

Reporter

Volume 47, August 2011, International



Fast and Accurate LC-MS Analysis of 25-Hydroxyvitamin D



Vitamin D is produced in the skin after exposure to sunlight. It improves our quality of life by promoting proper bone growth in children and preventing osteoporosis in adults.

HPLC/LC	
Fast and Accurate Analysis of Vitamin D Metabolites	3
LC-MS Analysis of Benzalkonium Chloride using HILIC	5
GC	
Analysis of Aromatic and Aliphatic Analytes in Gasoline on High Polarity SLB-IL111	7
GC Injection Port Issues: Two Case Studies	10
Sample Preparation	
Introducing Supel™-Inert Gas Sampling Bags with Thermogreen™ LB-2 Septa	12
Bioanalysis with SPME	14
Reagents	
High-Purity Solvents for Sensitive Analysis	16
Monthly Savings Programme	
30% on LC-MS Solvents	17
Chiral Chromatography	
Innovations in Chiral Chromatography	18
Standards	
NEW! USP Residual Solvent Standards	21
High-Purity PESTANAL® Standards	21
Accessories	
eVo® Hand-Held Automated Analytical Syringe	22

Multimedia Communication

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



Wayne Way
Market Segment Manager
HPLC/GC

Dear Colleague,

Communicating with customers is the most important aspect of a marketing programme. Effective communication provides a clear understanding of our products and services, and allows our customers to make informed decisions on how to choose and use them.

There are four common ways to deliver this communication: written, oral, physical and multimedia. Some examples include:

Written – Posters, Brochures, Email, Newsletters, Website

Oral – Telephone, Seminars, Tradeshows

Physical – Packaging, Products, Promotional Items

Multimedia – Webcasts (live and recorded), Podcasts, Videos

In today's busy world, multimedia presentations offer many advantages that are not possible with the other, more traditional methods. Multimedia methods allow easier and faster retention of information and an on-demand aspect, so that information is available when you need it. One doesn't need to look any further than YouTube™ to see the explosion and popularity of on-demand videos.

Our analytical team is working hard to provide more multimedia content than ever before. This content includes live and pre-recorded webcasts available on our BrightTALK™ channel – sigma-aldrich.com/brighttalk. Many of these presentations feature our innovative products such as Ascentis® Express Fused-Core® HPLC products, but please visit the site to see our entire offering.

We also have short analytical application presentations throughout our website – sigma-aldrich.com/videos. This part of our website is dedicated to providing you details on our HPLC products, SPME, HYDRANAL® and Flash product lines. We are always adding more videos and content based on your feedback. Take a moment and view any of these topics and let us know what else you would like to see added in the future.

In particular, I wanted to introduce **Nick the Hero**, a "customer of ours", who finds out how to boost HPLC productivity by searching the Sigma-Aldrich website and learning about Ascentis Express Fused-Core HPLC Columns. You can see Nick's story at sigma-aldrich.com/nick

In conclusion, we hope our multimedia programme provides clear communication that allows you to make an informed decision on your analytical and chromatography needs, and have some fun while doing it!

Kind regards,

Wayne K. Way

Market Segment Manager, HPLC/GC
wayne.way@sial.com

Reporter is published five times a year by Sigma-Aldrich
MarCom Europe, Industriestrasse 25, CH-9471 Buchs SG, Switzerland
Publisher: Sigma-Aldrich Marketing Communications Europe
Publication Director: Ingo Haag, PhD
Editor: Daniel Vogler

Fast and Accurate Analysis of Vitamin D Metabolites Using Ascentis® Express F5 HPLC Columns

Craig Aurand, David Bell, Anders Fridstrom

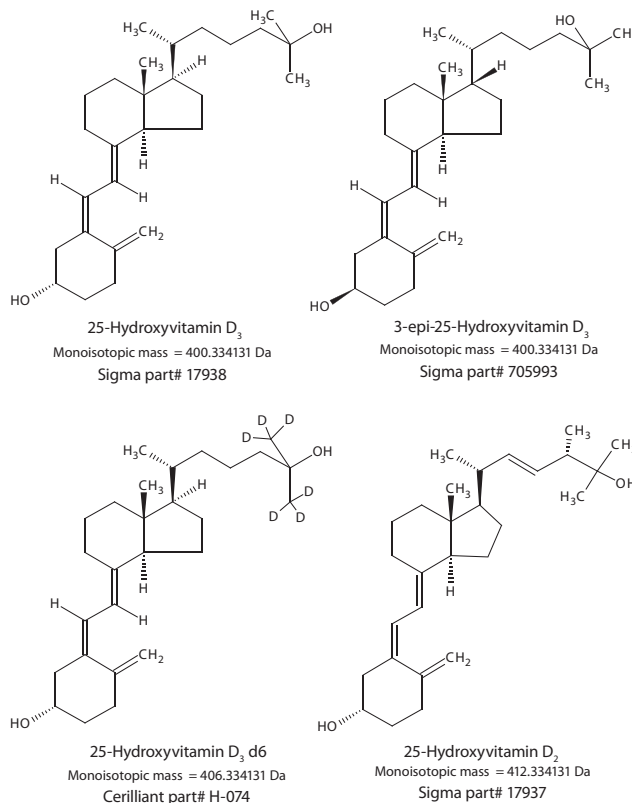
Vitamin D deficiency is still a topic of high interest 350 years after it was described in the literature.(1, 2) Vitamin D, along with calcium, promotes proper bone growth in children and aids in the prevention of osteoporosis in older adults. Vitamin D is present in two forms, Vitamin D₃ and Vitamin D₂. Vitamin D₃ is produced in the skin after exposure to ultraviolet light-stimulated conversion of 7-dehydrocholesterol in the skin. Vitamin D₂ is derived from plant sources. Both D₂ and D₃ vitamins are metabolised in the liver to form 25-Hydroxyvitamin D₂ (25-OH D₂) and 25-Hydroxyvitamin D₃ (25-OH D₃), respectively. In addition, biologically inactive 3-epi analogues of 25-OH D₂ and 25-OH D₃ have been reported, especially in young children.(3) The levels of the 25-Hydroxy metabolites are routinely measured for diagnostic assessment of vitamin D related diseases; however, recent studies have indicated that separation from the inactive 3-epi analogues may provide more accurate information for treatment and prevention. Analytical methods that can accurately quantitate both of the 25-Hydroxyvitamin D analytes in the presence of 3-epi analogues may become essential for diagnosis and monitoring of patients with vitamin D disorders.

HPLC analysis of 25-OH D₂ and 25-OH D₃ is classically performed using C18 stationary phases. On this phase, the 3-epi analogues are not resolved and thus are included in the overall reported value. Recently, Phinney, et al., reported the use of a cyano column for the effective separation of the 25-OH from the 3-epi forms for use in reference measurement procedures.(4) Although effective, the conditions necessitate a run time of better than 40 minutes, limiting its utility for routine high throughput analyses. During recent application development studies, it was observed that a pentafluorophenyl (PFP, Ascentis Express F5) stationary phase provided increased selectivity towards 25-OH D₃ and the corresponding 3-epi analogue relative to reported methods.

Method:

In this study, the analysis of 25-OH D₂, 25-OH D₃ and epi-25-OH D₃ is demonstrated on both C18 and pentafluorophenyl stationary phases. The structures of the vitamin D analytes are shown in **Figure 1**. Rat serum was spiked with 25-OH D₃, 25-OH D₂, epi-25-OH along with 25-OH D₃ d6 internal standard, 300 ng each. Protein precipitation was performed offline by adding 100 µL of spiked serum into a 500 µL 96-well collection plate, followed by 300 µL of 1% formic acid acetonitrile. Samples were mixed by performing five 300 µL draw/aspiration cycles with a pipette, and incubated five minutes before transferring 80 µL of supernatant into the HybridSPE®-Phospholipid Small Volume 96-well plate (52794-U). Samples were passed through by applying 10" Hg vacuum for three minutes; the resulting filtrate was then analysed directly.

