

# Reporter

Volume 29, January 2008, International



## Fatty Acid Analyses



Assisting the Food & Beverage Industry  
in Reporting Trans Fat Content

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# Reporter

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**Michael D. Buchanan**  
Product Manager  
Gas Separations

## Dear Colleague,

There is some talk that new labelling requirements will become effective that require food manufacturers to list trans fat content on the Nutritional Facts panel. In response, Supelco developed Discovery Ag-Ion SPE tubes, a product that allows the fractionation of cis and trans isomers. By bundling these tubes with an impressive selection of other specialized Supelco products, food analysts are able to quickly, accurately, repeatedly and confidently report their results.

To distinguish between the very slight polarity differences exhibited by unsaturated fatty acids in the cis configuration versus unsaturated fatty acids in the trans configuration, the carboxyl functional groups must first be neutralized. This is commonly performed by a transesterification reaction using derivatization reagents such as boron trifluoride in methanol (BF<sub>3</sub>-methanol) or boron trichloride in methanol (BCl<sub>3</sub>-methanol). Supelco has over 40 years' experience using these reagents in both our R&D and production areas.

One of our newest items, Discovery® Ag-Ion SPE tubes, is based on silver-ion chromatography work first pioneered in 1966. Silver ions anchored to the SPE support form specific polar complexes with the double bonds of unsaturated fatty acid methyl esters (FAMES). The differences in the strengths of these polar complexes between classes of FAMES and the silver ions can be exploited, allowing for fractionation of cis and trans isomers as the elution solvent changes.

Because the polarity differences between cis isomer and trans isomer FAMES are very small, very efficient capillary GC columns with a highly polar phase are required. The SP-2560 column, introduced by Supelco in 1983, possesses both the selectivity and column efficiency to provide high resolution cis/trans FAME isomer separation. No other column is able to provide the food analyst with the same level of cis/trans detail. A recent introduction is a Fast GC version of the SP-2560 column, which is ideal for increasing sample throughput without sacrificing quality.

Supelco's parent company, Sigma-Aldrich, offers many other items that a typical laboratory requires: solvents, glassware, chemicals and safety equipment, just to name a few.

To learn more about how Sigma-Aldrich/Supelco can help you achieve your FAME analysis goals, visit our website: [sigma-aldrich.com/fame](http://sigma-aldrich.com/fame). In addition to product listings, you will also find technical literature detailing how to use these products, chromatograms with peak IDs and conditions listed and peer-reviewed literature references.

Regards,

Michael D. Buchanan  
Product Manager, Gas Separations

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## GC-Like Performance using HPLC Column Coupling

High Resolution Liquid Chromatography with Conventional HPLC Systems and Ascentis® Express HPLC Columns

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### Abstract

In this study, high resolution HPLC with efficiencies greater than 100,000 plates/column was achieved under moderate conditions. Specifically, Ascentis Express columns were coupled and used on a standard Agilent® 1200 HPLC system. Both isocratic and gradient examples are illustrated.

### Introduction

High resolution liquid chromatography is important in many areas, including pharmaceutical product development, natural product chemistry and synthetic peptide mapping, just to name a few. A common need is LC methods that provide optimum assurance to the purity of peaks. For instance, in stress studies of active pharmaceutical ingredients (API), the ability to unambiguously quantitate and subsequently identify and potentially purify degradants of the API are paramount to the determination of the efficacy and safety of the pharmaceutical product. Modern hyphenated techniques such as LC-MS and LC-NMR often rely on the purity of a chromatographic peak for structural identification and confirmation.

To date, reducing the particle size of the packing in HPLC columns has been the strategy of many column manufacturers to provide higher resolution columns. Smaller particles have higher efficiencies and, therefore, can provide higher resolution. Unfortunately, column back pressure increases at a greater rate than efficiency as one decreases particle size. Therefore, high resolution LC with small particles (sub-2  $\mu\text{m}$ ) is difficult even with modern LC systems. For this reason, a particle with high efficiencies yet low back pressure would be a more suitable candidate for high resolution LC.

Ascentis Express columns provide a breakthrough in high resolution LC performance. Based on Fused-Core™ particle technology, Ascentis Express provides the high efficiency based benefits of sub-2  $\mu\text{m}$  particles but at much lower back pressure. Due to the high efficiencies at low back pressures, Ascentis Express can provide high resolution chromatography that was previously unattainable on commercial LC systems.

The Fused-Core™ particle consists of a 1.7  $\mu\text{m}$  solid core and a 0.5  $\mu\text{m}$  porous shell. A major benefit of the Fused-Core™ particle is the small diffusion path (0.5  $\mu\text{m}$ ) compared to conventional fully porous particles. The shorter diffusion path reduces axial dispersion of solutes and minimizes peak broadening. In fact, Ascentis Express columns are able to achieve efficiencies of 240,000 N/m, which is similar to that obtained with sub-2  $\mu\text{m}$  particle columns, even though the back pressures are only 50% of that achieved under similar conditions with sub-2  $\mu\text{m}$  particles. This means that Ascentis Express can turn almost any LC system into a high resolution workhorse for your lab.

Column coupling in HPLC is gaining interest since LC systems are being designed to withstand column back pressures of up to 15,000 psi. Column coupling is a simple and practical way to increase resolution by simply increasing column length. Because Ascentis Express HPLC columns provide higher efficiencies at any pressure compared to 3  $\mu\text{m}$  and sub-2  $\mu\text{m}$  particles, the coupling of Ascentis Express columns enables significantly higher resolution than any other column on any commercial HPLC system.

In this study, coupled Ascentis Express C18 columns were used on a standard Agilent 1200 HPLC system. Efficiencies greater than 100,000 plates/column are demonstrated in the isocratic separation of benzene and D6-benzene. Ascentis Express column coupling is further applied to the analysis of a synthetic hydrophilic peptide to separate the target peptide from its deletion side products.

### Separation of Benzene and Deuterobenzene

Figure 1 shows the efficiency obtained by coupling Ascentis Express columns together. Due to space limits in the column heater, three 15 cm x 4.6 mm columns and one 10 cm x 4.6 mm column were used to obtain a total column length of 55 cm.

Figure 2 shows a plot of efficiency as a function of column length. The linearity indicates that efficiency is not sacrificed due to coupling hardware. It should be noted that efficiencies of greater than 100,000 were achieved under isocratic conditions with a modest back pressure of 7,000 psi.

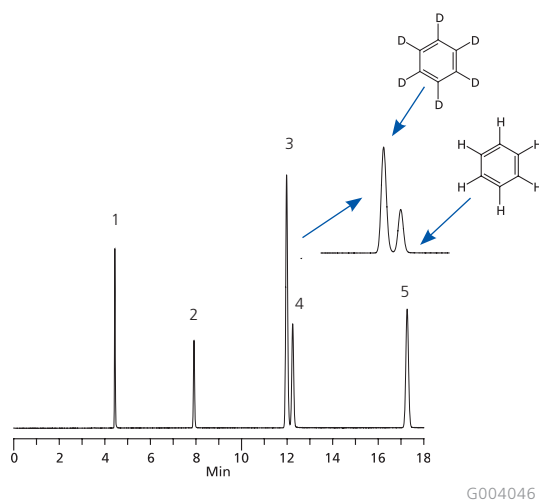
### Analysis of Synthetic Hydrophilic Peptide

Amino acid deletion products frequently result during the preparation of synthetic peptides. Since deletion may happen at any stage of the synthesis, the deletion products are diverse yet very similar to the target peptide. This process provides a unique and difficult separation challenge. The following is a study carried out on a hydrophilic synthetic peptide. The 12-mer peptide is composed of cysteine, proline, lysine, serine, phenylalanine and aspartic acid residues. Any one of the amino acids may be deleted at any position, thus a large number of possible deletion impurities may exist in the end product. Separation and identification of these side products is important.

Figure 3 demonstrates the effect of Ascentis Express column coupling on this separation. The column length was extended to 30 cm and compared to the 15 cm. The gradient rate was adjusted to account for the added column length. Comparison of the data shows the enhanced resolution obtained for several of the deletion products.

(continued on page 4)

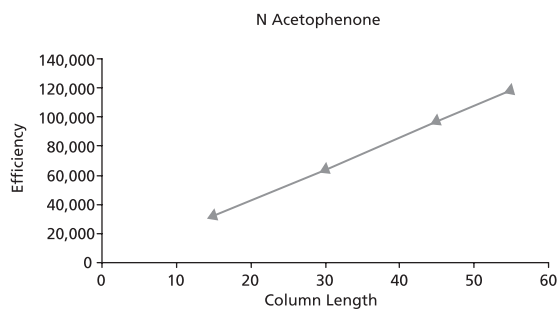
**Figure 1** Separation of Benzene from Deuterated Benzene using an Ascentis Express C18, 55 cm x 4.6 mm I.D.



Column: Ascentis Express C18, 55 cm x 4.6 mm I.D.  
 Mobile phase: 55:45, acetonitrile:water  
 Flow rate: 1.0 mL/min.  
 Temp.: 50 °C  
 Det.: 254 nm  
 Injection: 10 µL

2. Acetophenone = 120,045 plates  
 3. D<sub>6</sub> Benzene = 101,852 plates  
 4. Benzene = 104,463 plates  
 5. Toluene = 101,281 plates  
 Pressure = 480 bar (7,000 psi)

**Figure 2** Efficiency as a Function of Column Length



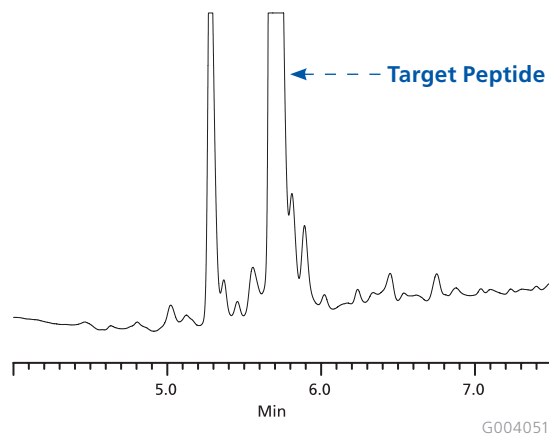
Efficiency is linear with respect to column length indicating no loss due to column coupling

### Conclusion

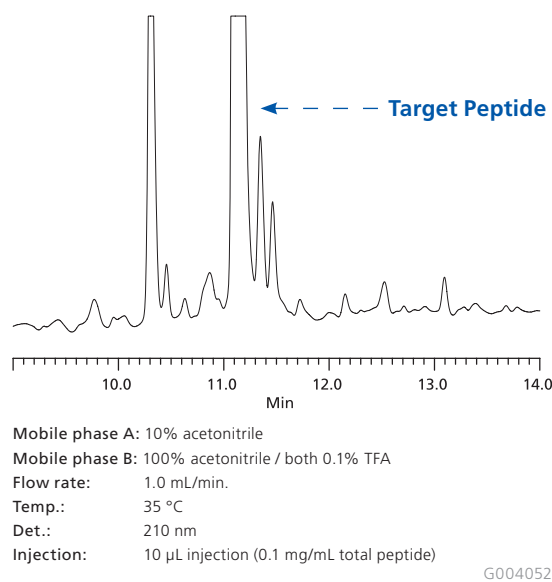
This study illustrates the potential for high resolution LC using Ascentis Express HPLC columns (using commercial instrumentation) under moderate conditions. Dramatic improvements in resolving power beyond that shown in this study are possible with elevated temperature and ultra-high pressure instrumentation.

