with UV-Vis (solid line) and fluorescence detection (dotted line) on a phenyl-modified column.



Apart from spectroscopic techniques, NBDPZ and its derivatives can also be detected by means of mass spectrometry. LC-MS experiments have been run using APCI(+) tandem MS [5] as well as APCI(-) with single quadrupole detection [6].

Using NBDPZ as derivatization reagent, the analysis of gaseous samples can either be done by active (pumped) [4] or by means of passive (diffusive) sampling methods [5]. Active methods comprise the use of impingers filled with NBDPZ dissolved in an organic solvent as well as the application of reagent-coated test tubes. Diffusive sampling devices - NBDPZ-impregnated filters in a dedicated housing - have been tested for the determination of methyl isocyanate and turned out to be useful for the monitoring of long-term exposure at workplaces.

### Summary

NBDPZ can ideally be applied as a reagent for the determination of mono- and diisocyanates in air samples. Due to versatile spectroscopic properties, selectivity of this method is superior to other reagents. The reagent and the respective urea derivatives can be separated by means of reversed-phase HPLC. Here, highest selectivity is obtained on phenyl-modified phase. The HPLC separation can be followed by fluorescence, UV-Vis or MS detection. Regarding sampling procedures, pumped as well as passive methods are successfully applied.

**Table 1. Product Range**

Product Name	Prod No.	Pack Size
4-Nitro-7-piperazinobenzofurazan (NBDPZ)	92614	100 mg
HMDI (HDI)	33490	2 ml
2,6-TDI	33493	100 mg
Butyl isocyanate	20070	10 ml, 50 ml, 250 ml
Phenyl isocyanate	78750	25 ml, 100 ml, 500 ml
Ethyl isocyanate	04200	10 ml, 50 ml
4,4'-Methylenebis(phenyl isocyanate) (4,4'-MDI)	33428	100 ml
Propyl isocyanate	P53373	5 ml, 25 ml

### Literature

1. Sangö, C.; Zimerson, E.; J. Liq. Chromatogr. 1980, 2, 971-990.
2. Karlsson, D.; Dahlin, J.; Skarping, G.; Dalene, M.; J. Environ. Monit. 2002, 4, 216-222.
3. Toyo'oka, T.; Ishibashi, M.; Takeda, Y.; Nakashima, K.; Akiyama, S.; Uzu, S.; Imai, K.; J. Chromatogr. 1991, 588, 61-71.
4. Vogel, M.; Karst, U.; Anal. Chem. 2002, 74, 6418-6426.
5. Henneken, H.; Lindahl, R.; Östin, A.; Vogel, M.; Levin, J.-O.; Karst, U.; J. Environ. Monit. 2003, 5, 100-105.
6. Hayen, H.; Jachmann, N.; Vogel, M.; Karst, U.; Analyst 2003, 128, 1365-1372.

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**Your Problem Solving Partner in Chromatography**

# Fatty Acid Analysis with FAME Columns from Supelco



## Consistent, Reproducible Performance

### Separation of

- Free Fatty Acids
- Fatty Acid Methyl Esters
- Cis/trans Fatty Acids
- Complex Mixtures

With our Special Purpose Columns:

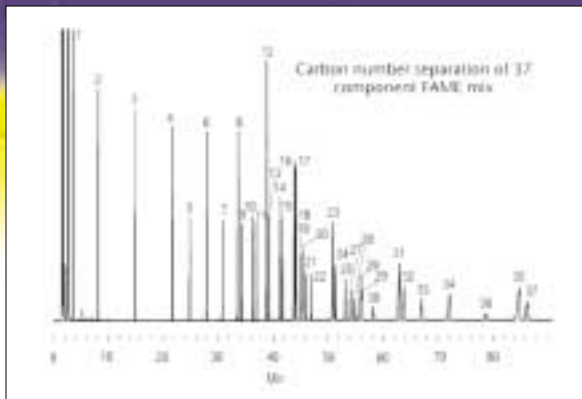
SUPELCOWAX 10

SPB-1000    SPB-2380

SPB-PUFA    SPB-2560

Omegawax

For a detailed product list of our FAME columns please refer to the Supelco catalogue, pp. 214



# FREE

**FAME standard**, buy one FAME Column and get 2 FAME Standards for FREE, listed on pages 602/603 of the Supelco Catalogue

Offer limited to one Standard per Column

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**i** Information Request.....1309




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