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EUROPE

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Sigma-Aldrich Standards CD

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## EDITORIAL

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The heart of the CD-ROM is a pdf version of the full Sigma-Aldrich Analytical Standards catalogue, bringing together Standards and Reference Materials from our keynote brands:

- Sigma
- Aldrich
- Fluka
- Riedel-de Haën
- Supelco
- Isotec

Supported by European Certified Reference Materials from BCR, ERM and EMPA.

But that is only the start. The pdf catalogue is fully indexed so that it is searchable, using the keyword search facility in Adobe Acrobat. On top of the Acrobat search facility the new Sigma Aldrich search facility, which includes multi keyword search options, will take you directly to the product. The search engine allows search by up to 4 keywords and search by the following categories:

- CAS Number
- Sigma Aldrich Catalogue Number
- Molecular formulae
- Brand – eg. Riedel-de Haën, Fluka.
- Standard type: more than 60 categories

Once a product of interest has been identified there is a direct link to the Sigma Aldrich main web page for the product, where more information is available. This includes:

- Price
- Availability
- Health and Safety Data
- MSDS
- Certificate of Analysis

As well as the key product and catalogue data the CD-ROM allows you to access a wealth of technical support data including:

- Technical notes: More than 50 notes describing procedures and applications, for example: "The Separation of Furosine in Dairy Products by RP-HPLC"
- Application Reports for specific product, for example the separation of Fusel Oils in Vodka using Supelco Equity 1701 HPLC Columns
- Bulletins: More than 70 background reports, including topics such as "Reference Standards: A competitive comparison"
- Product Information: Including additional specifications and background to products

Finally, there is a directory of Sigma Aldrich locations and full contact details.

It is not for nothing that at Sigma Aldrich we really believe we are truly "Setting the Standard for Analytical Standards!"

Sincerely,




Rainer Walz  
Product Manager Analytical Standards



## HPLC ARTICLE

# Analysis of Antiretrovirals Used in Combination HIV Therapy

Jacynth A. M. McKenzie, Carmen T. Santasania, and David S. Bell

### Abstract

The simultaneous determination of antiretrovirals from three therapeutic classes is demonstrated using the Supelco Ascentis™ RP-Amide column.

### Introduction

Antiretroviral agents are used in the treatment of human immunodeficiency virus (HIV) and acquired immuno deficiency syndrome (AIDS). To date, five therapeutic classes have been developed: nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), protease inhibitors (PIs) and fusion inhibitors (1). Effective treatment is usually accomplished using at least three drugs from more than one therapeutic class. For example, recommendations for initial treatment include the combination of two NRTIs or NtRTIs with either an NNRTI or a PI (2).

In the study of drug efficacy, pharmacokinetics, prevention and management of adverse reactions, therapeutic drug monitoring of antiretrovirals is performed. This is generally achieved by HPLC coupled with UV-diode array or mass spectrometry detection following solid phase extraction (3,4). Drawbacks of previous HPLC methods include the use of ion-pair reagents (5) and limited specificity for a single drug class (6). This report describes a simple HPLC method for the simultaneous determination of antiretrovirals from three drug classes using a new proprietary surface optimized embedded polar group (EPG) stationary phase, Ascentis RP-Amide. The Supelco Ascentis RP-Amide phase demonstrates advantages in polar analyte retention as well as improved selectivity when compared to traditional C18 stationary phases.

### Experimental Approach

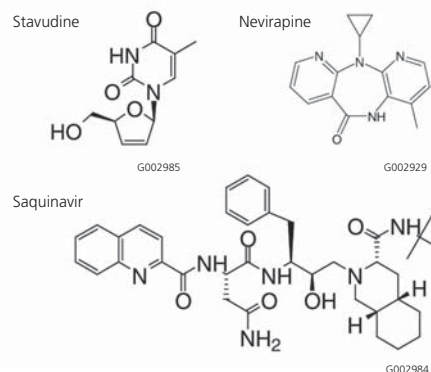
The USP assay method for nevirapine was modified by applying a gradient to include assay of other antiretrovirals. Alternately, if a MS detector is desired, minor adjustments to the mobile phase can be made. All chemicals utilized were obtained through Sigma-Aldrich and Riedel-de Haën. Analytes were obtained from USP or other sources.

### Results

Representative structures from three drug classes and an optimized chromatogram obtained on the Ascentis RP-Amide EPG phase for the simultaneous analysis of seven antiretrovirals are provided in Figures 1 and 2A, respectively. When zalcitabine is added to the analyte mixture (Figure 2B), a small interfering peak appears as a shoulder on peak 8. The shoulder appears only after zalcitabine is analyzed in combination with one or more of the protease inhibitors and thus appears to be a reaction product of the compounds.

For compatibility with mass spectrometric detection, the phosphate buffer initially employed was replaced with 0.1% ammonium acetate in water. Using this mobile phase in combination with acetonitrile, the elution of all 7 drugs was completed in 15 minutes (Figure 3).

**Figure 1.** Representative structures of an NRTI (Stavudine), an NNRTI (Nevirapine) and a PI (Saquinavir)

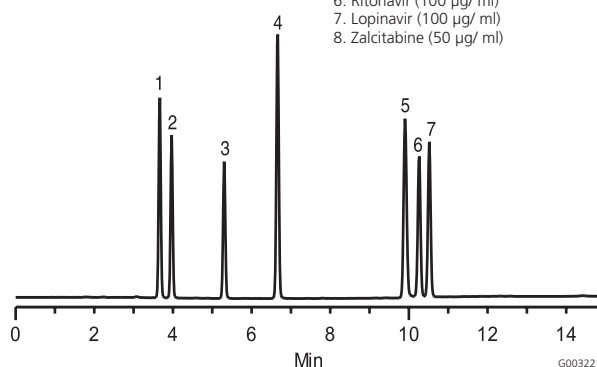


**Figure 2A.** Separation of Seven Antiretrovirals Using the Supelco Ascentis RP-Amide

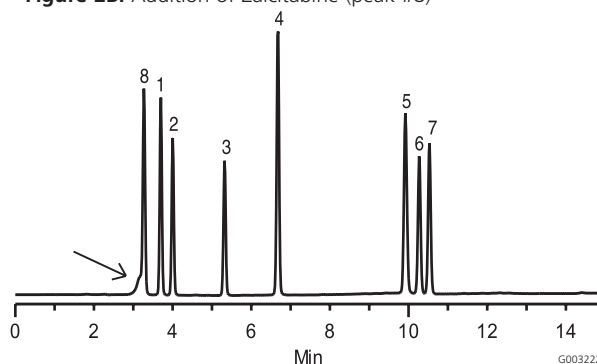
column: Supelco Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)  
 mobile phase A: A: 25 mM ammonium phosphate, pH 5.55 with phosphoric acid  
 mobile phase B: acetonitrile  
 flow rate: 1.0 ml/min.  
 temp.: 35 °C  
 det.: UV at 220nm  
 injection: 10 µl

sample: as indicated in 25 mM ammonium phosphate (pH 5.55)

Min.	%A	%B	1. Lamivudine (50 µg/ml)
0	95	5	2. Stavudine (50 µg/ml)
10	15	85	3. Zidovudine (50 µg/ml)
12	15	85	4. Nevirapine (35 µg/ml)
12.5	95	5	5. Saquinavir (100 µg/ml)
			6. Ritonavir (100 µg/ml)
			7. Lopinavir (100 µg/ml)
			8. Zalcitabine (50 µg/ml)



**Figure 2B.** Addition of Zalcitabine (peak #8)



## Conclusion

Simultaneous analysis of an NNRTI, nevirapine, NRTIs (lamivudine, zidovudine, stavudine), and PIs (ritonavir, lopinavir, saquinavir) was achieved in a single run without the use of ion-pair reagents utilizing a new EPG column, Ascentis RP-Amide. In comparison, alkyl type phases (C18 and C8) often require the use of ion pair reagents to achieve simultaneous monitoring of different therapeutic classes. Simultaneous monitoring of drugs used in combination therapy using the proprietary, surface optimized Ascentis RP-Amide stationary phase is advantageous in detecting interactions that can lead to clinical failures or shortfalls encountered in HIV treatment.

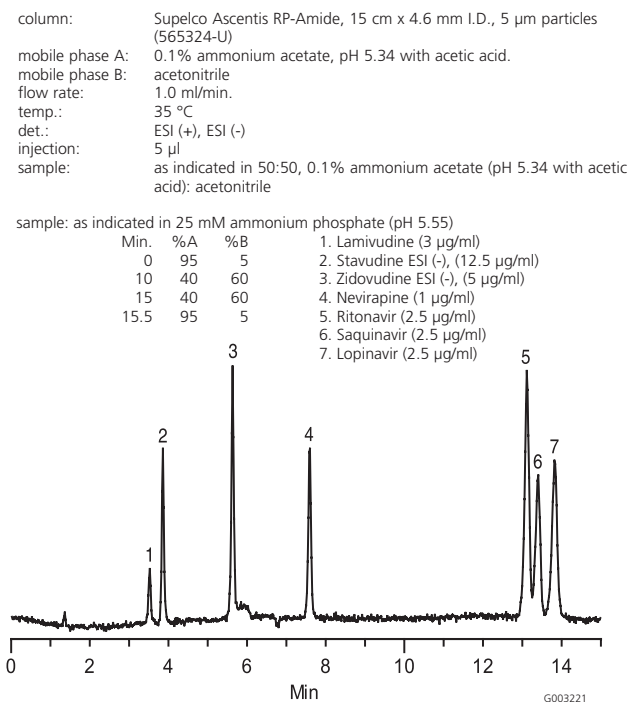
## References

1. C.P.W.G.M. Verweij-van Wissen, R.E. Aarnoutse and D.M. Burger, J. Chromatogr. B. 816 121-129 (2005).
2. Panel on Clinical Practices for Treatment of HIV Infection, Guidelines for the use of antiretroviral agents, [http://www.aidsinfo.nih.gov/guidelines/adult/AA\\_040705.pdf](http://www.aidsinfo.nih.gov/guidelines/adult/AA_040705.pdf)
3. M. Jenal, S. Rao, M. Gatz, D. Whigan, J. Chromatogr. B. 795 273-289 (2003).
4. B. Fan, J.T. Stewart, J. Liq. Chromatogr. Relat. Technolog. 24 3017 (2001).
5. G. Aymard, m. Legrand, T. Trichereau, B. Diquet, J. Chromatogr. B. 744 227-240 (2000).
6. V.A. Simon, M.D. Thiam, L.C. Lipford, J. Chromatogr. A. 913 447-453 (2001).