Figure 1. Vitamin D metabolite structures



Results:

Figure 2 demonstrates the coelution of the 25-OH D₃ and epi-25-OH D₃ on a reversed phase C18 phase. Because 25-OH D₃ and epi-25-OH D₃ are isobaric, this limits the reporting of 25-OH D₃, inflated due to presence of epi-25-OH D₃. **Figure 3** demonstrates that Ascentis Express F5 phase is effective for the resolution of 25-OH D₃, 25-OH D₂ and epi-25-OH D₃ in less than four minutes, enabling quantitation of all three components in one analysis. The unique selectivity of the Ascentis Express F5 allows for efficient isocratic separation without the need for time-consuming gradient elution. In **Table 1** the wrong positive result for 25-OH Vit D₃ on C18 columns due to overlapping peaks with the biological inactive epi-25-OH D₃ is obvious. Additionally, the high recovery rates for all compounds show the selectivity and efficiency of sample preparation by HybridSPE-Phospholipid.

(continued on page 4)

Summary:

Separation of the biologically inactive 3-epi analogue serves to provide improved data in support of vitamin D related clinical diagnostics and treatment. The pentafluorophenyl stationary phase has been shown to provide superior selectivity for the separation of the closely related 25-OH D₃ and 3-epi-25-OH D₃ compared with methods reported in the literature. The unique selectivity of the Ascentis® Express F5, combined with the selective phospholipid depletion of the HybridSPE®-Phospholipids 96-well plate, enable a fast and efficient method for the analysis of 25-Hydroxyvitamin D and related forms from serum samples. This approach demonstrates how selectivity, both chromatographic and sample preparation, allows for efficient analysis that would otherwise be unattainable with traditional reversed phase approaches.

Table 1. Comparison of 25-OH Vit D₃, epi-25-OH Vit D₃ and 25-OH Vit D₂ concentrations for samples spiked to a level of 300 ng for each of the analytes. Analysed with Ascentis Express C18 and Ascentis Express F5.

Analytical method	25-OH Vit D ₃ Results (ng/mL)	epi-25-OH Vit D ₃ Results (ng/mL)	25-OH Vit D ₂ Results (ng/mL)
Ascentis Express C18	745	---	165
Ascentis Express F5	325	332	240

References

- Whistler, Lugduni Batavorum, D. Morbo puerili Anglorum, quem patrio idiomate indigenae vocant The Rickets, 1–13 (1645).
- Higashi, T., Homma, S., Iwata, H., Shimada, K. Journal of Pharmaceutical and Biomedical Analysis 2002, 29, 947–955.
- Higashi, T., Shimada, K., Toyooka, T. Journal of Chromatography B 2010, 878, 1654–1661.
- Tai, S. S.-C., Bedner, M., Phinney, K. W. Analytical Chemistry 2010, 82, 1942–1948.

Figure 2. Analysis of 25-Hydroxyvitamin D₃ and Hydroxyvitamin D₂ on Ascentis Express C18

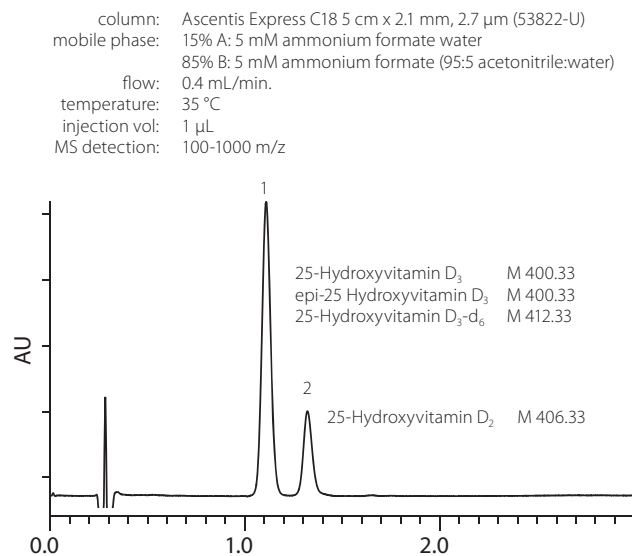
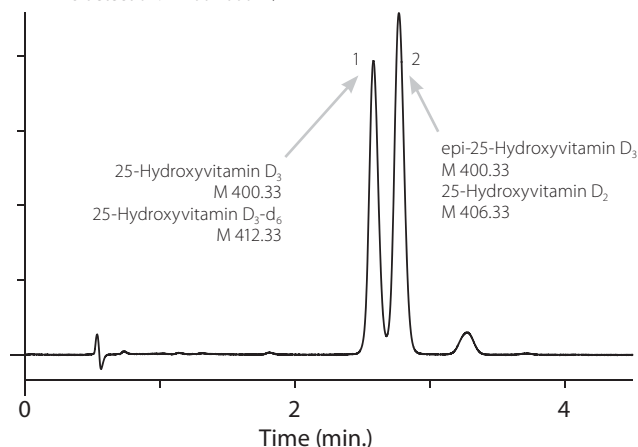


Figure 3. Analysis of 25-Hydroxyvitamin D₃ and epi-25-Hydroxyvitamin D₃ on Ascentis Express F5

column: Ascentis Express F5 10 cm x 2.1 mm, 2.7 μm (53569-U)
 mobile phase: 25% A: 5 mM ammonium formate water
 75% B: 5 mM ammonium formate methanol
 flow: 0.4 mL/min.
 temperature: 40 °C
 injection vol: 1 μL
 UV detection: 265 nm
 MS detection: 100-1000 m/z



+ Featured Products

Ascentis Express C18 and F5 Analytical Columns

Description	Cat. no.
Ascentis Express C18 5 cm x 2.1 mm, 2.7 μm	53822-U
Ascentis Express F5 10 cm x 2.1 mm, 2.7 μm	53569-U

Ascentis Express F5 Guard Holder and Cartridges

Description	Qty.	Cat. no.
Universal Guard Cartridge Holder	1	53500-U
Guard Cartridge, 2.1 x 5 mm	3	53594-U
Guard Cartridge, 3.0 x 5 mm	3	53597-U
Guard Cartridge, 4.6 x 5 mm	3	53599-U

Standards

Description	Qty.	Cat. no.
25-Hydroxyvitamin D ₃	1 mg	17938
3-epi-25-Hydroxyvitamin D ₃	1 mg	705993
25-Hydroxyvitamin D ₃ -d ₆	50 μg/ml	H-074 (Ceriliant)
25-Hydroxyvitamin D ₂	1 mg	17937

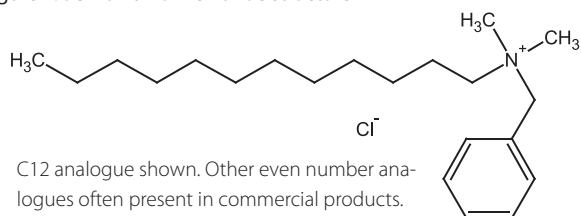
LC-MS Analysis of Benzalkonium Chloride using Hydrophilic Interaction Chromatography (HILIC)

David S. Bell and Jennifer Claus
wayne.way@sial.com

Introduction

Benzalkonium chloride (BAK, BAC), also known as alkyldimethylbenzylammonium chloride (ADBAC), is a mixture of alkylbenzyl-dimethylammonium chlorides of various even-numbered alkyl chain lengths (Figure 1). This product is a nitrogenous cationic surface-acting agent belonging to the quaternary ammonium group. It has three main categories of use: as a biocide, a cationic surfactant and phase transfer agent in the chemical industry.(1)

Figure 1. Benzalkonium chloride structure



The applications are extremely wide, ranging from disinfectant formulations to microbial corrosion inhibition in the oilfield sector and a multi-surface mould, algae and moss remover. It is used in:

