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The Reporter

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Fatty Acid Analyses



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Assisting the Food & Beverage Industry
in Reporting Trans Fat Content



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Dear Colleague,



Michael D. Buchanan
Product Manager,
Gas Separations

In 2006, new labeling requirements went into effect 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 (BF₃-methanol) or boron trichloride in methanol (BCl₃-methanol). Supelco has over 40 years experience with these reagents, using them 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.

For the food analyst, determining the fatty acid composition of a product is difficult because foods can contain a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids with a variety of carbon chain lengths. To confirm identification, the correct **chemical standards** must be used. One such standard is the Supelco 37 Component FAME Mix, very useful to food analysts since it can be used to identify key fatty acids in many different types of foods. Supelco also offers underivatized fatty acids, other FAME mixes, triglycerides, and highly characterized reference oils. With over 40 years of manufacturing chemical standards, Supelco has unsurpassed knowledge with regards to the stability, storage, and shipping of these products.

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, 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. 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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595 North Harrison Road, Bellefonte, PA 16823-0048.

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

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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 labeling 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 μ 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 μ L, 100:1 split
sample: 37-component FAME mix at concentrations listed in methylene chloride (47885-U)

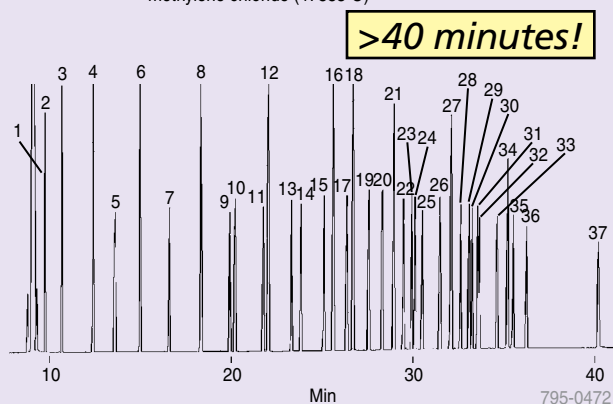
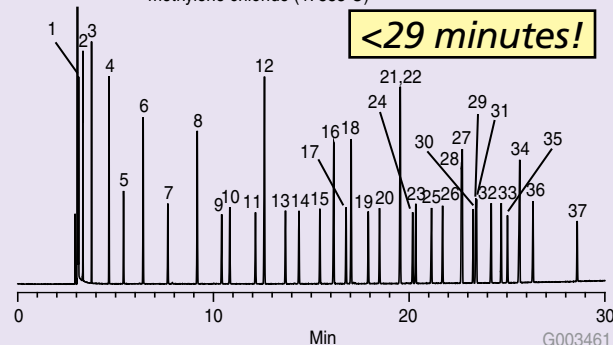


Figure 2. 37-Component FAME Mix on the 75 m SP-2560 Column

column: SP-2560, 75 m x 0.18 mm I.D., 0.14 μ 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 @ 175 °C
injection: 1 μ 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 μ 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 analysis

(continued on page 4)

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. γ -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-Docosaheptaenoic Acid Methyl Ester (C22:6n3) at 2 wt %

(continued from page 3)

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 μm) 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. Now, for analysts interested in improving throughput, a Fast GC version SP-2560 column in 75 m x 0.18 mm, 0.14 μm dimensions, offered exclusively by Supelco, provides both the high resolution and high speed needed to achieve high throughput with detailed cis/trans FAME analyses.

References

- Ascherio, A.; Willett, W. C. Health effects of trans fatty acids. *Am. J. Clin. Nutr.* 1997, 66 (suppl.), 1006S-1010S.
- 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.
- Official Methods of Analysis of AOAC International, 17th edition, Revision 1 (2002).

Featured Products

Description	Cat. No.
SP-2560, 100 m x 0.25 mm I.D., 0.20 μm	24056
SP-2560, 75 m 0.18 mm I.D., 0.14 μm	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. No.
SP-2560, 100 m x 0.25 mm I.D., 0.20 μm Wound on a 5" cage for Agilent 6850 GC	23362-U
Linoleic Acid Methyl Ester Isomer Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL	47791
C18:2 Δ 9c, 12c (10% w/w)	C18:2 Δ 9t, 12c (20% w/w)
C18:2 Δ 9c, 12t (20% w/w)	C18:2 Δ 9t, 12t (50% w/w)
Linolenic Acid Methyl Ester Isomer Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL	47792
C18:3 Δ 9c, 12c, 15c (~3% w/w)	C18:3 Δ 9t, 12c, 15c (~7% w/w)
C18:3 Δ 9c, 12c, 15t (~7% w/w)	C18:3 Δ 9t, 12c, 15t (~15% w/w)
C18:3 Δ 9c, 12t, 15c (~7% w/w)	C18:3 Δ 9t, 12t, 15c (~15% w/w)
C18:3 Δ 9c, 12t, 15t (~15% w/w)	C18:3 Δ 9t, 12t, 15t (~30% w/w)

Related Information

For more information on the analysis of FAMES, visit our website: sigma-aldrich.com/fame

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 μm (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 μL , 100:1 split
sample: mixture of C18:1, C18:2, and C18:3 FAMES
in methylene chloride