### Ordering information

Prod No.	Description
565324-U	Ascentis RP-Amide column, 15 cm x 4.6 mm I.D., 5 µm
467782	Ammonium phosphate
345245	Phosphoric acid
34851	Acetonitrile
34674	Ammonium acetate
33206	Acetic acid

**Figure 3.** Reconstructed Ion Chromatogram of Seven Antiretrovirals Using Supelco Ascentis RP-Amide and MS Detection



**i** Information Request ..... 1801

## APPLICATION NOTE 180

### Glyphosate Analysis Using SUPELCOSIL SAX1

Glyphosate (N -(phosphonomethyl) glycine) is among the most widely used herbicide in the United States and is marketed under such brand names as Roundup®, Rodeo®, Sonic® and Glifonox®. Common uses include control of broadleaf weeds and grasses in hay pastures, soybeans, field corn, ornamentals, lawns, turf, forest plantings, greenhouses and rights-of-way. Glyphosate is regulated by the US Environmental Protection Agency, which limits the amount that is acceptable in drinking water. EPA Method 547 is used for the determination of glyphosate in drinking water by direct aqueous injection HPLC, post column derivatization and fluorescence detection. The SUPELCOSIL™SAX1 HPLC column can be used for this method and has been shown to produce excellent results. The SUPELCOSIL SAX1 HPLC column offers superior resolution, minimal column bleed and is composed of non-fluorescing silica, which produces little to no baseline interference.

### Product Characteristics

stationary support:	silica	pH range:	2-7.5
particle size:	spherical	carbon (typical):	12%
particle size:	5 µm	frit pore size:	2 µm
pore size:	120 Å	approximate ion	
surface area (typical):	170m <sup>2</sup> /g	exchange capacity:	0.5meq/g 1.5meq/ column
pore volume (typical):	0.6 ml/g		

### Ordering information

Prod No.	Description
59138	SUPELCOSIL SAX1, 25 cm x 4.6 mm

### Glyphosate and Metabolite on SUPELCOSIL SAX1

column: SUPELCOSIL SAX1, 25 cm x 4.6 mm I.D., 5 µm particles (59138)  
 mobile phase A: 5 mM monobasic potassium phosphate (pH 1.9 with phosphoric acid)  
 flow rate: 0.5 ml/min.  
 temp.: 35 °C  
 det.: fluorescence, excitation 338nm, emission 455nm  
 injection: 100 µl

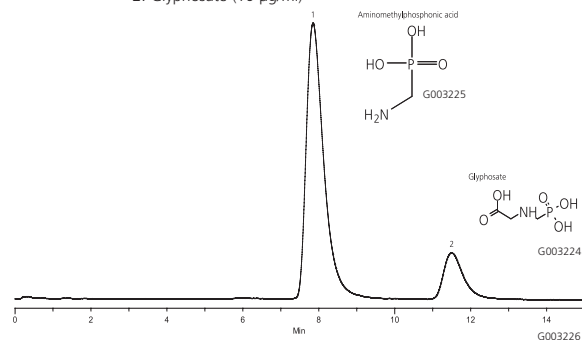
Post column conditions for the oxidation solution

flow rate: 0.2 ml/min.  
 mixer volume: 250 µl  
 temp.: ambient

Post column conditions for the derivatization solution

flow rate: 0.3 ml/min.  
 mixer volume: 500 µl  
 temp.: 38 °C  
 sample: as indicated below (in water)

1. Aminomethylphosphonic acid (10 µg/ml)
2. Glyphosate (10 µg/ml)



# Ascentis™

HPLC Products

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## Get MORE HPLC performance

Introducing the First Ultra Low Bleed  
Polar RP-HPLC Phase for LC-MS!

Supelco's new Surface-Optimized Technology yields the first amide based RP phase to exhibit ultra low bleed for LC-MS.

### Ascentis™ RP-Amide

Ascentis RP-Amide is a new generation, highly stable, polar embedded RP phase that provides unique selectivity compared to C18 phases and increased resolution for analysis of polar compounds.

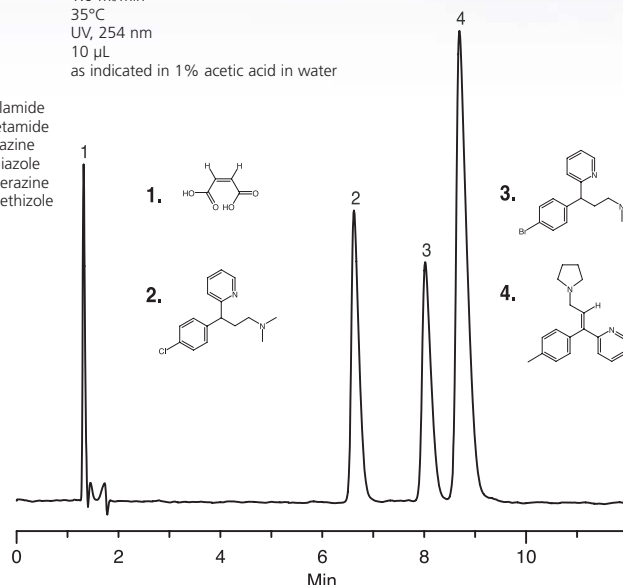
#### Phase Specifications

USP Code:	Pending
Bonded Phase:	Amido embedded reversed-phase
Endcapped:	Yes
Particle Shape:	Spherical
Particle Purity:	<5 ppm metals
Particle Size:	3 µm, 5 µm
Pore Size:	100 Å
Surface Area:	450 m <sup>2</sup> /g
Carbon Load:	19.5%
pH Range:	2 to 8
Temp. Range:	70 °C

Ascentis RP-Amide Delivers Excellent Performance in the Analysis of Antibiotic Sulfa Drugs

column:	Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase:	85:15, 1% acetic acid in water:CH <sub>3</sub> OH
flow rate:	1.0 ml/min
temp.:	35°C
det.:	UV, 254 nm
injection:	10 µL
sample:	as indicated in 1% acetic acid in water

1. Sulfanilamide
2. Sulfacetamide
3. Sulfadiazine
4. Sulfathiazole
5. Sulfamerazine
6. Sulfamethizole



## Trial Offer

**Free, no obligation 30-Day Trial on any analytical Ascentis RP-Amide column.**

Promotional code: U27

Offer valid until 31 December 2005

**i** Information Request ..... 1801

# HPLC Accessories: In-Line Filters



Protect your HPLC column by using in-line filters from Supelco

Avoid accumulation of particulate matter on the column frit, that can cause split peaks and high back pressure.

## Supelco Filter

Direct-connect; protects analytical and guard columns. Our precolumn filter can be connected directly, hand-tight, into any HPLC column or guard column that has Valco-compatible endfittings. PEEK cap and body, 2 µm stainless steel frit. For a metal-free system, order PEEK/Teflon replacement frits (Prod No. 57430-U).



### Ordering information

Prod No.	Description	Pack Size
Z227323	<b>Supelco Precolumn Filter</b>	1
<b>Replacement Frits</b>		
Z290874	0.5 µm pores	5
Z227331	2 µm pore	5
57430-U	PEEK/Teflon, 2 µm Biocompatible, metal-free.	5

## Upchurch Precolumn Filter

In-line installation. Stainless steel body with inert polyetherether-ketone (PEEK) endfittings and a 0.5 µm or 2 µm PEEK frit in one endfitting.



### Ordering information

Prod No.	Description	Pack Size
<b>Upchurch Precolumn Filter</b>		
55079	0.5 µm frit	1
55078	2 µm frit	1
<b>Replacement Frits</b>		
55080-U	0.5 µm	1
55081	2 µm	1

## Upchurch Precolumn MicroFilter

In-line filter connects capillary tubing (using Microtight tubing sleeves) or 1/16" OD tubing to female 10-32 fitting. 0.5 µm frit, 0.3 µl swept volume. Includes 5 frits (1 installed).



### Ordering information

Prod No.	Description
502677 502669	<b>MicroFilter for 0.025" OD Tubing</b> with PEEK Frits with Stainless Steel Frits
502693 502685	<b>MicroFilter for 1/16" OD Tubing</b> with PEEK Frits with Stainless Steel Frits
502790 502731 502723	<b>Replacement Frits, pk. of 10</b> PEEK, 0.5 µm Stainless Steel, 0.5 µm Stainless Steel, 2.0 µm

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- Accurately tested for LC-MS
- Very low amount of metal ions
- High UV-transmittance
- Excellent gradient baseline
- Particle tested

## Specifications:

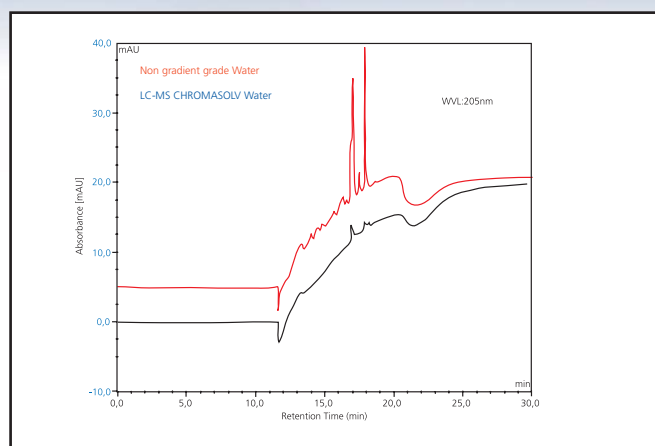
Acetonitrile and Methanol Blends

LC gradient testing in UV and MS, metal impurities (Na < 2 ppm, K, Mg, Ca < 0.5 ppm), UV-transmittance, additive content: 0.093-0.107% TFA, FA, AA (v/v), ammonium acetate (w/v), solvent content: (GC): > 99.0% (Prod No. 34669 – acetonitrile with 0.1% ammonium acetate: solvent content (GC) >98%)

Water blends

LC gradient testing in UV and MS, metal impurities (Na < 2 ppm, K, Mg, Ca < 0.5ppm), UV-transmittance, additive content: 0.093-0.107 TFA, FA, AA (v/v), ammonium acetate (w/v), pH: effective +/- 0.1

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



## Ordering information

Prod No.	Description	Pack Size
34978	Water with 0.1% TFA	2.5 L
34976	Acetonitrile with 0.1%R TFA	2.5 L
34974	Methanol with 0.1% TFA	2.5 L
34673	Water with 0.1% formic acid	2.5 L
34668	Acetonitrile with 0.1% formic acid	2.5 L
34675	Water with 0.1% acetic acid	2.5 L
34678	Acetonitrile with 0.1% acetic acid	2.5 L
34672	Methanol with 0.1% acetic acid	2.5 L
34674	Water with 0.1% ammonium acetate	2.5 L
34669	Acetonitrile with 0.1% ammonium acetate	2.5 L
34670	Methanol with 0.1% ammonium acetate	2.5 L

**i** Information Request ..... 1802

## Introductory Offer

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## SPE ARTICLE

# Mixed-Mode SPE Improves Extraction of Pharmaceutical Compounds from Biological Fluids

David Bell, An Trinh, Carmen Santasania, Yuhui Yang and Michael Ye

In an ideal SPE (solid phase extraction) scenario, under a single eluant system the analytes of interest are bound strongly but reversibly to the particle while impurities and other unwanted sample components pass through unretained. Unfortunately, this scenario is rarely realized using a single SPE tube. Typically, conditions strong enough to remove impurities also remove at least some of the analyte, reducing the overall recovery of the method. One way around this situation, a way that permits both enhanced analyte retention and reduced matrix impurity contamination, is by using mixed-mode SPE phases that take advantage of the differences between the retention mechanisms of analytes and unwanted components of the sample.