- Pharmaceuticals such as leave-on skin antiseptics
- Antiseptic to safely treat childhood scrapes and cuts
- Advanced, next-generation hand sanitisers
- Hygienic towelettes and wet wipes
- Cosmetics such as eye and nasal drops, as a preservative
- Cleaners for floor and hard surfaces, as a disinfectant
- High-level surgical instrument sterilising and disinfection solutions
- Air and surface sprayable disinfectants
- Over-the-counter herpes cold sore and fever blister single-application treatments (1)

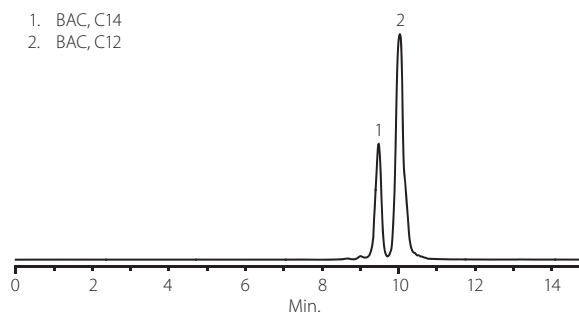
Objectives

Due to the highly diverse uses of benzalkonium chloride (BAC), objective of the study was to develop an LC-MS compatible set of conditions to analyse BAC in various pharmaceutical formulations and household products. MS detection would also allow for an additional separation mode in more complex matrices as well as provide supportive identification information. A literature search revealed

Figure 2. Separation of benzalkonium chloride standard using Ascentis® Express HILIC

XIC (m/z 304, 332 and 360) of 50% BAC in water (diluted 1000x with methanol). Conditions as per Experimental Section, 2 mM ammonium acetate in 90% acetonitrile.

1. BAC, C14
2. BAC, C12



several methods for the analysis of BAC; however, all those found utilised non-volatile buffers and/or strong ion-suppressing mobile phase modifiers.(2–5) Initial attempts to produce quality chromatography through simple alteration of the literature methods utilising MS-compatible mobile phases were unsuccessful.

In an attempt to take advantage of ion-exchange potential and the polarity of the target compounds, hydrophilic interaction chromatography (HILIC) conditions were studied. The resulting conditions were then applied to several potential separation challenges.

Experimental

instrument: Waters 2690/Waters Micromass ZQ, single quadrupole MS
 column: Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 μm (53981-U)
 mobile phase: 2, 5 or 10 mM ammonium acetate in 10:90 water:acetonitrile
 temperature: 35 °C
 flow rate: 1 mL/min.
 backpressure: ~1200 psi
 detection: ESI(+), scan range m/z 150-500, uv at 263 nm
 injection: 2 μL
 sample: various

Results and Discussion

Initial method development commenced using a commercially available BAC material that consists mainly of the C₁₂ and C₁₄ analogues, mass/charge (m/z) of 304 and 332, respectively. The elution order of the analogues is the opposite of that found using reversed-phase systems owing to the different interactions in the HILIC mode (Figure 2).

(continued on page 6)

For the analysis of BAC in ophthalmic formulations, the method is dependent on selective separation from other active and inactive components that may be present. To examine this, several anti-glaucoma pharmaceuticals ranging in pK_a and solubility values were run in the presence of BAC (Table 1). The impact of buffer concentration (2, 5 and 10 mM) was explored in each case.

Table 1. Physical properties and structures of representative antiglaucoma pharmaceuticals*

Compound	pK_a (MA)	pK_a (MB)	LogD(7.4)	LogP	MW
Latanoprost	14.84	N/A	4.28	4.28	432.5
Epinastine	N/A	11.98	1.54	3.51	249.3
Betaxolol	13.89	9.43	0.43	2.53	307.4
Epinephrine	9.6	9.16	-2.37	-0.54	183.2
Pilocarpine	N/A	7.02	-0.39	-0.24	208.2

* ACD/Labs PhysChem Database, v. 12

MA = most acidic MB = most basic

Epinephrine, a polar, strong base, and Pilocarpine, a polar, weak base, were shown to separate well from the BAC responses and were relatively insensitive to buffer concentration. Epinastine, a non-polar, strong base, and the moderately polar strong base, Betaxolol, show excellent selectivity from BAC, but retention is highly dependent on buffer concentration. It is apparent that the polar analytes retain primarily by a HILIC partitioning mechanism and that the retention of the non-polar bases is dominated by ionic interactions. Retention of the BAC components is easily manipulated using buffer concentration, increasing buffer concentration resulting in earlier elution. Latanoprost, a non-polar, non-ionic analyte, is unretained under the present conditions. Representative chromatograms from the study are presented in Figures 3–4.

Conclusions

HILIC has been shown to provide LC-MS compatible means to retain and separate components of widely used benzalkonium chloride. BAC is separated from some common pharmaceutical compounds that may be components of ophthalmic formulations. For polar strong bases, polar weak bases, moderately polar bases and non-polar bases, the system may be suitable for simultaneous analysis of the active components as well as BAC in the same run. Retention of both the BAC components and the more polar bases is easily manipulated using buffer concentration, making the conditions potentially applicable to a wide range of applications. Non-polar, non-basic compounds of interest are unretained; however, selectivity from BAC components remains.

References

1. Wikipedia, Benzalkonium chloride.
2. Dudkiewicz-Wilczynska, J., Tautt, J., Roman, I. Journal of Pharmaceutical and Biomedical Analysis 2004, 34, 909–920.
3. Gomez-Gomar, A., Gonzalez-Aubert, M. M., Garcés-Torrents, J., Costa-Segarra, J. Journal of Pharmaceutical and Biomedical Analysis Papers from the Second International Symposium on Pharmaceutical and Biomedical Analysis, April 1990, 8, 871–876.
4. Labranche, L.-P., Dumont, S. N., Levesque, S., Carrier, A. Journal of Pharmaceutical and Biomedical Analysis 2007, 43, 989–993.
5. Prince, S. J., McLaury, H.-J., Allen, L. V., McLaury, P. Journal of Pharmaceutical and Biomedical Analysis 1999, 19, 877–882.

Figure 3. Separation of epinephrine and epinastine from benzalkonium chlorides using Ascentis® Express HILIC

XIC (m/z 304, 332, 360, 180: epinephrine and 250: epinastine). Conditions as per Experimental Section, 5 mM ammonium acetate in 90% acetonitrile.

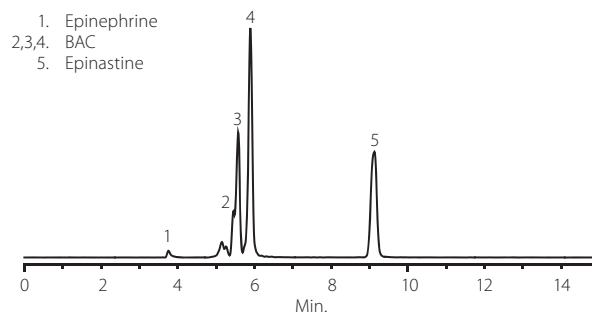
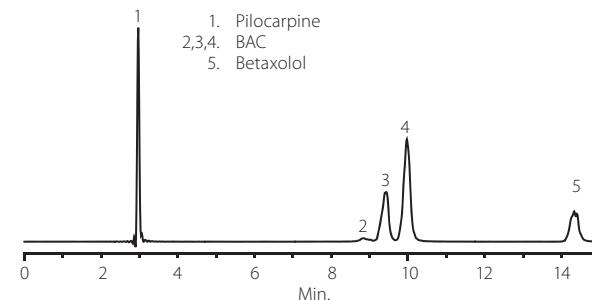


Figure 4. Separation of pilocarpine and betaxolol from benzalkonium chlorides using Ascentis Express HILIC

XIC (m/z 304, 332, 360, 308: betaxolol and 209: pilocarpine). Conditions as per Experimental Section, 2 mM ammonium acetate in 90% acetonitrile.