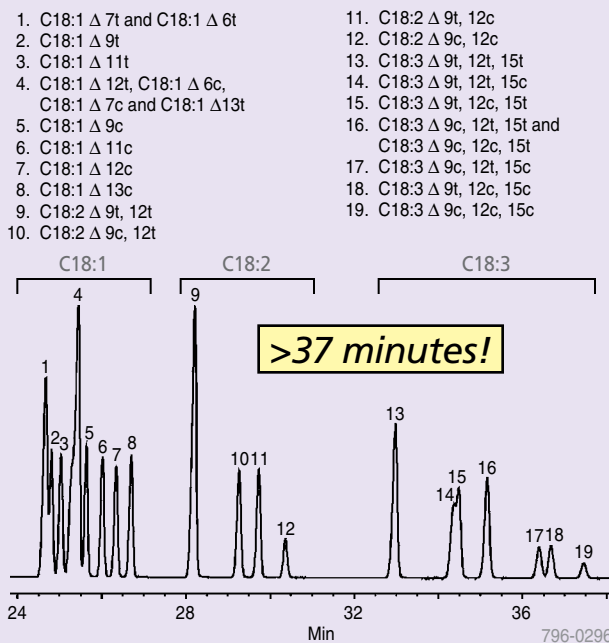
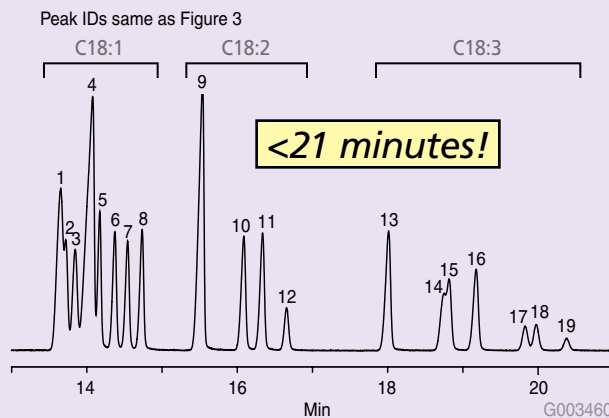


Figure 4. Detailed Analysis of C18 FAME Isomers on the 75 m SP-2560 Column

column: SP-2560, 75 m x 0.18 mm I.D., 0.14 μm (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 μL , 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



Analysis of Blood Alcohols on the SUPELCOWAX™ 10

Michael D. Buchanan and Robert F. Wallace
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Introduction

Gas chromatographic analysis of blood alcohols is preceded by one of several sample introduction techniques: direct injection, headspace, 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.

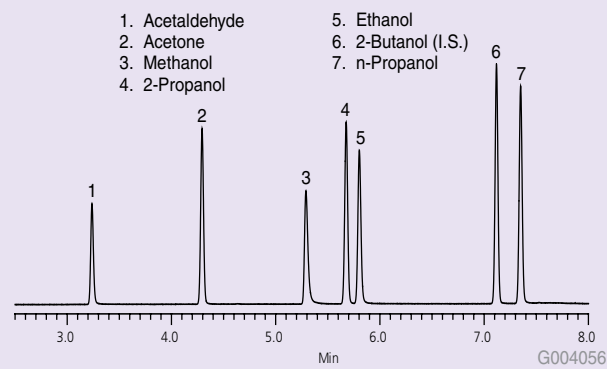
Discussion

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.

Figure 1. Blood Alcohols on the SUPELCOWAX 10

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



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. With an isothermal analysis, these compounds tend to accumulate in the system, 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 sizeable.

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 Product

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

Did you know...?

A chromatogram obtained from the use of solid phase microextraction (SPME) as a sample introduction technique for blood alcohols from human plasma is available in electronic format. This literature piece (T007515) can be obtained at no-charge by contacting Supelco Technical Service at 800-359-3041 (US and Canada only), 814-359-3041, or at techservice@sial.com. Please provide email address with your request.

Parabens in Topical Preparations Using SPME-GC-MS

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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 six 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.

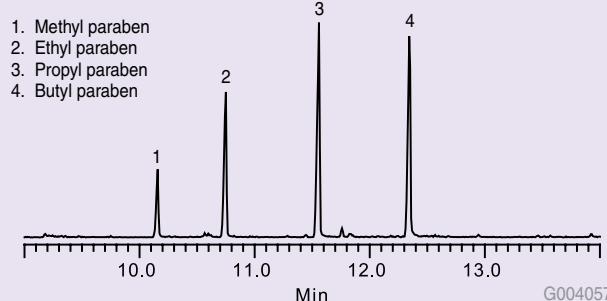
SPME-GC-MS was then used with three 'real-world' samples to confirm its applicability for complex matrices. These included a paraben-free ointment spiked with parabens, a paraben-containing arthritis lotion, and a paraben-containing anti-itch cream.

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 two 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 800-359-3041 (US and Canada only), 814-359-3041, or at techservice@sial.com

Figure 1. Analysis of Paraben Standard in Water

sample/matrix: parabens, each at 200 ppb in 3 mL water + 25% sodium chloride in a 4 mL vial
 SPME fiber: metal fiber assembly coated with 50/30 µm DVB/Carboxen/PDMS (57912-U)
 extraction: immersion with stirring, 25 °C (15 min.)
 desorption temp.: 260 °C, 2 min.
 column: SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm (28576-U)
 oven: 60 °C (2 min.), 15 °C/min. to 300 °C (5 min.)
 MSD interface: 275 °C
 scan range: m/z 40-450
 carrier gas: helium, 0.7 mL/min. constant
 liner: 0.75 mm I.D. SPME



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 three '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.

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.

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

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2. Lokhnauth, J.K.; Snow, N.H., Anal. Chem. 2005, 77, 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. For more information on Supelco Low Bleed SLB-5ms capillary columns, request T405130 (IKA) or visit sigma-aldrich.com/slb

NEW!