Using ion-exchange and reversed-phase SPE phases conjointly and manipulating the pH and organic modifier concentration of the eluant can isolate basic compounds from neutral and acidic components of the sample. Analytes with the appropriate charge interact with the ion-exchange bonded phase, locking them during the extraction process. With analyte molecules safely locked, the SPE tube can be washed with strong solvents to thoroughly remove impurities. Then, the pH of the eluant can be adjusted to reduce the charge on the analyte molecules and release them from the ion-exchange groups of the bonded phase. Since in the mixed-mode SPE system the compounds are also retained by a reversed-phase mechanism, the eluant's organic component percentage can also be adjusted to achieve selective elution.

## Mixed-mode SPE of basic pharmaceutical compounds

In the study reported here, basic pharmaceutical compounds and metabolites were extracted from biological fluids using Discovery DSC-MCAX, which comprises both C8 and SCX (benzenesulphonic acid) functional groups followed by analysis on a Discovery C18 HPLC column with UV detection. The method yielded recoveries greater than 90% across all compounds tested and relative standard deviations consistently less than 5%. We developed two different extraction protocols that targeted different analyte charge or polarity.

## Generic Extraction Protocol 1: Non-polar basic compounds

Protocol 1 is suitable for basic compounds that are ionized at pH 6.

1. Dilute 1 ml of sample (urine, plasma, serum) with 1 ml of 50 mM ammonium acetate (pH 6).
2. Condition a 100 mg/3 ml DSC-MCAX SPE tube with 1 ml methanol.
3. Equilibrate the SPE tube with 1 ml 50 mM ammonium acetate (pH 6).
4. Load diluted urine (or plasma) sample on the SPE tube at a flow rate of 1 ml/min.
5. Elute unwanted sample components with 1 ml each of the following sequence of solvents: 50 mM ammonium acetate (pH 6), 1M acetic acid, methanol.
6. Elute analytes with 5% ammonium hydroxide in methanol.

## Generic Extraction Protocol 2: Weakly basic and polar basic compounds

Protocol 2 is suitable for compounds not retained (not ionized) at pH 6, acidic compounds, or where the analyte has a  $pK_a$  of  $\sim 6$ . Protocol 2 is the same as Protocol 1, except 10 mM potassium phosphate (pH 3) or 10 mM acetic acid buffer (pH 3) is used in place of the pH 6 buffer. Also, the 1M acetic acid is not needed in step 5.

## Examples of mixed-mode SPE for basic pharmaceutical compounds in serum

Following Generic Extraction Protocol 1, mixed-mode SPE on Discovery DSC-MCAX SPE tubes was used to extract trace levels (10 ng/ml) of TCAs from serum. In Figure 1, pre-SPE and post-SPE samples analyzed by HPLC-UV are compared. Note that a near baseline level clean-up was achieved and average recovery values were greater than 95% with less than 5% RSD.

Employing Generic Extraction Protocol 2, high recoveries and excellent reproducibility were obtained for all of the compounds listed in Table 1. This emphasizes the versatility and suitability of the DSC-MCAX for drug screening in biological fluids.

## Conclusion

Mixed-mode SPE methods rely on two or more retention mechanisms to simultaneously extract a broad range of compounds from a single biological sample. Slight changes in eluant conditions bring about selective elution and high, reproducible recovery of trace levels of analytes free of contamination from the sample matrix. Discovery mixed-mode SPE phases, like the DSC-MCAX described here, put the perfect SPE scenario within reach.

## Product Information

The Sigma-Aldrich family of brands provides complete solutions to pharmaceutical analysis, including separation media, columns, mobile phases and drug standards.

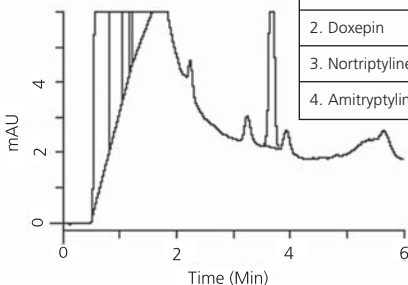
### Ordering information

Prod No.	Description	Pack Size
<b>SPE Tubes</b>		
52783U	Discovery DSC-MCAX, 100 mg/3 ml	Pack of 54
<b>HPLC Columns</b>		
50495521	Discovery C8, 15 cm x 2.1 mm I.D., 5 $\mu$ m particles	
<b>Solvents and Mobile Phase Additives for HPLC and SPE</b>		
17836	Ammonium acetate, puriss. p.a. for HPLC; >99.0% (NT)	50 g, 250 g
34877	Water, G CHROMASOLV® gradient grade	1 L, 2.5 L
34851	Acetonitrile, CHROMASOLV® gradient grade	1 L, 2.5 L, 7 L
34860	Methanol, CHROMASOLV®	1 L, 2.5 L, 7 L
<b>Solvents and Mobile Phase Additives for HPLC and SPE</b>		
N0392	Nordoxepin HCl	10 mg, 25 mg
D4526	Doxepin HCl	1 g, 5 g
N7261	Nortriptyline HCl	10 g
A8404	Amitriptyline HCl	10 g, 25 g, 100 g, 250 g

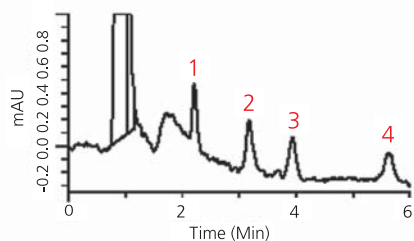
**Figure 1.** Extraction of 10 ng/ml Tricyclic Antidepressants from Serum. Generic Extraction Protocol 1

column: SPE: Discovery DSC-MCAX, 100 mg/3 ml HPLC: Discovery C8, 15 cm x 2.1 mm I.D., 5 µm particles  
 mobile phase: 10 mM ammonium acetate, (pH 4.5):acetonitrile (45:55)  
 flow Rate: 0.4 ml/min.  
 temp: 3 40 °C  
 det: UV, at 210nm,  
 injection: 10 µl

Compound (10ng/ml)	%Abs Recovery ± RSD (n=3)
1. Nordoxepin	99.4 ± 4.3%
2. Doxepin	102.1 ± 1.8%
3. Nortriptyline	95.6 ± 2.7%
4. Amitriptyline	101.3 ± 3.2%



Spiked Serum Sample Prior to SPE Cleanup



Spiked Serum Sample After DSC-MCAX Extraction

**Table 1. Absolute Recoveries (%) of Polar Compounds by DSC-MCAX Generic Extraction Protocol 2**

Analyte	Average Recovery (%) (mean ± RSD, n=3)
Acebutolol	91.4 ± 4.6
Alprenolol	99.4 ± 1.4
Amitriptyline	97.0 ± 0.7
Amphetamine	100 ± 1.9
R(+)-Atenolol	98.3 ± 2.1
Clomipramine	93.7 ± 1.8
Desipramine	105.4 ± 4.7
Diphenhydramine	93.4 ± 3.1
Doxepin	97.4 ± 0.9
Ephedrine	99.7 ± 1.8
Fluoxetine	98.8 ± 2.7
Imipramine	98.9 ± 0.9
Methamphetamine	103.4 ± 2.4
Metoprolol	93.2 ± 2.4
Oxprenolol	99.2 ± 0.7
Phentermine	106.5 ± 3.6
Propranolol	98.7 ± 2.3
Quinidine	93.2 ± 2.4
Verapamil	102.3 ± 2.8

# Discovery SPE - Solve your Reproducibility Problems



## Use Discovery SPE to...

- Achieve high recoveries of diverse compounds from difficult sample matrices
- Remove interfering sample components that cause high background, misleading, peaks and/or poor sensitivity
- Concentrate target analytes for increased sensitivity
- Remove endogenous interferences that can clog, foul, or otherwise damage your HPLC or GC column and system
- Change sample matrix (*i.e.*, solvent exchange) to improve compatibility with your analytical method

**i** Information Request ..... 1803



## SPE ARTICLE

## Celite® Analytical Filter Aid II (CAFA II)

Diatomaceous earth, a natural material comprising fossilized diatoms, has widespread commercial, industrial and agricultural applications. Diatoms themselves are single-celled or colonial eukaryotic algae found throughout both fresh water and marine environments. Their cell walls or frustules show a great diversity in form, with many being quite ornate (Figures 1 and 2).

**Figure 1.** Optical micrograph of various living diatoms (The Academy of Natural Sciences, Philadelphia, PA USA, used with permission)



**Figure 2.** SEM of *Thalassiosira* diatoms (Ivo Grigorov, Ph.D., www.sinia-planeta.com, used with permission)



In the chemical laboratory, diatomaceous earth is often employed as a filtration aid to remove particulate matter and clarify solutions. The silicate shell of the diatom and the calcining process provide high mechanical strength which reduces breakage and fines. The fusing process and the natural porosity of the frustules permit fast filtration rates.

The demands of analytical and other chemical applications dictate that the manufacturer pay strict attention to the composition, purity, extractables, and pore and particle size of the diatomaceous filtration media. One of the most respected and widely used commercial brands of diatomaceous earth is Celite®. Sigma-Aldrich offers Celite® Analytical Filter Aid II (CAFA II), a high purity material that replaces the original CAFA material. The production process has been refined to optimize the purity and consistency of CAFA II without compromising filtration effectiveness compared to the original CAFA material. In addition, World Minerals, the manufacturer of Celite®, moved the CAFA II process to a modern facility under ISO governance with batch tracking and record keeping designed for traceability and continuous quality monitoring.

CAFA II remains identical to the original material (CAFA) with respect to:

- Intended use as an analytical filter aid
- Filtration efficiency in the range of 0.5 - 1.0 micron
- Diatomite composition (marine plankton)
- Manufacturing location (World Minerals in Lompoc, CA, USA)

Any detectable changes in CAFA II over CAFA are within the boundaries of the original CAFA variability eliminating the need for change-control when switching to the new version.

Each batch of CAFA II is tested to ensure low extractables, optimal flow rate and clarifying properties as measured under standard conditions in special equipment. Test methodology and criteria are those defined by Advanced Minerals Corporation (the product development subsidiary of World Minerals) or the National Formulary. This extensive and relevant testing ensures that CAFA II will perform your most demanding filtration or clarification applications effectively and consistently.

#### Ordering information

Prod No.	Description	Pack Size
11484-U	Celite® Analytical Filter Aid II (CAFA II)	100 g
11485-U	Celite® Analytical Filter Aid II (CAFA II)	500 g
11486-U	Celite® Analytical Filter Aid II (CAFA II)	1 kg

Celite® is a registered trademark of World Minerals, Inc.