**Figure 3** Gradient Elution of a Synthetic Peptide and its Deletion Products: Comparison of an Ascentis Express C18 at 15 and 30 cm Column Lengths 1

Ascentis Express C18, 15 cm x 4.6 mm I.D.  
 Gradient: 0% B–20% B (10 min.)



Ascentis Express C18, 30 cm x 4.6 mm I.D.  
 Gradient: 0% B–20% B (20 min.)



Mobile phase A: 10% acetonitrile  
 Mobile phase B: 100% acetonitrile / both 0.1% TFA  
 Flow rate: 1.0 mL/min.  
 Temp.: 35 °C  
 Det.: 210 nm  
 Injection: 10 µL injection (0.1 mg/mL total peptide)

### Featured Products

Ascentis Express	C18 Cat. No.	C8 Cat. No.
3 cm x 2.1 mm I.D.	53802-U	53839-U
5 cm x 2.1 mm I.D.	53822-U	53831-U
7.5 cm x 2.1 mm I.D.	53804-U	53843-U
10 cm x 2.1 mm I.D.	53823-U	53832-U
15 cm x 2.1 mm I.D.	53825-U	53834-U
3 cm x 3.0 mm I.D.	53805-U	53844-U
5 cm x 3.0 mm I.D.	53811-U	53848-U
7.5 cm x 3.0 mm I.D.	53812-U	53849-U
10 cm x 3.0 mm I.D.	53814-U	53852-U
15 cm x 3.0 mm I.D.	53816-U	53853-U
3 cm x 4.6 mm I.D.	53818-U	53857-U
5 cm x 4.6 mm I.D.	53826-U	53836-U
7.5 cm x 4.6 mm I.D.	53819-U	53858-U
10 cm x 4.6 mm I.D.	53827-U	53837-U
15 cm x 4.6 mm I.D.	53829-U	53838-U

## When C18 is Not Quite Enough in HPLC...

a Phenyl Stationary Phase may do the Job

Denise Wallworth [denise.wallworth@sial.com](mailto:denise.wallworth@sial.com)

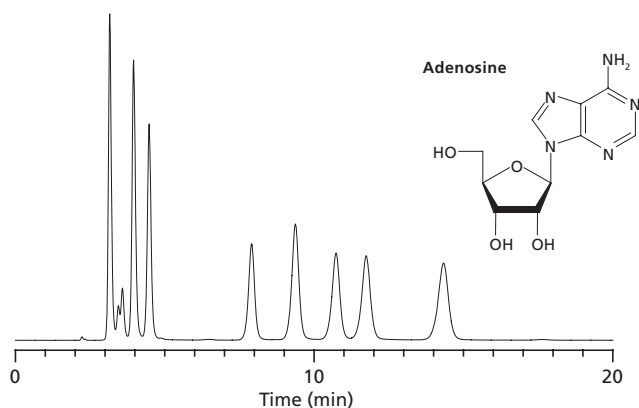
The automatic column choice in reversed phase HPLC is traditionally a C18 or perhaps a C8, but this does not always provide the optimum selectivity desired – it might not always have the best selectivity or the elution order that is required. Whether the column is struggling to retain polar molecules or requires a gradient to control a long retention, the alternative selectivity provided by a simple switch to Ascentis Phenyl may provide the ideal solution. This changes the mechanism from the predominantly hydrophobic interactions used in C18 to  $\pi$ - $\pi$  ones, taking advantage of  $\pi$ -accepting capabilities in polar aromatic and heterocyclic solutes. The result can be just the change in selectivity needed through enhanced retention and selectivity.

Because of the poorer wetting characteristics of hydrophobic phases, method reproducibility may also suffer on a C18 column if a highly aqueous mobile phase is in use. Ascentis Phenyl columns use pure phenyl chemistry so are highly compatible with 100% aqueous mobile phases.

### The secret of Ascentis Phenyl

- Low bleed for LC/MS and UV
- Greater selectivity for polar molecules
- Excellent peak symmetry and efficiency
- Compatible with 100% aqueous mobile phases
- Can be used in RP and HILIC (ANP) modes
- Proprietary trifunctional bonding chemistry for high column stability

**Figure 1** Separation of nucleosides in 100% aqueous mobile phases



**Column:** Ascentis Phenyl, 15 cm x 2.1 mm I.D., 5  $\mu$ m particles (581613-U)  
**Mobile Phase:** 10 mM ammonium formate (pH 3.0 with formic acid)  
**Temperature:** 35 °C  
**Flow Rate:** 0.2 mL/min  
**Detection:** UV at 270 nm; 750 psi back pressure regulator on outlet of flow cell

Cytidine (0.1 g/L)	Adenosine (0.1 g/L)
Contaminants from standards	Deoxyguanosine (0.1 g/L)
Deoxycytidine (0.1 g/L)	Deoxyadenosine (0.1 g/L)
Uridine (0.1 g/L)	Thymidine (0.1 g/L)
Guanosine (0.1 g/L)	

When using Ascentis Phenyl columns, it is also important to evaluate the use of methanol over the more typical acetonitrile as this can give additional benefits of increased retention. It is thought that ACN forms a multiple solvation layer over the stationary phase while MeOH forms a monolayer, increasing the  $\pi$ - $\pi$  effect<sup>1</sup>. Mixes of MeOH with ACN can also be explored.

Extensive studies<sup>2</sup> have shown that the butyl spacer used in Ascentis Phenyl is optimum for efficient interactions to occur between the solute and the phenyl moiety – allowing freedom of movement in the phenyl ring for interaction. This, combined with highly effective, proprietary end capping and a tightly controlled surface area specification, provides excellent performance and separation efficiency.

### Featured Products

- Ascentis Phenyl 5 $\mu$ m
- Ascentis Phenyl 3 $\mu$ m
- Additional table of columns available

### Did you know?

Changing from a C18 to an Ascentis Phenyl could not be easier. Just exchange the column using the same mobile phase – retention will be similar, but selectivity will be different. Then optimize the mobile phase from there.

### Availability

Column size	Ascentis Phenyl 3 $\mu$ m	Ascentis Phenyl 5 $\mu$ m
3cm X 2.1mm	581602-U	
3cm X 3.0mm	581606-U	
5cm X 2.1mm	581603-U	581611-U
5cm X 4.6mm	581608-U	581615-U
10cm X 1.0mm	581600-U	
10cm X 2.1mm	581604-U	581612-U
10cm X 3.0mm	581607-U	
10cm X 4.6mm	581609-U	
15cm X 1.0mm	581601-U	
15cm X 2.1mm	581605-U	581613-U
15cm X 4.6mm	581610-U	581616-U
25cm X 2.1mm		581614-U
25cm X 4.6mm		581617-U

Guard cartridges, prep HPLC columns and validation kits are also available.

### References:

- 1] I. M. Yang, S. Fazio, D. Munch, P. Drumm, *Journal of Chromatography A*, 1097 (2005), 124–129.
- 2] M. R. Euerby, P. Petersson, W. Campbell and W. Roe, *Journal of Chromatography A* (2007), 138–151.



## HPLC & GC Development and Purification – A Fast and Efficient Service

Dave Bell [dave.bell@sial.com](mailto:dave.bell@sial.com)

Newly expanded at our Supelco facility, our chiral HPLC & GC screening laboratory can speedily select the best column for optimum enantiomeric selectivity for a wide range of sample types.

The combination of Astec's 25 years of experience in chiral chromatography and Supelco's innovative approaches to bonding chemistry brings you a range of chiral stationary phases that provide practical solutions to chiral separation problems, especially in the current climate of the greater complexity of chiral molecules.

- HPLC and/or GC chiral method development screening
- HPLC and GC chiral method optimization
- Small-scale enantiomer purification

Automated, generic screening protocols process samples quickly and efficiently through polar, reversed and normal mobile phase types to find the column with the best selectivity. From the sample information given, the most appropriate method will be found, whether for bioanalysis, trace analysis, MS detection or for subsequent preparative HPLC. A Chiralyser™ polarimetric detector is used to identify the conformation of the individual enantiomers.

After some optimization, recommendations will be given as part of a method development report that will facilitate completion of the optimization to fully suit the application's objective. For chiral GC, a range of cyclodextrin chemistries are screened for selection, with derivatisation techniques optimized and verified by GC/MS.

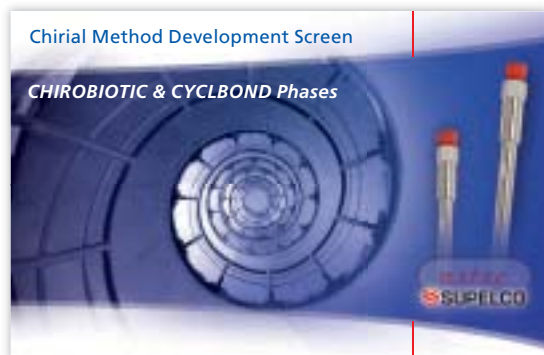
The additional service of purification for chiral molecules begins with an extensive solubility and loading study, determining the best solvent to achieve highest throughput and then calculating maximum capacity under those conditions for efficient scale up – an optimized compromise between sample solubility and chiral resolution. The result of this study is up to 100 mg of each enantiomer for evaluation and an optimized preparative method.

### Related Information

For more information on custom HPLC & GC chiral methods development and custom chiral purification services or to obtain a Chiral Sample Submission Form, please contact Technical Service [EurTechServ@sial.com](mailto:EurTechServ@sial.com) or go to [sigma-aldrich.com/astec](http://sigma-aldrich.com/astec). Product and ordering information as well as technical resources are also available from this webpage.