GC Literature from Supelco

Analytical Tools Designed to Accelerate Your Success

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Fast GC (JTW):

A Practical Guide for Increasing Sample Throughput without Sacrificing Quality

- Explains how to decrease costs while increasing revenue
- Practical considerations are covered in detail
- Includes a section on theoretical aspects
- Common applications are presented in 26 chromatograms, complete with peak IDs and conditions
- Literature references and further reading also included

sigma-aldrich.com

Maximize Performance! (JWE):

Gas Chromatography Accessories and Gas Purification/Management Products

- A convenient source for the most commonly replaced items
- Pictures and technical specifications
- Easy-to-read format
- Includes septa, liners, seals, ferrules, nuts, guard columns, connectors, hand tools, PID lamps, syringes, vials, purifiers, gas generators, regulators, flow meters, leak detectors, tubing, and fittings

SUPELCO

High Resolution Liquid Chromatography with Commercial HPLC Systems and Ascentis® Express HPLC Columns: Increasing Resolution by Column Coupling

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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 μm) is difficult even with modern LC systems. For this reason, a particle with high efficiencies yet low backpressure 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 μm particles but at much lower backpressure. 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 μm solid core and a 0.5 μm porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.5 μm) 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 μm particle columns, even though the backpressures are only 50% of that achieved under similar conditions with sub-2 μm 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 μm and sub-2 μm 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. 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

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. D₆ Benzene = 101,852 plates
4. Benzene = 104,463 plates
5. Toluene = 101,281 plates
Pressure: = 480 bar (7,000 psi)

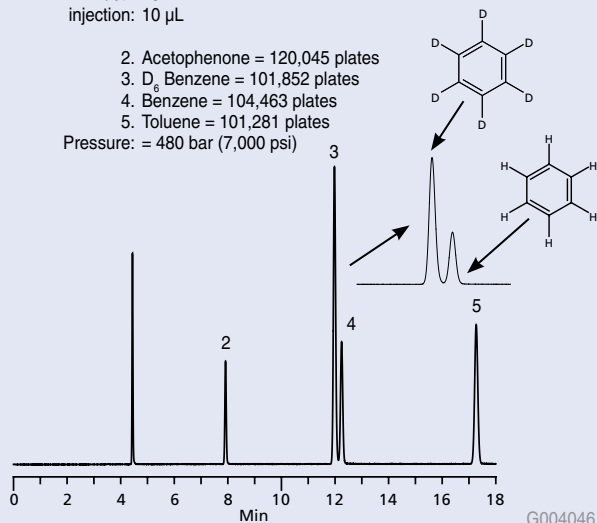
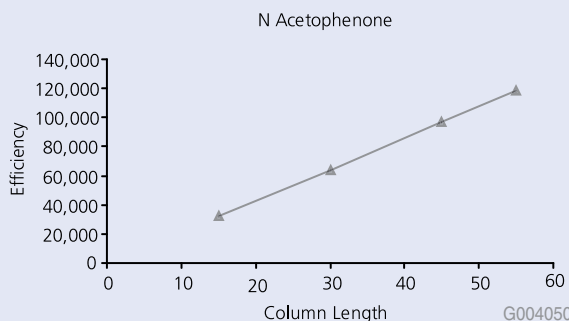


Figure 2. Efficiency as a Function of Column Length

Efficiency is Linear with Respect to Column Length
Indicating no Loss Due to Column Coupling



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.

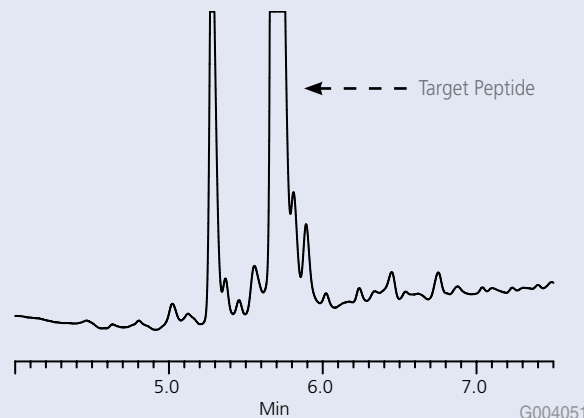
Conclusion

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

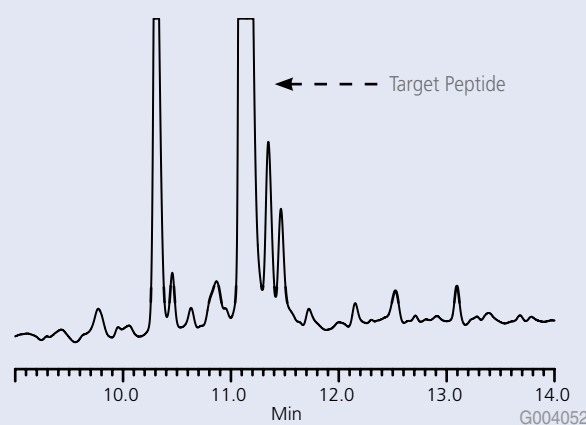
Figure 3. Gradient Elution of a Synthetic Peptide and its Deletion Products: Comparison of an Ascentis Express C18 at 15 and 30 cm Column Lengths

column: Ascentis Express C18
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)

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.)



+ Featured Products

Column Dimensions	C18	C8
Ascentis Express	Cat. No.	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

Chiral Screening of Pharmaceutical Compounds

Ric Cone

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Racemic switches are the result of reformulations in single enantiomeric form of drugs that were first approved as racemates (ie. mixtures of enantiomeric forms). To assist with the early stage identification and successful chiral HPLC separation of enantiomers in racemic compounds during drug development, data collected by Astec (Table 1) is summarized for a number of enantiomeric compounds developed as racemic switches.