#### Celite® Analytical Filter Aid (CAFA II) Test Parameters

- |                                 |                                     |
|---------------------------------|-------------------------------------|
| - Wet density method)           | - Permeability (Darcy               |
| - Percent retained on 150 mesh  | - Percent moisture                  |
| - Specific resistivity          | - Conductivity                      |
| - Endotoxins                    | - Loss on drying                    |
| - Loss on ignition              | - Acid-soluble iron                 |
| - Other acid-soluble substances | - Leachable arsenic                 |
| - Leachable lead                | - Limit of non-siliceous substances |

## VERSA FLASH ARTICLE

# Comparison of Spherical and Irregular Silicas in Flash Chromatography

Michael Ye, Craig Aurand, Charles Mi, Boris Polanuyer and Daniel Vitkuske

Flash chromatography is an efficient, rapid and economical technique for the purification of organic compounds. Introduced over thirty years ago, it has gained popularity with the introduction of disposable pre-packed flash cartridges. These cartridges provide safe, reproducible and economical alternatives to in-lab packed glass columns and solve many practical problems associated with flash purification. However, care must be taken to choose the right cartridge to maximize your success.

### Silica particles: The heart of flash chromatography

Even though it is a relatively “quick and dirty” technique, flash is still a form of liquid chromatography and all the rules regarding speed, capacity and resolution that apply to HPLC columns apply to it as well. The heart of flash chromatography is the cartridge which is typically packed with unmodified or C18-modified silica gel. The physical properties of the silica gel play a significant role in the quality of the flash separation. In this article we will discuss the effects of three physical aspects of the silica particle:

- Particle shape
- Particle size and size distribution
- Surface area

These properties affect the resulting chromatography in terms of:

- Backpressure, which affects speed of the separation and the types of pumps required
- Capacity and retention, which affect how much sample can be separated in one injection
- Efficiency and resolution, which effect final sample concentration and purity

Four different silicas were evaluated: two spherical and two irregular. Physical properties of the silicas are found in Table 1 while photomicrographs appear in Figure 1. Spherical and irregular silica were obtained from various flash chromatography suppliers. VersaFlash™ cartridges packed with spherical silica were from Supelco.

Table 1. Particle size, surface area, pore size and particle size distribution of the four silicas

Silica	Particle Size (µm)	Surface Area (m <sup>2</sup> /g)	Pore Size (Å)	Particle Size Distribution (SD, µm)
Spherical 1	64	480	70	10.13
Spherical 2	31	480	70	7.73
Irregular 1	68	490	60	18.42
Irregular 2	56	kg	70	16.3

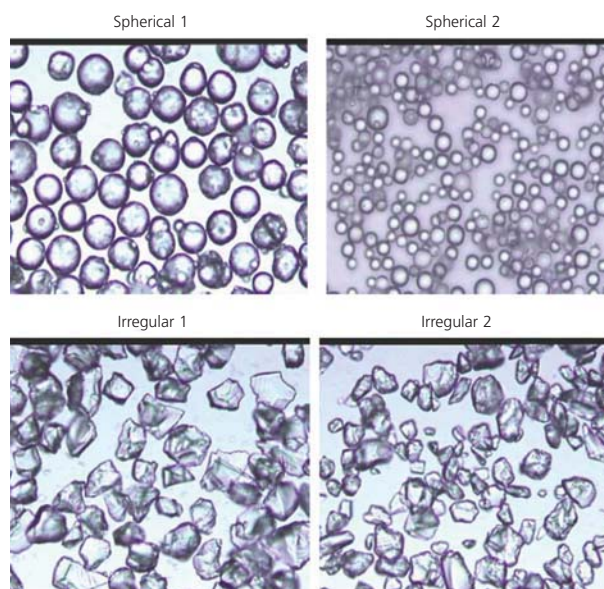
### The VersaFlash™ system

The Supelco VersaFlash™ Station used in these experiments accepts cartridges of different sizes and lengths. Cartridge change-out is quick and easy and all materials that are in contact with the sample and eluants are inert and durable Teflon®, PEEK or polypropylene.

### Backpressure

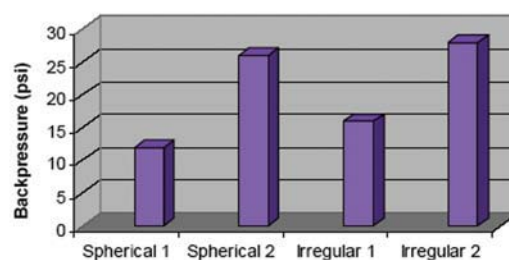
Particle shape, size and size distribution affect backpressure (along with compressibility and porosity which are not

Figure 1. SEM of *Thalassiosira* diatoms (Ivo Grigorov, Ph.D., www.sinia-planeta.com, used with permission)



considered here). To evaluate the effect of these parameters on backpressure, methanol was pumped through the cartridges at 100 ml/min. The pressure was measured with an in-line pressure gauge from Supelco. Results are presented in Figure 2. Two observations are noteworthy. First, Spherical 1 with twice the particle size of Spherical 2 gave roughly half the backpressure. Second, although the particle sizes of Spherical 1 and Irregular 1 are similar, the backpressure of Spherical 1 is 33% lower. Surprisingly, the backpressure of Spherical 2 is even lower than that of Irregular 2 although its particle size is 45% smaller, possibly due to the presence of fines in Irregular 2.

Figure 2. Backpressure of irregular and spherical silica under 100 ml/min flow of methanol in 53 x 23 mm I.D. cartridges



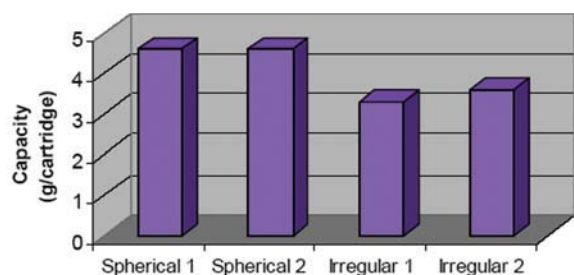
### Capacity and retention

Preparative materials should have high capacity to maximize the yield and concentration of purified compounds. The breakthrough method was used to measure the capacity of each type of silica in this study. Silica was packed into a standard cartridge and a solution of benzyl alcohol in dichloromethane was pumped through. Once breakthrough of benzyl alcohol was detected, the flow was stopped and the amount of benzyl alcohol adsorbed in the cartridge was calculated. Results are shown in Figure 3.

Although the surface areas of the four silicas are similar, their capacity for benzyl alcohol is quite different. The two spherical silicas have more than 40% higher capacity than the two irregular silicas. Two factors likely contribute to this difference. First, because of the high packing density of the spherical particles, more material can be packed into each cartridge. Second, the silicas may differ in the degree of microporosity (<20Å). Micropores contribute to the total surface area, but surface inside the micropores is not actually accessible to analyte molecules.

**Figure 3.** Capacity of benzyl alcohol on different flash silica particles

Cartridge: Flash cartridge, 75 x 40 mm I.D.  
 Mobile phase: benzyl alcohol in dichloromethane (70 mg/ml)  
 Flow rate: 50 ml/min  
 Det.: UV 254nm  
 Breakthrough cutoff: 0.2A

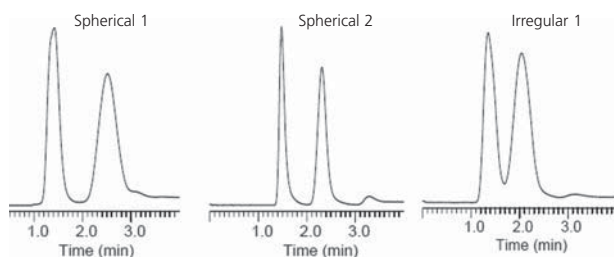


### Efficiency and resolution

Efficient preparative columns deliver high sample concentration (less peak dilution) and high sample purity (less overlap of peaks). The separation of toluene and benzyl alcohol shown in Figure 4 and toluene, 2,6-dinitrotoluene and benzanilide shown in Table 2 show that spherical silica particles gives higher efficiencies and better resolution than irregular silicas with similar pore and particle size. The highest efficiencies and best resolution were obtained on the smaller spherical silica, Spherical 2.

**Figure 4.** Separation of Toluene and Benzyl Alcohol

Cartridge: Flash cartridge, 75 x 40 mm I.D.  
 Mobile phase: dichloromethane: methanol (10:1, v/v)  
 Sample: 1 ml, 7 mg/ml of each compound in dichloromethane  
 Flow rate: 50 ml/min  
 Det.: UV 254nm



**Table 2.** Efficiency and resolution of toluene, 2,6-dinitrotoluene and benzanilide on spherical and irregular silicas (75 x 40 mm I.D. cartridge)

	Spherical 1	Spherical 2	Irregular 1	Irregular 2
<b>Efficiency:Toluene</b>	244	989	258	351
<b>Efficiency: 2,6-Dinitrotoluene</b>	254	979	246	196
<b>Resolution</b>	3.11	5.93	2.92	3.02
<b>Efficiency: Benzanilide</b>	292	820	252	152
<b>Resolution</b>	5.45	8.81	4.88	4.31

### Benefits of spherical silica particles in VersaFlash™ cartridges

Compared to irregular particles, spherical silica particles generate lower backpressure and have greater mechanical stability, higher efficiency and better reproducibility. Surface areas and chemical properties are comparable. In spite of their benefits, Supelco is one of the very few suppliers of cartridges packed with spherical silica particles. Choose Supelco's VersaFlash™ system of cartridges and equipment for fast and efficient flash separations.

#### Ordering information

Prod No.	Description	Pack Size
<b>VersaPak™ Silica Cartridges*</b>		
97704-U	40 mm x 75 mm	12/pk
97705-U	40 mm x 75 mm	96/pk
97706-U	40 mm x 150 mm	6/pk
97707-U	40 mm x 150 mm	48/pk
97708-U	80 mm x 150 mm	2/pk
97709-U	80 mm x 150 mm	12/pk
97710-U	80 mm x 300 mm	1/pk
97711-U	80 mm x 300 mm	6/pk
97712-U	110 x 300 mm	1/pk
97713-U	110 x 300 mm	1/pk
<b>VersaPak™ C18 Cartridges*</b>		
97700-U	40 mm x 75 mm	2/pk
97701-U	40 mm x 150 mm	1/pk
97702-U	80 mm x 150 mm	1/pk
97703-U	80 mm x 300 mm	1/pk

\*Smaller cartridges are currently under development. Check the website or call us for dimensions and availability.

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## SPME ARTICLE

Measurement of acetic acid vapor in the workplace using passive sampling SPME and on-fiber derivatization with 1-pyrenyldiazomethane: Comparison with active sampling<sup>1</sup>**Introduction**

Workplace exposures to acetic acid vapors can cause irritation to eyes and mucous membranes, chronic bronchitis and asthma (1,2,3). The American Conference of Governmental Industrial Hygienists (ACGIH) proposed 25 mg/m<sup>3</sup> TLV-TWA (time-weighted average) and 37 mg/m<sup>3</sup> TLV-STEL (short-term exposure limit) threshold limit values (TLVs) for workers exposed to acetic acid. The American Industrial Hygiene Association (AIHA) accepted a 0.37 mg/m<sup>3</sup> (0.15 ppm) lowest detectable limit for the human nose, and an irritative threshold of 105 mg/m<sup>3</sup> (42 ppm) (4). Concentrations higher than 50 ppm are usually intolerable (1). The deleterious effects of acetic acid at relatively low levels prompted the development of analytical tools that can sample and detect it in workplace situations.