## For chiral chromatography on the web, visit [sigma-aldrich.com/astec](http://sigma-aldrich.com/astec)

Your best web source for Astec chromatography products is [sigma-aldrich.com/astec](http://sigma-aldrich.com/astec). One site for all your chiral needs.



To download our Chiral Methods Development Screen, go to [sigma-aldrich.com/astec](http://sigma-aldrich.com/astec), select Technical Resources, then Technical Notes.

TRADEMARKS: Agilent – Agilent Technologies; Ascentis, Carboxen, Chiralyser, CHIROBIOTIC, CHROMASOLV, CYCLOBOND, Discovery, P-CAP, Sigma-Aldrich, SLB, SP, Supelco, SUPELCOWAX, SupelMIP, Thermoseal, TraceCERT – Sigma-Aldrich Biotechnology LP; Certan – Promochem GmbH; Interseal – Integrated Liner Technologies; Fused-Core – Advanced Materials Technology, Inc.; Mininert – Valco Instruments Co., Inc.; Viton – E.I. Du Pont De Nemours and Company  
SPME – Technology licensed exclusively to Supelco. US patent #5,691,206, European patent #523,092.  
P-CAP and P-CAP-DP are patent pending and manufactured under license from La Sapienza, Università degli Studi di Roma.

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- use 2 selectivities inline (e.g. Silica & C18)
- connect a solid sample cartridge inline
- use Rev-Elution for stronger retained compounds to save solvent

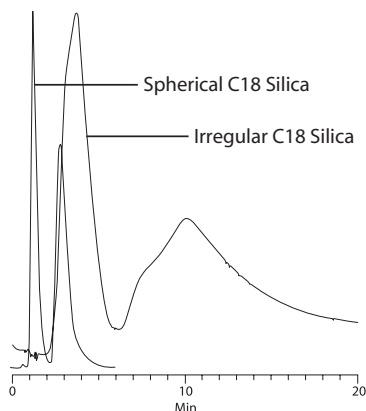


Stand setup

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## Class-Selective Extraction and Analysis of $\beta$ -Receptor Agonists and Antagonists using Molecularly Imprinted Polymer SPE

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<sup>2</sup> Supelco, Bellefonte, PA, USA

Beta-adrenergic blocking agents (beta blockers) are a class of drugs used to treat various cardiac disorders such as hypertension, angina, congestive heart failure and arrhythmia. Beta-2-adrenergic receptor agonists (beta agonists) have been clinically used to treat asthma and other breathing disorders. However, because of key side effects associated with the drugs, they are heavily regulated by government agencies worldwide. Beta blockers have been used as a performance enhancer among athletes by lowering heart rate and reducing tremor. Consequently, the International Olympic Committee has banned the use of beta-blockers. Beta agonists are illegal muscle growth promoters due to their anabolic effects. As a result, the drugs have been internationally banned for use in humans, livestock and racehorses. Also, because these drugs are not completely eliminated from the body after ingestion for therapeutic use, they are often excreted in wastewaters. As a result, there has been concern over the longterm subtle and chronic effects of these drugs on humans and the ecosystem.

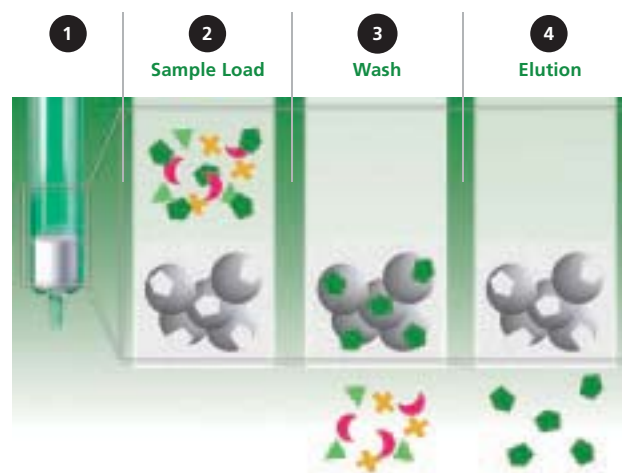
Because the drugs are heavily regulated and often analyzed in difficult sample matrixes such as biological fluids and wastewater, a highly selective and sensitive extraction and analytical method is required to achieve targeted lower limits of detection and quantitation. For example, maximum residue limits for beta agonists in Europe are 0.1 and 0.3 ppb (EU Council Regulation (EEC) No. 2377/90).

In previous issues of the Reporter, we demonstrated the use of molecularly imprinted polymer (MIP) SPE for the highly selective extraction of single analytes such as chloramphenicol, clenbuterol and NNAL from difficult sample matrixes such as biological fluids. These applications are thoroughly discussed in *Reporter* issues No. 26, 27 and 28, respectively. In this report, we describe the use of MIP-based SPE for the simultaneous extraction (class-selective) of both beta agonists and beta blockers for subsequent LC-MS-MS analyses.

### Improving Selectivity with SupelMIP SPE

MIPs are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related compounds with high selectivity. By careful design of the imprinting site, the binding cavities can be engineered to offer multiple interactions with the analyte(s) of interest (a combination of hydrogen bonding, hydrophobic and ionic interactions allows for stronger and more specific analyte retention). Improved selectivity is introduced through the use of harsher wash conditions during sample prep methodology (Figure 1). Because extraction selectivity is significantly improved, lower background is observed allowing analysts to achieve lower detection limits relative to other less selective sample prep techniques.

Figure 1 Overview of SupelMIP SPE Procedure



- 1 Condition and equilibrate SupelMIP SPE
- 2 Sample load
- 3 Application of a series of vigorous wash steps that will selectively retain analyte(s) of interest but elute interfering components
- 4 Analyte elution

### Using SupelMIP SPE for Class-Selective Retention

Although the specificity and selectivity of MIPs are often compared to the interactions observed in antibody-antigen interactions, the MIP binding site often offers a range of interaction types (e.g. ion exchange, reversed-phase, hydrogen bonding, etc.) that can be exploited to offer selective retention during sample load and/or wash. Very often, selective interaction between a MIP phase and analyte occurs at the substructure for the analyte. When conducting class-selective extraction, the MIP-analyte interaction occurs with a substructure common between a class of analytes. In the case of beta agonists and beta blockers, selective MIP retention is dominated by ion exchange and hydrogen bonding and specifically targeted towards the beta-alcohol and secondary amine common across both of these classes of compounds (Figure 2).

### Extraction and Analysis of Beta Blockers and Beta Agonists using SupelMIP SPE

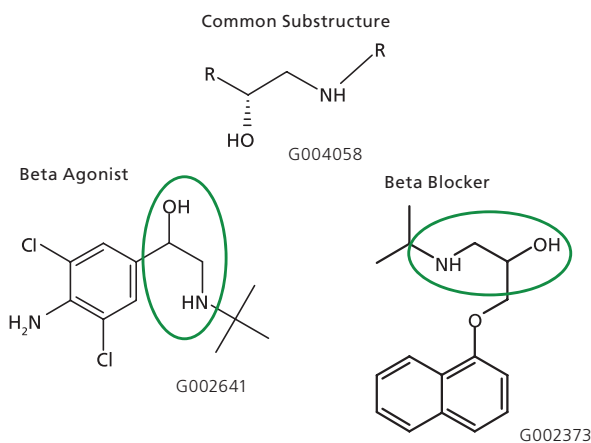
In this study, a selection of 10 beta blockers and beta agonists were extracted from both horse urine and wastewater using SupelMIP SPE-Beta-Receptor via the extraction procedure described in Table 1. Analysis of the resulting eluate was conducted by LC-MS-MS using the procedure described in Table 2.

(continued on page 9)

## Lower Limits of Quantitation in Horse Urine and Wastewater

Using the SupelMIP SPE and LC-MS-MS described in Tables 1 and 2, trace levels of beta agonists and beta blockers were determined in spiked urine and wastewater samples and lower limits of quantitation (LLOQ) values were determined for each of the analytes tested relative to sample matrix in which the signal-to-noise ratio of each analyte response was 10. The LLOQ values are summarized in Table 3 and an example chromatogram of a spiked urine sample is depicted in Figure 3. Using the SupelMIP SPE protocol and LC-MS-MS conditions described in this report, lower quantitation limits of 0.1 ng/mL and 0.01 ng/mL were achieved for horse urine and wastewater, respectively. Lower limits of detection for beta blockers were estimated to be < 0.03 ng/mL for urine and < 0.003 ng/mL for wastewater.

**Figure 2** Beta-Alcohol and Secondary Amine Substructure Common between Beta Agonists and Beta Blockers



An actual wastewater sample was collected from a sewage treatment plant located in Sweden and extracted and analyzed using the SupelMIP procedure and LC-MS-MS conditions described in this report. Using this procedure, 4 analytes were detected and quantitated. The other analytes were below the limits of quantitation for this assay. Determined concentration values are described in Figure 4.

**Table 1** SupelMIP Extraction Procedure for Beta Agonists and Beta Blockers

### Sample Pre-Treatment:

Horse urine was centrifuged at 3,000 g for 10 min., diluted with DI water 1:1 (v/v), adjusted to pH 7.

Wastewater was filtered with 1 µm filter paper and adjusted to pH 6-7.