+ Featured Products

Ascentis Express HILIC and Guard Cartridges

I.D. (mm)	Length (cm)	Cat. no.
2.1	5	53934-U
2.1	10	53939-U
2.1	15	53946-U
4.6	5	53975-U
4.6	10	53979-U
4.6	15	53981-U

Description	I.D. (mm)	Pack size	Cat. no.
HILIC	2.1	3	53520-U
HILIC	3.0	3	53521-U
HILIC	4.6	3	53523-U

Description	I.D. (mm)	Pack size	Cat. no.
Guard cartridge holder	Fits all	1	53500-U

Analysis of Aromatic and Aliphatic Analytes in Gasoline on the Extremely Polar SLB™-IL111

Katherine K. Stenerson
katherine.stenerson@sial.com

Gas chromatography is commonly employed for the analysis of gasoline. The challenge with the analysis lies in the complex composition of gasoline, which consists of hundreds of different compounds that include aliphatic, aromatic, and oxygenated constituents. To resolve benzene (and other aromatics) from the aliphatic portion of gasoline, a highly efficient column with a very polar phase is required.

The amount of benzene in gasoline is of concern because it is a known human carcinogen, and exposure to it has been linked to leukaemia.(1) On 1 January, 2011, a new rule limit was instituted by the US EPA requiring the benzene content of gasoline to be <0.62%,(2) a decrease from previous regulation, which allowed for a maximum benzene content of 1%.

Reformulated Gasoline and Ethanol

Reformulated gasoline contains additives to produce more complete combustion and results in lower emissions of harmful compounds. These additives are compounds that boost the oxygen content of the gasoline and are commonly referred to as "oxygenates". Methyl tertiary-butyl ether (MTBE) was a popular oxygenate for a number of years, but is no longer widely used. Ethanol has replaced the use of MTBE in many cases, and is now the most common oxygenate used in gasoline. Presently, 77% of US gasoline contains ethanol.(3) The level of ethanol used in reformulated gas varies, but can be as high as 10%. From an analytical standpoint, the presence of ethanol presents a problem in the detection of benzene.

Current Methodology

As stated previously, a very polar stationary phase must be used to resolve benzene and other aromatics from the aliphatic compounds in gasoline. The stationary phase traditionally used for this analysis is 1,2,3-tris(2-cyanoethoxy)propane, also known as TCEP. This phase is highly polar and can separate aliphatics and aromatics, plus provide some resolution of ethanol and benzene. However, the low maximum temperature of the phase (145 °C) precludes it from eluting the heavier constituents of gasoline in a timely fashion. For this reason, it is used in combination with a non-polar polydimethylsiloxane column using a switching valve. The sample enters the non-polar column first, where compounds retain, based on their boiling points. After the elution of n-octane, flow through this column is reversed and constituents heavier than n-octane are back-flushed out of the system. The components eluting prior to n-octane pass into the TCEP column, which separates the aromatics from the aliphatics. However, if ethanol is present in the gasoline sample, it can interfere with the detection of benzene.

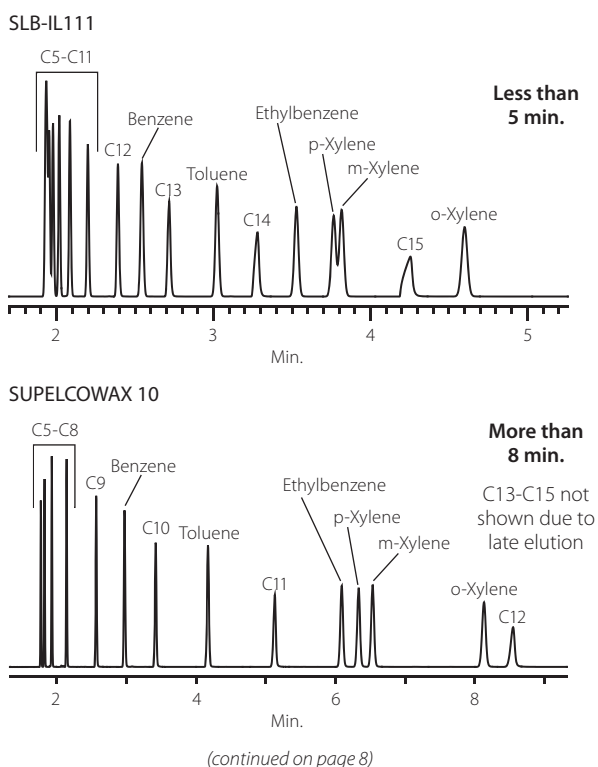
The SLB-IL111

The SLB-IL111 is an ionic liquid column that is extremely polar, and has demonstrated selectivity indicating it to be even more polar than TCEP. It is inherently more stable than TCEP, such that it can be used to a substantially higher temperature (270 °C). The extreme polarity of this phase, in combination with the high maximum temperature, makes the SLB-IL111 useful in the analysis of benzene in gasoline.

Figure 1 illustrates the selectivity of this phase in the separation of several aromatics found in gasoline (including benzene), and their elution in relation to the range of alkanes common to gasoline. The extreme polarity of this phase results in low retention of the alkanes, as evidenced by the elution of benzene between C12 and C13. (By contrast, on a non-polar polydimethylsiloxane column, benzene elutes between C6 and C7.) The alkanes most prevalent in gasoline, C5-C12, elute prior to benzene. Toluene, which is another analyte of interest in gasoline, also elutes after the C5-C12 hydrocarbon range.

Figure 1. C5-C15 Hydrocarbons and BTEX compounds on the SLB-IL111 and SUPELCOWAX 10

column 1: SLB-IL111, 30 m x 0.25 mm I.D., 0.20 µm (28927-U)
column 2: SUPELCOWAX 10, 30 m x 0.25 mm I.D., 0.25 µm (24079)
oven: 65 °C
inj.: 250 °C
det.: FID, 265 °C
carrier gas: helium, 30 cm/sec
injection: wet needle, 200:1 split
liner: 4 mm I.D. FocusLiner™ inlet liner with taper
sample: neat mixture of C5-C15 hydrocarbons + BTEX, equal volumes



The poor peak shape of the C15 alkane is due to the low solubility of this long hydrocarbon chain in the SLB™-IL111 phase. Peak shape can be improved if a higher isothermal oven temperature or temperature programme is used. However, when making temperature adjustments, it should be noted that highly polar and extremely polar phases, such as the SLB-IL111, can show changes in elution patterns at different temperatures. For example, at the temperature chosen for this analysis (65 °C), toluene elutes between C13 and C14. At an analysis temperature of 110 °C, it will co-elute with C14 on the SLB-IL111.

For comparison to the SLB-IL111, the same mixture was analysed on a traditional polar column with a similar maximum temperature, the SUPELCOWAX™ 10 (Figure 1). On this less polar phase, benzene elutes between C9 and C10, and the elution range of the C5-C12 hydrocarbons overlaps with benzene and toluene. Retention of the C13-C15 hydrocarbons was extremely long under the conditions used, with C15 eluting after >50 minutes.