Two problems with acetic acid quantification are its low molecular weight and high volatility. In 1995 Pan *et al* (5) proposed making the 1-pyrenyldiazomethane (PDAM) derivative on a solid phase microextraction (SPME) fiber as a passive sampling technique for acetic and other carboxylic acid vapors. The PDAM-esters could then be analyzed by GC. The PDAM, synthesized by Nimura *et al* (6) in 1988 is an ideal derivatization agent for acetic acid. The reaction occurs readily at ambient temperatures without catalysts and the PDAM-ester derivative is stable over a wide temperature and humidity range.

To make the sampling reproducible and permit measurement of the TLV-TWA for VOCs (including carboxylic acids), Martos and Pawliszyn (7) recommended setting the PDMA-coated SPME fiber in the needle housing at a defined and constant of 0.1 to 3.5 cm. This provides the opportunity to increase the sampling time of the vapors without saturating the SPME fiber, as well as to create a small diffusion chamber (0.00086 cm<sup>2</sup>) that is not affected by variation in air speed flow.

The aim of the work presented here is to measure the exposure to acetic acid vapors in a workplace environment and compare the results obtained with SPME (passive) and activated charcoal (active) sampling techniques.

**Acetic acid exposure in cytology laboratories**

Workers in cytology labs are exposed to large amounts of acetic acid vapor because it is a commonly used fixative agent. Air samples were taken below the breathing zone from ten cytology lab workers over a 240 minute period during the preparation of the 1:3 v/v acetic acid:ethanol fixing solution and the slides themselves.

**Passive sampling technique using SPME**

An 85 µm polyacrylate SPME fiber (Supelco) was treated for 60 minutes with a solution of 1-pyrenyldiazomethane (PDAM), 5 mg/ml in n-hexane. The treated fiber was withdrawn a fixed distance (Z = 0.3 cm) into the needle housing. The treated fiber assemblies were then affixed to the workers at a prescribed distance below their breathing zone.

After the 240-minute exposure time the PDAM-acetic acid derivatives were desorbed from the SPME fiber at 300 °C in a GC injector (Varian model CP-3800). For GC/MS detection, analytes were resolved on an Equity-5 capillary column, 30m x 0.25 mm ID, 0.25 µm df (Supelco) and detected on a

Saturn 2200 EI-Cl MS (Varian). For GC/FID detection, a Supelco SPB-5 capillary column, 30m x 0.25 mm ID x 1.0 µm df was used. Conditions appear in the Table 1 below. The limit of quantification (LOQ) for acetic acid was found to be 0.2 ng for the MS/EI method and 3.0 ng for the GC/FID method. Both values subtracted the contribution from the blank reagent baseline.

**Active sampling technique using ORBO 32 activated charcoal tubes**

Solvent desorption tubes (ORBO 32, Supelco) packed with activated charcoal were used in the active sampling system. The sampling apparatus was affixed to the workers proximal to the SPME assembly. The personal air sampling pump was set at 200 ml/min and air was sampled for 240 minutes (48L sampled). After sampling, the ORBO tubes were treated with thionyl chloride and aniline to form the corresponding anilide derivative of acetic acid. Analysis of the anilide derivative was by capillary GC on an instrument equipped with FID and thermoionic specific detector (TSD) joined with a Y-connector to a Supelco SPB-5 capillary column, 30m x 0.25 mm ID x 1.0 µm df. Conditions appear in Table 1 below. The resulting LOD for the active sampling technique was 104 mg/m<sup>3</sup> with GC-FID and 0.021 mg/m<sup>3</sup> with GC-TSD.

**Table 1. GC conditions for PDAM and Anilide Analysis**

Injector	PDAM: splitless (2 min) then split 20:1 (liner I.D. 0.75 mm) at 300 °C Anilide: split 4:1 at 220 °C
Desorption/ Injection	PDAM: 10 minutes Anilide: 3.0 µl injection
Carrier gas	PDAM: Helium 2 cc/min (FID); 1 cc/min (MS) Anilide: Helium 1.2 cc/min
Oven temperature	PDAM-FID: 40 °C (6 min) 30 °C /min-265 °C -25 °C / min-300 °C (10 min) PDAM-MS: 100 °C (2 min) 20 °C /min-280 °C (1 min)-2 °C / min-310 °C Anilide: 100 °C (1 min) 10 °C /min-300 °C
Detector	PDAM-FID: 270 °C PDAM-MS: full scan E.I. 100-300 m/z (molecular ion acquisition 274 m/z) PDAM-MS: full scan C.I. 100-300 m/z (acquisition 275 m/z) Anilide: FID-TSD: 300 °C

**Results**

Results of the analysis of workplace acetic acid using the passive (SPME) and active (charcoal ORBO 32) methods are reported in Table 2. Using the Student's T-test for paired samples, there appears to be no significant difference between the two methods (p<0.05). Acetic acid levels in the air breathed by the workers ranged from 1.5 to 4.1 mg/m<sup>3</sup> (mean 2.8±1.05) by the SPME method and 1.1 to 4.2 mg/m<sup>3</sup> (mean 2.7±0.98) with the charcoal method. Of importance to the workers, all acetic acid values were well below the TLV-TWA of 25 mg/m<sup>3</sup> suggested by the ACGIH.

The SPME technique is also characterized by good analytical reproducibility and repeatability as tested with four different fiber lots (C.V. inter-day = 4.3%, C.V. intra-day = 3.8%). We found that over 200 analyses could be performed on the same fiber. Also, the PDAM and PDAM-ester derivative adsorbed on the fiber are stable for at least 7 days if stored at or below -20 °C .

**Table 2. Concentration of Acetic Acid environmental values (mg/m<sup>3</sup>) detected with the two sampling system.**

Worker	SPME Method Passive sampling Acetic acid (mg/m <sup>3</sup> )	ORBO 32 (Charcoal) Method Active sampling Acetic acid (mg/m <sup>3</sup> )
1	4.0	4.2
2	3.2	3.3
3	1.9	2.2
4	1.7	1.1
5	4.1	4.0
6	3.0	2.7
7	1.5	1.7
8	2.7	2.3
9	1.5	2.3
10	3.9	3.2
<b>Mean</b>	<b>2.75</b>	<b>2.70</b>
<b>S.D.</b>	<b>1.05</b>	<b>0.98</b>
<b>Minimum</b>	<b>1.50</b>	<b>1.10</b>
<b>Maximum</b>	<b>4.10</b>	<b>4.20</b>

## References

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- 7) Martos P.A. and Pawliszyn J.; "Time-weighted average sampling with solid-phase microextraction device: implications for enhanced personal exposure monitoring to airborne pollutants." *Anal. Chem.*, 71, 1513-1520 (1999).
- 8) Vohra K. And Gaid S.V.; "Gas chromatographic determination of airborne organic acids via their anilides." *Analyst*, 117, 1567-1570 (1992).

## Ordering information

Prod No.	Description
57304	85 µm Polyacrylate SPME fiber, pack of 3 fibers
24035	SPB-5 capillary column, 30 m x 0.25 mm ID x 1.0 µm df
28094U	Equity-5 capillary column, 30 m x 0.25 mm ID x 1.0 µm df

<sup>1</sup> M. Pacenti, P. Boccalon, S. Dugheri and L. Focardi, U.O. Medicina Preventiva dei Lavoratori, Azienda Ospedaliero Universitaria Careggi, Firenze, Italia (Presented at the 12th ICOH International Congress on Occupational Health (Modena, 13-16 October 2004)

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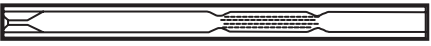



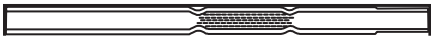


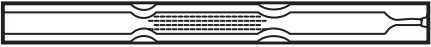






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### Ordering information

Prod No.	Description	Dimension	Pack Size
<b>Agilent</b>			
2879901-U	for HP5890, HP6890,		1
2879905-U	HP6850 & HP4890		5
2879925-U	Split/Splitless FocusLiner Single Taper, packed with quartz wool		25
2879801-U	Split/Splitless FocusLiner, packed with quartz wool		1
2879805-U			5
2879825-U			25
2879601-U	Split/Splitless FAST FocusLiner, packed with quartz wool		1
2879605-U			5
2879625-U			25
2879501-U	Split/Splitless FAST FocusLiner, Single taper, packed with quartz wool		1
2879505-U			5
2879525-U			
<b>PerkinElmer</b>			
2879201-U	Split/Splitless FocusLiner for AutoSystem and Clarus™ 500, packed with quartz wool		1
2879205-U			5
2879101-U	Split/Splitless FocusLiner for AutoSystem and Clarus 500, Single Taper, packed with quartz wool		1
2879105-U			5
2878901-U	Split/Splitless FocusLiner for PSS Injector and AutoSystem XL, packed with quartz wool		1
2878905-U			5
<b>Varian</b>			
2875701-U	for 1078 & 1079		1
2875705-U	Split/Splitless FocusLiner, Single Taper, packed with quartz wool		5
2875501-U	Split/Splitless FocusLiner, Dual Taper, packed with quartz wool		1
2875505-U			5
2875401-U	for 1075 & 1077		1
2875405-U	Split FocusLiner, packed with quartz wool		5
2874801-U	Split FocusLiner, tapered, packed with quartz wool		1
2874805-U			5
2874701-U	Split FAST FocusLiner, tapered, packed with quartz wool		1
2874705-U			5
2874901-U	Split FocusLiner, with top-end restriction, packed with quartz wool		1
2874905-U			5
2874601-U	Splitless FocusLiner, with top-end restriction, packed with quartz wool		1
2874605-U			5

GC ARTICLE

# Using Fast GC and Column Selectivity to Optimize Trans FAME Analysis

Len Sidisky, Kathy Stenerson, Rodney George and Greg Baney

## Introduction

There is a confirmed link between consumption of foods that contain trans fatty acids and LDL or so-called bad cholesterol levels. High LDL levels are associated with increased risk of coronary heart disease, the leading cause of death in the US and a growing concern in other parts of the world. *Trans* fatty acids, also known as *trans* fat, are made by hydrogenation of liquid oils which solidifies the oils and increases the shelf life and flavor stability of the oils and foods that contain them, including vegetable oils, crackers, candies, baked goods, cookies, snack foods, fried foods, salad dressings and many other processed foods. The US Food and Drug Administration (FDA) recently amended its regulations on nutritional labeling to require that the amount of *trans* fatty acids in a food be included in the Nutrition Facts panel. (See US FDA 21 CFR Part 101, Sec. 101.62 "Nutrient content claims for fat, fatty acid, and cholesterol content of foods.") Because of their impact on health, the measurement of *trans* fatty acid levels requires a reliable analytical method.

## FAME analysis by capillary GC

Whereas the fatty acid *trans* isomers have adverse health affects, the *cis* isomers typically do not. Therefore it important to be able to resolve positional *cis-trans* isomers in food samples to ensure the food conforms to label requirements. Capillary GC is by far the most common analytical tool to measure fatty acids in food and other matrices. (The fatty acids are first derivatized to the corresponding methyl esters (FAME) to improve their volatility prior to GC analysis.) Capillary GC columns provide the necessary selectivity to resolve *cis-trans* pairs and can provide rapid analysis, which is important since more samples are continually analyzed on a day-to-day basis.

