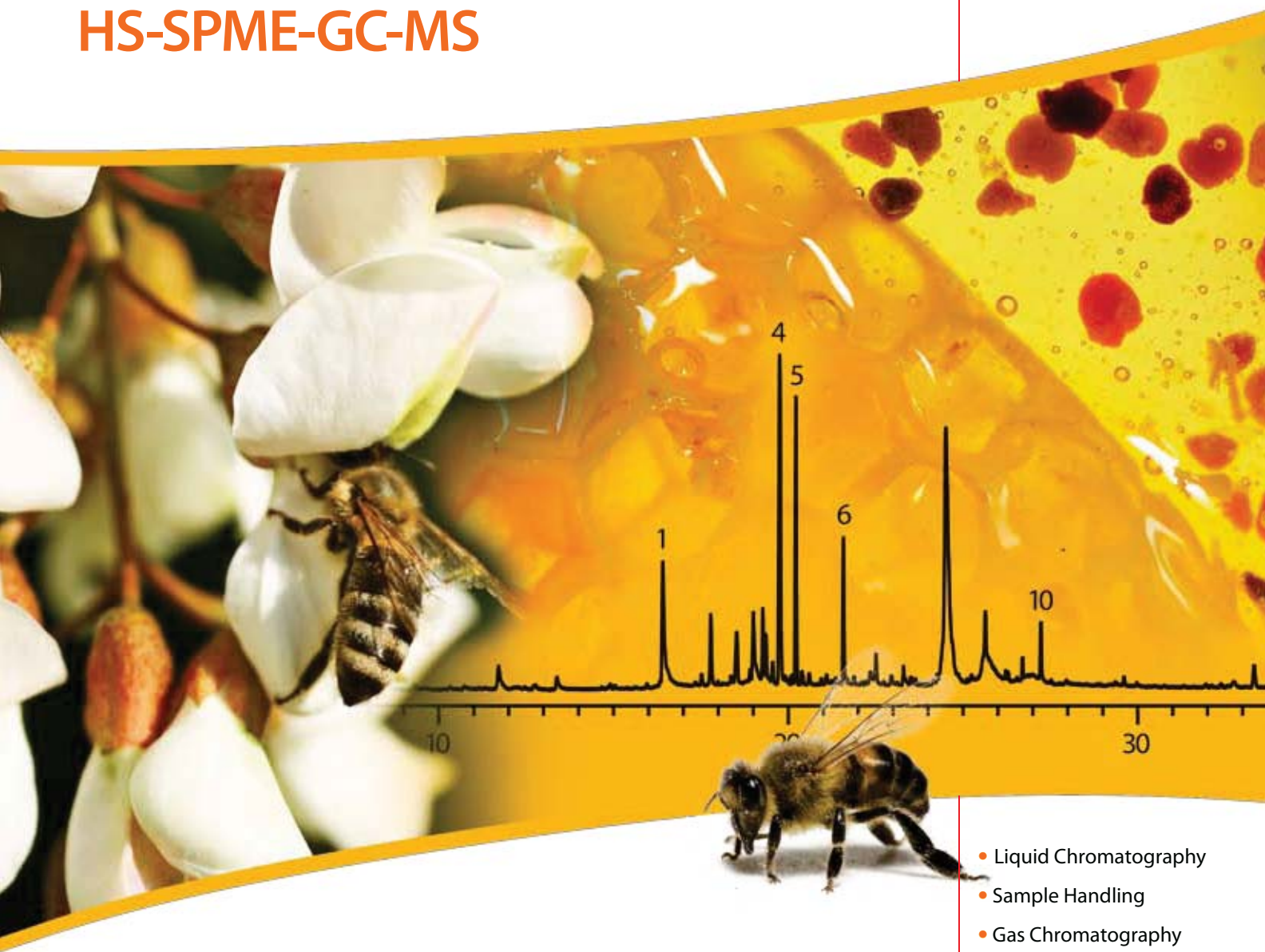
Reporter

Volume 28.1

SUPELCO® Analytical

Volatile Profiles of Honeys Using HS-SPME-GC-MS



The applicability of GC for determining botanical origin of honey

- Liquid Chromatography
- Sample Handling
- Gas Chromatography
- Standards
- Accessories
- Chiral Chromatography

Reporter
Volume 28.1

Visit us on the web at
sigma-aldrich.com/thereporter

Table of Contents

Reporter and eReporter – Two *different* vehicles containing our latest offerings about chromatographic analysis and purification.

Don Hobbs
Director of Marketing

Dear Colleague:

You may already be the recipient of our monthly electronic newsletter, eReporter, which is sent by e-mail. Perhaps you have wondered how the e-newsletter differs from the printed Reporter you are currently reading.

Below is a brief overview indicating the highlights of each vehicle, which will hopefully impress upon you the need to subscribe to both of these complimentary publications in order to obtain the greatest benefit from what we have to offer.

Reporter

Our printed Reporter newsletter – in press since 1982 – is guaranteed to keep you informed on the latest innovations, advances, and new products that we offer to the chromatography consumables marketplace.

The 24-page printed Reporter, as you are probably aware, is delivered 5 times a year and contains full-length articles on chromatography topics written by experts from our R&D group as well as guest authors. The content, rich in application notes and technical details, is designed to be as useful and relevant to the bench chemist as the lab director.

You can view digital versions of current and archived Reporter issues at any time on our website: ***sigma-aldrich.com/thereporter***

eReporter

Our monthly electronic newsletter, eReporter, provides snippets of information about important web content under HPLC, GC, Standards, and Sample Handling categories. In each issue, you also get notified about discounted pricing offers that have potential for huge savings – an exclusive to eReporter subscribers!

This e-newsletter is designed to keep you updated routinely and quickly. It only takes a couple of minutes to scan through an issue and receive a quick update on all the newsworthy items and to click on individual links to view details about specific topics that interest you.

The eReporter content is prepared by our product managers, and relates to:

- Recently added products, literature, and interactive web tools
- Products that meet the needs of current "hot" topics or new trends in chromatography
- Discount offers for a new selection of products each month!

To summarize, our printed Reporter has been and continues to be your resource for in-depth technical chromatography articles, whereas the recently launched monthly eReporter serves to alert you by email about key online updates and discount offers.

Visit ***sigma-aldrich.com/newsletters*** to subscribe to the Reporter and the eReporter, if you have not already done so! You can sign up to receive other Analytical newsletter publications using the same link.

Hope the new year is going well for you.

Regards,

Don Hobbs
Direct of Marketing
don.hobbs@sial.com

Reporter is published 5 times a year by Supelco Marketing, 595 North Harrison Road, Bellefonte, PA 16823-0048.

Volatile Profiles of Honeys Using HS-SPME-GC-MS

Contributed Article

The following was generated with the assistance of an outside source using Sigma-Aldrich products. Technical content was generated and provided by:

Federica Bianchi[1] and Marilena Musci[1]

1. Università degli Studi di Parma, Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Viale Usberti 17/A, 43100 Parma, Italy

mike.buchanan@sial.com

Introduction

The assessment of the authenticity of honey is a subject of primary importance both for consumers and industries. Usually, the determination of the botanical origin of honey is carried out by melissopalynological analysis, based on the identification of pollen by microscopic examination (1). However, data interpretation is very difficult and does not always lead to correct identification. The characterization of the volatile profile of honey has proven to be a reliable alternative to pollen analysis for the assessment of its botanical origin (2,3). In fact, the volatile profile represents a chemical fingerprint of honey, since both the nature and the amount of volatile compounds are characteristics of the floral source.

Solid phase microextraction (SPME) (4) is a rapid, solvent-less, and easy-to-use extraction technique widely applied in the determination of volatile compounds in several kinds of food. In this work we evaluate the applicability of headspace SPME (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) for the characterization of the volatile fraction of some honey samples.

Materials and Methods

Four samples of unifloral honeys (milk thistle, citrus, eucalyptus, and acacia) and a sample of a multifloral honey were analyzed. Dynamic headspace (DHS) extraction was performed following the procedure described in a previous work (2). A brief description of this DHS process can be found in Figure 1. HS-SPME was carried out per the procedure described in Figure 2. GC-MS conditions are listed in both Figures 1 and 2.

Compounds were identified by comparing the mass spectra obtained with those stored in the National Institute of Standards and Technology (NIST) library. In addition, retention indices (RIs) were calculated for each

(continued on page 4)

Table 1. Most Abundant Compounds Identified in Honey Headspace

Peak ID	Compound	Peak ID	Compound
1.	Nonanal	7.	Phenylacetaldehyde
2.	Furfural	8.	Nonanol
3.	Decanal	9.	3-Phenyl furan
4.	Benzaldehyde	10.	Phenylethyl alcohol
5.	α-Linalool	11.	Nonanoic acid
6.	Hotrienol		

Figure 1. Milk Thistle Honey Volatiles Using DHS

sample/matrix: 25 g milk thistle honey in a 200 mL Erlenmeyer flask at 45 °C, purged with nitrogen at 60 mL/min. for 30 min. as a stripping gas to collect onto a Tenax® TA tube
thermal desorption tube: 20/35 Tenax TA, 90 mg
dry-purge: back-flushed with nitrogen, 60 mL/min. at room temperature for 5 min.
desorption process: helium, 10 mL/min. at 280 °C for 10 min. to cryofocus onto a glass-lined tube at -120 °C, which was subsequently heated to 240 °C for transfer to the GC column
transfer line/valve temp.: 250 °C, transfer line directly coupled to the GC column using a press-fit connector
column: SUPELCOWAX™ 10, 30 m x 0.25 mm I.D., 0.25 µm (24079)
oven: 35 °C (8 min.), 6 °C/min. to 60 °C, 4 °C/min. to 160 °C, 20 °C/min. to 200 °C (1 min.)
MSD interface: 230 °C
scan range: 35-300 m/z
carrier gas: helium, 1 mL/min.

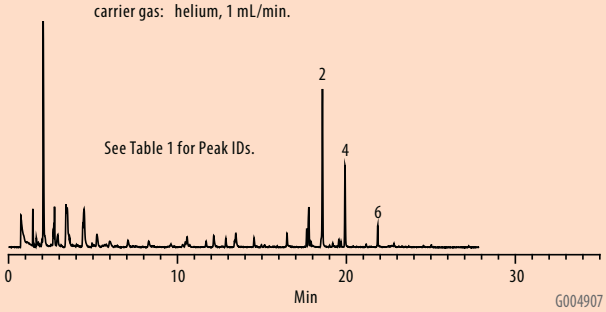


Figure 2. Milk Thistle Honey Volatiles Using SPME

sample/matrix: 5 g milk thistle honey + 5 mL bidistilled water in a 20 mL vial, allowed to equilibrate at 50 °C for 15 min. with magnetic stirring
SPME fiber: 2 cm 50/30 µm divinylbenzene/carboxen on polydimethylsiloxane (57348-U)
extraction: headspace, 50 °C for 40 min. with magnetic stirring
desorption process: 250 °C for 2 min.
column: SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.25 µm (24079)
oven: 35 °C (8 min.), 6 °C/min. to 60 °C, 4 °C/min. to 160 °C, 20 °C/min. to 200 °C (1 min.)
MSD interface: 230 °C
scan range: 35-300 m/z
carrier gas: helium, 1 mL/min.
liner: 0.75 mm I.D., SPME

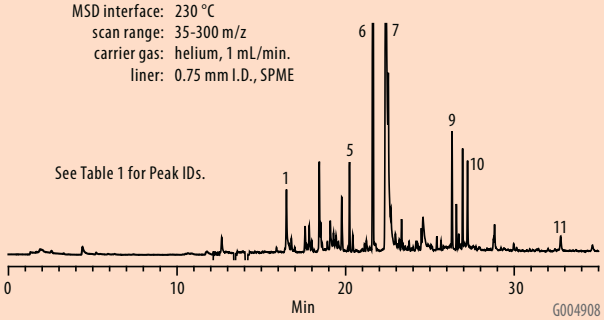


Figure 3. Citrus Honey Volatiles Using SPME

sample/matrix: 5 g citrus honey + 5 mL bidistilled water in a 20 mL vial, allowed to equilibrate at 50 °C for 15 min. with magnetic stirring

Other conditions the same as Figure 2.
See Table 1 for Peak IDs

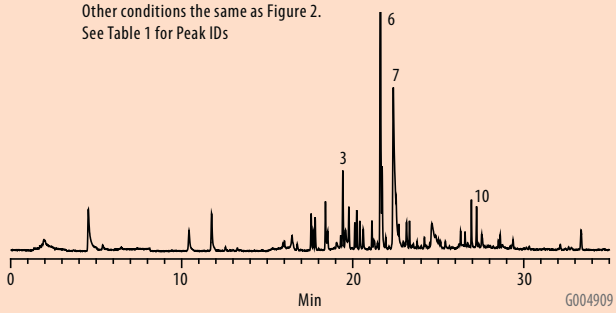


Figure 4. Eucalyptus Honey Volatiles Using SPME

sample/matrix: 5 g eucalyptus honey + 5 mL bidistilled water in a 20 mL vial, allowed to equilibrate at 50 °C for 15 min. with magnetic stirring