Figure 2. Reformulated gasoline on the SLB-IL111

column: SLB-IL111, 30 x 0.25 mm I.D., 0.20 µm (28927-U)
 oven: 50 °C (3 min.), 15 °C/min. to 260 °C (5 min.)
 inj.: 250 °C
 det.: FID, 265 °C
 carrier gas: helium, 30 cm/sec constant
 injection: 0.5 µL, 100:1 split
 liner: 4 mm I.D. FocusLiner inlet liner with taper
 sample: reformulated gasoline

- | | |
|---------------------|--------------------------------|
| 1. C5-C11 n-alkanes | 8. o-Xylene |
| 2. Ethanol | 9. 1,2,4-Trimethylbenzene |
| 3. Benzene | 10. 1,2,3-Trimethylbenzene |
| 4. Toluene | 11. 1,2,4,5-Tetramethylbenzene |
| 5. Ethylbenzene | 12. Naphthalene |
| 6. p-Xylene | 13. 2-Methylnaphthalene |
| 7. m-Xylene | 14. 1-Methylnaphthalene |

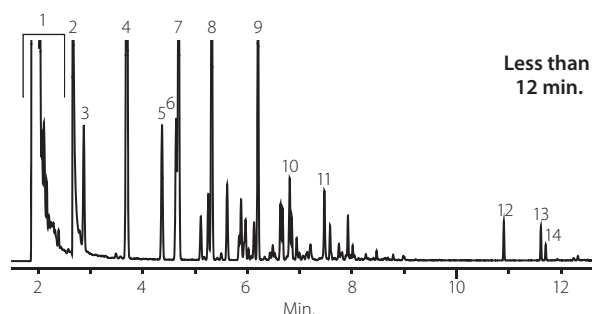
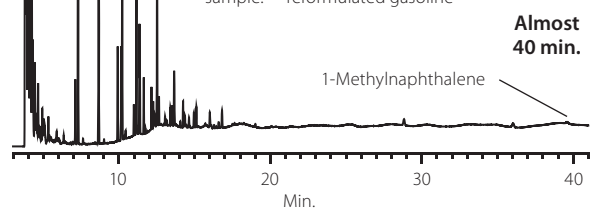


Figure 3. Reformulated gasoline on TCEP

column: TCEP, 60 x 0.25 mm I.D., 0.44 µm (24153)
 oven: 50 °C (3 min.), 10 °C/min. to 140 °C (35 min.)
 inj.: 220 °C
 det.: FID, 170 °C
 carrier gas: helium, 30 cm/sec constant
 injection: 1 µL, 100:1 split
 liner: 4 mm I.D. FocusLiner inlet liner with taper
 sample: reformulated gasoline



Reformulated Gasoline on the SLB-IL111

The GC analysis of a sample of reformulated gasoline on the SLB-IL111 is presented in Figure 2. Even with a 30 m column length, the extreme polarity of the phase was able to provide some resolution of benzene and ethanol, and elute the aliphatic portion of the gasoline prior to the aromatic portion. A starting oven temperature of 50 °C provided the best resolution of benzene, and the high maximum temperature of the phase allowed a temperature program to 260 °C to be used to elute the naphthalenes in <12 minutes. By comparison, on a capillary column version of the TCEP (Figure 3), similar resolutions were achieved, but an unstable baseline was observed during the temperature program portion of the run. The low maximum temperature of the TCEP resulted in an analysis time of almost 40 minutes to elute the naphthalenes.

Conclusion

The SLB-IL111 phase has the selectivity necessary for the analysis of benzene in reformulated gasoline. Specifically, it will elute C5-C12 aliphatics prior to benzene, and will provide some resolution of benzene and ethanol. The phase stability of the SLB-IL111 gives it

+ Related Products

Description	Cat. no.
SLB-IL111, 15 m x 0.10 mm I.D., 0.08 µm	28925-U
SLB-IL111, 100 m x 0.25 mm I.D., 0.20 µm	29647-U

Standards

Description	Cat. no.	Brand	Pack size
Pentane	76870	Fluka®	10 mL
Hexane	52750	Fluka	10 mL
Heptane	51730	Fluka	5 mL
Octane	74820	Fluka	5 mL
Nonane	74250	Fluka	50 mL
Decane	30540	Fluka	5 mL
Undecane	94000	Fluka	5 mL
Dodecane	44010	Fluka	5 mL
Tridecane	91490	Fluka	5 mL
Tetradecane	87139	Fluka	5 mL
Pentadecane	76509	Fluka	5 mL
Pentane	76870	Fluka	10 mL
Benzene	12540	Fluka	5 mL
Toluene	89680	Fluka	5 mL
Ethylbenzene	03079	Fluka	5 mL
m-Xylene	95670	Fluka	5 mL
o-Xylene	95660	Fluka	5 mL
p-Xylene	95680	Fluka	5 mL
1,2,4-Trimethylbenzene	45996	Fluka	250 mg
1,2,3-Trimethylbenzene	45935	Fluka	250 mg
1,2,4,5-Tetramethylbenzene	74658	Fluka	5 g
Naphthalene	91489	Fluka	100 mg
1-Methylnaphthalene	45795	Fluka	250 mg
2-Methylnaphthalene	45796	Fluka	250 mg

a distinct advantage over the TCEP phase in that it exhibits a stable baseline when subjected to a temperature ramp, and can be used up to 270 °C, allowing the timely elution of the heavy constituents in gasoline. This makes the SLB-IL111 a candidate for the use of a single capillary column for this application and the possibility of eliminating the need for a two-column back-flush system. In addition to the analysis of benzene in gasoline, the extreme polarity of this column makes it a candidate for related applications such as measuring aromatic impurities in toluene and mineral spirits.

References

1. Benzene; Material Safety Data Sheet, Sigma-Aldrich, ver. 4.1, revised 1/6/2011.
2. Hogue, Cheryl. "Less Benzene in Gasoline, EPA Rule Aims to Cut Toxic Emissions from Cars", Chem. Eng. News 2007, Feb. 19, p. 8.
3. Weaver, James W., Exum, Linda R., Prieto, Lourdes M. "Gasoline Composition Regulations Affecting LUST Sites", EPA/600/R-10/002, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency Office of Research and Development, Washington, DC 20460, January 2010.

+ Featured Products

Description	Cat. no.
SLB™-IL111, 30 m x 0.25 mm I.D., 0.20 µm	28927-U
SUPELCOWAX™ 10, 30 m x 0.25 mm I.D., 0.25 µm	24079
TCEP, 60 m x 0.25 mm I.D., 0.44 µm	24153

SPECIAL INTRODUCTORY OFFER:

40% off SLB-IL111 columns

Use **Promo Code 962** when placing your order.

Offer expires 30 September, 2011.



Maximise Your GC Performance!

Keep your GC running at its optimum by performing preventative maintenance on a **regular schedule**. Our Maximize Performance! Gas Chromatography Accessories and Gas Purification/Management Products brochure (T407103 JWE) is a "must-have" for all GC labs! It includes all the common replacement items, such as septa, liners, ferrules, solvents, syringes, vials, purifiers, and much more.

Request your copy on the attached card, by email (EurTechServ@sial.com) or download at sigma-aldrich.com/gc

SIGMA-ALDRICH®

GC Injection Port Issues: Two Case Studies

Robert F. Wallace
bob.wallace@sial.com

Introduction

For the vast majority of uses, samples pass through an injection port at the start of the gas chromatography (GC) process. Therefore, it is very important to ensure that the proper injection port items are selected based on the application to be performed. Once the proper items are selected, a simple, routine preventative maintenance programme will help prevent simple problems from turning into major problems. This article will highlight two recent calls to our Technical Service Chemists concerning GC performance problems, and the solutions that resolved them.

Case Study 1 – Peak Tailing

An analyst at a large environmental laboratory contacted our Technical Service group. She was running routine assays on two GCs, and noticed the chromatograms generated by one of the GCs contained tailing peaks (**Figure 1**). Additionally, analyte response seemed low. Believing the column had deteriorated, she enquired about the proper replacement column. To determine if the problem could be something other than the column, the Technical Service Chemist began to ask questions concerning the type of routine maintenance performed in the customer's lab. It was discovered that the analyst was relatively new to her position and had not performed any maintenance on the GC inlet system. The Technical Service Chemist then explained that peak tailing and low response could be symptoms of a dirty inlet liner. At this point our chemist instructed the analyst to do an inspection of the inlet liner. Upon inspection, the analyst found the inlet liner had a brown coating with what appeared to be small fragments on the inside. It was determined that the small fragments were bits of cored injection port septa, and that the brown coating was non-volatile residue that had accumulated over time. Both these phenomena create adsorption sites that interact with the sample as it passes through the inlet liner.