For analysts employing capillary GC for FAME analysis, two critical aspects of the method need to be optimized:

- Speed of the FAME analysis -- to maximize throughput
- Selectivity of GC stationary phase -- to ensure resolution and reliable quantification of critical pairs

To address these issues we evaluated approaches to decreasing the analysis times of trans FAMES while using selective capillary

columns to provide the desired sample resolution.

## Fast GC in FAME analysis

Reducing the run time of a GC separation can be achieved by the use of shorter capillary columns, decreased column internal diameter, thinner stationary phase films, H<sub>2</sub> carrier gas, higher carrier gas velocities, optimized  $\alpha$  values, selective detectors, faster oven temperature programming rates and combinations of the parameters in this list. However, one must be careful to understand the impact of these changes on the resolution of the method.

In our work, we focused on using narrow bore capillary columns (<250  $\mu$ m I.D.) in combination with higher carrier gas linear velocities and fast temperature programming rates using H<sub>2</sub> as the carrier gas in order to reduce analysis times while maintaining necessary sample resolution.

## Column selectivity in FAME analysis

Selectivity was evaluated by preparing polar and highly polar capillary columns. Polar polyethylene glycol columns resolve FAMES by degree of unsaturation, with minimal overlap of the carbon chain lengths. They also resolve *cis* and *trans* isomers. Highly polar cyanosilicone columns, depending upon the column type, will also resolve *cis* and *trans* isomers and positional geometric isomers.

## Separation of FAMES by carbon chain length and degree of unsaturation

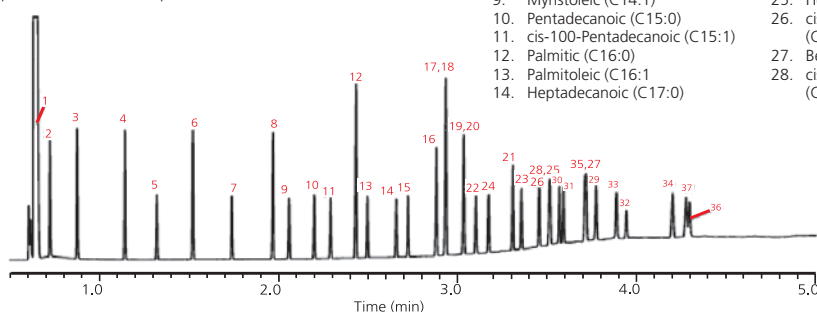
Polar Omegawax and SUPELCOWAX (polyethylene glycol) capillary columns are the choice for resolving FAME isomers according to carbon chain length and degree of unsaturation, with minimal carbon chain length overlap. Figure A demonstrates the fast GC analysis of a 37-component FAME mix on this polar column. The analysis time is about 5 minutes. The elution order within a carbon chain length is: saturated, monoene, diene, triene, etc. Minimal resolution of the *cis* and *trans* isomers is achieved on this column as shown by the resolution of methyl eladate (peak 17) from methyloleate (peak 18) and methyl linolelaidic (peak 19) from methyl linoleate (peak 20). In both cases, the *cis* isomer elutes prior to the *trans* isomer.

Figure A. 37-component FAME MIX on Supelcowax 10

Column: SUPELCOWAX 10, 15 m x 0.10 mm I.D., 0.10  $\mu$ m  
 Oven: 140  $^{\circ}$ C, 40  $^{\circ}$ C/min. to 280  $^{\circ}$ C (2 min.)  
 Inj: 250  $^{\circ}$ C  
 Det.: FID, 260  $^{\circ}$ C  
 Carrier gas: H<sub>2</sub>, 50 cm/sec, constant  
 Injection: 0.2  $\mu$ l, 200:1 split  
 Liner: 4 mm I.D., cup split  
 Sample: 37-component FAME Mix

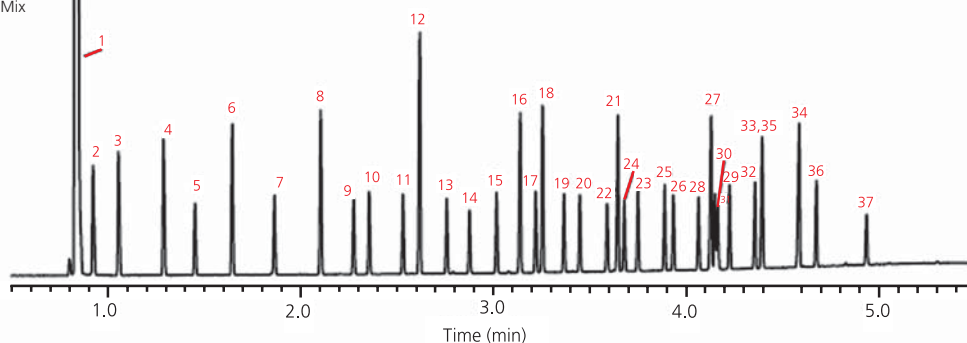
### Peak List

- |                                   |  |   |
|-----------------------------------|--|---|
| 1. Butyric (C4:0)                 | 15. cis-10-Heptadecanoic (C17:1)         | 29. Erucic (C22:1n9)                              |
| 2. Caproic (C6:0)                 | 16. Stearic (C18:0)                      | 30. cis-11,14,17-Eicosatrienoic (C20:3n3)         |
| 3. Caprylic (C8:0)                | 17. Elaidic (C18:1n9t)                   | 31. Arachidonic (C20:4n6)                         |
| 4. Capric (C10:0)                 | 18. Oleic (C18:1n9c)                     | 32. Tricosanoic (C23:0)                           |
| 5. Undecanoic (C11:0)             | 19. Linolelaidic (C18:2n6t)              | 33. cis-13,16-Docosadienoic (C22:2)               |
| 6. Lauric (C12:0)                 | 20. Linoleic (C18:2n6c)                  | 34. Lignoceric (C24:0)                            |
| 7. Tridecanoic (C13:0)            | 21. Arachidic (C20:0)                    | 35. cis-5,8,11,14,17-Eicosapentaenoic (C20:5n3)   |
| 8. Myristic (C14:0)               | 22. $\gamma$ -Linolenic (C18:3n6)        | 36. Nervonic (C24:1)                              |
| 9. Myristoleic (C14:1)            | 23. cis-11-Eicosenoic (C20:1)            | 37. cis-4,7,10,13,16,19-Docosahexaenoic (C22:6n3) |
| 10. Pentadecanoic (C15:0)         | 24. Linolenic (C18:3n3)                  |   |
| 11. cis-100-Pentadecanoic (C15:1) | 25. Heneicosanoic (C21:0)                |   |
| 12. Palmitic (C16:0)              | 26. cis-11, 14-Eicosadienoic (C20:2)     |   |
| 13. Palmitoleic (C16:1)           | 27. Behenic (C22:0)                      |   |
| 14. Heptadecanoic (C17:0)         | 28. cis-8,11,14-Eicosatrienoic (C20:3n6) |   |



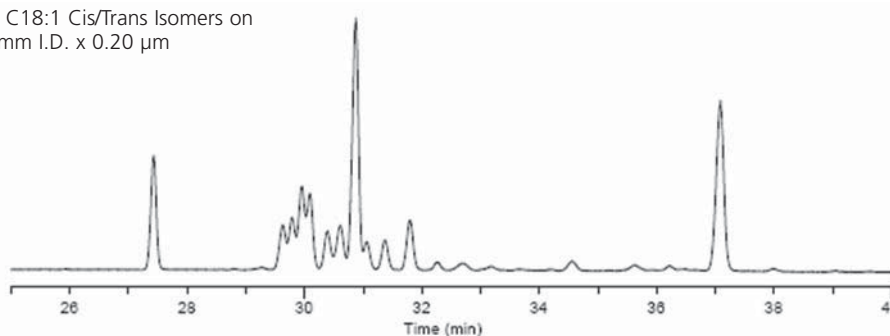
**Figure B.** Fast GC analysis of FAMES on SP-2380, 15m x 0.10 mm I.D., 0.08  $\mu$ m

column: SP-2380, 15 m x 0.10 mm I.D., 0.08  $\mu$ m  
 oven: 125 °C (0 min.), 25 °C /min. to 245 °C (1 min)  
 inj.: 200 °C  
 det.: FID, 260 °C  
 carrier gas: H<sub>2</sub>, 45 cm/sec, constant flow  
 injection: 0.1  $\mu$ l, split 300:1  
 liner: 4 mm I.D., cup split  
 sample: 37-component FAME Mix



**Figure C.** Resolution of C18:1 Cis/Trans Isomers on SP-2560, 100m x 0.25 mm I.D. x 0.20  $\mu$ m

176 °C, 17 cm/sec, He



### Cis and trans FAME isomer separation

Highly polar cyanosilicone based capillary columns are needed to provide *cis* and *trans* FAME isomer resolution. Standard cyanosilicones such as SP-2330, SP-2340, and SP-2380 provide group *cis* and *trans* isomer resolution, where the *trans* isomers typically elute first as a group followed by the *cis* isomer group. Figure B demonstrates the fast GC analysis of a 37-component FAME mixture on an SP-2380 0.10 mm I.D. column. C18:1 *cis* and *trans* FAMES are included in the sample. Note the improved resolution of the *cis* and *trans* pairs (peaks 17/18 and 19/20) compared to the previous SUPELCOWAX 10 column separations.

### Positional geometric FAME isomers on SP-2560

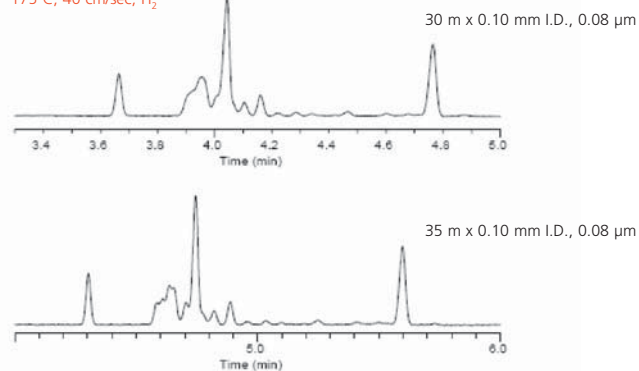
A specially prepared and tested 100m x 0.25 mm I.D., 0.20  $\mu$ m SP-2560 resolves the positional *cis* and *trans* isomers. Figure C demonstrates this analysis under standard run conditions using helium carrier. Simply switching to H<sub>2</sub> carrier gas will reduce analysis time.

### Fast GC on 0.1 mm I.D. columns

In developing a fast GC alternative to this long (100m) column, we investigated decreasing the column internal diameter in order to provide similar resolution in a faster analysis time. Figure D demonstrates the analysis on 30m and 35m x 0.10 mm I.D. x 0.08  $\mu$ m df SP-2560 columns. As shown, these columns do not provide the resolution demonstrated on the 100m column. The 0.10 mm I.D. columns have limited sample capacity, resulting in the columns being easily overloaded. We also investigated various temperature program rates (Figure E) to try and improve the resolution. Some improvements were noted, but results comparable to the 100m columns were not achieved.