Partial separation or better of the enantiomers in these racemates (ie 'hits') was achieved on one or more CHIROBIOTIC™ or CYCLOBOND™ columns as listed in Table 1, using one of the primary screen mobile phase conditions recommended in the Chiral Method Development Screen brochure (T405089 - IEY). The brochure is available upon request (sigma-aldrich.com/astec or through Technical Service). For the racemates tested in

Table 1. Enantiomeric Separations

Classification	Compound	Chiral Column
Basic		
Analgesic:	Methadone Nefopam (R,R)-Tramadol	βCD HP-RSP V2/V/βCD DNP/βCD HP-RSP βCD DMP
CNS stimulant:	Methylphenidate	V2/V/T/T2
Gastroprokinetic:	Mosapride	V2/V
Anthelmintic:	Oxamniquine	V2/V
Decongestant:	(S,S)-Pseudoephedrine	T2/βCD
Antipsychotic:	Thioridazine	βCD
Muscle relaxant:	Tolperisone	V2/V
Sedative:	(S)-Zopiclone	βCD
Bronchodilator:	R(-)-Albuterol R(-)-Epinephrine Clenbuterol (R,R)-Formoterol Isoproterenol; Terbutaline	T2/T/TAG βCD HP-RSP T2/T/P-CAP T2 T2/T/TAG
Antihypertensive:	(-)-Amlodipine; Lercanidipine Metoprolol Nicardipine Propranolol Sotalol	V2/V T/T2 V2/V T/T2/TAG/βCD/P-CAP T/T2
Antifungal:	Miconazole	βCD HP-RSP
Anesthetic:	S(-)-Bupivacaine	V2/V
Antihistaminic:	(+)-Chlorpheniramine	βCD/P-CAP
Antidepressant:	(S)-Citalopram; Fluoxetine Mianserin Nefopam (S,S)-Sertraline Trimipramine	V2/V βCD DNP/βCD HP-RSP βCD/βCD HP-RSP V2/V
Acidic		
Antiinflammatory:	S(+)-Ibuprofen S(+)-Ketoprofen S(+)-Naproxen	V2/V R/V2/V/P-CAP V2/V
Neutral		
Antiulcerative:	S(-)-Omeprazole	R/βCD DMP
Anxiolytic:	Lorazepam Oxazepam	T/TAG/T2 T/TAG/T2/βCD DNP
Pediculicide:	cis-Permethrin	TAG
Anticoagulant:	S(-)-Warfarin	V2/V/βCD
Chiral Column Abbreviation Key		
βCD: β-Cyclobond I-2000; V2: CHIROBIOTIC V2; V: CHIROBIOTIC V; T: CHIROBIOTIC T; T2: CHIROBIOTIC T2; TAG: CHIROBIOTIC TAG; R: CHIROBIOTIC R		

this study, 80% were hits on CHIROBIOTIC columns, and 40% were hits on CYCLOBOND columns. There was a 20% overlap between the two classes of chiral stationary phase (CSP).

Those CSPs that produced 'hits' upon initial screening are listed in Table 1 with each enantiomer. Enantiomers were subsequently separated to baseline following mobile phase optimization using the column and primary screen conditions offering the best selectivity. The resolution of the optimized separations was from 1.5 to 11.0, largely with mass spectrometry-compatible mobile phases. Several recommended optimization procedures are listed in the Chiral Method Development brochure. To obtain additional information regarding suggested optimal column and mobile phase conditions for the compounds in Table 1, please contact Technical Service (techservice@sial.com; 800-359-3041/814-359-3041).

There are 8 chiral HPLC columns included in this screening study that are listed in the Chiral Method Development

brochure. These columns are now available in CHIROBIOTIC (10300AST; 10305AST) and CYCLOBOND (2005AST) Method Development kits.

Enantiomers of some of the compounds screened have also been separated with either a P-CAP™ or P-CAP-DP™ polymeric column using Supercritical Fluid Chromatography (based on information provided in a poster presentation by Amgen at Chirality, 2006). Information for P-CAP is noted in Table 1. These polymeric columns also demonstrate improved enantiomeric selectivity and loading capacity in normal phase HPLC mode, and show great promise for screening racemic drug candidates.



Featured Products

Description	Length (cm)	I.D. (mm)	Cat. No.
CHIROBIOTIC - 100 Kit	10	4.6	10300AST
CHIROBIOTIC - 250 Kit	25	4.6	10305AST
CYCLOBOND - 250 Kit	25	4.6	20005AST
CHIROBIOTIC V2	25	4.6	15024AST
CHIROBIOTIC T	25	4.6	12024AST
CHIROBIOTIC TAG	25	4.6	14024AST
CHIROBIOTIC R	25	4.6	13024AST
CYCLOBOND I 2000	25	4.6	20024AST
CYCLOBOND I 2000 DNP	25	4.6	25024AST
CYCLOBOND I 2000 DMP	25	4.6	20724AST
CYCLOBOND I 2000 HP-RSP	25	4.6	24024AST
(R,R) P-CAP, 5µm	25	4.6	31024AST
(S,S) P-CAP, 5µm	25	4.6	33024AST
(R,R)P-CAP-DP, 5 µm	25	4.6	35024AST
(S,S) P-CAP-DP, 5 µm	25	4.6	37024AST

Chiral Analytical Service at Supelco/Sigma-Aldrich

Dave Bell

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Supelco/Sigma-Aldrich is pleased to announce operational status for our Chiral Analytical Service laboratory offering:

- HPLC chiral column screening
- GC chiral column screening
- HPLC and GC chiral method optimization
- Small-scale enantiomeric purification

Our HPLC chiral column screening protocol includes 4 mobile phase conditions run using 6 different chiral stationary phase chemistries. Positive separation is verified on a separate analytical system, and may include minimal optimization. Enantiomers are identified as (+) and (-) using the Chiralysers™ optical rotation detection system. In some situations, manual method development may be conducted.

GC column screening involves manual exploration of 3-4 GC column chemistries. Samples that require derivatization are verified by GC-MS.

To establish the most efficient means of chromatographically purifying enantiomers, a methods optimization and loading study can be conducted from a method demonstrating partial separation. The output of a loading study is approximately 100 mg of purified material and the methodology utilized to obtain such material.

When more than 100 mg of enantiomerically pure material is required, larger scale purifications are necessary. The output from a small-scale purification project is 1-2 grams of enantiomerically pure material to customer specifications (typically > 98%), and verification of enantiomeric excess percent purity by analytical methods established in the screening study.