Figure 1. Poor chromatography before liner & septum are changed

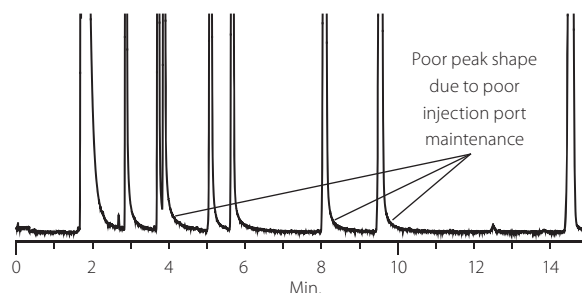
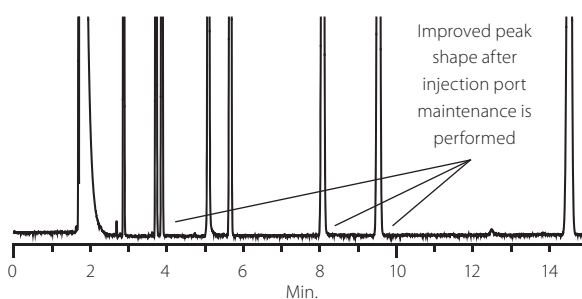


Figure 2. Normal chromatography after liner & septum are changed



Case Study 1 Solution

The Technical Service Chemist suggested that the current septum and inlet liner should be changed. A moulded Thermogreen™ LB-2 septum was recommended due to its bleed-temperature-puncturability-optimised nature. Once injection port maintenance was completed, peak shape and overall chromatography improved (**Figure 2**). The analyst also learned that changing the septum daily, especially if the instrument is in heavy use, will save costly downtime, rework and inaccurate results.

Injection Port Septa Use, Maintenance, Storage and Handling

A GC septum is located at the top of the injection port and serves two functions: 1) providing a leak-free seal to maintain carrier gas pressure inside the system, and 2) handling repeated puncturing by a syringe needle for sample introduction purposes without severe coring or leaking.

Temperature Programming: When performing temperature-programmed analyses, you may observe ghost peaks or a baseline rise not traceable to the sample or to column bleed. These disturbances are often caused by septum bleed. Volatile materials from the

septum can accumulate at the head of the column during the cool-down portion of the programme. When the column is heated for the next sample, these accumulated volatiles are eluted, producing peaks, a general baseline rise, or both.

Routine maintenance: To reduce the risk of leaks and contamination, injection port septa should routinely be replaced. Change the septum daily, especially if the instrument is in heavy use. Repeated use of the same septum may result in increased coring, resulting in a leak. Septum fragments in the inlet liner can

also lead to ghost peaks and/or loss of response due to adsorption of analytes as they pass through.

Storage and handling: Septa can become contaminated by volatile compounds in the room air, or by finger oils. To ensure cleanliness, it is recommended that septa be stored in their shipping container with the lid securely closed, and that clean forceps be used for handling the septa during installation.

Inlet liners: choosing the best for your injection method

An injection port liner is used to make the connection between sample introduction and the GC column. Four primary injection techniques are used in GC; split, splitless, direct, and on-column. Inlet liners should be selected based on the injection technique being used to ensure optimal sample transfer to the column.

Split injection: Wide-bore 2 or 4 mm I.D. inlet liners are necessary for solvent expansion. Cups, baffles or twists are often used to

facilitate sample mixing. Wool may be used to improve vaporisation, and/or to keep non-volatile material from entering the column.

Splitless injection: Similar to split inlet liners but without cups, baffles or twists. Tapers (either at the bottom or at both the top and bottom) may be incorporated to help focus analytes onto the column. Wool may be used to improve vaporisation, and/or to keep non-volatile material from entering the column.

Direct injection: Often used for gas phase samples, such as with purge-and-trap and solid phase microextraction (SPME) techniques. Narrow-bore 0.75 or 1 mm I.D. inlet liners are necessary to maintain a high linear velocity through the injection port, minimising band broadening. All of the sample is transferred to the column. Also known as flash vaporisation.

Figure 3. Customer's first chromatogram – low response

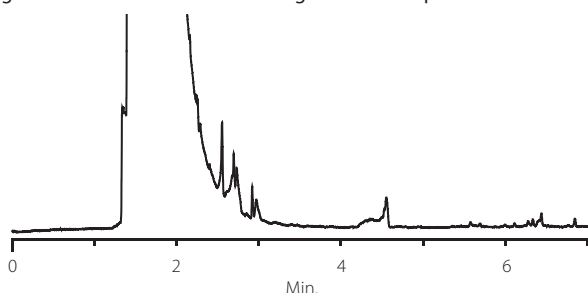
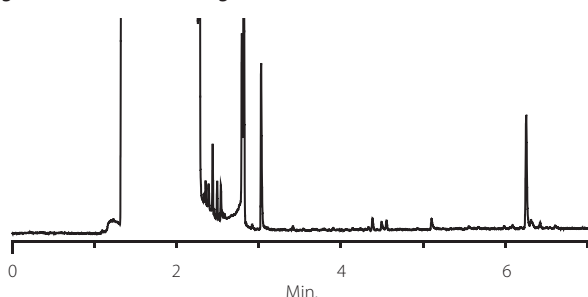


Figure 4. Result of installing the correct liner



Case Study 2 – Low Response

A customer was working with a new method for volatile compounds. After installing a column, he proceeded to set up a splitless injection method and obtained the results shown in **Figure 3**. He had a skewed solvent peak and lower analyte responses than shown in the sample chromatogram of the method. The customer confirmed he was using the correct solvent, initial temperature, hold time and split vent time, as indicated by the method. A call was placed to Supelco® Technical Service. They questioned if the customer had changed the inlet liner and cleaned out the injection port. The customer acknowledged he had changed the inlet liner but had not cleaned out the injection

port. The Supelco Technical Service Chemist asked the customer to do so, suspecting that the inlet liner may not have been sealing correctly. This suggestion helped but did not solve the problem. After further questioning, it was discovered that the customer was using a split rather than a splitless inlet liner. This was the major cause of the solvent tailing. The split inlet liner did not allow efficient transfer of the sample onto the column. Plus, some of the sample was lost when the split vent opened.

Case Study 2 Solution

For trace analysis that includes volatile components, it is recommended to use a < 2 mm I.D. inlet liner. The reduced volume of this diameter increases the linear velocity of the carrier gas through the liner. This produces a more rapid introduction of analytes onto the column in a narrow band. The improved focusing provides a better response, especially for lighter analytes. Following a suggestion to install a splitless liner, the customer obtained the chromatogram as shown in **Figure 4**.

Conclusion

The importance of proper product selection and preventative maintenance for the GC inlet are vital to the chromatographic process. A proactive approach and system awareness will reduce the risk of problems, saving both time and money.

+ Related Information

For more information on Supelco inlet liners or to locate catalogue numbers, request "Capillary GC Inlet Liner Selection Guide" (T196899, BBB) or visit sigma-aldrich.com/inletliners

For more information on Supelco GC septa or to locate catalogue numbers, request "Molded Thermogreen LB-2 Septa" (T407082, JQV) or visit sigma-aldrich.com/moldedsepta

Introducing Supel™-Inert Gas Sampling Bags with Thermogreen™ LB-2 Septa

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



Model 1062

Introduction

Gas sampling bags are used for whole air sampling and are recognised as economical alternatives to canisters for sampling VOCs and other gases. Until recently, Tedlar® was the most recognised film used to manufacture sampling bags. Recently, DuPont informed its customers that they would no longer be supplying Tedlar film to the gas sampling bag market. We

have been successful in sourcing a suitable replacement and are pleased to offer our new Supel-Inert film, a proprietary fluoropolymer developed specifically for air sampling applications. The Supel-Inert Gas Sampling bags are available with two valve options incorporating our exclusive Thermogreen LB-2 septa. The Thermogreen LB-2 polymer has the industry's lowest bleed, preventing sample contamination from the septum. **Table 1** provides a comparison of Supel-Inert Film to Tedlar.