Other conditions the same as Figure 2.
See Table 1 for Peak IDs

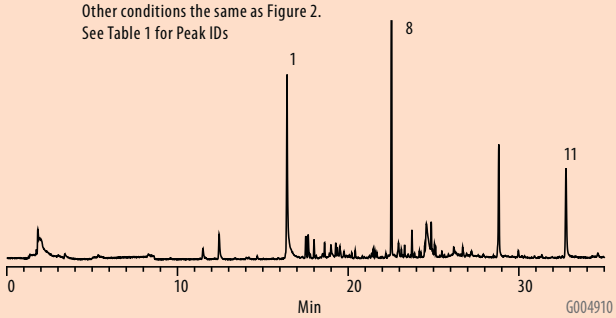


Figure 5. Acacia Honey Volatiles Using SPME

sample/matrix: 5 g acacia honey + 5 mL bidistilled water in a 20 mL vial, allowed to equilibrate at 50 °C for 15 min. with magnetic stirring

Other conditions the same as Figure 2.
See Table 1 for Peak IDs

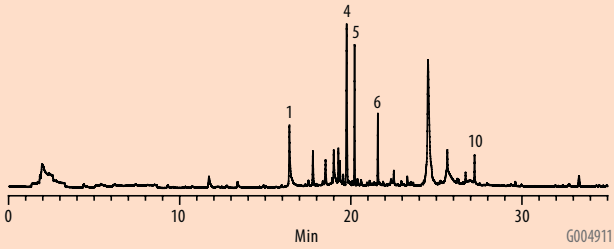
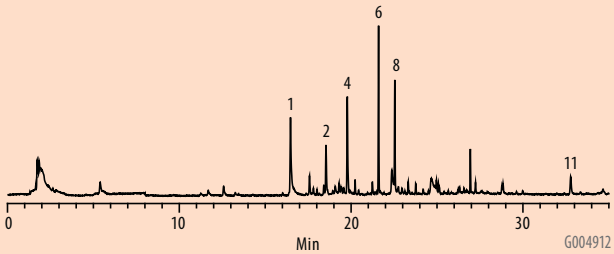


Figure 6. Multifloral Honey Volatiles Using SPME

sample/matrix: 5 g multifloral honey + 5 mL bidistilled water in a 20 mL vial, allowed to equilibrate at 50 °C for 15 min. with magnetic stirring

Other conditions the same as Figure 2.
See Table 1 for Peak IDs



Chromatograms for Figures 1-6 courtesy of Dr. Federica Bianchi and Prof. Marilena Musci (Univ. of Parma, Italy)

(continued from page 3)

peak and compared with literature data (5). Table 1 lists the most abundant compounds identified during this work. The chromatograms in Figures 1-6 are shown on the same abundance scale for ease of visual comparison. In order to evaluate quantitative differences in the aromatic profile of the samples investigated, gas chromatographic peak areas were calculated as Total Ion Current (TIC).

Results and Discussion

Preliminary experiments were carried out in order to compare the performances of the DHS technique, used in a previous study dealing with the characterization of the volatile profile of honey (2), with the SPME technique. Chromatographic profiles for a milk thistle honey obtained by DHS and SPME are shown in Figure 1 and Figure 2, respectively. As can be seen, the SPME profile not only shows a greater number of peaks with respect to that obtained by DHS, but also presents higher signal intensities. Less volatile compounds were better extracted using SPME, owing to its coating properties, whereas the more volatile compounds were better extracted by DHS. Due to the greater number of peaks observed, SPME was chosen as the extraction technique for the remainder of our study.

Figures 2-6 show the chromatographic profile of five honey samples obtained by the SPME technique. From a qualitative point of view, the chromatographic profiles of honey samples of different botanical origins were quite similar, as some of the same volatile compounds were detected in different samples. So, it was not possible to define specific volatile compounds as markers characteristic for a defined botanical origin.

However, significant differences were observed from a quantitative point of view, since different amounts of the volatile compounds were observed depending upon the floral source. For example, the milk thistle honey (Figure 2) contained a high content of hotrienol (35%), phenylacetaldehyde (16%), and 3-phenyl furan (5%), the last compound being detected only in milk thistle honey. Citrus honey (Figure 3) was characterized by a high content of hotrienol (30%) and phenylacetaldehyde (10%). Alternatively, neither hotrienol nor phenylacetaldehyde were observed in eucalyptus honey (Figure 4), which was characterized by

high amounts of nonanol (32%), nonanal (12%), and nonanoic acid (7%). Acacia honey (Figure 5) was characterized by high amounts of benzaldehyde (18%), α-linalool (11%), nonanal (6%), and hotrienol (6%). The chromatographic profile of multifloral honey (Figure 6) presents a high number of compounds, the most abundant being hotrienol (14%), benzaldehyde (10%), nonanol (10%), nonanal (8%), and furfural (5%).

Conclusion

Considering both the nature and the relative amount of the volatile compounds extracted by SPME, it was possible to obtain and distinguish the chemical fingerprints of different types of honey. Therefore, it is possible that SPME may prove to be a useful tool for determining the botanical origin of honeys to achieve authenticity assessment.

References

1. Loveaux, J., Maurizio, A., & Vorwhohl, G. (1978) Bee World, 59, 139-157.
2. Radovic, B.S., Careri, M., Mangia, A., Musci, M., Gerboles, M., & Anklam, E. (2001) Food Chemistry, 72 511-520.
3. Cuevas-Glory, L.F., Pino, J.A., Santiago, L.S., & Sauri-Duch, E. (2007) Food Chemistry, 103, 1032-1043.
4. J. Pawliszyn, Solid Phase Microextraction; Wiley-VCH: New York, 1997.
5. Bianchi, F., Careri, M., Mangia, A. & Musci, M. (2007) Journal of Separation Science, 30, 563-572.

Featured Products

Description	Cat. No.
SPME fiber, 2 cm 50/30 µm divinylbenzene/carboxen on polydimethylsiloxane	**57348-U**
Capillary GC column, SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.25 µm	**24079**

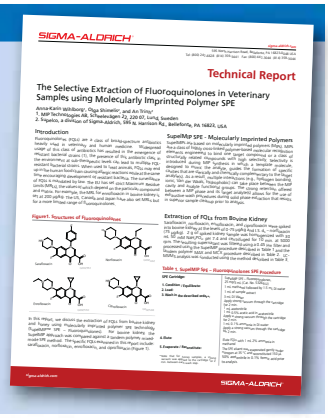
Related Information

The 7th edition of our **SPME CD-ROM** - T199925 (CJQ) is now available. This popular item contains the best of our SPME literature, plus an application guide with references to peer-reviewed journal articles. Request yours today!

Supelco also offers a full-range of GC columns and accessories. To learn more, request T407133 (KCX) *GC Column Selection Guide* or T407103 (JWE) *Maximize Performance! Gas Chromatography Accessories and Gas Purification/Management Products*.

Did you know…?

Supelco offers other literature pieces for the analysis of other compounds of interest related to honey. Request Technical Report T408177 (LCC) - *The Selective Extraction of Fluoroquinolones in Veterinary Samples Using Molecularly Imprinted Polymer SPE*, and/or poster T407125 (KDS) - *Detection of Low Level of Chloramphenicol in Milk and Honey with MIP SPE and LC-MS-MS* to learn more. Both of these are available electronic only.


SIGMA-ALDRICH

Technical Report

The Selective Extraction of Fluoroquinolones in Veterinary Samples using Molecularly Imprinted Polymer SPE

SUPELCO Analytical

Seminars on Innovative Technologies for Chromatography & Sample Prep (That Work)

Supelco and Fluka – Sigma-Aldrich's Analytical & Chromatography brands – invite you to our complimentary half day, open seminars in your region. Delivered by our technical experts and special guest speakers, the seminars show how our practical, innovative products and technologies can help you today.

Seminar Abstracts

HPLC & LC-MS: Techniques to Improve Speed, Resolution and Sensitivity without Investing in Capital Equipment

Chromatography column suppliers have responded to analysts' need for higher throughput, better sensitivity, greater resolution and longer lifetimes through an array of innovations in particles, surface chemistries and column formats. While choice is good, its scope can often cloud the important underlying fundamentals: What do all these techniques mean? Why do they work? When would you choose one over the other? What about instrument compatibility? What role does mobile phase play? This talk will attempt to clarify the situation, demystifying the recent approaches the column industry has taken, and putting the innovations in historical and physicochemical context. Intended as an instructional talk, the scientific principles behind today's innovative technology, such as Fused-Core™ particles, will be described and demonstrated using industry-specific applications.

Special Topics in Chromatography and Sample Prep

In this segment we will use special guest speakers to introduce current chromatography and sample prep topics. Title and speaker will be announced closer to the time of the event. Please consult the web site for up-to-date information.

Sample Prep for Chromatography: Sorbents, Devices and Techniques to Improve Sensitivity, Specificity and Throughput

Sample prep can be a significant bottleneck, especially when dealing with difficult or complex matrixes like biological fluids, tissues, food and agricultural products, grease and oils. This talk deals with unique combinations of innovative sorbents, devices and methodologies that address specific sample prep challenges. Topics include HybridSPE™-PPT technology for the low-level MS detection of pharmaceuticals and other small molecules in serum, solid phase microextraction (SPME), which has now been applied to bioanalysis, molecular imprinted polymers (SupelMIP™) and others. We will describe the scientific principles behind these technologies and demonstrate them using market-specific applications derived from the registrants' areas of interest.

Date	City
March 23, 2010	Houston, TX
March 25, 2010	Dallas/Ft. Worth, TX
April 20, 2010	Kansas City, MO
April 22, 2010	St. Louis, MO
May 18, 2010	Cambridge, MA
May 20, 2010	Wallingford, CT
June 15, 2010	Chicago, IL
June 16, 2010	Indianapolis, IN
June 17, 2010	Cincinnati, OH
July 20, 2010	Seattle, WA
July 22, 2010	Portland, OR
August 31, 2010	Philadelphia, PA
September 2, 2010	Long Island, NY
September 21, 2010	Princeton, NJ
September 23, 2010	Rahway, NJ
October 19, 2010	RTP, NC
October 21, 2010	Greensboro, NC
November 9, 2010	Miami, FL
November 11, 2010	Atlanta, GA
December 7, 2010	Nashville, TN

Coming to your area in 2010!

Seminar Registration & Schedule Information:

sigma-aldrich.com/analytical-seminars

Questions? Call (814) 359-5910 or e-mail *ac_seminars@sial.com*

Ascentis Express Peptide ES-C18 Expands the Fused-Core Particle Platform into Bioseparations

Wayne K. Way and William Campbell
wayne.way@sial.com

Ascentis Express Peptide ES-C18 columns were specifically engineered to separate higher molecular weight compounds such as peptides and small proteins. These columns contain advanced Fused-Core particles that have bigger pores (160 Å versus 90 Å in standard Ascentis Express), which greatly expands the application range for Ascentis Express columns.

Key Applications for Ascentis Express Peptide ES-C18:

- Pharmaceutical/therapeutic peptides
- Peptide mapping
- Natural and synthetic peptide analysis
- Oligonucleotide analysis

Key Advantages:

- Higher peak capacity providing greater resolution
- Amenable to higher flow rates for faster analysis
- Exceptional ruggedness providing long column lifetime

Ascentis Express Peptide ES-C18 columns utilize a steric-protected C18 bonded-phase with extremely high resistance to acid-catalyzed hydrolysis of the siloxane bond that attaches the C18 chain to the surface. Thus, the combination of low pH and elevated temperature operation of the column is well tolerated. Peptide separations are efficiently conducted using low pH mobile phase modifiers, often at 0.01-0.1% concentration, most popularly employing trifluoroacetic acid (TFA), and the related perfluorocarboxylic acids, pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA). These acids exhibit desirable low UV transparency, volatility, and peptide ion-pairing properties. Additional opportunities for low pH operation include the normal short chain carboxylic acids, formic acid and acetic acid, as well as mineral acids, such as phosphoric acid (0.001-0.02 M).