### SPE Procedure:

SupelMIP SPE – Beta-Receptor, 25 mg/10 mL (LRC) (Cat. No.53223-U)

1. Condition and equilibrate MIP phase with 1 mL acetonitrile and 1 mL DI water.
2. Load 1 mL pre-treated urine sample.
3. Wash (elute interferences) using the following wash scheme:
  - 3 x 1 mL DI water (elution of salt and matrix interferences)
  - Apply 2 min. of full vacuum to dry the tube
  - 1 mL acetonitrile (selective removal of hydrophobic interferences)
  - 1 mL 60% acetonitrile/40% DI Water (selective removal of hydrophilic interferences)
  - Apply 2 min. of full vacuum to dry the tube
4. Elute beta agonists and beta blockers with 2 x 1 mL 1% formic acid in acetonitrile. Evaporate and reconstitute with LC mobile phase prior to analysis.
5. Evaporate under nitrogen and reconstitute with 150 µL 5% acetonitrile in 10 mM ammonium acetate, pH 4.6 prior to LC-MS-MS analysis

**Table 2** LC-MS/MS Conditions for Beta Agonists and Beta Blockers

Column: C18, 5 cm x 3 mm I.D., 3 µm,

Instrument: API3200 MS-MS

Mobile phase: (A) 10 mM ammonium acetate, pH 4.6 (adjusted with acetic acid); and (B) acetonitrile

Gradient:	Min.	% A	% B
	0	95	5
	2	90	10
	5	50	50
	6	50	50
	7	95	5

Detection (MS/MS):	Analyte	Rt (min.)	Q1/Q3	DP	EP	CEP	CE	CXP
	Atenolol	3.0	267.2/145	45	5	15	38	4
	Carazolol	6.2	299.1/194.2	50	5	20	37	5
	Metoprolol	5.6	268.2/133	45	4	15	35	4
	Propranolol	6.5	260.2/154.9	50	4	15	34	4
	Timolol	5.5	317.2/188.1	50	7	20	32	6
	Clenbuterol	5.6	277.1/202.9	26	3	10	22	7
	Ritodrine	3.9	288.2/121	39	5	11	31	4
	Salbutamol	2.6	240.2/147.9	38	4	12	24	4
	Terbutaline	2.6	226.2/152	36	4	10	24	4
	Tulobuterol	5.6	228.2/154.1	41	5	10	20	5

Dwell time (MS):	50
Ion mode:	Positive
Ion source:	Turbospray
Ion spray voltage:	5500 V
Source temperature:	500 °C
Curtain gas:	10 psi
Gas 1:	50 psi
Gas 2:	60 psi
Injection:	20 µL

(continued on page 10)

## Offer

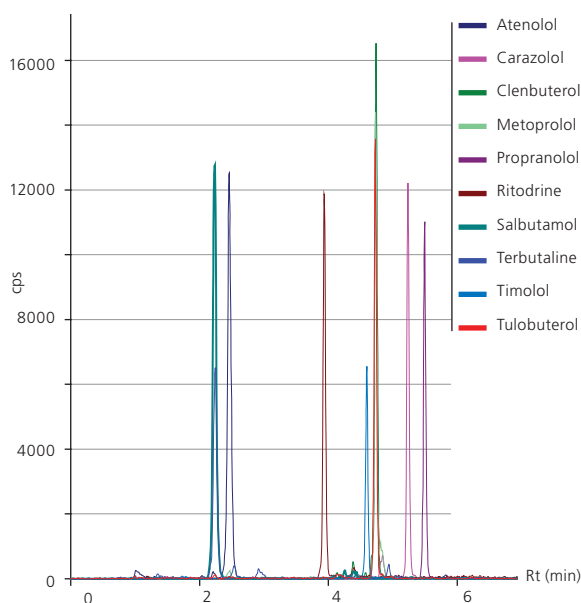
Sample packs for the SupelMIP products for beta blocker, agonists and receptors are available on request. Please contact your local Sigma-Aldrich office for more details or visit our web site [sigma-aldrich.com/supelmip](http://sigma-aldrich.com/supelmip) and check under "Request a FREE SupelMIP SPE MutliPak sample".



## Conclusion

In this report, we demonstrated the trace level determination of beta agonists and beta blockers in both horse urine and wastewater using class-selective molecularly imprinted polymer SPE phase. The SupelMIP SPE – Beta Receptor assay described in this report took less than 2 hours to complete and offered the selectivity necessary to achieve quantitation limits of 0.1 ng/mL and 0.01 ng/mL for horse urine and wastewater, respectively. This procedure was further demonstrated by analyzing an actual wastewater sample where 4 out of 10 beta-receptor agonists and antagonists were determined and quantified.

**Figure 3** Total Ion Chromatogram of Urine Sample Spiked with 1 ng/mL Beta Blockers and Beta Agonists

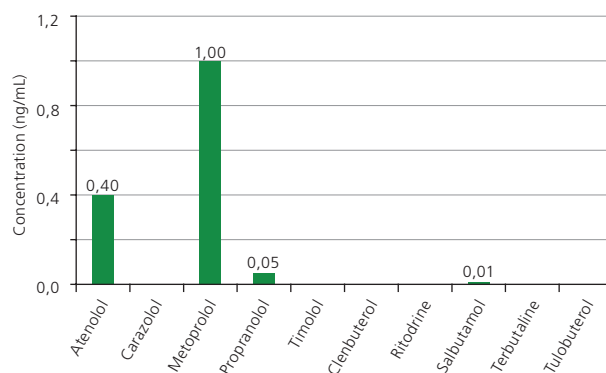


Note: Clenbuterol and Tulobuterol were spiked at the levels of 0.1 ng/mL.

**Table 3** LLOQ Values of Beta Agonists and Beta Blockers in Urine and Wastewater

Analyte	Lower Limit of Quantitation (ng/mL, ppb or µg/kg)	
	1 mL Horse Urine	10 mL Wastewater
Atenolol	0.1	0.01
Carazolol	0.1	0.01
Metoprolol	0.1	0.01
Propranolol	0.1	0.01
Timolol	0.1	0.01
Clenbuterol	0.02	0.002
Ritodrine	0.05	0.005
Salbutamol	0.1	0.01
Terbutaline	0.2	0.02
Tulobuterol	0.005	0.0005

**Figure 4** Determined Concentrations of Beta Agonist and Beta Blockers in Wastewater



## Featured Products

+	SupelMIP SPE Cartridges	Sorbent Mass (mg)	Cartridge Volume (mL)	Cartridges/Box	Cat. No.
	Full beta receptor (beta agonists and beta blockers)	25	10	50	53223-U
	Full beta receptor (beta agonists and beta blockers)	25	3	50	53224-U
	Clenbuterol	25	10	50	53201-U
	Beta agonists (class selective)	25	10	50	53202-U
	Beta agonists (class selective)	25	3	50	53225-U
	Beta blockers (class selective)	25	10	50	53218-U
	Beta blockers (class selective)	25	3	50	53213-U
	Chloramphenicol	25	10	50	53210-U
	Chloramphenicol	25	3	50	53209-U
	NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25	10	50	53206-U
	NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25	3	50	53203-U
	TSNAs (4 different Tobacco specific Nitrosamines: NNK, NNN, NAB, NAT)	50	10	50	53221-U
	TSNAs (4 different Tobacco specific Nitrosamines: NNK, NNN, NAB, NAT)	50	3	50	53222-U
	Riboflavin (vitamin B2)	25	10	50	53207-U
	Triazines (class selective)	25	10	50	53208-U

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- **Discovery Ag-Ion SPE** – SPE tubes for the fractionation of cis & trans FAMES prior to GC

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## Selecting the Appropriate SPME Fiber for your Application

### The Effect of Sample Concentration and Complexity on SPME Fiber Selection

Bob Shirey bob.shirey@sial.com

Previously in the Reporter (Vol. 23), we discussed how to select the appropriate fiber assembly, focusing on fiber core, needle gauge and assembly design. In the Reporter (Vol. 28), we discussed the affect of analyte molecular weight and polarity on fiber selection. This article discusses the affect of sample concentration and complexity on fiber selection.

There are 2 mechanisms for the extraction of analytes into fiber coatings, absorption and adsorption. Absorbent coatings (film fibers) are primarily bonded gums that act somewhat like a sponge. The analytes partition in and out of the coating. The primary parameter for retention of the analytes is the coating thickness and the size of the analyte. The coating type determines the affinity of an analyte for the phase. There is usually a little competition of analytes for the fiber coating unless one analyte is in a much greater concentration than the other analytes.

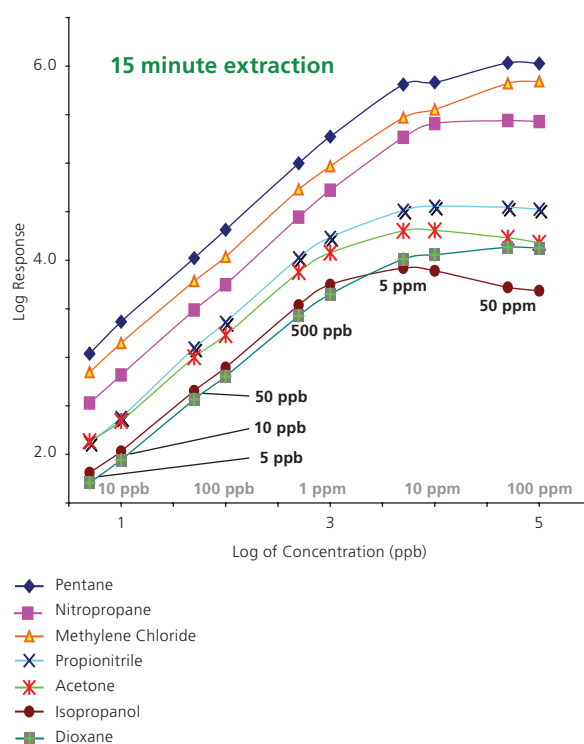
Adsorption type fibers more physically or chemically retain the analytes. Usually, adsorption coatings contain particles (adsorbents) that have pores. These particles are typically suspended in an adsorbent phase to bind the particles to the fiber. Analytes will typically migrate to the particles and either be retained by interaction with the surface, such as pi-pi interactions, or migrate into the pores where they are more tightly retained. The size of the pore determines whether a given analyte will be retained. Because there are a limited number of pores per given fiber, there is a limited analyte capacity that the fiber can retain. If a given analyte has a higher affinity for a pore site, one analyte could possibly be displaced by another analyte. This is based upon Langmuir's Isotherm for uniform pores.

By monitoring a group of analytes over a wide concentration range, one can determine the type of mechanism and the capacity of the fibers for the analytes. In this study, we primarily focused on small polar and non-polar analytes.

Carboxen™ 1006 used in the Carboxen Polydimethylsiloxane (PDMS) fibers has tapered pores. Since the pores are not uniform, analytes with different size and shapes fit in different regions in the pores. **Figure 1** shows the log-log plot of 7 analytes across a wide concentration range.

The results show that all of the analytes were extracted at 5 ppb. The polarity of the analytes increases in order from top to bottom. The responses for all of the analytes began to level off at 5 ppm, indicating the maximum capacity for analytes with this fiber coating. Responses remained constant between 10 ppm and 100 ppm. Where lines crossover each other, this could be an indicator of displacement. Generally when displacement occurs, the response for the displaced analyte will decrease and the analyte doing the displacement will increase. The decreased response for isopropanol may simply be a solubility issue as the concentration of the other analytes increased.

**Figure 1** Plot of Analyte Response using Carboxen PDMS Fiber



The Divinylbenzene (DVB) fibers have larger micropores and a more uniform mesopore that could lead to displacement. **Figure 2** shows a plot of the extraction of the same analytes under the same conditions using a PDMS-DVB coated fiber.

The results indicate that the response for the polar analytes is not detected until the concentration increases to 50–100 ppb, while the non-polar analytes could be extracted at 5–10 ppb. The response begins to level off for some analytes between 10–50 ppm and there appears to be some displacement. The response for methylene chloride continues to climb, while the slopes of the response lines for dioxane and acetone begin to decline.