Our mission is to establish, maintain, and nurture chiral analytical services that are valued and preferred by our customers. We will provide:

- Fast, efficient, and effective analytical services
- Consistent, informative, and friendly communications before, during, and following each study
- Timely assistance with technical and legal issues
- A streamlined, easy-to-use system
- A fair pricing structure

! Related Information

For more information on custom chiral methods development and custom chiral purification services, or to obtain a **Chiral Sample Submission Form**, please contact Technical Service at 800-359-3041 (US and Canada only), 814-359-3041, techservice@sial.com, or go to 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

Your best web source for Astec chromatography products is sigma-aldrich.com/astec. One site for all your chiral needs.



To download our Chiral Methods Development Screen, go to sigma-aldrich.com/astec, select **Technical Resources**, then **Technical Notes**.

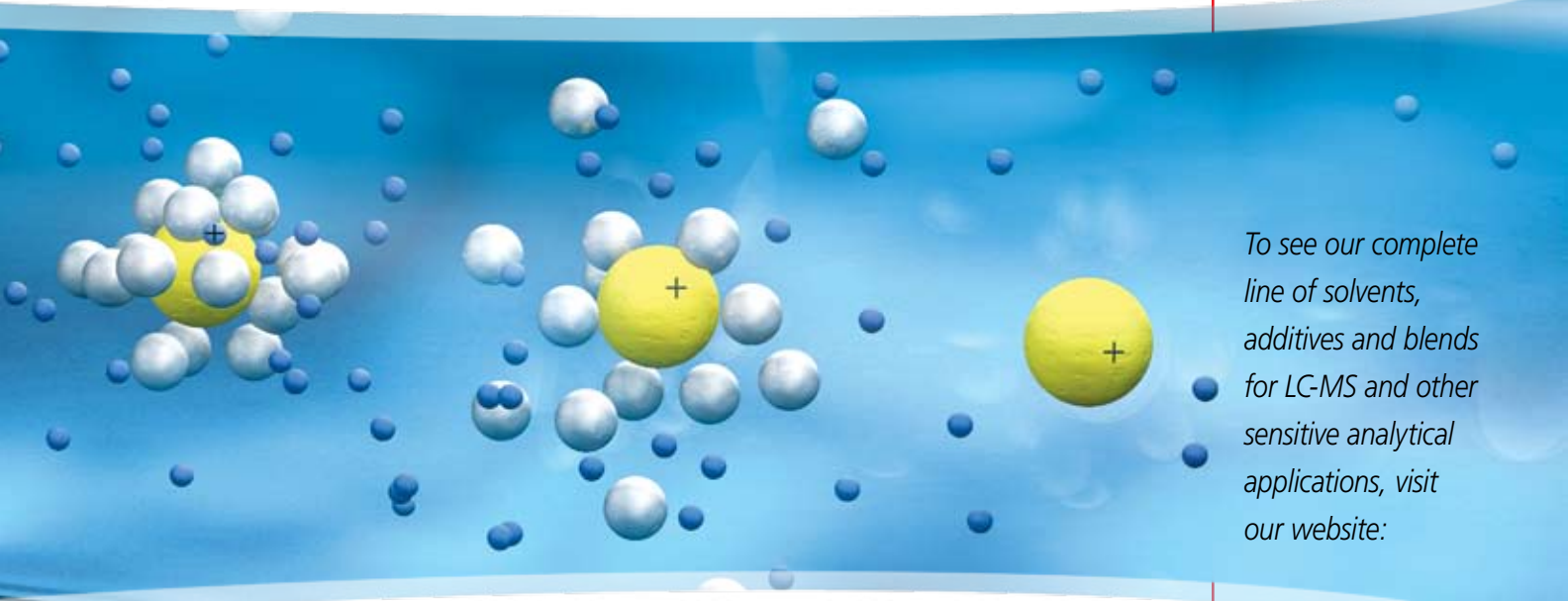
TRADEMARKS: Agilent - Agilent Technologies; Ascentis, Carboxen, Chiralysers, CHIROBIOTIC, CHROMASOLV, CYCLOBOND, Discovery, P-CAP, Sigma-Aldrich, SLB, SP, Supelco, SUPELCOWAX, SupelMIP, Thermoseal, TraceCERT - Sigma-Aldrich Biotechnology LP; Certan - Promochem GmbH; Interseal - Integrated Liner Technologies; 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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The Class-Selective Extraction and Analysis of β -Receptor Agonist and Antagonists using Molecularly Imprinted Polymer SPE

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2. 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 an illegal muscle growth promoter due to its 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 upon ingestion, they are often excreted in wastewaters after therapeutic use. As a result, there has been concern for the longterm subtle and chronic effects of these drugs on humans and the ecosystem.

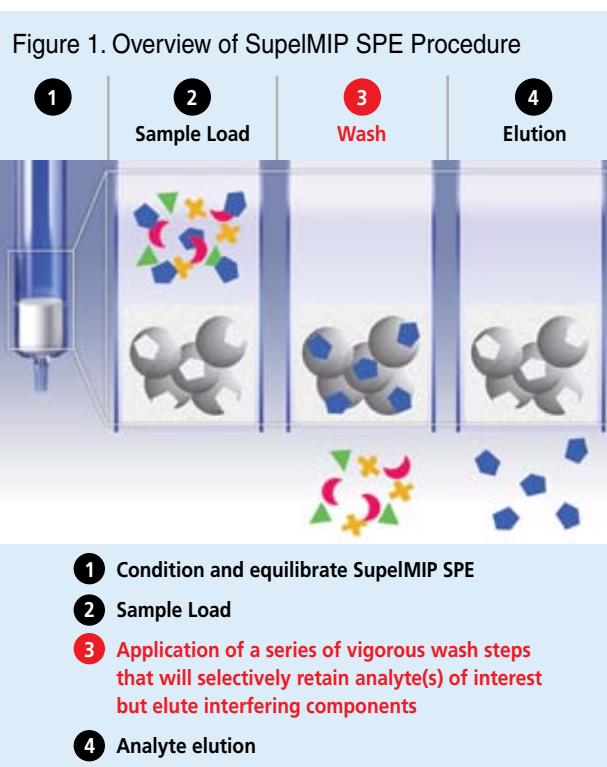
Because the drugs are heavily regulated and often analyzed in difficult sample matrixes such as biological fluids and waste water, a highly selective and sensitive extraction and analytical method are required to achieve targeted lower limits of detection and quantitation. For example, maximum residue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

In previous issues of the Reporter we demonstrate 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 US Reporter Issues 25.1, 25.2, and 25.3, 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 compounds 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 (combination of hydrogen bonding, hydrophobic and ionic interactions, and Van der Waals) allowing 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.