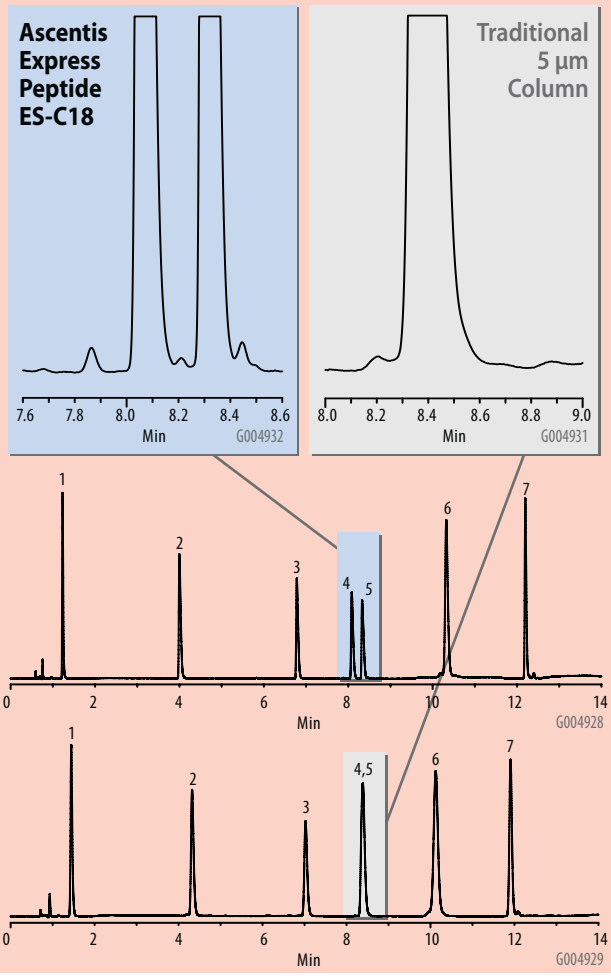
Shown in Figure 1 is the chromatographic separation of a peptide mix. The peptide mix contains a range of peptides in terms of molecular weight, basicity, and hydrophobicity. Excellent peak shape and peak width are achieved with a standard acetonitrile gradient and 0.1% TFA modifier. The resolution of small baseline impurities are shown in the inset, demonstrating the resolving power of the Ascentis Express Peptide ES-C18 column versus a traditional 5 µm column.

Figure 1. Comparison of Peptide Test Mix with Ascentis Express Peptide ES-C18 and Traditional Column

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 10% acetonitrile / 90% water / 0.1% trifluoroacetic acid
mobile phase B: 75% acetonitrile / 25% water / 0.1% trifluoroacetic acid
gradient: 0% to 50% B in 15 min
flow rate: 1.5 mL/min.
det: UV at 220 nm
temp: 30 °C
injection: 5 µL

Peptide Test Mix

1. Gly-Tyr MW = 252
2. Val-Tyr-Val MW = 379
3. Met Enkephalin MW = 574
4. Angiotensin II MW = 1032
5. Leu-Enkephalin MW = 555.62
6. Ribonuclease MW = 13,700
7. Bovine Insulin MW = 5733



Related Information

For more information on the Ascentis Express Peptide ES-C18, request brochure T410043 (MII).

Related Products

Ascentis Express Peptide ES C18 Columns

I.D. (mm)	Length (cm) 3	5	7.5	10	15
2.1	53299-U	53301-U	53304-U	53306-U	53307-U
3.0	53308-U	53311-U	53312-U	53313-U	53314-U
4.6	53316-U	53318-U	53323-U	53324-U	53328-U

Flexible Method Development Using a New pH Stable HPLC Column, Kromasil Eternity C18

Jared Benedict, Marketing and Sales Manager, Kromasil
jared.benedict@akzonobel.com

Introduction

The separation of new generation pharmaceutical compounds has increasingly become more challenging. There is a need for stationary phases that can withstand a wide pH range, from very acidic to very basic conditions, while maintaining peak shape, symmetry and lifetime. Utilizing pH as a method development tool has now allowed more flexibility in altering retention times of ionizable compounds. More basic compounds are being synthesized during the discovery of pharmaceuticals and thus there is a great need for robust stationary phases when running at high pH. This article shows how Kromasil Eternity provides solutions for the separation of ionizable compounds using extreme pH conditions, from pH 1 to pH 12.

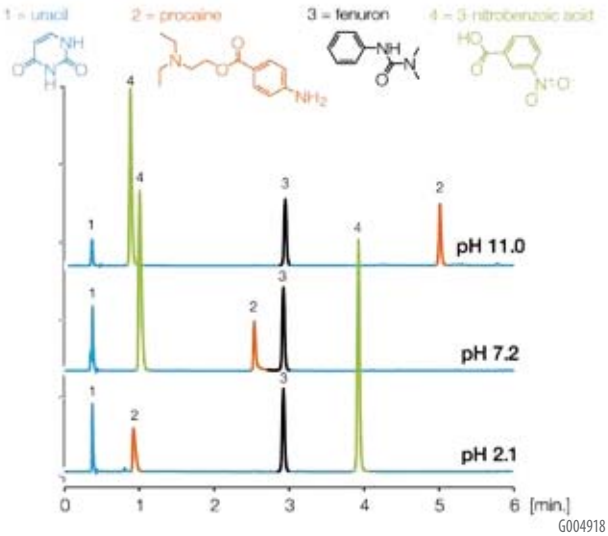
Results

In this study, we show the retention time of amines and acids can be altered by changing the pH, utilizing a single column (Figure 1). Running under basic (pH 11.0) conditions will destroy classic C18 phases very quickly, thus limiting the range of pH that can be utilized. However, when running basic pH (11.0) conditions on a Kromasil Eternity C18, procaine, an amine, is strongly retained whereas the acidic nitrobenzoic acid is weakly retained. The reverse is true under acidic conditions. Therefore, not only can you utilize the solvent strength and temperature as a method development tool but you can now also utilize nearly the full pH range. The most striking conclusion from Figure 1 is that these extreme pH conditions can be run on a single column. Kromasil Eternity can be used at an extended pH range while maintaining a very long lifetime.

In addition to utilizing the flexibility in pH in analytical method development one can also employ the robustness of these columns for semi-preparative HPLC. When doing high throughput purification of pharmaceutical compounds one needs a column that can run under extreme pH conditions to maximize the separation factor and thus the loadability. Kromasil Eternity C18 provides a solution for high loadability and flexibility under extreme pH conditions using a single column which also maintains a long lifetime that gives the maximum total economy for your purification.

Figure 1. Separation of Ionizable Compounds at Three Different pH Conditions

column: Kromasil Eternity C18, 5 cm x 4.6 mm, 2.5 µm
mobile phase: acetonitrile:20 mM sodium phosphate, pH 2.1, 7.2, and 11.0, respectively
gradient: 0-0.5 min: 10% acetonitrile, 5.5 min: 50% acetonitrile
flow rate: 1.5 mL / min
temp.: 25°C
det.: UV 254 nm
sample: 1. Uracil, 2. Procaine, 3. Fenuron, 4. 3-nitrobenzoic acid



Conclusion

Kromasil Eternity provides flexible method development and long column lifetime through the use of unique modified silica. Kromasil Eternity C18 is available as a 2.5 µm particle size for UHPLC or HPLC applications. The small particle size allows for fast runs that maximize efficiency and minimize solvent consumption. Additionally, a 5 µm particle size is available for analytical to semi-preparative HPLC up to 30 mm I.D. columns. The loadability and lifetime of the semi-preparative columns are excellent.

Featured Products

Kromasil Eternity C18 Columns

Particle Size (µm)	I.D. (mm)	Length (cm)	Cat. No.
2.5	2.1	5	K08670894
2.5	2.1	10	K08670895
2.5	4.6	5	K08670896
2.5	4.6	10	K08670897
5	2.1	5	K08670898
5	2.1	15	K08670899
5	4.6	5	K08670900
5	4.6	10	K08670901
5	4.6	15	K08670902
5	4.6	25	K08670903

Related Information

For more information on Kromasil Eternity, request the *Kromasil Eternity Brochure* (MFR) or visit our website: ***sigma-aldrich.com/kromasil***

Kromasil analytical HPLC columns are now available through the sole distributor, Sigma-Aldrich/Supelco

SUPELCO Analytical

Kromasil®

Kromasil Products, including analytical, prep, and bulk*, now available through Sigma-Aldrich/Supelco in the USA, Canada and Puerto Rico include:

Kromasil Eternity – a pH stable (pH 1 – 12) silica based C18 for complete method development flexiblity

Kromasil 100Å – Silica, C1, C4, C8, C18, Phenyl and Amino phases

Kromasil 60Å – Silica, Diol, and Cyano phases

Kromasil 300Å – Silica, C1, C4, C8, and C18 phases

Kromasil Chiral Phases – cellulose and amylose based CelluCoat™ and AmyCoat™ phases as well as the immobilized TBB and DMB phases

30% off
Kromasil Eternity Analytical Columns.

Use Promo Code 099 when placing your order to receive the discounted price.
Offer Valid through 05/31/2010.

The Sigma-Aldrich Difference

You provide the spirit of scientific innovation. We'll supply the resources to make your vision a reality.

For more information about Kromasil Products including ordering visit ***sigma-aldrich.com/kromasil***

** up to 5kg*

SIGMA-ALDRICH®

Accelerated Bioanalytical LC-MS-MS Using Ascentis Express Fused-Core HPLC Columns

Wayne K. Way and Carmen T. Santasania
wayne.way@sial.com

Fast bioanalysis requires the use of short columns packed with high efficiency particles. The use of HPLC columns packed with Fused-Core particles and sub-2 µm porous particles have led the way in these demanding analyses. They significantly enhance resolution and speed by producing either higher efficiency for the same column length or equivalent efficiency with a shorter column length. The use of ultra-high performance liquid chromatography (UHPLC) comes at a price of much higher pressure when sub-2 µm particles are employed. As an attractive alternative, the revolutionary Ascentis Express 2.7 µm Fused-Core silica particle has quickly become accepted because it is equivalent in performance to particles in the sub-2 µm range. With a very narrow particle size distribution, Ascentis Express columns employ conventional 2 µm frits and operate ruggedly at much lower pressures that are within the operating limits of conventional HPLC instruments.

The goal of this work was to demonstrate the ease of converting traditional multi-minute assays on a 50 mm Discovery® C18 column to one-minute assays using 20 mm Ascentis Express columns on an HPLC configured for LC-MS-MS bioanalysis (Agilent 1100/ABI 3200 Q Trap MS-MS). The assay integrity and quality had to be maintained in the conversion. In addition, samples were extracted from plasma based on a published method (1) and analyzed by LC-MS-MS. These extraction conditions are shown in Table 1.

Table 2 shows the compounds, their MS-MS transitions, columns and chromatographic conditions used. The slopes of each gradient were adjusted to accommodate the 20 and 50 mm column lengths. Flow rates differ because the Discovery C18 5 µm column is typically used at 0.5 mL/min in these analyses while a 1 mL/min flow rate would be more likely used with the Ascentis Express to take advantage of the higher efficiency that can be obtained at this higher flow rate.

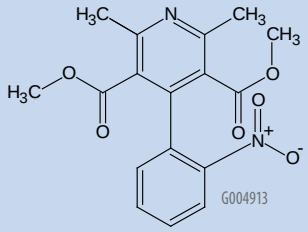
Figure 1a shows the compound mix separated in less than 0.5 minutes on the Ascentis Express HPLC column. Figure 1b shows the separation taking about 2 minutes. Excellent resolution is seen in both bases. Table 3 shows the peak width at half height measurement for both compounds. As expected the Ascentis Express phase shows much narrower peak width, indicating better efficiency for this column, even at the flow rate of 1 mL/min in a 2.1 mm column.

Table 1. Sample Preparation Procedure (1)

1. Prepare 5000 ng/mL sample in rat plasma by taking 5 µL of a mix carbamazepine and dehydronifedipine (1mg/mL each in methanol) and spike 1 mL of rat plasma.
2. Vortex 1 min, take 25 µL of plasma and 200 µL of acetonitrile to crash proteins.
3. Centrifuge at 10, 000 rpm for 1 minute. Remove 100 µL supernatant and add 200 µL of water. Vortex 1 minute.
4. Inject 2 µL.