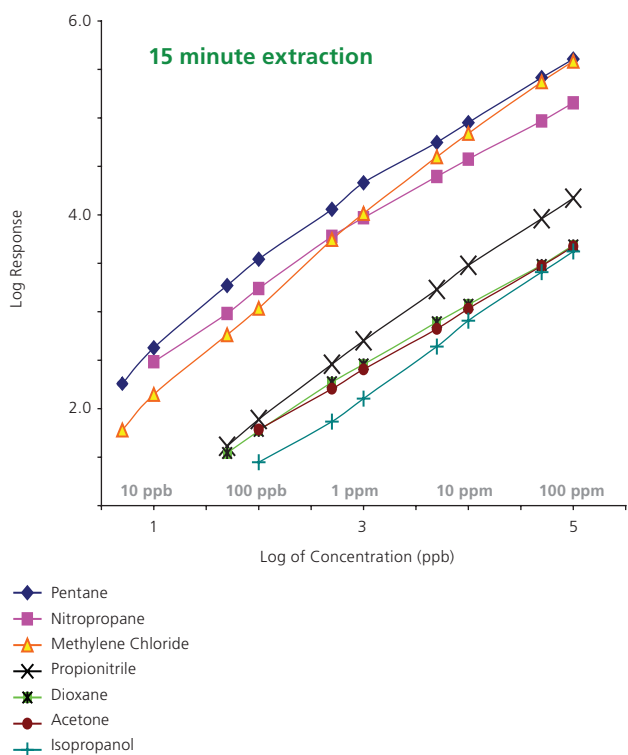
A longer extraction time would show this effect more dramatically. **Figure 3** shows the same analysis using the absorbent 100  $\mu$ m PDMS coated fiber.

Results show that the minimum detection limits are much higher for these smaller analytes, but the linearity is excellent up to 100 ppm. This was the highest concentration level evaluated. No displacement of the analytes was observed.

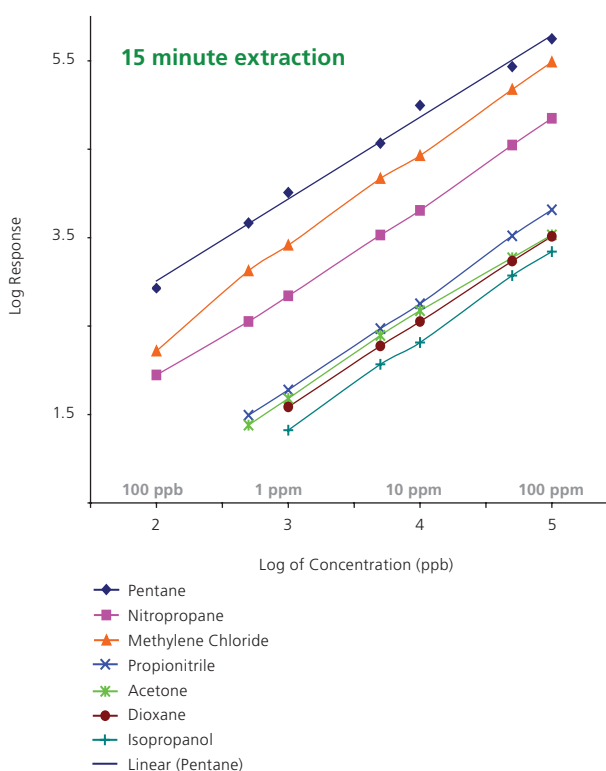
The following summary can be made based upon the results of this study and can be used as a guideline for selecting the appropriate SPME fiber.

(continued on page 13)

**Figure 2** Plot of Analyte Response using PDMS-DVB Coated Fiber



**Figure 3** Plot of Analyte Response using 100  $\mu$ m PDMS Fiber



1. Adsorbent (particle) fibers are better for analytes at low concentration levels.
2. Adsorbent (particle) fibers have a limited capacity so linear range for each analyte needs to be determined.
3. It is best to keep extraction times under 30 min for particle adsorbent fibers to reduce displacement.
4. Adsorbent (film) are better for complex samples with varied concentration ranges.
5. DVB/Carboxen/PDMS fiber is good for complex samples at low concentration levels due to the 2 adsorbent layers.
6. Adsorbent (film) fibers are better for screening samples at high concentration levels.
7. Adsorbent (film) fibers are a better option for dirty samples that may contain multiple unknown compounds.

## Featured Products



SPME fiber assortment kits consist of one fiber each of the types listed below and are ideal for method development and screening.

Kit	Fibers	Manual	Auto-sampler
Fiber Kit 1	85 $\mu$ m polyacrylate, 100 $\mu$ m PDMS, 7 $\mu$ m PDMS	57306	57307
Fiber Kit 2	85 $\mu$ m polyacrylate, 65 $\mu$ m PDMS/DVB, 75 $\mu$ m PDMS/Carboxen	57320-U	57321-U
Fiber Kit 3	85 $\mu$ m polyacrylate, 60 $\mu$ m PDMS/DVB, 100 $\mu$ m PDMS	-	57323-U
Fiber Kit 4	100 $\mu$ m PDMS, 65 $\mu$ m PDMS/DVB, 75 $\mu$ m PDMS/Carboxen	57324-U	57325-U
Fiber Kit 5	85 $\mu$ m polyacrylate, 65 $\mu$ m PDMS/DVB, 100 $\mu$ m PDMS, 50/30 DVB/Carboxen/PDMS	-	57362-U



## Related Information

For more information on SPME, request the SPME Applications Reference Guide T199925 (CJQ). The guide includes over 2,200 applications references for SPME, is searchable by analyte and includes video demonstrations on the use of SPME.



## Parabens in Topical Preparations using SPME-GC-MS

Katherine K. Stenerson [katherine.stenerson@sial.com](mailto:katherine.stenerson@sial.com)

### Introduction

Parabens are commonly used preservatives found in many commercial products, such as pharmaceutical and cosmetic topical preparations. While these compounds are generally considered safe, there is growing concern due to their detection in breast tumor tissue (1).

Due to the sample matrices (creams, lotions and ointments) that must be investigated, sample preparation tends to be complex. The use of solid phase microextraction (SPME) as a simpler sample preparation / sample introduction technique for parabens in these matrices prior to analysis via ion mobility spectrometry (IMS) has been demonstrated (2).

In this article, the use of SPME in conjunction with capillary gas chromatography-mass spectrometry (GC-MS), a more common analytical technique than IMS, for the analysis of parabens was investigated. While GC analysis times cannot approach the quickness obtained with IMS, GC has the advantage of being available in many laboratories.

### Experimental

A series of 6 calibration standards ranging in concentrations from 25 to 300 µg/L of each analyte were prepared. SPME was used to extract the parabens from each standard prior to GC-MS analysis on an SLB™-5ms capillary GC column. Conditions used and the resulting chromatogram of the 200 µg/L standard are shown in Figure 1.

SPME-GC-MS was then used with 3 'real-world' samples to confirm its applicability for complex matrices. These included a paraben-free ointment spiked with parabens, a paraben-containing arthritis lotion and a paraben-containing anti-itch cream.

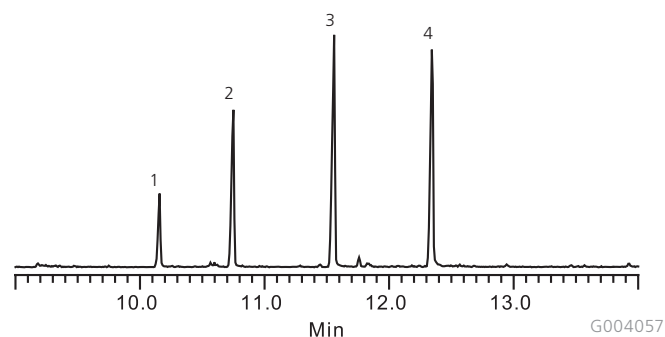
### Results

**Calibration.** All R2 values from the calibration were in the range from 0.9811 to 0.995, indicating good linearity. These calibration curves were subsequently used to determine values for the 3 'real-world' samples analyzed as part of this work.

**Spiked ointment.** A paraben-free betamethasone valerate ointment was spiked with each paraben, then processed. Each analyte was well resolved from other components in the ointment. Percent recoveries ranging between 79 and 109 were obtained.

**Arthritis lotion and anti-itch cream.** SPME-GC-MS results from both a paraben-containing arthritis lotion and a paraben-containing anti-itch cream were compared to results obtained from traditional high pressure liquid chromatography (HPLC) analyses. The results obtained by SPME-GC-MS were comparable to those obtained by traditional HPLC.

Figure 1 Analysis of Paraben Standard in Water



1. Methyl paraben
2. Ethyl paraben
3. Propyl paraben
4. Butyl paraben

<b>Sample/matrix:</b>	parabens, each at 200 ppb in 3 mL water + 25% sodium chloride in a 4 mL vial
<b>SPME fiber:</b>	metal fiber assembly coated with 50/30 µm DVB/Carboxen/PDMS (57912-U)
<b>Extraction:</b>	immersion with stirring, 25 °C (15 min.)
<b>Desorption temp.:</b>	260 °C, 2 min.
<b>Column:</b>	SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm (28576-U)
<b>Oven:</b>	60 °C (2 min.), 15 °C/min. to 300 °C (5 min.)
<b>MSD interface:</b>	275 °C
<b>Scan range:</b>	m/z 40–450
<b>Carrier gas:</b>	helium, 0.7 mL/min. constant
<b>Liner:</b>	0.75 mm I.D. SPME

### Discussion

The use of GC has distinct benefits over HPLC for complex matrices, namely due to the non-target components in the sample. With GC, simple sample preparation, such as SPME, can be used. As the inlet liner / head of the column become contaminated, they can easily be replaced / clipped. With HPLC, the column must be replaced when the front of the packing material in the column becomes contaminated. Therefore, more rigorous and time-consuming sample preparation may be necessary to adequately remove non-target compounds from complex matrices prior to HPLC analyses.

#### > Did you know?

The 2006 poster, "The Application of Solid Phase Microextraction to the Analysis of Pharmaceutical Products" (T406117) contains many details not covered in this article. Included are R2 data from the calibration, a chromatogram and recovery data from a paraben-free ointment spiked with parabens and chromatograms plus results (compared to HPLC analysis) from 2 paraben-containing products – an arthritis lotion and an anti-itch cream. An electronic file of this poster can be obtained by contacting Supelco Technical Service at [EurTechServ@sial.com](mailto:EurTechServ@sial.com)

(continued on page 15)

### Conclusion

In this article, it has been shown that the determination of parabens in topical products can be successfully performed using SPME-GC-MS. SPME is a simple and effective sample preparation / sample introduction technique. The SLB-5ms capillary column is an excellent choice due to its low bleed characteristics (up to 360 °C), highly inert nature and impressive durability.