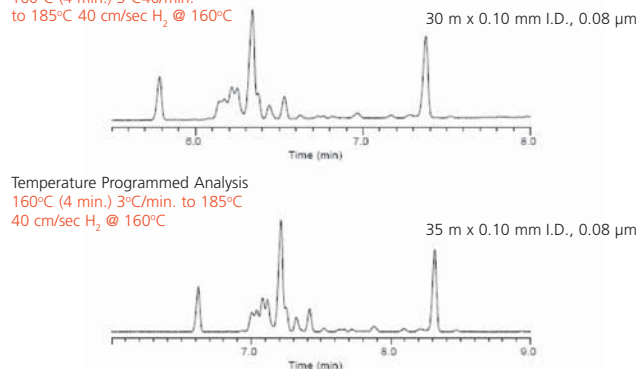
**Figure D.** C18:1 Cis/Trans Isomers on the 0.10 mm I.D. SP-2560

Isothermal Analysis  
 175°C, 40 cm/sec, H<sub>2</sub>



**Figure E.** C18:1 Cis/Trans Isomers on the 0.10 mm I.D. SP-2560

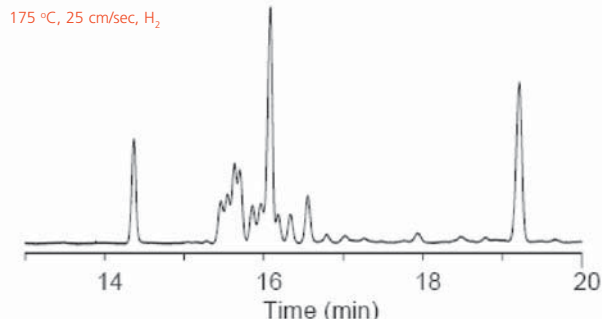
Temperature Programmed Analysis  
 160°C (4 min.) 3°C/min. to 185°C  
 to 185°C 40 cm/sec H<sub>2</sub> @ 160°C



**Fast GC on 0.18 mm I.D. columns**

Since the 0.10 mm I.D. columns did not provide the necessary resolution and decreased analysis time, we focused our attention on 0.18 mm I.D. columns. Theoretical calculations predict that a 75m x 0.18 mm I.D. x 0.14 μm SP-2560 should provide similar resolution to the 100m x 0.25 mm I.D. column. Figure F shows the results of this analysis.

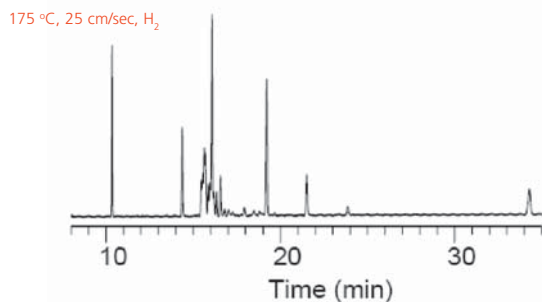
**Figure F.** C18:1 Cis/Trans Isomers on the SP-2560, 75 m x 0.18 mm I.D. x 0.14 μm df



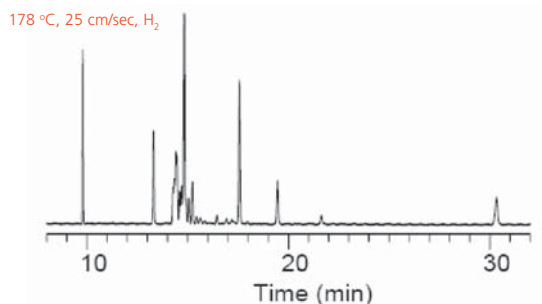
**Analysis temperature**

We also investigated the effect of analysis temperature on the resolution of the *cis* and *trans* C18:1 isomers. Figures G through I show that slight adjustments in the temperature can affect the resolution of the isomers and the overall analysis time.

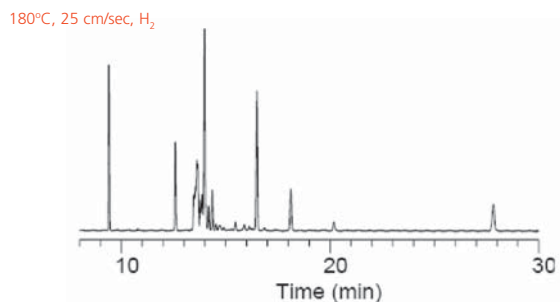
**Figure G.** Temperature Effects on the SP-2560, 75 m x 0.18 mm I.D. x 0.14 μm df



**Figure H.** Temperature Effects on the SP-2560, 75 m x 0.18 mm I.D. x 0.14 μm df



**Figure I.** Temperature Effects on the SP-2560, 75m x 0.18 mm I.D. x 0.14 μm df



**Summary**

Fast GC analysis is typically performed using short, 0.10 mm I.D. capillary columns with H<sub>2</sub> carrier gas and rapid temperature programming rates. FAMES can be analyzed with a variety of 0.10 mm I.D. columns, depending upon the information required. SUPELCOWAX 10 (polyethylene glycol) polar columns resolve FAME isomers according to carbon chain length and degree of unsaturation. SP-2380 and SP-2560 (cyanosilicone) highly polar columns provide *cis* and *trans* resolution of FAME isomers. SP-2560 column length will impact the resolution of positional geometric C18:1 FAME isomers. A 0.10 mm I.D. version of the SP-2560 column does not provide the necessary resolution. However, the 75 m x 0.18 mm I.D. x 0.14 μm df SP-2560 offers the best alternative to the 100 m x 0.25 mm I.D. x 0.20 μm df SP-2560 column for resolving positional geometric isomers in a shorter analysis time. Analysis temperature can affect the resolution and overall analysis time for *trans* FAME analysis. It is important when reducing the analysis time to consider the impact on overall resolution.

**Ordering Information**

Prod No.	Description
<b>Capillary GC Columns</b>	
24343	SUPELLOWAX 10, 15 m x 0.10 mm I.D., 0.10. μm df
24317	SP-2380, 100 m x 0.25 mm I.D., 0.20 μm df (specially tested for fatty acid analysis)
24056	SP-2560, 100 m x 0.25 mm I.D., 0.20 μm df
23348-U	SP-2560, 75 m x 0.18 mm I.D. x 0.14 μm df
Custom	SP-2380, 15 m x 0.10 mm I.D., 0.08 μm df
Custom	SP-2560, 30 m x 0.10 mm I.D., 0.08 μm df
Custom	SP-2560, 35 m x 0.10 mm I.D., 0.08 μm df
<b>FAME Standards</b>	
47885U	37-component FAME Mix (each ampoule contains 10 mg/ml of the FAME reference standard mix in methylene chloride) 1 ml

The Sigma-Aldrich family of brands provides you with complete solutions to fatty acid analysis, including columns, GC accessories, reagents and standards.

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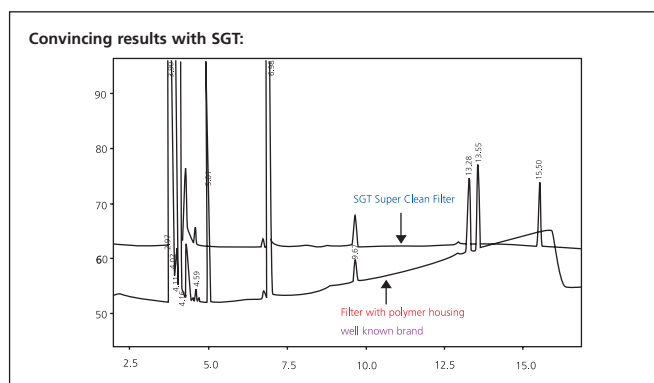


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Prod No.	Description
<b>Hardware for Super Clean-Gas Filters®</b>	
SU861011	GC-1-Position Baseplate
SU861012	GC-2-Position Baseplate
SU861013	GC-3-Position Baseplate
SU861014	GC-4-Position Baseplate
SU861015	LC-Base plate - high flow - for 2 filters (N <sub>2</sub> -purification)
<b>Standard Classic Super-Clean Gas Filter® extra-large capacity cartridges</b>	
SU861021	Moisture GC-Filter Ultra Capacity; with indicator
SU861022	Oxygen GC-Filter Ultra Capacity; with indicator
SU861023	Hydrocarbon Filter (Charcoal); Ultra Capacity without indicator
SU861025	Combi GC-Filter replacement (charcoal/moisture) fuel gas
SU861026	Triple GC-Filter Replacement (Oxygen/Moisture/Charcoal), 1ea.
SU861027	Triple GC-Filter Replacement for Helium (Oxygen/Moisture/Charcoal), 1ea.
SU861029	LC-Filter bundle of 2 (charcoal 2x for LC-MS: N <sub>2</sub> purification) - high flow: without indicator
SU861028	HIGH-FLOW Special Moisture Filter; bundle of 2 replacement cartridges
<b>Super-Clean Kits</b>	
SU861040	GC-MS Kit for He - gas specific (Baseplate + 1 triple filter)
SU861041	GC-MS, ECD,FID,NPD-carrier gas Kit (Baseplate + 1 triple filter)
SU861044	GC-FID Fuel Gas Purification System (2 x charcoal/moisture)
SU861046	LC-MS Kit for 2 filters/baseplate (2 x charcoal: N <sub>2</sub> purification) - HIGH FLOW capacity, without indicator
SU861045	HIGH-FLOW Special Moisture Filter kit for 2 filters/baseplate
SU861043	GC-FID Kit for 3 filters, Triple + 2 x Combi Cartridge-(Charcoal/moisture)
SU861042	GC-FID Kit for 4 filters, (O <sub>2</sub> , Moisture, 2 x Charcoal)

## HPLC STANDARDS ARTICLE

Use of Certified Reference Materials Thomas Linsinger, EC-JRC, Institute for Reference Materials andMeasurements (IRMM), Geel, Belgium: Thomas.Linsinger@cec.eu.int With introduction by Rainer Walz, PhD, Product Manager, Fluka/Riedel-de Haën

Reference materials, especially certified reference materials, are indispensable tools in ensuring analytical quality. They are used in calibration, method validation, routine quality control and establishing traceability.

Analytical chemists in industrial QC and international and regional governmental regulatory agencies are important players in the enforcement of quality and safety regulations of both domestic and imported products. The quality and reliability of their efforts, however, is in turn dependent on the quality and reliability of the reference standards used in their analytical methods. Sigma-Aldrich, through its Fluka brand, offers a wide selection of Certified Reference Materials (CRM) sanctioned by the Institute for Reference Materials and Measurements (IRMM) to ensure that our customers have available the most accurate, precise, stable, traceable and reliable single component and matrix reference standards. In this article, an official from the IRMM discusses some basic aspects of CRMs and how to use them to full advantage.