Table 2. Experimental Conditions

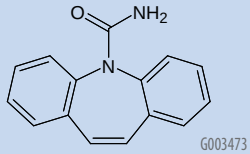
1. Compounds Used in this Study:



Dehydronifedipine

Monoisotopic Mass = 344.100836 Da

MS/MS Transition: 345.1 → 284.2



Carbamazepine

Monoisotopic Mass = 236.094963 Da

MS/MS Transition: 237.18 → 194.2

2. LC-MS-MS Conditions:

column	Ascentis Express C18, 2 cm x 2.1 mm I.D., 2.7 µm particles (53799-U)
	Discovery C18, 5 cm x 2.1 mm I.D., 5 µm particles (577507-U)
mobile phase A:	0.1% acetic acid in 95:5 water:acetonitrile
mobile phase B:	0.1% acetic acid in acetonitrile
flow rate:	see gradient table below
temp.:	40 °C
det.:	ABI 3200 Q Trap MS-MS
injection:	2 µL
sample:	carbamazepine, dehydronifedipine
back pressure:	both less than 3000 psi

3. Gradient Tables:

column: Ascentis Express, 2 cm x 2.1 mm, 2.7 µm
flow rate: 1 mL/min

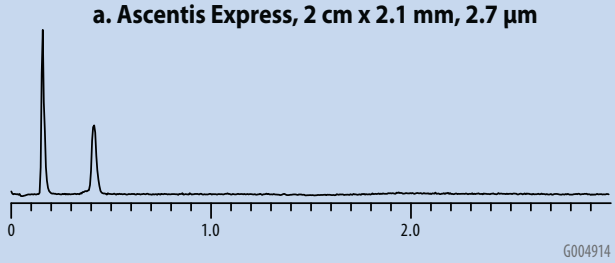
Min	%A	%B
0.00	70	30
0.75	5	95
0.85	5	95
0.90	70	30
1.00	70	30

column: Discovery C18, 5 cm x 2.1 mm, 5 µm
flow rate: 0.5 mL/min

Min	%A	%B
0.0	70	30
1.5	5	95
1.7	5	95
1.8	70	30
2.0	70	30

Figure 1. Chromatographic Comparison of Fused-Core vs. Porous Particles

a. Ascentis Express, 2 cm x 2.1 mm, 2.7 µm



b. Discovery C18, 5 cm x 2.1 mm, 5 µm

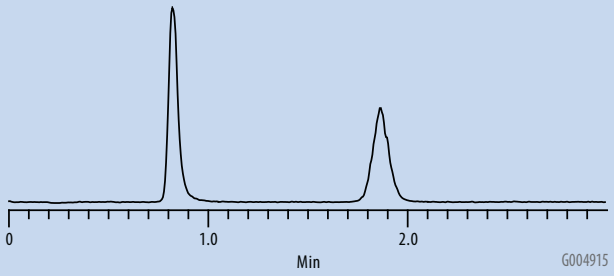
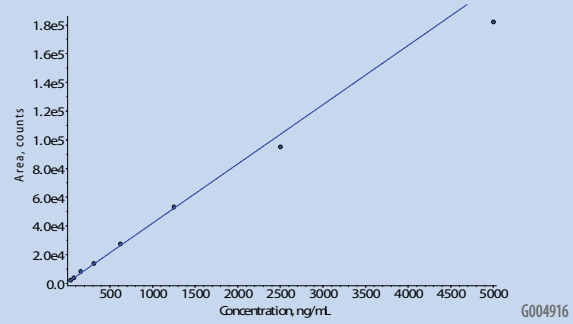


Table 3. Peak Width Comparison

Column	Flow Rate	Peak Width (carbamazepine)	Peak Width (dehydronifedipine)
Ascentis Express C18, 2 cm x 2.1 mm, 2.7 µm	1 mL/min	0.019	0.028
Discovery C18, 5 cm x 2.1 mm, 5 µm	0.5 mL/min	0.049	0.095

Figure 2. Calibration Curves

a. Carbamazepine



b. Dehydronifedipine

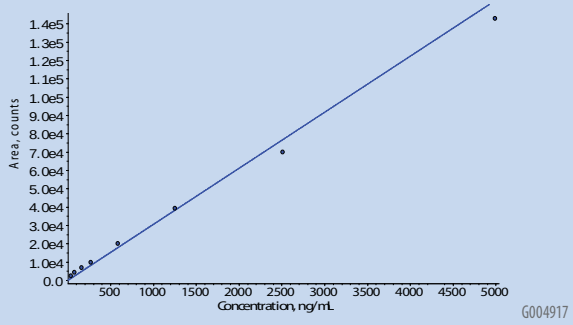


Figure 2 shows a calibration curve for the dehydronifedipine and carbamazepine. The calibration standards were prepared in plasma and extracted using the method in Table 1. Good linearity was obtained over the range studied. Calibration curves are shown using a linear plot with $1/x^2$ weighting.

The demands of increased speed and resolution are vital factors to scientists that work in the area of bioanalytical research. In this work presented here, the benefits of short Fused-Core columns have been demonstrated. The integrity and quality of the analysis was maintained in transferring from totally porous to Fused-Core particles, fast gradients were possible using traditional LC pumps and the Ascentis Express columns have been shown to be rugged and provide good results with greater than 2 years of use (1) in these types of bioanalytical assays.

Reference

1. Recent Advancements in Accelerated Bioanalytical LC/MS Using Fused-Core Columns. Richard L. Beardsley, Ethan R. Badman, Zhenmin Liang, Surendra Bansal ASMS Presentation 2009. Philadelphia, PA.

Did you know…?

Supelco's HybridSPE–PPT sample prep cartridges and 96-well plates for the removal of phospholipids are the perfect complement to Ascentis Express for bioanalytical LC-MS-MS assays. If you have an interest in these products, please complete the survey at ***sigma-aldrich.com/bioanalysis-request***

Featured Products

Particle Size (µm)	I.D. (mm)	Length (cm)	Cat. No.
Ascentis Express C18 Columns			
2.7	2.1	2	**53799-U**
2.7	2.1	3	**53802-U**
2.7	2.1	5	**53822-U**
2.7	2.1	7.5	**53804-U**
2.7	2.1	10	**53823-U**

Related Product

Particle Size (µm)	I.D. (mm)	Length (cm)	Cat. No.
Discovery C18 Column			
2.7	2.1	5	**53799-U**

Increased Bioanalytical Throughput and Recovery Utilizing HybridSPE-PPT Small Volume Plates

Craig Aurand
craig.aurand@sial.com

Often a major concern in developing bioanalytical methods is addressing the affect of biofluid matrix on the detection of desired analytes. The impact of matrix affects in bioanalysis has been well documented. In the majority of cases, co-extracted interferences directly affect the quantitation of analytes due to ionization effects induced by the extracted matrix. This extracted matrix can impact the chromatographic analysis, but more often results in a chromatographic build-up that leads to irregularities in both retention and quantitation. To address these issues, organic gradient elution is often utilized to 'wash' adsorbed contaminants from the column. In most cases gradient elution is not required for resolution of desired analytes, but instead required only to elute extracted matrix from the analytical column.

Performing a more thorough sample cleanup enables faster chromatographic analysis and thus increases the overall sample throughput. Using the HybridSPE-PPT platform for selective phospholipid depletion eliminates the need for gradient elution of adsorbed matrix from the analytical column, resulting in the ability to perform isocratic chromatographic separation with a dramatic increase in throughput.

HybridSPE-Small Volume 96-Well Plate and Closeup of Tips

E001087

Figure 1. HybridSPE-Small Volume 96-Well Schematic Diagram

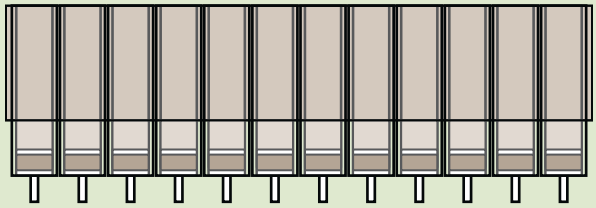
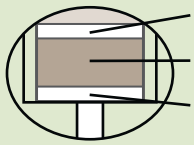


Plate: 96-well format employs special frits at the top and bottom of the same selective bed; proteins **can be removed on-line** for added speed and convenience.

Single Well Explosion:



5 µm PTFE Frit

15 mg Zirconia-Coated Silica Bed

0.45 µm Hydrophobic Graded Filter/ Frit Yields Ultra-Clean Sample

This study evaluates the performance of the newly developed HybridSPE-PPT Small Volume 96-well plate for preparation of small volumes of rat plasma. The HybridSPE-PPT Small Volume plate accommodates plasma volumes of 20-40 µL, ideally suited for bioanalytical testing of mouse plasma. It makes use of the zirconia-coated silica stationary phase as is also used in the standard HybridSPE-PPT plate for phospholipid depletion. The HybridSPE-PPT Small Volume plate is a scaled down version with a 1 mL well volume and a 15 mg packed stationary bed. A 0.45 µm polishing filter is also used for fine particle removal. The narrow internal well diameter of the plate, along with small packed bed, results in minimal holdup volume (typically 20-40 µL). This enables sufficient volume recovery when handling small volume plasma samples.

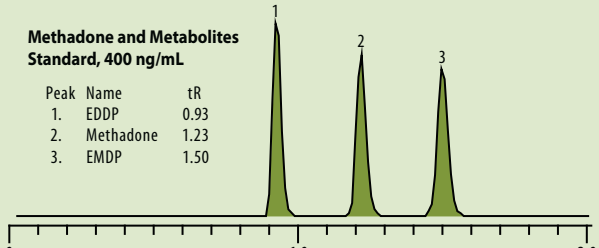
In this study, rat plasma samples spiked with methadone and metabolites 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) were processed with the HybridSPE-PPT Small Volume 96-well plate and compared against standard protein precipitation methods. The analysis was conducted on an Agilent® 1200SL Rapid Resolution system coupled to an Agilent 6210 TOF LC/MS. Chromatographic separation was performed on the Ascentis Express RP-Amide. The high sensitivity of methadone and metabolites enable for direct small volume injection of the processed sample without the need for evaporation or reconstitution.

Figure 2. Chromatographic Conditions and Conditions for Sample Preparation

column: Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm, (53913-U)
mobile phase: 10mM ammonium formate (65:35 water:acetonitrile) pH 3.6
flow rate: 0.4 mL/min
system pressure: 275 bar
temp: 35 °C
inj vol: 0.5 µL
instrument: Agilent 1200SL Rapid Resolution, Agilent 6210 TOF LC/MS
detection: ESI+
sampling rate: 1.5 spectra/sec, 6309 transients/spectra

Methadone and Metabolites Standard, 400 ng/mL

Peak	Name	tR
1.	EDDP	0.93
2.	Methadone	1.23
3.	EMDP	1.50



Sample Preparation

Standard Solutions:
Standard solutions were prepared from a stock standard in (3:1) 1% formic acid acetonitrile: water at a level of 25, 50, 100, 200, 300 ng/mL. These standards were used to establish recovery of methadone and metabolites from the HybridSPE-Small Volume without interference from plasma interactions

Plasma:
Rat plasma stabilized with K_2EDTA was acquired from Lampire Biological Laboratories, (Pipersville PA). Plasma was spiked directly from stock standard to a level of 100, 200, 400, 800, 1200 ng/mL

HybridSPE-small volume Plasma Samples: apply 20 µL of plasma to plate, followed by 60 µL of 1% formic acid acetonitrile. Agitate via vortex for 1 minute, place on vacuum manifold and apply 10"Hg vacuum for 2 minutes. Collect filtrate and analyze directly

HybridSPE-small volume Standard Solution: apply 80 µL of standard prepared in (3:1) 1% formic acid acetonitrile:water. Agitate via vortex for 1 minute, place on vacuum manifold and apply 10"Hg vacuum for 2 minutes. Collect filtrate and analyze directly. Samples were prepared n=8

Standard Protein Precipitation: apply 100 µL of plasma to centrifuge vial, followed by 300 µL of 1% formic acid acetonitrile. Agitate via vortex for 1 minute, place into centrifuge for 2 minutes at 15000 rpm. Collect supernatant and analyze directly

Each spiked level sample was prepared n=8 for both the HybridSPE-PPT Small Volume technique and the standard protein precipitation method. Samples processed using the HybridSPE-PPT Small Volume technique were collected directly into an Agilent low volume 96-well collection plate, average sample volume recovery from the plate was 40 µL. To ensure that sufficient sample was drawn into the injector, the autosampler was set for bottom well sensing. Samples were assayed for content of methadone and metabolites along with matrix monitoring for phospholipids. In this particular study, monitoring for 1-palmitoylglycerophosphatidylcholine, m/z 496.3375, was conducted as a representative phospholipids matrix ion.