### References

- 1] P.D. Darbe, A. Alijarrah, W.R. Miller, N.G. Coldham, M.J. Sauer, G.S. Pope, *J. Applied Toxicology*, 24 (2004), 5–13.
- 2] J.K. Lokhnauth, N.H. Snow, *Anal. Chem.*, 77 (2005), 5938–5946.

### Featured Products

Description	Cat. No.
SPME Metal Fiber, 50/30 DVB/Carboxen/PDMS	57912-U
SPME Fiber Holder for CTC Autosampler	57347-U
SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm	28576-U

### Related Products

Description	Cat. No.
SPME Metal Fiber, 2 cm, 50/30 DVB/Carboxen/PDMS	57914-U
SPME Fiber Holder for Varian Autosampler	57331

### Related Information

For more information on SPME, request T199925 (CJQ) or visit [sigma-aldrich.com/supelco-spme](http://sigma-aldrich.com/supelco-spme). For more information on Supelco Low Bleed SLB-5ms capillary columns, request T405130 (IKA) or visit [sigma-aldrich.com/slb](http://sigma-aldrich.com/slb).

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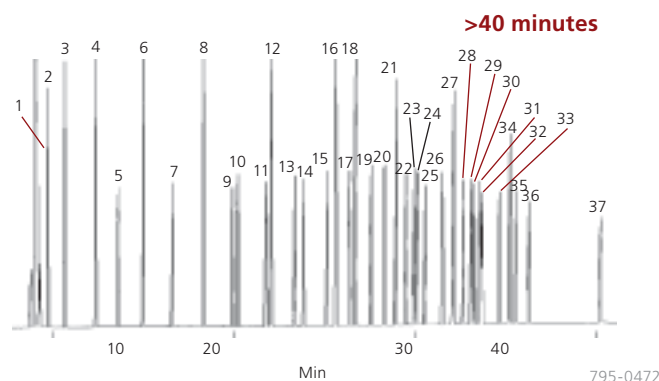
## Fast GC Analysis of Detailed Cis/Trans Fatty Acid Methyl Esters (FAMES) on the 75 m SP™-2560 Capillary Column

Michael D. Buchanan [mike.buchanan@sial.com](mailto:mike.buchanan@sial.com)

### Trans Fat Analysis

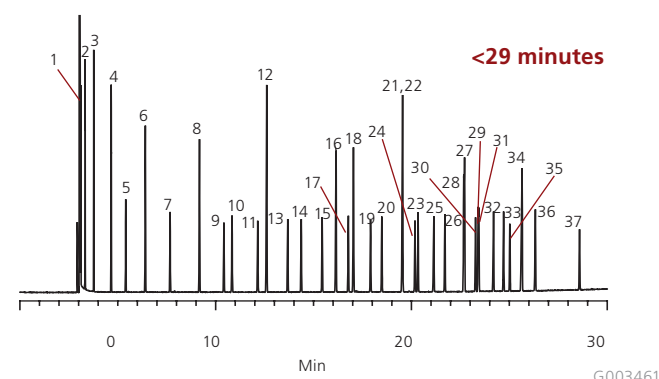
Because of the adverse health effects of trans fats, the United States Food and Drug Administration (FDA) requires that food manufacturers list trans fat content on the food's Nutrition Facts panel (1, 2). These labelling requirements have placed added pressure on food analysts to process more samples, which in turn creates the need for a rapid analytical method.

**Figure 1** 37-Component FAME Mix on the 100 m SP-2560 Column



**Column:** SP-2560, 100 m x 0.25 mm I.D., 0.20  $\mu$ m (24056)  
**Oven:** 140 °C (5 min.), 4 °C/min. to 240 °C (15 min.)  
**Inj.:** 260 °C  
**Det.:** FID, 260 °C  
**Carrier gas:** helium, 20 cm/sec @ 175 °C  
**Injection:** 1  $\mu$ L, 100:1 split  
**Sample:** 37-component FAME mix at concentrations listed in methylene chloride (47885-U)

**Figure 2** 37-Component FAME Mix on the 75 m SP-2560 Column



**Column:** SP-2560, 75 m x 0.18 mm I.D., 0.14  $\mu$ m (23348-U)  
**Oven:** 140 °C (5 min.), 4 °C/min. to 240 °C (2 min.)  
**Inj.:** 250 °C  
**Det.:** FID, 250 °C  
**Carrier gas:** hydrogen, 40 cm/sec at 175 °C  
**Injection:** 1  $\mu$ L, 100:1 split  
**Liner:** 4 mm I.D split, cup design  
**Sample:** 37-component FAME mix at concentrations listed in methylene chloride (47885-U)

### SP-2560: The Best GC Phase Available for Detailed Cis/Trans FAME Analyses

Cis/trans selectivity increases with increasing column polarity (percentage of biscyanopropyl). The 100 m x 0.25 mm internal diameter (I.D.), 0.20  $\mu$ m SP-2560 column is the longest, most polar column currently available. By combining both selectivity of the phase and column efficiency (by virtue of long column length), highly polar 100% biscyanopropyl SP-2560 capillary GC columns provide high resolution cis/trans FAME isomer separation. The SP-2560 column is specified in the Association of Official Analytical Chemists (AOAC) cis/trans FAME method (3).

### Analytical Challenge: Improved Throughput of Detailed Cis/Trans FAME Analyses

To increase throughput of the detailed cis/trans FAME analysis, Fast GC principles were applied by reducing column length, column I.D., film thickness and carrier gas viscosity. The result is a significant reduction in analysis time compared to the 100 m column method: 30% reduction of the 37-component FAME sample (Figures 1 and 2) and nearly 50% reduction of the detailed

#### Peak IDs for Figures 1 and 2

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

#### References

- 1] A. Ascherio, W. C. Willett, Health effects of trans fatty acids. *Am. J. Clin. Nutr.*, 66 (suppl.) (1997), 1006–1010.
- 2] US Food and Drug Administration. Questions and Answers about Trans Fat Nutrition Labeling. <http://www.cfsan.fda.gov/~dms/qatrans2.html>. Accessed May 17, 2007.
- 3] *Official Methods of Analysis of AOAC International*, 17th edition, Revision 1 (2002).

(continued on page 17)

analysis of the C18 isomer mix (Figures 3 and 4). Note that in both cases, peak shape and resolution does not suffer even with the shorter analysis times.

In both examples shown here, the loss of total theoretical plates by reducing the column length from 100 m to 75 m is offset by the narrower column I.D. (0.18 vs. 0.25 mm), thinner film (0.14 vs. 0.20  $\mu\text{m}$ ) and the higher diffusivity, lower viscosity carrier gas (hydrogen vs. helium). Simply put, the 75 m x 0.18 mm I.D. SP-2560 column does what the 100 m x 0.25 mm I.D. column does, but in a much shorter time. The 0.18 mm I.D. column is compatible with both conventional and Fast GC instruments.

## Conclusion

Supelco offers food analysts performing detailed cis/trans FAME analyses solutions in terms of both resolving power and speed. The 100 m SP-2560 column provides excellent resolution and is a workhorse column for detailed cis/trans FAME analyses. For analysts interested in improving throughput, a Fast GC version SP-2560 column in 75 m x 0.18 mm, 0.14  $\mu\text{m}$  dimensions, offered exclusively by Supelco, now provides both the high resolution and high speed needed to achieve high throughput with detailed cis/trans FAME analyses.

## Featured Products

Description	Cat. No.
SP-2560, 100 m x 0.25 mm I.D., 0.20 $\mu\text{m}$	24056
SP-2560, 75 m 0.18 mm I.D., 0.14 $\mu\text{m}$	23348-U
Supelco 37-Component FAME Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL See Figure 1 for a list of components	47885-U

## Related Products

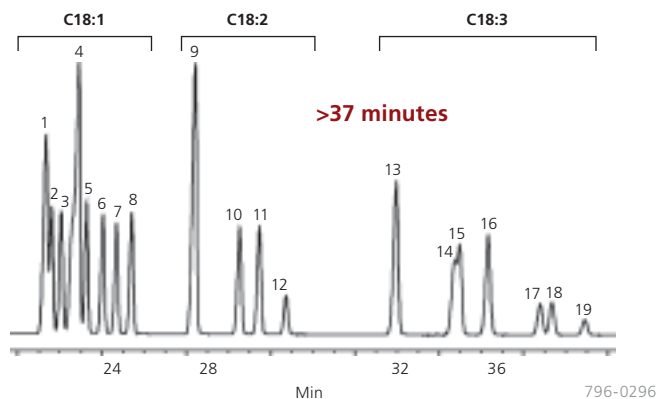
Description	Cat.
SP-2560, 100 m x 0.25 mm I.D., 0.20 $\mu\text{m}$	23362-U
Wound on a 5" cage for Agilent	6850 GC
Linoleic Acid Methyl Ester Isomer Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL C18:2 D 9c, 12c (10% w/w) C18:2 D 9t, 12c (20% w/w) C18:2 D 9c, 12t (20% w/w) C18:2 D 9t, 12t (50% w/w)	47791
Linolenic Acid Methyl Ester Isomer Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL C18:3 D 9c, 12c, 15c (~3% w/w) C18:3 D 9t, 12c, 15c (~7% w/w) C18:3 D 9c, 12c, 15t (~7% w/w) C18:3 D 9t, 12c, 15t (~15% w/w) C18:3 D 9c, 12t, 15c (~7% w/w) C18:3 D 9t, 12t, 15c (~15% w/w) C18:3 D 9c, 12t, 15t (~15% w/w) C18:3 D 9t, 12t, 15t (~30% w/w)	47792

## Related Information

For more information on Fast GC request your copy of the Fast GC brochure. Additional information about FAME analysis is available under [sigma-aldrich.com/fame](http://sigma-aldrich.com/fame). For fractionation of cis and trans fatty acids prior to GC via Ag-Ion SPE please check [sigma-aldrich.com/ag\\_ion](http://sigma-aldrich.com/ag_ion) or request publication T406062 (IRV) or the „Fast GC Brochure“ (code: JTW).