(continued on page 14)

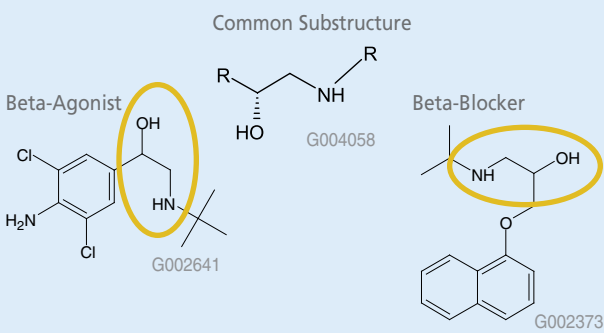


(continued from page 13)

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 these classes of compounds (Figure 2).

Figure 2. Beta-Alcohol and Secondary Amine Sub-structure Common Between Beta-Agonists and Beta-Blockers



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.

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 were summarized in Table 3, and an example chromatogram of a spiked urine sample is depicted in Figure 3.

Table 1. SupelMIP Extraction Procedure for Beta-Agonists and Beta-Blockers

Sample Pre-Treatment:

Horse urine was centrifuged at 3000 g for 10 min., diluted with DI water 1:1 (v/v), adjusted to pH 7.

Wastewater was filtered with 1 μ m 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 μ L 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 μ m,
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

flow rate: 0.5 mL/min.

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

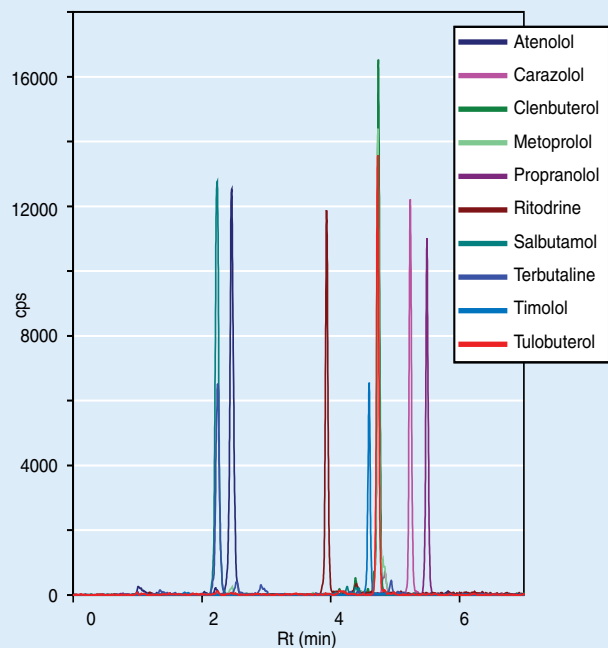
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ion mode: Positive
ion source: Turbospray
ion spray voltage: 5500 V
source temperature: 500 °C
curtain gas: 10 psi
gas 1: 50 psi
gas 2: 60 psi
injection: 20 μ L

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 < 0.1 μ g/L for urine and 0.01 μ g/L for wastewater.

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

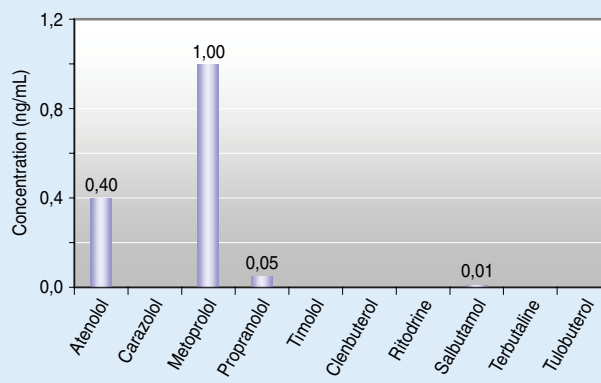
Analyte	Lower Limit of Quantitation (ng/mL, ppb, or µg/kg)	
	1 mL Horse Urine	10 mL Wastewater
Atenolol	0.1	0.01
Carazolol	0.1	0.01
Metoprolol	0.1	0.01
Propranolol	0.1	0.01
Timolol	0.1	0.01
Clenbuterol	0.02	0.002
Ritodrine	0.05	0.005
Salbutamol	0.1	0.01
Terbutaline	0.2	0.02
Tulobuterol	0.005	0.0005

Figure 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 4. Determined Concentrations of Beta-Agonist and Beta-Blockers in Wastewater



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, four analytes were detected and quantitated. The other analytes were below the limits of quantitation for this assay. Determined concentration values are described in Table 4.

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 two 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.

Featured Products

SupelMIP SPE Cartridges	Sorbent Mass (mg)	Cartridge Volume (mL)	Cartridges/Box	Cat. No.
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
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
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 B ₂)	25	10	50	53207-U
Triazines (class selective)	25	10	50	53208-U

Selecting the Appropriate SPME Fiber for your Application

The Effect of Sample Concentration and Complexity on SPME Fiber Selection

Bob Shirey

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Previously, in the Reporter (Vol 24.3) we discussed how to select the appropriate fiber assembly, focusing on fiber core, needle gauge and assembly design. In the Reporter (Vol 25.3) 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.