### Reference Materials, Certified Reference Materials and Standard Reference Materials

The use of highly accurate reference materials is an important aspect of quality assurance in analytical chemistry laboratories. Consequently, ISO 17025 [1] dedicates the whole paragraph 5.6.3 to reference materials. The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre of the European Commission (EC-JRC) is responsible for storage, production, management and distribution of certified reference materials (CRM) for the European Commission. Although ISO guidelines exist for the use of CRM [2], the IRMM realizes that many questions and misunderstandings still remain.

All reference materials must have the following characteristics specified and confirmed:

- Homogeneity
- Stability
- Assigned values (concentration, purity, etc.)

Certified Reference Materials (CRM) are a subgroup of reference materials with a higher degree of characterization, assigned and traceable values and written certification than the latter. Calibration standards are also reference materials. Arguably they should even be certified reference materials, since a standard without guaranteed purity and concentration is of very limited use. Recently, the IRMM launched a new brand of CRMs, European Reference Materials (ERM®) to address the need for CRMs of even higher quality. Other brands of CRMs available from IRMM include BCR® and IRMM branded materials.

### Applications of RMs and CRMs

Every analytical chemistry application requires a reference material of some form. The term reference material gives only some basic requirements and therefore describes a wide range of materials. For several intended uses the higher degree of characterization achieved for CRMs is required.

### Method Calibration

Calibration standards relate instrument response to the analyte concentration. The uncertainty of the stated analyte concentration or purity should be as low as possible, because this uncertainty is incorporated fully in the final analytical result. If, for example the concentration of the calibration standard is given as  $10 \pm 0.5$  mg/l, the final uncertainty of the analytical result cannot be lower than 5% (0.5/10 mg/l). Using CRMs of pure powders or pure solutions as calibration standards is preferred. Typically, matrix reference materials are not suitable for calibration; uncertainties on the certified values are usually higher than for pure solutions due to the more difficult value assignment process.

### Validation and Performance Assessment

A typical method validation study evaluates the repeatability, intermediate precision (within-laboratory reproducibility), robustness and trueness\* of the method. Repeatability and intermediate precision could be assessed using normal laboratory samples (if they are homogeneous and stable over the period of the study). However, valuable personnel and instrument time can be saved by using CRMs also for this purpose as the assessment of trueness can be done using the data from these experiments.

In the assessment of trueness, results of measurements on a CRM are compared to the uncertainty of the certified value. Total uncertainty of the measurement ( $u_t$ ) is found from the uncertainty of measurement ( $u_m$ ) and the certified value of the reference material ( $u_{CRM}$ ):

$$u_t = \sqrt{u_m^2 + u_{CRM}^2}$$

When the uncertainty of the certified value ( $u_{CRM}$ ) is very small, the total uncertainty is essentially the uncertainty contributed by the experiment. The standard deviation of the measurements on the CRM divided by the square root of the number of measurements can be used as rough estimation for  $u_m$ . These measurements should be spread over several days to achieve conditions of intermediary precision.

**Example:** ERM-BB445 (PCBs in pork fat):

PCB 52: certified value =  $12.9 \pm 0.9$  µg/kg.

Footnote 2 states that a coverage factor of 2 was applied.  $u_{CRM}$  is therefore  $0.9 / 2 = 0.45$  µg/kg.

The validation study gave an average of  $14.3 \pm 1.8$  (single standard deviation of 6 measurements spread over 3 days).  $u_m$  is therefore estimated at  $1.8/\sqrt{6} = 0.73$  µg/kg.

$$u_t = \sqrt{u_m^2 + u_{CRM}^2} = \sqrt{0.73^2 + 0.45^2} = 0.86 \text{ µg/kg}$$

The expanded uncertainty is  $2 u_t = 1.7$  µg/kg. This is larger than the difference between the certified and the found value ( $14.3 - 12.9 = 1.4$  µg/kg). Therefore, the method does not have a significant bias and  $u_t$  is used as uncertainty contribution of trueness in the total uncertainty calculation.

## Routine Quality Control

Using CRMs to produce quality control charts offers significant advantages over ordinary, laboratory prepared standards:

- CRMs have been tested for homogeneity. Switching from one bottle to the other is possible without having to re-establish the control chart.
- CRMs have been stabilized so that even if opened they can be assumed to be more stable than normal laboratory samples.
- CRMs are consistent and accurate allowing every result on the control chart to be checked against a true value.
- Labor costs in preparing, validating and rechecking home-made reference materials quickly exceed the additional cost of a CRM.

## Establishment of Traceability

Most analytical techniques involve sample pre-treatment, dilution, extraction, etc. Each step breaks the traceability chain. Matrix CRMs are the tool of choice for proving that no significant errors in the sample preparation steps occurred, thereby maintaining traceability.

## Reliable Sources of BCR® and ERM® and IRMM® Reference Materials

For quality-minded analytical laboratories, CRMs are indispensable tools to ensure the quality of the analytical results, and aid in compliance to ISO standards and guidelines. Fluka is among the few authorised distributors of CRMs produced by the European Commission, and in particular by the IRMM. These materials fulfill the highest requirements regarding uncertainty, homogeneity, stability and traceability.

## References:

- [1] ISO 17025 "General requirements for the competence of testing and calibration laboratories", ISO, Geneva, 2000
- [2] ISO Guide 33 "Uses of Certified Reference Materials", ISO, Geneva, 2000

## Training course

### „Selection and Use of Reference Materials“

Reference materials are considered key tools for achieving traceability of measurements, proving accuracy of methods and demonstrating proficiency of laboratories. The aim of this course is the practical demonstration of selection and use of reference materials:

- Selection of appropriate materials
- Evaluation of analytical results on reference materials
- Establishing traceability
- Uncertainty estimation
- Demonstrating trueness
- Proof of laboratory proficiency
- Proper material handling
- Making full use of existing information

## Who should attend

Laboratory managers and practitioners in analytical laboratories who use reference materials for statistical quality control, method validation and calibration.

## Course layout

Lectures as well as practical exercises for material selection and data evaluation.

For more information, please visit:  
[www.irmm.jrc.be/html/events/](http://www.irmm.jrc.be/html/events/)

\* Trueness is defined as the "closeness of agreement between the average value obtained from a large series of test results and an accepted reference value" (ISO 5825:1994). Accuracy is defined as "closeness of agreement between a test result and the accepted reference value" (ISO 5725:1994), and is therefore a combined parameter comprising both precision and trueness. This means if a method is accurate, one gets the true result with a low variability. Inaccurate methods have poor precision, insufficient trueness or both.

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We are pleased to announce the addition of six new acid herbicide mixes to our chemical standards product line. They are appropriate for use in monitoring soil, drinking water, and groundwater for contamination, as well as acid herbicide residue on foods. All raw materials and solvents used in the preparation of Supelco brand mixes are screened for purity. Mixtures are gravimetrically prepared and then chromatographically assayed. Each acid herbicide mix is supplied with a certificate of analysis. Data packets are also available, free of charge, upon request. Each data packet documents the rigorous analytical methods we use to verify raw material identity and purity, and provides certification as to the purity and final concentration accuracy.

### Ordering information

Prod No.	Description	Concentration	Composition
861264	Methyl Herbicide Mix 1	1x1 ml, Hexane, Varied Conc.	
			2,4,5-T Methyl Ester 2,4-D Methyl Ester 2,4-DB Methyl Ester Dalapon Methyl Ester Dicamba Methyl Ester
			Dichloroprop Methyl Ester Dinoseb Methyl Ester MCPA Methyl Ester MCPP Methyl Ester Silvex® (2,4,5-TP) M.E.

### Ordering information

Prod No.	Description	Composition
861164	Acid Herbicide Mix	1x1 ml, Methanol, Varied Conc.
		2,4,5-T Dalapon MCPA 2,4,5-TP Dicamba MCPP 2,4-D Dichloroprop Pentachlorophenol 2,4-DB Dinoseb Picloram
861386-U	Acid Herbicide Spiking Mix	1x1 ml, Methanol, Varied Conc.
		2,4,5-T Dalapon MCPA 2,4,5-TP Dicamba MCPP 2,4-D Dichloroprop Pentachlorophenol 2,4-DB Dinoseb
861194	Acid Herbicide Mix	1x1 ml, Methanol, Varied Conc.
		2,4,5-t Dalapon Dinoseb 2,4-D Dicamba MCPA 2,4-DB Dichloroprop MCPP
861258	Herbicide Spiking Mix 1	1x10 ml, Acetone, Varied Conc.
		2,4,5-T 2,4-Dichlorophenylacetic acid
861259	Acid Herbicide Spiking Mix 2	1x10 ml, Acetone, Varied Conc.
		2,4,5-T 2,4,5-TP 2,4-D
861263	Acid Herbicide Spike Mix 3	1x10 ml, Acetone, 50 µg/ml
		2,4,5-TP Dalapon 2,4-D Dinoseb

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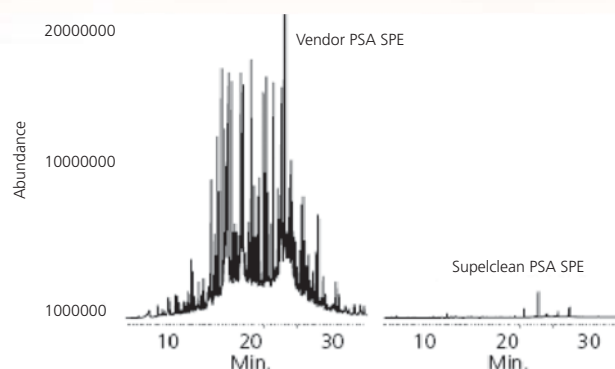
The Supelclean™ ENVI-Carb™-II/PSA SPE product line consists of multi-layer SPE cartridges that were developed for superior cleanup when conducting multi-residue pesticide analysis in agricultural products (fruits, vegetables, meat, shellfish, grains, and dairy products). The technology acts as a chemical filter in which each layer plays a specific role for removing key interferences when conducting pesticide analysis using GC. The example to the right shows the superior cleanup of an orange juice sample by the Supelclean method versus a competitive SPE product.

### Ordering information

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<b>Supelclean ENVI-Carb-II/PSA SPE Tube</b>		
54058-U	300 mg/600 mg/6 ml	30
54067-U	500 mg/500 mg/6 ml	30
55119-U	500 mg/300 mg/6 ml	30
<b>Supelclean ENVI-Carb-II/SAX/PSA SPE Tube</b>		
52574-U	500 mg/500 mg/500 mg/12 mg	20
<b>Supelclean SAX/PSA SPE Tube</b>		
52576-U	250/ mg/250 mg/6 ml	30
52577-U	500 mg/500 mg/6 ml	30

### GC-MS Analysis of Orange Juice Extracted with the Canadian Method and Clean Up with ENVI-CARB-II/PSA SPE

column: Equity-1. 30m x 0.25 mm I.D., 0.25 µm (28046-U)  
oven: 50 °C (5 min.), 25 °C/min. to 125 °C, 10 °C/min. to 300 °C (8 min.)  
inj: 200 °C, aux: 325 °C  
det.: MSD, scan range 45-450 amu  
carrier gas: helium, 0.9 ml/min, constant flow mode  
injection: 1 µl, splitless (splitter open at 1 min.)  
liner: 4 mm ID, single taper



G002998, G003000

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