Samples prepared using the HybridSPE-PPT Small Volume plate demonstrated high recovery across the concentration range. These samples were not affected by the matrix buildup due to the complete depletion of phospholipids. No signal suppression was observed using the HybridSPE-PPT Small Volume technique. As shown in Tables 1, 2, and 3 nearly equivalent calibration slopes between the standard solution

Table 1. Calibration Table: Methadone

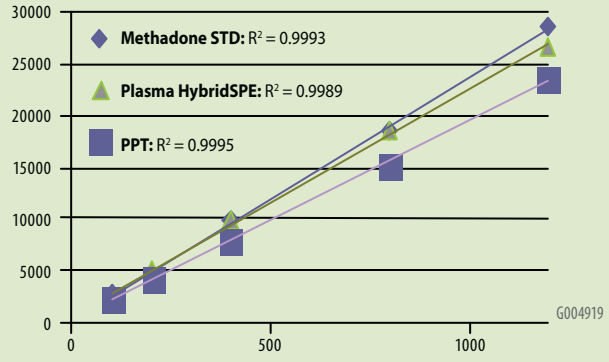


Table 2. Calibration Table: EMDP

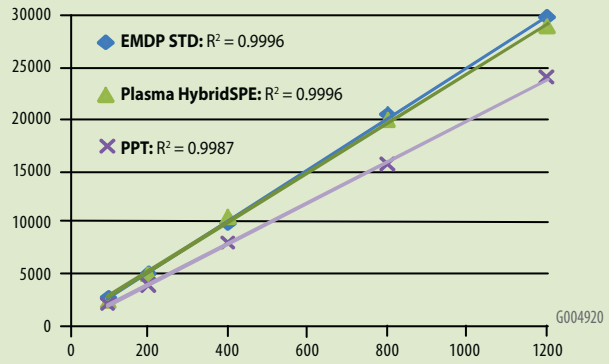
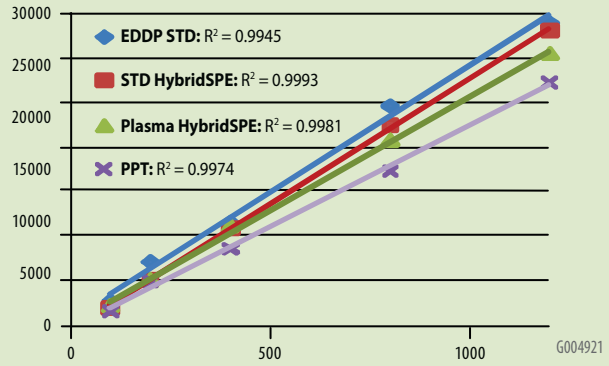


Table 3. Calibration Table: EDDP

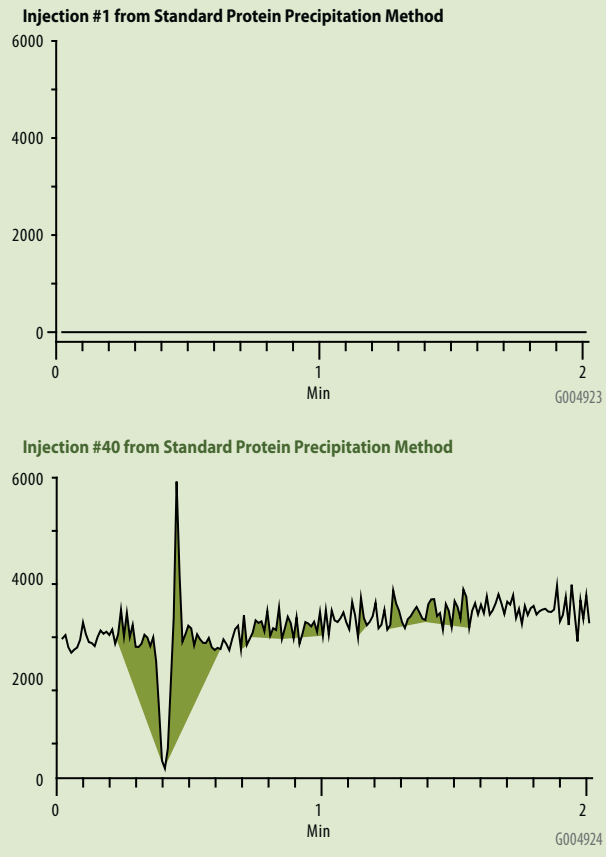


and spiked plasma processed with the HybridSPE-PPT small volume technique was achieved. In Table 3, some drug protein binding was observed for the EDDP metabolite, resulting in the slight decrease in response. The recovery of the standard solution (red) was included in this chart to show the high recovery of EDDP from the HybridSPE-PPT Small Volume plate.

Phospholipid matrix affect was evident with samples prepared using the standard protein precipitation technique. Often when performing ballistic gradient methods, the high organic content elutes a portion of the matrix from the column in a broad range. When performing isocratic

(continued on page 14)

Figure 3. Phospholipid Buildup on Column from Standard Protein Precipitation Method



(continued from page 13)

methods, phospholipid buildup is continuous and results in an overall background increase, due to phospholipids gradually leaching from the column. As demonstrated in Figure 3, monitoring of m/z 496.3375 representing 1-palmitoylglycerophosphatidylcholine, an increase in background from none detected to over 3000 count was observed over the 40 sample injection range of the standard protein precipitation technique. The gradual increase in background phospholipids is the cause for the dramatic decreased signal response with increasing spike level samples. Samples were analyzed in order from lowest spike level to highest spike level. The highest level spiked samples were then subject to the highest amount of chromatographic buildup of phospholipid. Significant signal reduction was observed for the standard protein precipitation method due to the background phospholipids.

This study has demonstrated the detrimental effect of phospholipid buildup and resulting matrix ionization effect when performing standard protein precipitation techniques. By utilizing the HybridSPE-PPT Small Volume plate, excellent recovery of methadone and associated metabolites across the concentration range along with depletion of proteins and phospholipids from the plasma samples was achieved. The combination of facile protein precipitation/phospholipid depletion and fast analysis using modern chromatographic particles shows great promise in increasing the throughput for bioanalytical methods. The ability to perform selective matrix removal enables the use of optimized chromatographic elution conditions without the need for gradient elution of sample matrix, resulting in shorter run times and more rugged bioanalytical methods.

Featured Products

Description	Cat. No.
HybridSPE-PPT – Small Volume 96-well plate, 15 mg/well	52794-U
96-Square/Deep Well Collection Plates, 1 mL, PP	575652-U
IKA® MS 3 Digital Orbital Shaker	Z645036-1EA

Related Products

Description	Cat. No.
Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm	53913-U
HybridSPE-Precipitation 96-well Plate, 50 mg/well	575656-U
HybridSPE-Precipitation Cartridge, 30 mg/1 mL	55261-U
96-well Protein Precipitation Filter Plate	55263-U
Supelco PlatePrep Vacuum Manifold	57192-U
96-Square/Deep Well Collection Plates, 0.35 mL, PP	575651-U
96-Square/Deep Well Collection Plates, 2 mL, PP	575653-U
96-Square Well Pierceable Cap Mats	575655-U
Methadone, 50 mg and 1 g	M0267
EDDP, 10 mg	E5264

Related Information

For more information on the HybridSPE-PPT products, request brochure T409095 (LOP) - *HybridSPE-Precipitation Technology.*

Did you know…?

Supelco's Ascentis Express is a perfect complement to HybridSPE™-PPT sample prep cartridges and 96-well plates for bioanalytical LC-MS-MS assays. If you have an interest in these products, please complete the survey at ***sigma-aldrich.com/bioanalysis-request.***

Introducing Supelco Glass-Fritted Thermal Desorption Tubes with Barcode

Kristen Schultz and Jamie Brown
kristen.schultz@sial.com

E001084

Supelco now offers glass-fritted thermal desorption tubes in the dimension ¼" (6.35 mm) O.D. x 3.5" (89 mm) length for DANI, Markes, OI Analytical, and Shimadzu Instruments. ***These tubes are specifically designed to fit any thermal desorber accepting these tube dimensions.*** In addition to the glass frit at the inlet, each tube is preconditioned, sealed in a TDS^3 storage container, and supplied with a unique code-128 barcode. The barcode can easily be read through the TDS^3 storage container. The final product is QA tested and packaged in a new field friendly box.

It is a well-documented problem in thermal desorption that glass wool plugs at the inlet have a tendency to blow out of the tube and into the instrument, resulting in sample loss, lost instrument time, contamination of the thermal desorber and instrument repair. Glass frits fused at the optimum position at the inlet prevent this problem from occurring. Other advantages of the glass frit are:

- Optimized position at the inlet maintains the adsorbent(s) in the instruments heated zone. (More consistent recovery of higher molecular weight compounds.)
- Consistent backpressure from tube-to-tube.
- Adsorbent(s) bed(s) stay intact, providing longer serviceable life of the tubes and prevents channeling during sampling.

Note: Over multiple uses, the glass-frit will darken but this does not affect the performance of the tube.

A new feature is the addition of a unique number code-128 barcode, which is easily scanned with any standard barcode reader. If you do not have a barcode reader, the unique number is located directly underneath the barcode and can be manually entered into your system. Having the barcode standard on every tube streamlines sample processing from tube receipt/inventory to deployment and sampling in the field and lastly, to the final steps of sample processing-sample analysis and reporting. It also reduces errors due to unreadable tube markings. Our barcode is Laboratory Information Management System (LIMS) or like systems friendly and can be integrated into your workflow like other products you currently process with a barcode.

All tubes are preconditioned and QA tested to ensure conformance with our high-quality standards. As the world leader in carbon adsorbent technology and over 20 years of expertise in thermal desorption tube manufacturing and applications, our goal is to ensure you receive the highest quality product.

Each tube is sealed in our TDS^3 storage container upon thermal conditioning to preserve the conditioned integrity of the adsorbents. The TDS^3 system is unique to Supelco and is an alternative to brass Swagelok® end-fittings, which require tooling for both sealing and removal. The TDS^3 storage container does not require any tooling, eliminates internal dead volume, minimizes the risk of contamination from outside sources, and protects the tubes from damage. Another benefit we often hear from our customers is that the tubes are lighter in weight without the Swagelok® end-fittings and are easier to transport because they are not weighted at the ends. The TDS^3 Storage Container System is well-tested in the market with over 15 years of use in various other Supelco thermal desorption tube products such as GERSTEL and Dynatherm and are available for all TD tube sizes.

E001088

Featured Products

Description	Qty.	Cat. No.
Tenax® TA Fritted 89 mm Glass TD Tube, w/TDS^3	10	29530-U
Carbopack™ B Fritted 89 mm Glass TD Tube, w/TDS^3	10	29535-U
Carbopack X Fritted 89 mm Glass TD Tube, w/TDS^3	10	29537-U
Carboxen™ 569 Fritted 89 mm Glass TD Tube, w/TDS^3	10	29534-U
Carboxen 1016 Fritted 89 mm Glass TD Tube, w/TDS^3	10	29536-U
Carbotrap™ 217 Fritted 89 mm Glass TD Tube, w/TDS^3	10	29531-U
Carbotrap 300 Fritted 89 mm Glass TD Tube, w/TDS^3	10	29532-U
Carbotrap 349 Fritted 89 mm Glass TD Tube, w/TDS^3	10	29533-U
Empty Fritted 89 mm Glass TD Tube	10	29538-U

SPME Fast Fit Assemblies (FFA) & Multi-Fiber Exchanger (MFX)

Daniel Vitkuske and Kaj Peterson[1]
daniel.vitkuske@sial.com

1. GERSTEL GmbH & Co.KG, Eberhard-Gerstel-Platz 1, D-45473 Mülheim an der Ruhr

The success of SPME over the past two decades can be attributed to a long list of reasons. Some key points are listed here: SPME is easily automated; a wide range of high-quality fibers are available covering a broad selectivity range; SPME covers analytes ranging from VOCs to SVOCs; additional selectivity in terms of volatility range and matrix interference elimination can be applied by extracting either from the headspace or directly from the liquid sample phase (which can easily be modified to release the maximum amount of VOCs); and last, but not least, the extraction is solvent-free, eliminating dilution and offering a substantial concentration effect along with a reduction in solvent background in the laboratory air.

One thing that many users have sought for quite some time has been the ability to automatically change SPME fibers during the analysis sequence. New SPME Fast Fit Assemblies and the Multi-Fiber Exchanger for the GERSTEL® MultiPurpose Sampler (MPS) now make this possible, adding extra power to SPME automation.

Why Would Anyone Want or Need Automation Power?

There are many benefits to having the ability to automatically exchange SPME fibers. For example, SPME analysis can be automatically performed using fibers with different polarities, covering a wider range of analytes and extracting the maximum amount of information from a sample without manual intervention. MFX enables faster method development by allowing the analyst to modify both method parameters and fiber polarity during an automated sequence. Routine analysis of large batches of difficult samples or samples with a high matrix load can now be performed overnight or through the weekend since fibers can be replaced at regular intervals as the fiber performance deteriorates.