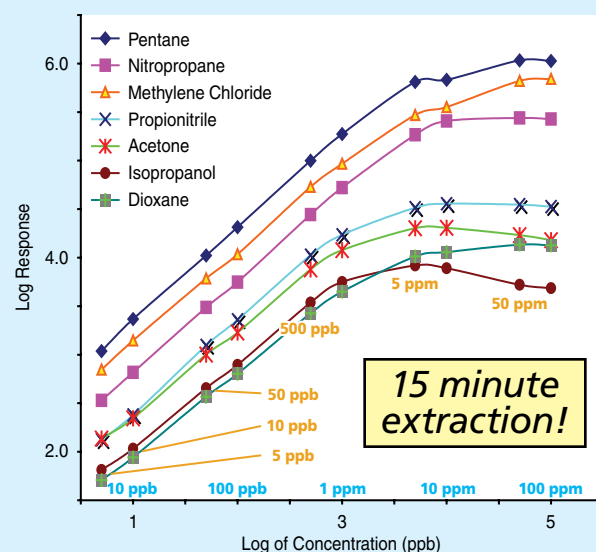
There are 2 mechanisms for the extraction of analytes into fiber coatings, absorption and adsorption. Absorbent coatings are primarily bonded gums that act somewhat like a sponge. The analytes partition in and out of the coating. The primary mechanism for retention of the analytes is the coating thickness and the size of the analyte. The coating type determines the affinity of an analyte for the phase. Usually there is 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 that have pores (adsorbents). 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 they migrate into the pores where they are more tightly retained. The size of the pore determines if 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 Polydimethyl siloxane (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.

Figure 1. Plot of Analyte Response using Carboxen-PDMS Fiber

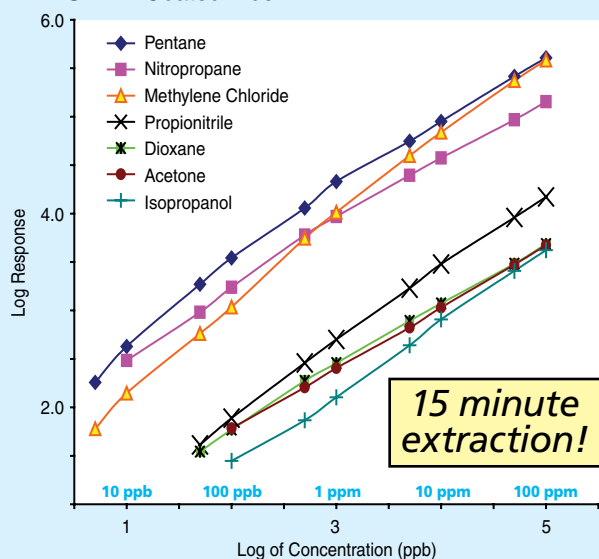


The results show that all of the analytes were extracted at 5 ppb. The polarity of the analytes increases in order from top to bottom. The responses for all of the analytes began to level off at 5 ppm, indicating that the maximum capacity for the analytes with this fiber coating. Between 10 ppm and 100 ppm the responses remained constant. 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.

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.

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



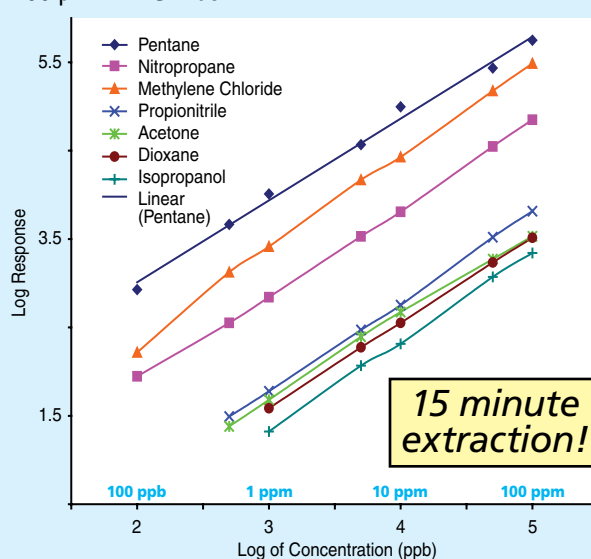
A longer extraction time would show this effect more dramatically. Figure 3 shows the same analysis using the absorbent 100 μ m PDMS coated fiber.

The 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.

1. Adsorbent fibers are better for analytes at low concentration levels
2. Adsorbent 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 adsorbent fibers to reduce displacement
4. Adsorbent fibers 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 beds.
6. Adsorbent fibers are better for screening samples at high concentration levels
7. Adsorbent fibers are a better option for dirty samples that may contain multiple unknown compounds.

Figure 3. Plot of Analyte Response using 100 μ m PDMS Fiber



+ Featured Products

The 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	Autosampler
Fiber Kit 1	85 μ m polyacrylate, 100 μ m PDMS, 7 μ m PDMS	57306	57307
Fiber Kit 2	85 μ m polyacrylate, 65 μ m PDMS/DVB, 75 μ m PDMS/Carboxen	57320-U	57321-U
Fiber Kit 3	85 μ m polyacrylate, 60 μ m PDMS/DVB, 100 μ m PDMS	—	57323-U
Fiber Kit 4	100 μ m PDMS, 65 μ m PDMS/DVB, 75 μ m PDMS/Carboxen	57324-U	57325-U
Fiber Kit 5	85 μ m polyacrylate, 65 μ m PDMS/DVB, 100 μ 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 2200 applications references for SPME, is searchable by analyte and includes video demonstrations on the use of SPME.

Reference Standards for Analyzing Polyphenol Catechins

James S. Walbridge & Kathleen Kiefer

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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
- Prevention of kidney stones
- Reducing the chance of developing rheumatoid arthritis
- Producing stronger bones
- Reducing inflammation

The catechins are a group of polyphenolic compounds exhibiting strong anti-oxidant, 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 *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.