Table 1. Physical properties of Supel-Inert film compared to Tedlar

	Supel-Inert	Tedlar
thickness	3 mil (76.2 µm)	2 mil (50.8 µm)
tensile strength	6100 psi (42 Mpa)	8000 psi (55 Mpa)
max. operating temp.	150 °C (302 °F)	204 °C (400 °F)
specific gravity	1.78	1.70
oxygen permeability	58 mL/(m ² x d)	50 mL/(m ² x d)
water vapour permeability	12–15 g/(m ² x d)	9–57 g/(m ² x d)
carbon dioxide permeability	172 mL/(m ² x d)	172 mL/(m ² x d)

Performance

Several factors are important for selecting a gas sampling bag for an application. The most important is preservation of the sample. *Sample loss* (leaks in the bags) is the single most problematic issue with other replacement films available in the market, followed by contaminant background levels, and stability of the compounds of interest. In this issue a background level comparison will be discussed between Supel-Inert film compared to Tedlar and a competitor's film. The next issue of *The Reporter* will provide details regarding compound stability and storage.

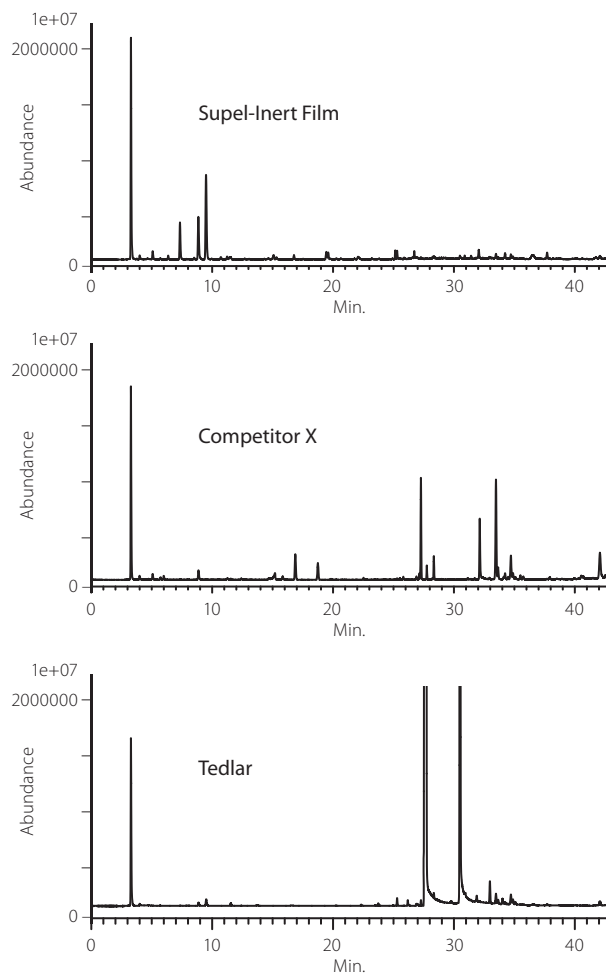
It is well-known that Tedlar film contains the contaminants dimethylacetamide (DMAC) and phenol as part of the film composition. Supel-Inert film does not contain these compounds. Typically, gas sampling bags are used to sample atmospheres in the ppmv range. The Supel-Inert bags can also be used to sample in the ppbv range,

but may require flushing of the bag with clean nitrogen or air to further reduce background levels to meet ppbv analysis requirements.

The following analysis demonstrates that our Supel-Inert film contains the lowest background compared to the competitor's proprietary film and Tedlar.

Figure 1 illustrates the background levels from five-litre gas sampling bags. The bags were filled with clean nitrogen and stored at ambient temperatures for 24 hours; one litre was extracted from each bag and concentrated on a multi-bed thermal desorption tube (Carbotrap™-300). The two large peaks from the Tedlar bags are DMAC and phenol.

Figure 1. Comparison of background levels on Supel-Inert film, competitor X alternative film, and Tedlar



Key Features and Benefits:

- Low VOC and sulphur background levels; no detectable background levels of DMAC (Dimethylacetamide) or phenol, common with Tedlar® bags
- Inertness properties similar to Tedlar for a wide range of compounds.
 - Suitable for sampling and analysis for most VOCs within two days and many sulphur compounds for up to 24 hours
 - Chemically inert to most acids, aliphatic and aromatic organic compounds, chlorinated solvents and alcohols
- Abrasion resistant
- Hermetically heat-sealed bags being leak free
- Two valve fitting options with Thermogreen™ LB-2 septa available; these high-quality valves provide leak free performance
- 5 sizes available: 1 L, 2 L, 5 L, 10 L, and 25 L
- More economical packaging and easier access to bags compared to the competition

Gas Sampling Bag Valves



Push-Lock Valve (PLV)

Supelco® now offers two types of sampling valves: Push-Lock Valve (PLV or 2-n-1) and Screw Cap Valve (SCV). The body of both valves is composed of inert polypropylene; our Thermogreen LB-2 septum is incorporated into each valve type. The original valve, the Push-Lock Valve (PLV) is designed with a septum sandwiched between the bag's film and the valve body, so the sample in the bag is not exposed to the septum until it

is punctured to remove the sample. This valve design will not dead-head the sampling pump, preventing an immediate inrush of air when the valve is opened. When the valve is in the closed position, sample flow travels down the stem of the valve and exits a small hole above the o-ring seal of the valve. When the stem is pushed in to open the valve, the flow is directed into the bag. This valve is an excellent choice when exact flow rates are required. The Push-Lock Valve is securely fastened in the centre of the bag, and the stem of the valve is perpendicular to the bag's surface.



Screw-Cap Valve (SCV)

The new valve is a screw cap design. The valve is opened and closed by only turning the cap (not the body) a half turn. When the valve is closed, the Thermogreen LB-2 septum makes the seal to maintain the integrity of the sample. This screw-cap valve is securely fastened in the upper two-thirds of the bag, and the stem of the valve is parallel to the bag. It is an

excellent choice when the application requires a replacement of the septum.

Method Suitability

Like Tedlar, Supel™-Inert gas sampling bags are suitable for use for the following methods and applications. To draw a sample with a syringe or to prepare a calibration mix, Supel-Inert is the most suitable option for this purpose, owing to its low background film and low-bleed Thermogreen LB-2 septa.

Method /Application	Compounds
EPA 18	Gaseous Organic Compounds; VOCs by GC
EPA 0040	Volatile Organic Compounds (VOCs)
EPA TO-3	Volatile Organic Compounds (VOCs)
EPA TO-12	Non-Methane Organic Compounds (NMOC)
EPA TO-14A/TO-15 mod.*	Volatile Organic Compounds (VOCs) by GC/MS
NIOSH 3704	Perchloroethylene (Tetrachloroethylene)
Vapour intrusion	DCE, TCE, 1,1,1-TCA, PCE, Benzene, Toluene
Calibration Mixes	Preparation of Gas Phase Standards/ Gas Mixtures

* Supel-Inert film is not recommended for storing hydrogen sulphide

Summary

Supel-Inert Gas Sampling Bags are an ideal replacement for Tedlar film owing to low background contaminant levels, no sample loss, and our low-bleed Thermogreen LB-2 septa. The Screw-Cap Valve (SCV) and Push-Lock Valve (PLV) are easy to operate during sampling and analysis. An additional benefit is the inventory-friendly product packaging.

+ Related Products & Information

For filling gas sampling bags without risk of sample contamination, use dedicated bag sampler (see picture of Model 1062 sampler on previous page).

Visit us at sigma-aldrich.com/air_monitoring to learn more.