Figure 1. Fast Fit Assembly versus a Standard SPME Fiber

E001090

Figure 2. FFA Barcode Guide

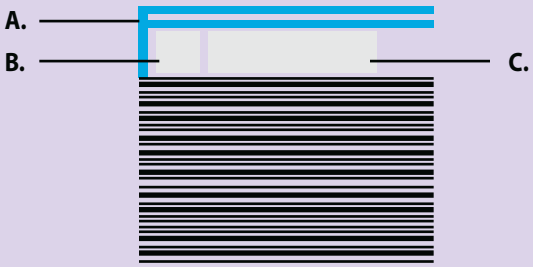


Figure 3. Multi-Fiber Exchanger on the GERSTEL MPS

E001097

The key to automating fiber exchange is the new SPME Fast Fit Assemblies, which are modified SPME fiber assemblies that allow the MFX system to pickup and operate the fiber assembly without manual intervention. Eliminating the threaded hub and adding certain features (Figure 1) allows the fiber exchanger to readily pick up, expose and retract the fiber, and exchange it with other fiber assemblies.

Special FFA barcoding (Figure 2) provides the user and the autosampler with a variety of information about the properties of the fiber assembly:

A. **The color code indicates the phase type. The coding is the same for the colored screw hub of the traditional SPME fiber assemblies**
B. **The first 2 digits of the barcode is the fiber type identity incorporating the phase type & the needle gauge size.**
C. **The remaining digits represent a unique tracking number for sample traceability.**

The MFX system is available with a three-fiber tray or a 25-fiber tray, in which conditioned SPME FFA's are kept in sealed compartments ready for use. Well-conditioned SPME fibers provide the best possible analytical results and lowest limits of detection. A bake-out station or the GC inlet can be specified for conditioning. If derivatization is needed, pre- or post-extraction derivatization can also be specified.

Stirred, Not Shaken, Sample Extraction

Sample agitation accelerates the SPME process and improves overall throughput, but agitation can lead to mechanical stress on the fiber causing breakage. Sample stirring in the MPS 007 Agitator/Stirrer is based on magnetic stir bars, which do not cause fiber stress and therefore lead to longer fiber life expectancy and improved instrument up-time. The PrepAhead function of the MAESTRO software allows samples to be prepared in advance while the GC run is ongoing for improved throughput and system utilization. In addition, samples can be heated or cooled during storage and extraction, which is highly useful, for example, for fresh food samples.

Summary

The new SPME fast fit assemblies and Multi-Fiber Exchanger were developed in partnership with Chromline srl in Prato, Italy. They offer a unique automated fiber exchange option for the GERSTEL MPS. Currently installed MPS systems, with the MPS L model as the only exception, can be updated to MFX productivity.

For more information about MFX and other GERSTEL solutions, please contact GERSTEL: ***gerstel@gerstel.com*** or visit their website: ***www.gerstel.com***

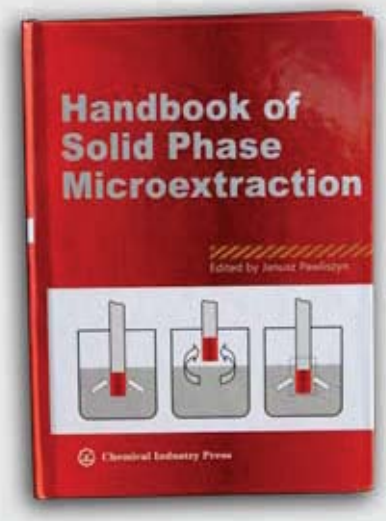
Featured Products

Description	Qty.	Cat. No.
SPME Fast Fit Assembly		
100 µm PDMS (Fused silica), 23 ga	3	**FFA57341-U**
100 µm PDMS (Fused silica), 24 ga	3	**FFA57301**
30 µm PDMS (Fused silica), 23 ga	3	**FFA57289-U**
30 µm PDMS (Fused silica), 24 ga	3	**FFA57309**
7 µm PDMS (Fused silica), 23 ga	3	**FFA57291-U**
7 µm PDMS (Fused silica), 24 ga	3	**FFA57302**
85 µm Polyacrylate (Fused silica), 23 ga	3	**FFA57294-U**
85 µm Polyacrylate (Fused silica), 24 ga	3	**FFA57305**
60 µm PEG (Metal), 23 ga	3	**FFA57354-U**
65 µm PDMS-DVB, 23 ga	3	**FFA57293-U**
65 µm PDMS-DVB, 24 ga	3	**FFA57327-U**
85 µm CAR/PDMS, 23 ga	3	**FFA57295-U**
85 µm CAR/PDMS, 24 ga	3	**FFA57335-U**
50/30 µm DVB/CAR/PDMS, 23 ga	3	**FFA57298-U**
50/30 µm DVB/CAR/PDMS, 24 ga	3	**FFA57329-U**

Featured Products (Contd.)

SPME FFA Assortment Kits		
StableFlex™ Kit, 23 ga *65 µm PDMS/DVB, 50/30 µm DVB/Carboxen/PDMS, 85 µm Carboxen/PDMS, and 85 µm polyacrylate coated fibers*	4	FFA57284-U
Kit 1 (Fused silica), 23 ga *For Volatiles and Semi-volatiles: 85 µm polyacrylate, 100 µm PDMS, and 7 µm PDMS coated fibers.*	3	FFA57285-U
Kit 2 (Fused silica), 23 ga *For Volatile or Polar Organics in Water: 75 µm Carboxen/PDMS, 65 µm PDMS/DVB, and 85 µm polyacrylate coated fibers.*	3	FFA57286-U
Kit 4 (Fused silica), 23 ga *For Flavors and Odors: 100 µm PDMS, 65 µm PDMS/DVB, and 75 µm Carboxen/PDMS coated fibers.*	3	FFA57287-U
Kit 5 (Fused silica), 23 ga *For Flavors and Odors: 100 µm PDMS, 65 µm PDMS/DVB, 85 µm Carboxen/PDMS, and 50/30 µm DVB/PDMS coated fibers.*	4	FFA57362-U

NEW! *Handbook of SPME by Janusz Pawliszyn*



This new 400-page book contains comprehensive descriptions of the fundamental principles of solid phase microextraction (SPME), recent applications, SPME devices and procedures published to date. SPME protocols are presented in a step-by-step fashion, providing useful tips and potential pitfalls. The important steps in SPME method development and optimization including calibration are clearly discussed to assist new users of the technology. This handbook enables researchers at all stages of their careers to effectively apply this convenient and solvent free sample preparation technique to solve their analytical challenges in an effective way. This up-to date handbook contains 13 chapters with topics including: Theory of SPME, SPME devices and fiber coatings, commercial devices and coatings, automated SPME systems, calibration of extraction step, SPME method development, ligand-receptor binding, in-vivo SPME, review of different application areas including: environmental, food and fragrance, forensic and drug analysis as well as SPME protocols.

Description	Cat. No.
Handbook of SPME	Z569046

FAME Standard for Optimizing GC System Performance

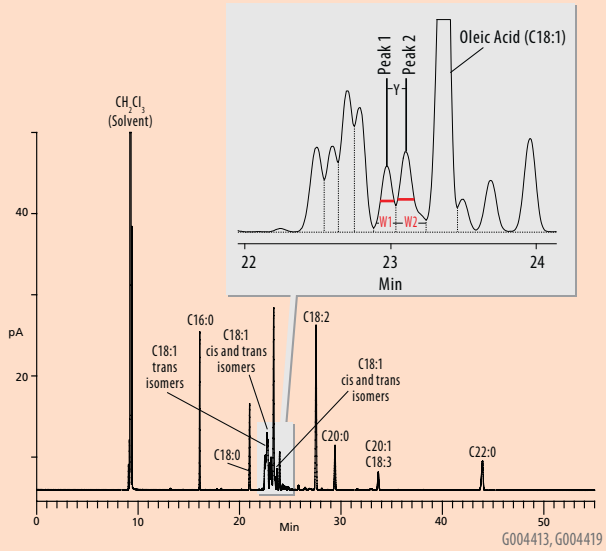
Vicki Yearick and Katherine Stenerson
vicki.yearick@sial.com

AOCS and AOAC methods for determining the trans fatty acid composition of foods by gas chromatography require the use of a highly polar cyanosilicone capillary column to provide the best peak resolution attainable of the numerous geometric (cis & trans) and positional isomers. Due to the highly polar nature of these phases, subtle changes in the GC system can alter the chromatographic separation of the positional cis & trans isomers. Therefore, it is important to continually monitor column performance and correct for unexpected changes in the column flow, oven temperature, column degradation and/or sample concentration as they occur.

AOCS Method Ce 1h-05 recommends using a well-characterized mixture of fatty acid methyl esters (FAMEs) covering the range of fatty acids under investigation to monitor changes in column performance over time. Sigma-Aldrich's new cis/trans FAME Column Performance Mix is an excellent choice for monitoring these changes. This qualitative mix is specifically designed to optimize and to monitor performance changes per AOCS Method Ce 1h-05 and AOAC Method AOAC 996.06. The mix contains C18:1 cis/trans positional isomers of critical importance to the food industry.

Figure 1. cis/trans FAME Column Performance Mix

column: SP™-2560, 100 m x 0.25 mm I.D., 0.20 µm (24056)
oven: 180 °C (isothermal)
inj: 250 °C
det.: FID, 250 °C
carrier gas: helium, 1 mL/min. @ 180 °C
injection: 1 µL, 100:1 split
sample: cis/trans FAME column performance mix (40495-U)



G004413, G004419

Because some cis/trans positional FAMEs are difficult to separate and baseline resolution may not be possible, regular use of the Sigma-Aldrich FAME Column Performance Mix is of utmost importance to achieve the required column performance. Small changes in the sample size, sample concentration, or oven temperature may be required to achieve the best resolution between the C18:1 cis/trans isomers. Once optimal resolution for the column is established, weekly analysis of the mix allows monitoring of the column's performance.

The analyst injects the mix and identifies the peaks labeled as "1" and "2" in Figure 1. The resolution between these peaks is then calculated using the equation shown in Figure 2. It is important to run the mix upon initial installation of a new capillary column to ensure that conditions are established to optimize resolution. Optimum resolution between two peaks is defined as "baseline" resolution. Full baseline resolution is achieved when the R-value calculated using the equation in Figure 2 is approximately 1.5 or greater. The example chromatogram shown in Figure 1 illustrates the measurements required when calculating the resolution value - specifically peak width and retention time. As is evident in Figure 1, baseline resolution of peaks 1 and 2 will not be attained under normal testing conditions. The minimum acceptable resolution value will be described in a future revision of AOCS Method Ce 1h-05. For now, laboratories must establish their own criteria.

Figure 2. Resolution Equation

$$R=1.18\,(t_{R2}-t_{R1})/\,(w_{(1/2)1}+w_{(1/2)2})$$

t_{R1} = the retention time of peak 1

t_{R2} = the retention time of peak 2

$w_{(1/2)1}$ = peak width at half height of peak 1 in mm

$w_{(1/2)2}$ = peak width at half height of peak 2 in mm

For example:

$$R=1.18(23.053-22.922)/(0.0846+0.0912)$$

$$R=0.88$$

At present, no column will fully resolve all C18:1 cis/trans positional isomers. By utilizing the cis/trans FAME Column Performance Mix, analysts can optimize column performance before assaying complex mixtures. Regular use also makes it possible to monitor column degradation and to recognize when to replace the capillary column.

References

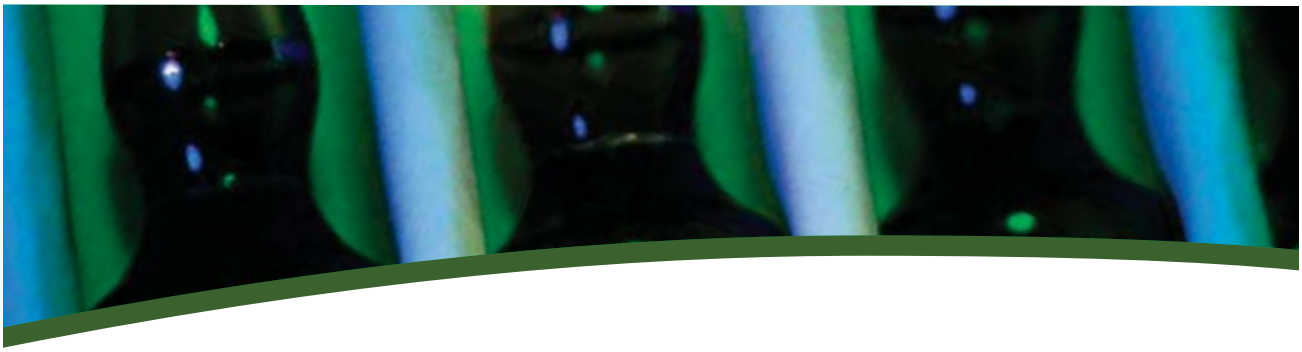
1. AOCS Official Method Ce 1h-05. Determination of cis-, trans-, Saturated Monosaturated and Polysaturated Fatty Acids in vegetable or Non-ruminant Animal Oils and fats by Capillary GLC, 2005.
2. AOAC Official Method Ce 996.06. Fat (Total, Saturated, and Unsaturated) in Food, Hydrolytic Extraction Gas Chromatographic Method, 2001.