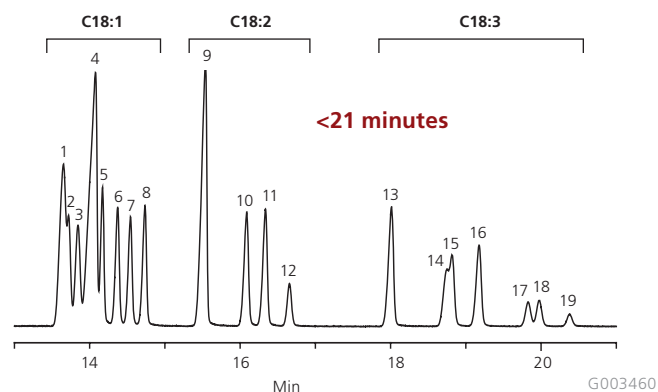
**Figure 3** Detailed Analysis of C18 FAME Isomers on the 100 m SP-2560 Column



Column: SP-2560, 100 m x 0.25 mm I.D., 0.20  $\mu\text{m}$  (24056)  
Oven: 175  $^{\circ}\text{C}$ , isothermal  
Inj.: 210  $^{\circ}\text{C}$   
Det.: FID, 250  $^{\circ}\text{C}$   
Carrier gas: helium, 20 cm/sec. @ 175  $^{\circ}\text{C}$   
Injection: 1.0  $\mu\text{L}$ , 100:1 split  
Sample: mixture of C18:1, C18:2 and C18:3 FAMES in methylene chloride

- |   |  |
|---|--|
| 1. C18:1 $\Delta$ 7t and C18:1 $\Delta$ 6t  | 11. C18:2 $\Delta$ 9t, 12c   |
| 2. C18:1 $\Delta$ 9t  | 12. C18:2 $\Delta$ 9c, 12c   |
| 3. C18:1 $\Delta$ 11t   | 13. C18:3 $\Delta$ 9t, 12t, 15t                                    |
| 4. C18:1 $\Delta$ 12t, C18:1 $\Delta$ 6c,<br>C18:1 $\Delta$ 7c and C18:1 $\Delta$ 13t | 14. C18:3 $\Delta$ 9t, 12t, 15c                                    |
| 5. C18:1 $\Delta$ 9c  | 15. C18:3 $\Delta$ 9t, 12c, 15t                                    |
| 6. C18:1 $\Delta$ 11c   | 16. C18:3 $\Delta$ 9c, 12t, 15t and<br>C18:3 $\Delta$ 9c, 12c, 15t |
| 7. C18:1 $\Delta$ 12c   | 17. C18:3 $\Delta$ 9c, 12t, 15c                                    |
| 8. C18:1 $\Delta$ 13c   | 18. C18:3 $\Delta$ 9t, 12c, 15c                                    |
| 9. C18:2 $\Delta$ 9t, 12t   | 19. C18:3 $\Delta$ 9c, 12c, 15c                                    |
| 10. C18:2 $\Delta$ 9c, 12t  |  |

**Figure 4** Detailed Analysis of C18 FAME Isomers on the 75 m SP-2560 Column



Column: SP-2560, 75 m x 0.18 mm I.D., 0.14  $\mu\text{m}$  (23348-U)  
Oven: 180  $^{\circ}\text{C}$ , isothermal  
Inj.: 220  $^{\circ}\text{C}$   
Det.: FID, 220  $^{\circ}\text{C}$   
Carrier gas: hydrogen, 25 cm/sec. @ 180  $^{\circ}\text{C}$   
Injection: 0.5  $\mu\text{L}$ , 100:1 split  
Liner: 4 mm I.D. split, cup design  
Sample: mixture of C18:1, C18:2 and C18:3 FAMES in methylene chloride

Peak IDs same as Figure 3

## GC Analysis of Blood Alcohols on the SUPELCOWAX™ 10

Michael D. Buchanan, Robert F. Wallace [mike.buchanan@sial.com](mailto:mike.buchanan@sial.com)

### Introduction

Gas chromatographic analysis of blood alcohols is preceded by one of several sample introduction techniques: direct injection, head-space or solid phase microextraction (SPME). Each of these techniques has distinct advantages over the others. Regardless of which sample introduction technique is selected, the column choice must result in both sharp peaks and complete resolution of all peaks of interest.

### SUPELCO WAX 10 Column

In this article, the use of a polar SUPELCOWAX 10 capillary column will be evaluated for the GC analysis of blood alcohols. Because this column offers higher polarity than any of the phenylsilicone phases, it is widely used for the separation of many polar compounds, including alcohols. The SUPELCOWAX 10 column will often resolve critical pairs that may not otherwise separate by boiling point alone.

Selectivity of the SUPELCOWAX 10 column provides ample room, chromatographically, for the analysis of samples with large ethanol concentrations.

### Experimental

An alcohol sample containing each analyte at 0.08% was prepared in water. The alcohol 2-butanol was included for use as an internal standard. Using a 4 mm I.D. split, cup design liner, a 0.5 µL injection with a split of 100:1 was performed onto a 30 m x 0.25 mm I.D., 0.50 µm SUPELCOWAX 10 column. To achieve good resolution and a short analysis time, a thin film (0.50 µm) was chosen for this analysis.

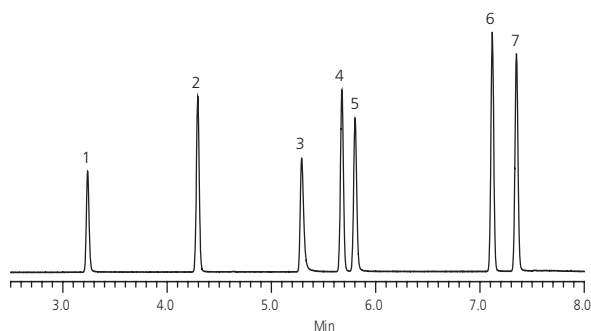
### Discussion

As shown in Figure 1, an analysis time of less than 8 minutes was achieved with excellent peak shapes and complete separation for all blood alcohol components, their metabolites and the internal standard. All peaks eluted at less than 80 °C oven temperature. A final oven temperature of 125 °C was used to ensure that any water present in the sample eluted from the column.

A distinct advantage of the SUPELCOWAX 10 for this application is its selectivity. This is most evident when looking at the ethanol peak. Ethanol elutes such that it does not have another peak close behind it. This is critical when analyzing 'real-world' samples in which the ethanol peak would be expected to be much larger in size, resulting in a wider peak to the right. The selectivity of the SUPELCOWAX 10 column provides ample room, chromatographically, for the analysis of samples with large ethanol concentrations.

An advantage of using an oven temperature programmed analysis over an isothermal analysis is that the system tends to be kept clean. That is, non-target compounds are forced through the system during each analytical run as the oven temperature rises.

Figure 1 Blood Alcohols on the SUPELCOWAX 10



G004056

Column: SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.50 µm (24284)  
 Oven: 35 °C (1 min.), 10 °C/min. to 125 °C (1 min.)  
 Det.: FID, 200 °C  
 Carrier gas: helium, 1.0 mL/min. constant  
 Injection: 0.5 µL, 100:1 split  
 Liner: 4 mm I.D. split, cup design  
 Sample: blood alcohols, each analyte at 0.08% in water

- |                 |                     |
|-----------------|---------------------|
| 1. Acetaldehyde | 5. Ethanol          |
| 2. Acetone      | 6. 2-Butanol (I.S.) |
| 3. Methanol     | 7. n-Propanol       |
| 4. 2-Propanol   |                     |

With an isothermal analysis, these compounds tend to accumulate in the system and are seen in subsequent analyses as carry over and/or ghost peaks. Depending on the sample preparation and sample introduction techniques being employed, the amount of non-target compounds being transferred to the GC column may be sizable.

### Conclusion

With the SUPELCOWAX 10 column, ethanol, methanol, n-propanol, 2-propanol and their metabolites acetaldehyde and acetone can be analyzed in less than 8 minutes. This column has distinct advantages for this application over other commercially available columns. In particular, its applicability for samples that contain high concentrations of ethanol.

### Featured Products

Description	Cat. No.
SUPELCO WAX 10, 30 m x 0.25 mm I.D., 0.50 µm	24284

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Vial Size (mL)	2	2	2	4	7	15	22	40/60
Septa Diameter (mm)	8	9	10	11	13	16	18	22
Package Size	100	100	100	100	100	100	100	100
PTFE/silicone, Max. Temp.: 250 °C								
tan/white	27095-U	–	–	27144	27155	27166	27177	27188-U
blue/white	27510-U	–	–	27511	27512	27513	27514	27515
red/white	27097-U	29039-U	27277	–	–	–	–	–
white/white	–	–	–	27356	–	–	–	–
red/white w/slit	27098-U	–	27279	–	–	–	–	–
PTFE/silicone/PTFE	27096-U	29041-U	27275	27122-U	–	–	–	–
Thermoseal, Max. Temp.: 300 °C (high temperature)	27191	–	–	27192	27193	27194	27195	27196
PTFE/rubber, Max. Temp.: 100 °C	27132	29038-U	–	27145	27156	27167	27178	–
PTFE liner, Max. Temp.: 225 °C	27133	–	27281	27146	27157	27168	27179	–
Barrier, Max. Temp.: 350 °C (aluminum/silicone)	24882-U	–	–	24883	24884	24885-U	24886-U	27190-U
Viton, Max. Temp.: 260 °C	27350-U	–	–	27351	27352	–	27354	27355



PTFE/Rubber

P000879



PTFE/Silicone

P000874



Thermoseal



PTFE Liner

P000881



Barrier

P000882



Viton

P000883



## Reference Standards for Analyzing Polyphenol Catechins

James S. Walbridge & Kathleen Kiefer [EurTechServ@sial.com](mailto:EurTechServ@sial.com)

Catechins have become a hot topic in today's health conscious world. Current research suggests that catechins aid health by:

- Reducing formation of atherosclerotic plaques
- Suppressing the growth of tumors by inhibiting enzymes involved in the spread of cancer cells, eradicating tumor promoting substances and blocking chemical carcinogens
- Reducing high blood pressure
- Protecting against digestive and respiratory infections
- Lowering cholesterol levels
- Lowering blood glucose levels
- Preventing kidney stones
- Reducing the chance of developing rheumatoid arthritis
- Producing stronger bones
- Reducing inflammation

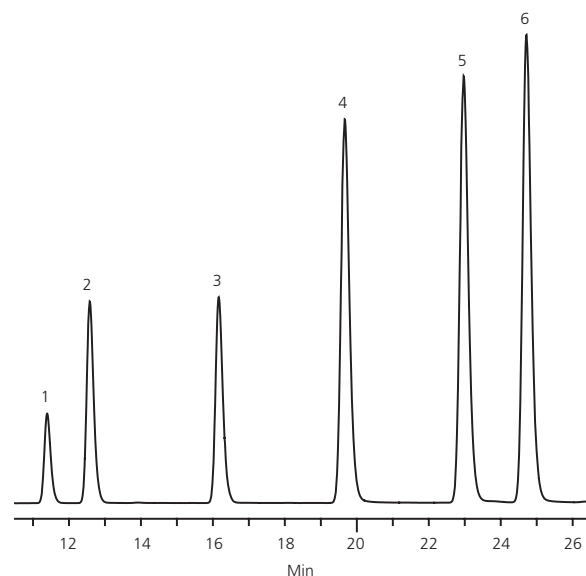
The catechins are a group of polyphenolic compounds exhibiting strong antioxidant, as well as remarkable antibacterial, antiviral, and anti-inflammatory properties. They are found in high concentrations in the leaves of *Camellia sinensis* (tea) and in smaller amounts in chocolate, grapes, raspberries, apples, pears and wine. The very young leaves and buds of the *Camellia sinensis* used to make white tea have the highest concentrations, followed by the slightly more mature leaves used to make green tea. Older leaves used to make Oolong and black teas are more oxidized and contain higher concentrations of other polyphenols including theaflavins and thearubigins.