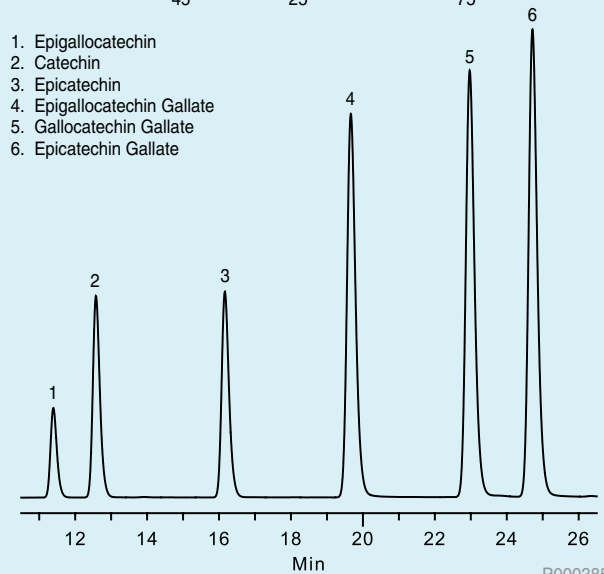
Reactive oxygen species damage cells and have been implicated in the slow chain reaction of



Figure 1. HPLC Analysis of Six Primary Green Tea Catechins

column: Ascentis RP-Amide, 15 cm x 4.6 mm I. D., 5 μ m particles (565324-U)
 mobile phase A: 10 mM ammonium formate, pH 3.0 with conc. formic acid
 mobile phase B: methanol
 flow rate: 1 mL/min.
 temp.: 35 $^{\circ}$ C
 det.: UV at 273 nm
 injection: 10 μ L
 sample: catechins solution 300 μ g/mL methanol
 gradient:

Time	% A Mobile Phase	% B Mobile Phase
0	100	0
1	55	45
30	55	45
45	25	75



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 eight polyphenol catechins. The six predominant catechins in tea leaves are catechin, galocatechin gallate (GCG), epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), with EGCG being the most abundant.

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 com-

pounds. 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

Sigma-Aldrich Solution

To meet this need, eight 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 solvent 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.

! Related Information

For more information, please contact Technical Service at techservice@sial.com

References

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+ Featured Products

Catechin Reference Solutions Each Prepared at 2000 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

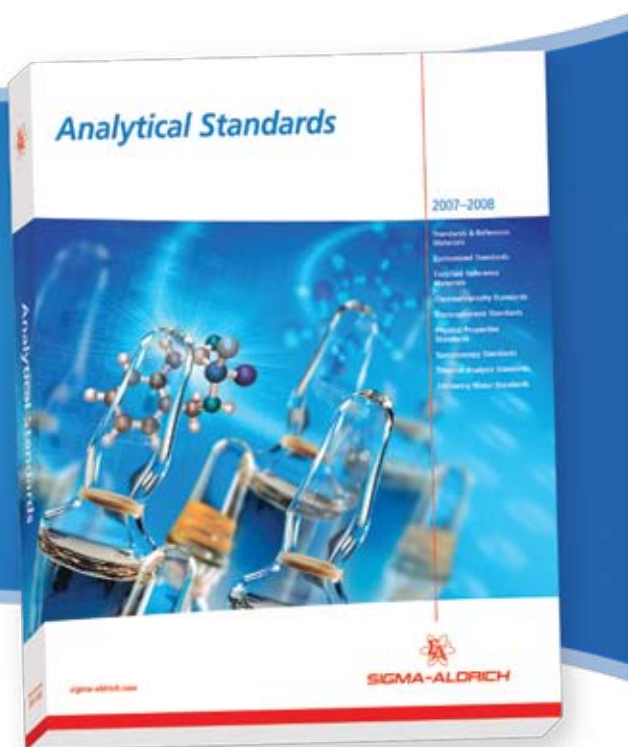
Description	Cat. No.
Ascentis RP-Amide, 15 cm x 4.6 mm I. D., 5 µm	565324-U

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Standards

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Pat Myers

techservice@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 two lines of defense against sample loss. Mininert valves have a PTFE valve backed up by a cylindrical red rubber septum. Certan 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 of 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.

Related Products

Description	Capacity	Dimensions	Cat. No.
Certan Capillary Vials			
	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
Micro Reaction Vessels			
	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
Mininert Valves			
	For 15 mm screw cap		33301
	For 20 mm screw cap		33303



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Ca, K, Na: $\leq 10 \mu\text{g}/\text{kg}$ each; and NH_4^+ : $\leq 50 \mu\text{g}/\text{kg}$ each

ORGANIC TRACES:

Acetate, formate, glycolate, oxalate: $\leq 10 \mu\text{g}/\text{kg}$ each

CONDUCTIVITY:

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Related Product:

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sives. Septa remain secured in place, eliminating sample evaporation due to poor seating in the closure.

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Material	Cap (Open-Top)	Thread	Cat. No.
PTFE/silicone	blue	9 mm	29019-U
PTFE/silicone with slit	blue	9 mm	29025-U
PTFE/silicone	black	9 mm	29026-U
PTFE/silicone with slit	black	9 mm	29028-U
PTFE/silicone	white	8 mm/425	29099-U
PTFE/silicone with slit	white	8 mm/425	29101-U
PTFE/silicone	black	8 mm/425	29102-U
PTFE/silicone with slit	black	8 mm/425	29103-U
PTFE/silicone	black	10 mm/425	29112-U
PTFE/silicone with slit	black	10 mm/425	29113-U
PTFE/silicone	white	10 mm/425	29114-U
PTFE/silicone with slit	white	10 mm/425	29115-U
PTFE/silicone	white	24 mm/400	SU860005
PTFE/silicone	white*	24 mm/400	SU860006

*solid top cap

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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

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