Featured Products

Description	Cat. No.
cis/trans FAME Column Performance Mix, 2.5 mg/mL	
1 x 1mL	40495-U
10 x 1mL	4M0495-U

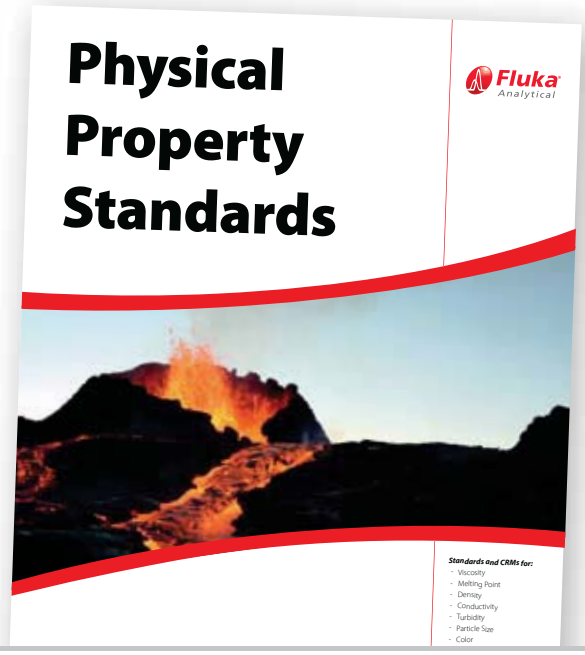
Related Products

Description	Cat. No.
SP-2560 Column	
100 m x 0.25 mm I.D., 0.20 µm	24056
75 m x 0.18 mm I.D., 0.14 µm	23348-U



NEW! Analytical Standards Resources

Certified reference materials for AAS, IC & ICP applications (code MBZ)



Comprehensive offering of calibration standards for monitoring physical values of chemicals (code LPD)

Request Your Copies Today!

Use the attached card or visit ***sigma-aldrich.com/standards-literature***

Molded Thermogreen LB-2 Septa Durability

Robert F. Wallace and Gregory A. Baney
bob.wallace@sial.com

P001208

Introduction

The injection port septa selected for gas chromatography (GC) use should exhibit low bleed, resist leaks, and be easy to penetrate. Molded Thermogreen LB-2 septa have all these characteristics. Using the same exclusive rubber formulation as the traditional Thermogreen LB-2, molded Thermogreen LB-2 septa allow for stricter tolerances of both diameter and thickness, resulting in much easier installation and better sealing. A previous article discussed the low bleed characteristics of molded Thermogreen LB-2 septa (1). This article will focus on their resistance to leaks.

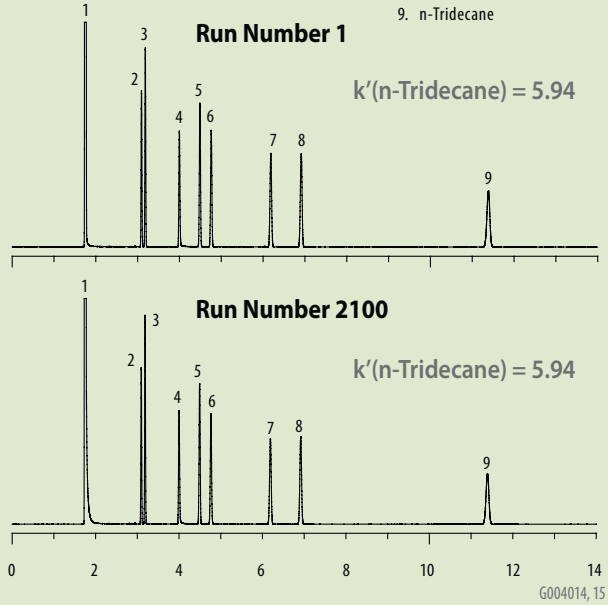
Durability Requirements

A GC septum should resist fragmentation. This is because septum fragments in the inlet liner bleed (observed as extraneous peaks) and adsorb active sample components (causing loss of analyte response). Molded Thermogreen LB-2 septa are specially engineered to offer excellent resistance to fragmentation. With its high elasticity, molded Thermogreen LB-2 septa ensure that multiple injections can be obtained with a wide range of needle compatibility. In fact, the entire Thermogreen LB-2 family is widely accepted as the industry standard in GC septa because of their great durability.

Figure 1. Molded Thermogreen LB-2 Septum Durability Test

column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven: 115 °C (15 min.)
inj.: 250 °C
det.: FID, 360 °C
carrier gas: helium, 30 cm/sec. @ 115 °C
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split, cup design, unpacked
sample: Nonpolar Column Test Mix (47300-U), each analyte at 500 µg/mL in methylene chloride

1. Methylene chloride (solvent)
2. 2-Octanone
3. n-Decane
4. 1-Octanol
5. n-Undecane
6. 2,6-Dimethylphenol
7. 2,6-Dimethylaniline
8. n-Dodecane
9. n-Tridecane



G004014, 15

Puncturability Test

As a measure of durability, a molded Thermogreen LB-2 septum with injection hole was subjected to repeated injections. Column head pressure and carrier gas linear velocity/flow rate were monitored throughout the test to determine if the septum developed a leak. An autosampler was used for the test, ensuring that the syringe needle (tapered 23s-26s gauge) punctured the same location repeatedly. Figure 1 shows chromatograms from the initial run and after 2100 injections. As demonstrated, no artifacts were present in either the initial or final runs to indicate any problems with the chromatography. Additionally, retention, head pressure, and column flow remained constant, indicating that a leak-free seal was maintained.

Conclusion

The quality traits to look for in a GC septum are low bleed, resistance to leaks, and easy penetration. The strict tolerances of the mold used to manufacture molded Thermogreen LB-2 septa result in septa that are easier to install and consistently fit better. These septa exhibit an ultra low bleed profile, are temperature stable to 350 °C, are very resistant to both slivering and coring, and have a high puncture tolerance.

Reference

1. M.D. Buchanan, *"NEW! Molded Thermogreen LB-2 Septa"* The Reporter 25.3 (2007) p. 15.

Featured Products

Diam. (mm)	Type	Qty.	Cat. No.
Molded Thermogreen LB-2 Septa			
9.5 (3/8 in.)	with injection hole	50	**28331-U**
9.5 (3/8 in.)	with injection hole	250	**28332-U**
9.5 (3/8 in.)	solid disc	50	**28670-U**
9.5 (3/8 in.)	solid disc	250	**28671-U**
10 (13/32 in.)	with injection hole	50	**28333-U**
10 (13/32 in.)	with injection hole	250	**28334-U**
10 (13/32 in.)	solid disc	50	**28673-U**
10 (13/32 in.)	solid disc	250	**28675-U**
11 (7/16 in.)	with injection hole	50	**28336-U**
11 (7/16 in.)	with injection hole	250	**28338-U**
11 (7/16 in.)	solid disc	50	**28676-U**
11 (7/16 in.)	solid disc	250	**28678-U**

Related Products

Description	Cat. No.
Hook septum puller	**20352**
Screw septum puller	**20353**

SGE Introduces Color-Coded Syringes

E001098, E001099, E001100

SGE has implemented significant changes to their chromatographic syringe line to make it easier to distinguish between the different volumes of syringes while experiencing improved technical performance.

The glass barrels of SGE autosampler syringes are now color-coded according to volume to make it easier and faster for an analyst to select the proper volume of syringe for different applications. For example, the 50 µL syringe has a purple barrel while the 10 µL syringe can be distinguished by its orange barrel. This color-coding also makes it easier for quick volume identification of syringes already installed in an autosampler.

The new color-coded syringes are now packaged in a clear 100% recyclable protective inner sleeve with a fully recyclable outer box with a window for easy visual identification of the product when stored in a drawer.

E001101

Newly introduced tighter physical specifications and design changes have greatly reduced or eliminated common problems including syringe adhesive contamination of the sample, sample carryover, and mechanical problems with the needle, plunger and barrel. These improvements provide superior technical performance for the analysts and have the added benefit of extending the life of the syringe.

Longer Life

Modifications to the inner surface finish of the syringe glass barrel and the design of the syringe components provide greater solvent resistance, a wider working-temperature range and improved operational smoothness. As a result of these modifications, the lifetime of the syringe can be as much as ten times greater when compared to SGE's current syringes.

Superior Performance

Elimination of syringe adhesive from the fluid path removes the possibility of the sample being contaminated by adhesive, sample carryover, or the possibility of the sample matrix dissolving the adhesive that holds syringe components together. This is accomplished by the addition of a press fit PTFE seal that fits tightly against the inner walls of the syringe and is flush to the end of the needle leaving no gaps and preventing contact of the sample with the syringe adhesive.

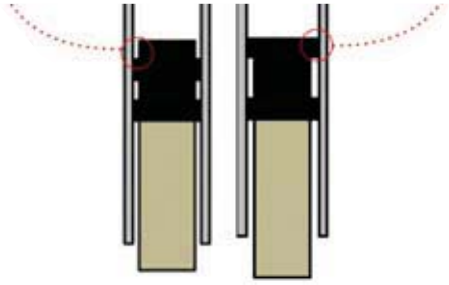
Additionally, the use of a new adhesive chemistry in the manufacturing of SGE syringes allows the syringe to be operated over a wider temperature range.

Improvements have also been made to the needle design. The needle and hub are permanently fused together, increasing the strength of the parts.

Reduced Carryover

Design improvements in the PTFE plunger tips and the PTFE seal inserts mentioned previously create a much tighter fit between the plunger tip of the barrel and the PTFE seal. A smoother glass surface ensures that the fluid is flushed out and no carryover occurs.

Superior Plunger Tip



Other tip designs make it impossible to completely expel sample.

SGE 2010 plunger tip sits flush against syringe insert, minimizing any potential carryover.

SGE has made significant changes to improve their syringe line for 2010. The changes in both packaging and syringe design make these products better with extended life, easier identification, and overall better performance for syringe customers.

For more information on any of our syringes, email our Technical Service Department at *techservice@sial.com* or visit us on the web at ***sigma-aldrich.com/syringes***.

The Utility of Headspace Grade Solvents for the Analysis of Organic Volatile Impurities

Katherine Stenerson and Shyam Verma
katherine.stenerson@sial.com

Introduction

Organic volatile impurities or "OVIs" sometimes remain behind in pharmaceutical preparations as a result of synthetic and manufacturing procedures. For reasons of health and safety, testing is done to ensure that these solvents are not above concentration limits listed by the United States Pharmacopeia (USP) and in the International Conference on Harmonization (ICH) guidelines (1,2). Static headspace GC (SH-GC) is a commonly used technique in the analysis of these OVIs. This technique concentrates volatile analytes, and allows for their analysis free from sample matrix.

Samples to be analyzed by SH-GC must be dissolved in a suitable solvent; and in addition to being able to dissolve the sample, the solvent chosen must allow for sufficient sensitivity of the analytes of interest in the headspace. Partition coefficient affect the ability of an analyte to enter into the headspace from the liquid sample, and those with lower values will have higher vapor pressure and thus greater sensitivity in the headspace (3). Thus, for low-level detection, the analytes of interest must have low partition coefficients in the dissolution solvent chosen. In addition, the vapor pressure of the dissolution solvent itself should be sufficiently low so it will not affect detection of OVI analytes by "flooding" the headspace.

Water offers a very low partition coefficient for analytes and has a low vapor pressure, but cannot be used in all cases. USP <467> and European Pharmacopeia (EP) methodologies list procedures for both water soluble and water-insoluble samples. For water-insoluble samples, USP <467> designates the use of the solvents dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). Other dissolution solvents that have been found to be useful for headspace analysis of water-insoluble samples include dimethylacetamide (DMAC) and 1,3-dimethyl-2-imidazolidinone (DMI), and the later is described for use in EP Method 2.4.24 (4). These solvents elute later than most OVI analytes in chromatographic analyses, and have significantly lower vapor pressure than many other high boiling-point organic compounds.