Catechins, like other antioxidants, help protect cells from oxidative stress. Oxygen is vital to life, however; it is also incorporated into reactive oxygen species, including hydrogen peroxide, hypochlorous acid and free radicals, such as the hydroxyl radical and the superoxide anion. Reactive oxygen species damage cells and have been implicated in the slow chain reaction of damage leading to heart disease, cancer and many other ailments. Antioxidants function by preventing the formation of reactive oxygen species or by reacting with them before they can damage cells.

Leaves of *Camellia sinensis* contain at least 8 polyphenol catechins. The 6 predominant catechins in tea leaves are catechin, gallic catechin gallate (GCG), epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), with EGCG being the most abundant.



**Figure 1** HPLC Analysis of 6 Primary Green Tea Catechins



P000385

1. Epigallocatechin
2. Catechin
3. Epicatechin
4. Epigallocatechin Gallate
5. Gallic catechin Gallate
6. Epicatechin Gallate

<b>Column:</b>	Ascentis RP-Amide, 15 cm x 4.6 mm I. D., 5 µm particles (565324-U)	
<b>Mobile phase A:</b>	10 mM ammonium formate, pH 3.0 with conc. formic acid	
<b>Mobile phase B:</b>	methanol	
<b>Flow rate:</b>	1 mL/min.	
<b>Temp.:</b>	35 °C	
<b>Det.:</b>	UV at 273 nm	
<b>Injection:</b>	10 µL	
<b>Sample:</b>	catechins solution 300 µg/mL methanol	
<b>Gradient:</b>	<b>Time</b>	<b>% A Mobile Phase</b>
	0	100
	1	55
	30	55
	45	25
		<b>% B Mobile Phase</b>
		0
		45
		45
		75

### Analytical Challenge

Analysts determining catechin concentrations are challenged by the lack of commercially-available catechin reference solutions. One option is to prepare reference standards in-house from the individual catechin compounds.

This is typically not a cost-effective solution due to the following:

- High-purity catechin compounds are often difficult to find and are very expensive.
- Catechins, both neat and in solution, require proper storage.
- They are sensitive to heat, light and air.
- Preparing standards is a time-consuming process.

(continued on page 21)

### The Sigma-Aldrich Solution

To meet this need, 8 catechin analytical reference standard solutions were developed. These Supelco-brand standards are prepared, dispensed, packaged and stored to minimize chemical degradation and provide maximum shelf life. Each standard comes with a Certificate of Analysis that includes a purity determination. Catechins may also be prepared in specifically tailored combinations of concentration, components and solvents utilizing our custom chemical standards program. Additionally, a simple, robust LC-UV method using the Ascentis RP-Amide column was developed (Figure 1). The method is also compatible with MS detection. It provides reproducible analytical results and excellent peak shape.

#### References

- 1] Xiaolan Zhu, Bo Chen, Ming Ma, Xubiano Luo, Fei Zhang, Shouzhou Yao, Zutian Wan, Dajin Yang, Hongwei Hang, *Journal of Pharmaceutical and Biomedical Analysis*, 34 (2004), 695–704.
- 2] David S. Bell, William Campbell, Hugh M. Cramer, Molecular Interactions Contributing to Alternative Selectivity of Polar-Embedded HPLC Stationary Phases. Supelco Publication T405014.
- 3] Ya Lun Su, Lai Kwok Leung, Yu Huang and Zhen-Yu Chen, *Food Chemistry*, Volume 83, Issue 2 (November 2003), 189–195.
- 4] Mendel Friedman, Hella S. Jurgens, *J. Agric. Food Chem*, 48 (6) (2000) 2101–2110.
- 5] URL: <http://pubs.acs.org/journals/jafcau/index.html>
- 6] URL: <http://pubd.scs.org/journals/jacau/index.html>
- 7] Li He, S. Penzotti, F. Bedu-Addo, Cardinal Health Pharmaceutical Development, 14 Schoolhouse Road, Somerset, NJ 08873.

### Featured Products

Catechin Reference Solutions each Prepared at 2,000 µg/mL in Methanol

Description	Package Size	Cat. No.
Epigallocatechin	0.5 mL	49037-U
Catechin	0.5 mL	49040-U
Epigallocatechin Gallate	0.5 mL	49044-U
Epicatechin	0.5 mL	49045-U
Gallocatechin Gallate	0.5 mL	49047-U
Epicatechin Gallate	0.5 mL	49060-U
Catechin Gallate	0.5 mL	49061-U
Gallocatechin	0.5 mL	49069-U

### Related Product

Description	Cat. No.
Ascentis RP-Amide, 15 cm x 4.6 mm I. D., 5 µm	565324-U

### Related Information

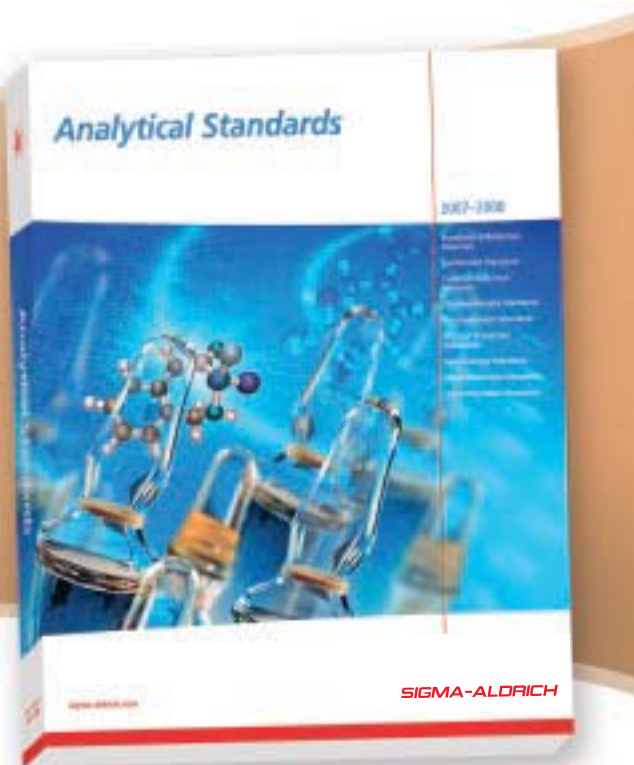
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## The Proper Storage and Handling of Volatile Analytical Standards

Pat Myers [EurTechServ@sial.com](mailto:EurTechServ@sial.com)



Properly storing and handling standards is critical to achieving accurate and reproducible analytical results. This is especially true when the analytical standard contains very volatile or gaseous components. All standards supplied by Supelco are packaged in containers that are suitable, if unopened, for long-term storage, as indicated on the label. However, once opened, standards must be transferred

to new containers. We recommend using either micro reaction vessels with Mininert® valves or Certan® bottles to maximize shelf life and minimize possible component loss. Choose amber glass vessels if any components are light sensitive or clear glass vessels for better visibility. The size of the container should be matched to the volume of the standard to minimize evaporation of volatile components into the headspace. Both recommended options provide 2 lines of defense against sample loss: 1) mininert valves have a PTFE valve backed up by a cylindrical red rubber septum and 2) certain vials have a capillary tube opening backed up by a PTFE-lined cap.

Handling procedures can have a large impact on standard integrity. A big factor affecting analyte loss from volatile standards is evaporation into the headspace of the container. The only parameter influencing evaporation that can be manipulated by the analyst is temperature. It is important to keep volatile standards at the recommended storage temperature until the container is opened for transferring the contents to a new container or removing an aliquot for dilution. Volatile standards should not be allowed to warm to room temperature before opening. Warming will lead to evaporative loss of volatile and gaseous components into the headspace of the container and out of the container once it is opened. Additionally, it is recommended that new vials for storing volatile standards be cooled before the transfer. This can be done by filling the vial with dry nitrogen and chilling it in a refrigerator. Take care to wipe any external condensation from the vial before opening.

Finally, while it is generally a good practice to thoroughly mix standards before use, mixing may lead to a loss of gases and volatile components from a standard because agitation increases the surface area of the liquid, increasing evaporation rate. Therefore, shaking volatile standards should be avoided immediately before opening.

Sigma-Aldrich is a trusted source for a broad range of analytical and reference standards.

Our standards include neat, single components and multi-component calibration mixtures. All raw materials used in the production of these products have been tested for purity. Documentation is shipped with most standard purchases. Please visit our website for a complete listing of all available analytical standards.

### Featured Products

Description	Dimensions	Cat. No.
<b>Certan Capillary Vials</b>		
1.5 mL	1.2 x 28 mm	44419
4.5 mL	1.2 x 28 mm	44420
10 mL	1.2 x 28 mm	44421
<b>Micro Reaction Vessels</b>		
0.1 mL	16.5 x 32 x 16 mm	33289
0.3 mL	16.5 x 34 x 23 mm	33291
1 mL	16.5 x 40 x 33 mm	33293
2 mL	16.5 x 58 x 48 mm	33295
3 mL	20.5 x 42 x 42 mm	33297
5 mL	20.5 x 61 x 58 mm	33299
<b>Mininert Valves</b>		
For 15 mm screw cap		33301
For 20 mm screw cap		33303



P000385

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Al, Ba, Bi, Cd, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sr, Zn : ≤ 5 µg/kg each;  
Ca, K, Na: ≤ 10 µg/kg each; and NH<sub>4</sub><sup>+</sup>: ≤ 50 µg/kg each

### ORGANIC TRACES:

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≤ 2 µS/cm

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