The purity of dissolution solvents is essential to avoid extraneous peaks in the chromatographic analysis, and prevent interference with the analytes of interest. Many protocols followed by laboratories doing OVI analysis require the analysis of an acceptable blank, and some published methodologies, such as EP Method 2.4.24, require the analysis of a blank to verify the absence of interfering peaks. Fluka brand high purity headspace grade solvents are now available which are specially tested to ensure suitability for SH-GC analysis.

Experimental

In this evaluation, the utility of Fluka brand DMSO headspace grade solvent was evaluated for use in the SH-GC analysis of OVIs. The purity of the headspace solvent was evaluated by preparing sample blanks using headspace and alternative grades of DMSO. The retention times of peaks present in the blanks were then compared to an OVI standard prepared in headspace grade DMSO solvent.

The blanks were prepared by pipetting 1 mL of DMSO into a 10 mL headspace vial, and subjecting the sealed vial to SH-GC analysis. The OVI working standard included a variety of common process solvents, representing various classes as described in USP <467> and ICH guidelines. It was prepared from a stock solution in DMAC by dilution with headspace grade DMSO. The composition and final concentration of the OVI working standard is summarized in Table 1. The working standard was prepared for SH-GC analysis in a similar manner

Table 1. Composition of OVI Standard + Peak IDs for Figure 3

Peak #	Compound	Class	Concentration (µg/mL)	Cat. No.
1	Methanol	2	237	82762
2	Ethanol	3	395	–
3	Acetone	3	235	90872
4	Isopropanol	3	390	–
5	Acetonitrile	2	39	45983
6	Methylene chloride	2	66	02575
7	tert-Butanol	NC	237	50621
8	Methyl-tert-butyl ether	3	221	08603
9	n-Hexane	2	33	52750
10	n-Propanol	3	240	96566
11	Methyl ethyl ketone	3	240	02469
12	Ethyl acetate	3	271	58958
13	Tetrahydrofuran	2	88	78445
14	Cyclohexane	2	232	–
15	Isobutanol	3	241	82059
16	Isopropyl acetate	3	262	90871
17	2-Methyl-tetrahydrofuran	NC	86	85488
18	n-Heptane	3	68	51730
19	1-Methoxy-2-propanol	NC	192	65692
20	1,4-Dioxane	2	52	76887
21	4-Methyl-2-pentanone	3	239	02474
22	Toluene	2	86	89680
23	Diisopropyl ethylamine	NC	94	–
24	Dimethylformamide	2	74	72438
25	m-Xylene	2	86	95670
26	o-Xylene	2	88	95660
27	Tetramethyl urea	NC	387	–
28	N-Methylpyrrolidone	2	614	78769

NC = solvent not classified

to the blanks. Both blanks and the standard were analyzed by SH-GC using the parameters listed in Table 2.

Results

Chromatograms of the SH-GC blanks using headspace and organic synthesis grades of DMSO are presented in Figures 1 and 2. Overall, the headspace grade blank had fewer peaks in the OVI elution range than the organic synthesis grade blank. Comparing the blanks with the chromatogram of the working OVI standard prepared in headspace-grade DMSO (Figure 3), both blanks were found to contain some DMF. The organic synthesis blank contained a peak eluting close to the retention time of ethanol. This peak could potentially interfere with the proper detection and analysis of ethanol as an OVI. A peak corresponding to the retention time of DMI was detected in the SH-GC blank. This same peak was also detected in the OVI working standard, which was prepared in headspace grade DMSO.

Table 2. SH-GC Parameters

Headspace Parameters		
Temperatures:	**Times:**	**Pressures:**
Oven: 100 °C	Vial Equilibration: 10 min.	Vial: 15 psi
Loop: 110 °C	Pressurization: 0.2 min.	Transfer Line: 25 psi
Transfer Line: 150 °C	Loop Fill: 0.2 min.	Loop Volume: 1.0 mL
	Loop Equilibration: 0.05 min.	Inject Time: 1 min.

GC Parameters	
column:	SPB™-624, 30 m x 0.32 mm I.D., 1.8 µm (28464-U)
oven:	40 °C (4 min.), 8 °C/min. to 60 °C, 5 °C/min. to 85 °C (2 min.), 30 °C/min. to 220 °C (2 min.)
inj.:	225 °C
det.:	FID, 270 °C (FID)
carrier gas:	Helium, 1.5 mL/min, constant
injection:	1.0 mL, 5:1 split
liner:	2 mm I.D.

Figure 1. SH-GC Blank, DMSO – Organic Synthesis Grade

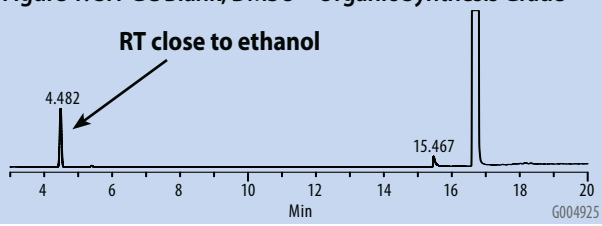
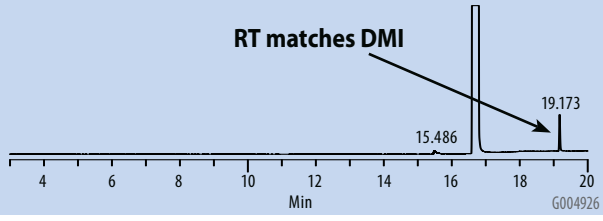


Figure 2. SH-GC Blank, DMSO – Fluka Headspace Grade



Conclusions

The Fluka headspace grade produced a "cleaner" blank for SH-GC analysis than a lesser grade of DMSO. The peaks that did appear in the headspace grade solvent blank eluted outside of the retention range of the OVIs analyzed. Additional headspace grade solvents (DMF, DMAC, and DMI) suitable for this application are also available. Experimental data on these solvents, similar to that presented here for DMSO, can be obtained by requesting Supelco Publication T409180 (only available electronically).

Acknowledgements

We would like to thank Amanda Quiroga and Michael Dong at Genentech USA, for providing the data for this evaluation, and for their invaluable input.

References

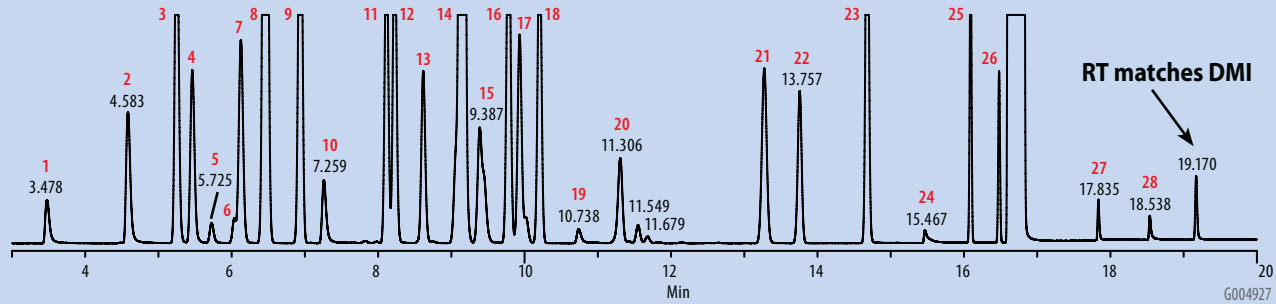
1. United States Pharmacopoeia (USP), 31st Edition (2008), <467> Residual Solvents.
2. ICH Guidelines for Industry, Q3C Impurities: Residual Solvents, US Dept. of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), ICH December, 1997.
3. Y. Sitaramaraju, A. van Hul, K. Wolfs, A. Van Schepdael, J. Hoogmartens, E. Adams, Static headspace gas chromatography of (semi-)volatile drugs in pharmaceuticals for topical use. J. of Pharmaceutical and Biomedical Analysis. 47: 834-840, 2008.
4. European Pharmacopoeia (EP) 5.0, Vol.1, (2004), 2.4.24 Identification and Control of Residual Solvents.
5. A. Quiroga, M. Dong, K. Stenerson, S. Verma, The Utility of Headspace Grade Solvents in the Analysis of Organic Volatile Impurities. Supelco Publication T409180, November 2009.

Featured Products

Description	Boiling Point	Pkg. Size	Cat. No.
N,N-Dimethylacetamide	166 °C	1 L	**44901**
Dimethyl sulfoxide	189 °C	1 L	**51779**
N,N-Dimethylformamide	153 °C	1 L	**51781**
1,3-Dimethyl-2-imidazolidinone	225 °C	1 L	**67484**
Water	100 °C	1 L	**53463**

All products are puriss. p.a.

Figure 3. SH-GC Analysis of OVI Working Standard Prepared in Fluka Headspace Grade DMSO



Sigma-Aldrich® Worldwide Offices

Argentina
Free Tel: 0810 888 7446
Tel: (+54) 11 4556 1472
Fax: (+54) 11 4552 1698

Australia
Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria
Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium
Free Tel: 0800 14747
Free Fax: 0800 14745
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil
Free Tel: 0800 701 7425
Tel: (+55) 11 3732 3100
Fax: (+55) 11 5522 9895

Canada
Free Tel: 1800 565 1400
Free Fax: 1800 265 3858
Tel: (+1) 905 829 9500
Fax: (+1) 905 829 9292

Chile
Tel: (+56) 2 495 7395
Fax: (+56) 2 495 7396

China
Free Tel: 800 819 3336
Tel: (+86) 21 6141 5566
Fax: (+86) 21 6141 5567

Czech Republic
Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark
Tel: (+45) 43 56 59 00
Fax: (+45) 43 56 59 05

Finland
Tel: (+358) 9 350 9250
Fax: (+358) 9 350 92555

France
Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 88
Fax: (+33) 474 95 68 08

Germany
Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1160

Hungary
Ingyenes telefonszám: 06 80 355 355
Ingyenes fax szám: 06 80 344 344
Tel: (+36) 1 235 9063
Fax: (+36) 1 269 6470

India
Telephone
Bangalore: (+91) 80 6621 9400
New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 2570 2364
Hyderabad: (+91) 40 4015 5488
Kolkata: (+91) 33 4013 8003
Fax
Bangalore: (+91) 80 6621 9650
New Delhi: (+91) 11 4358 8001
Mumbai: (+91) 22 4087 2364
Hyderabad: (+91) 40 4015 5488
Kolkata: (+91) 33 4013 8000

Ireland
Free Tel: 1800 200 888
Free Fax: 1800 600 222
Tel: (+353) 402 20370
Fax: (+ 353) 402 20375

Israel
Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4100
Fax: (+972) 8 948 4200

Italy
Tel: (+39) 02 3341 7310
Fax: (+39) 02 3801 0737

Japan
Tel: (+81) 3 5796 7300
Fax: (+81) 3 5796 7315

Korea
Free Tel: (+82) 80 023 7111
Free Fax: (+82) 80 023 8111
Tel: (+82) 31 329 9000
Fax: (+82) 31 329 9090

Malaysia
Tel: (+60) 3 5635 3321
Fax: (+60) 3 5635 4116

Mexico
Free Tel: 01 800 007 5300
Free Fax: 01 800 712 9920
Tel: (+52) 722 276 1600
Fax: (+52) 722 276 1601

The Netherlands
Free Tel: 0800 022 9088
Free Fax: 0800 022 9089
Tel: (+31) 78 620 5411
Fax: (+31) 78 620 5421

New Zealand
Free Tel: 0800 936 666
Free Fax: 0800 937 777
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Norway
Tel: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10

Poland
Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal
Free Tel: 800 202 180
Free Fax: 800 202 178
Tel: (+351) 21 924 2555
Fax: (+351) 21 924 2610

Russia
Tel: (+7) 495 621 5828
Fax: (+7) 495 621 6037

Singapore
Tel: (+65) 6779 1200
Fax: (+65) 6779 1822

Slovakia
Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

South Africa
Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain
Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden
Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland
Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2828
Fax: (+41) 81 755 2815

United Kingdom
Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 1747 833 000
Fax: (+44) 1747 833 313

United States
Toll-Free: 800 325 3010
Toll-Free Fax: 800 325 5052
Tel: (+1) 314 771 5765
Fax: (+1) 314 771 5757

Vietnam
Tel: (+84) 3516 2810
Fax: (+84) 6258 4238

Internet
sigma-aldrich.com

Accelerating Customers' Success through Innovation and Leadership in ***Life Science, High Technology*** *and* ***Service***

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
Technical Service (800) 325-5832 • *sigma-aldrich.com/techservice*
Development/Custom Manufacturing Inquiries SAFC® (800) 244-1173
Safety-related Information *sigma-aldrich.com/safetycenter*

World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

MEU
T210001