+ Featured Products

Supel-Inert Gas Sampling Bags with Thermogreen LB-2 Septa

Capacity	Dimensions	Pk	Push Lock Valve (PLV)	Screw Cap Valve (SCV)
1 L	7 x 7 in. (17.8 x 17.8 cm)	10	30213-U	30221-U
2 L	9 x 9 in. (22.9 x 22.9 cm)	10	30214-U	30222-U
5 L	12 x 12 in. (30.5 x 30.5 cm)	10	30215-U	30223-U
10 L	12 x 19 in. (30.5 x 48.3 cm)	10	30216-U	30224-U
25 L	18 x 24 in. (45.7 x 61 cm)	5	30217-U	30225-U

Bioanalysis with SPME

Contributed Article

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

H. Lord, E. Cudjoe, D. Vuckovic, P. Togunde, F.M. Musteata, S.N. Zhou, X. Zhang, Md E. Hoque, J. Pawliszyn

University of Waterloo, Waterloo, ON, Canada

Solid phase microextraction (SPME) offers rapid sample preparation both in the laboratory and field.⁽¹⁾ The basic concept of the technology is a sorbent-coated rod that is put into contact with a sample (gaseous, liquid, semi-solid) or the headspace of liquids or solids. The sorbent is selected to have good affinity for the analyte of interest in the sample. After a pre-defined exposure time, sufficient analyte will have moved from the sample to the sorbent to permit quantitative analysis. The amount extracted is proportional to the original concentration of analyte in the sample, permitting simple determination of sample concentration.

Figure 1 illustrates the basic concept of the commercial SPME device introduced by Supelco® in 1993 (1) that has seen wide application in a variety of fields.

A new line of SPME devices has been recently introduced to better address bioanalytical sampling (**Figure 2**). These devices employ C18 bonded porous silica sorbent particles, similar to particles typically used in HPLC columns or as SPE sorbents, in a proprietary biocompatible binder. The binder used is a non-swelling polymer which resists fouling by biological matrix components. After extraction, solvent desorption is performed in a small volume (50-100 µL) and the desorption solution directly injected, typically to LC or LC-MS. The solid support used for these probes is a flexible metal alloy (0.008"/203 µm diameter), offering both robustness and an inert support, and the coatings are

Figure 1. Design and enlarged view of the first commercial SPME device made by Supelco

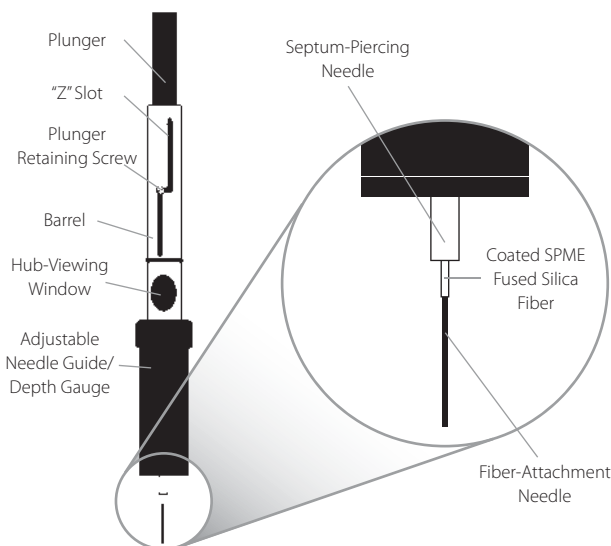
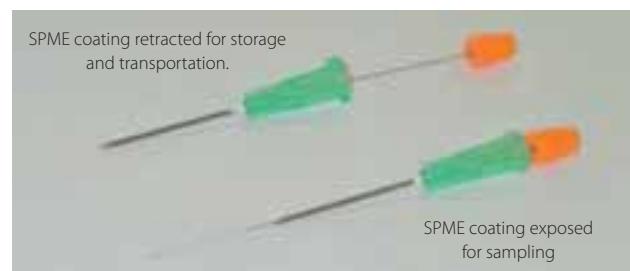


Figure 2. New biocompatible SPME devices for bioanalysis and *in vivo* sampling (45 µm thickness, 15 mm length of the coating, Cat. No. 57281-U)



housed inside a 22-gauge hypodermic needle with an incorporated seal to prevent sample wicking. Because of the C18 extraction phase, they behave as an absorptive phase. They may be employed as single-use devices and are ideally suited for either *in vitro* sampling directly from whole blood or plasma in sample vials sealed with hole caps and septa, or through an injection bulb on an intravenous catheter for *in vivo* analysis. Devices without the attached hypodermic needle are also available where sealing is not critical, e.g. tissue sampling or sampling from open vials. The operating principles are analogous to conventional SPME devices.

An important advantage of SPME, particularly for on-site sampling, is the possibility of performing analyses without pre-defining a specific sample size. For SPME from small volumes of sample, the amount of an analyte extracted from a sample is given by Equation 1.⁽¹⁾

$$n = \frac{C_0 K_{fs} V_s V_f}{K_{fs} V_f + V_s} \quad (1)$$

where C_0 is the initial sample concentration of the analyte, n is the amount of analyte extracted, V_s is the sample volume, V_f is the fibre volume and K_{fs} is the analyte distribution constant between the fibre and sample matrix. However, when sample size is large relative to the fibre capacity ($V_s \gg V_f K_{fs}$), Equation 1 reduces to Equation 2, which renders the amount of analyte extracted by SPME independent of the sample volume.

$$n = C_0 K_{fs} V_f \quad (2)$$

This simplification is valid for most on-site analyses and eliminates the need to remove a representative sample from the system under study in order to perform the analysis. From a bioanalytical perspective, this permits the use of SPME to directly sample blood or tissue of animals *in vivo*, without having to first withdraw a biofluid/tissue sample. New calibration approaches, including internal standardisation by pre-loading standards on the SPME device, allow rapid pre-equilibrium sampling and control of variability in complex matrixes.

Initially, *in vivo* SPME was used to study the pharmacokinetics (PK) of various drugs directly from the veins of animals. A specialised interface was developed to permit monitoring of small rodents (mice and rats). More recently, *in vivo* SPME was successfully applied to fish to study bioaccumulation of pharmaceuticals, pesticides and other environmental pollutants, using direct muscle or adipose tissue sampling. A survey of a number of additional sorbents has broadened the range of analyte polarities that can be extracted to include highly polar compounds (logP to -8) and has permitted the application of both *in vivo* and *in vitro* SPME for non-targeted metabolomics analysis.(2)

The use of *in vivo* SPME offers important advantages over conventional methods, such as simplified sample cleanup, fast stabilisation of unstable analytes, elimination of enzymatic degradation after extraction, and reduced ion suppression for mass spectrometry analyses. Furthermore, because both sampling and sample cleanup are combined into one

step, the number of sample preparation steps is minimised, reducing the potential for analyte loss or accidental contamination. A recent article in *Nature Protocols* details the steps involved in performing *in vivo* SPME for intravenous drug and metabolite monitoring.(3)

References

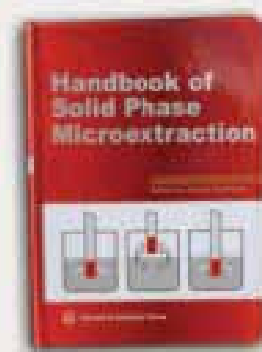
1. Pawliszyn, J. *Handbook of SPME*, Chemical Industry Press, Beijing, 2009.
2. Vuckovic, D., Pawliszyn, J. *Anal. Chem.* 2011, 83, 1944–54.
3. Lord, H.L., Zhang, X., Musteata, F. M., Vuckovic, D. Pawliszyn, J. *Nature Protocols*, in press.

+ Featured Products

Description	Cat. no.
C18 SPME-LC Fibre Probes, pk. of 5	57281-U

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 optimisation including calibration are clearly discussed to assist new users of the technology. The handbook contains 13 chapters with topics including: theory of SPME, SPME devices and fibre coatings, automated SPME systems, calibration of the extraction step, SPME method development, ligand-receptor binding, *in vivo* SPME, and a 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

Meet Supelco® and Fluka® at ...

Being one of the market leaders for Analytical and Chromatography products, Sigma-Aldrich is present at the major scientific conferences in Europe. Focusing on its two analytical brands Supelco and Fluka, Sigma-Aldrich will participate as exhibitor with posters and oral presentations from our R&D group.

We look forward to meeting you at one of the following events:

Exhibition dates for your diary:

10–13 July 2011	Chirality 2011, Liverpool, UK www.liv.ac.uk/chirality-2011
21–23 July 2011	In Vino Analytica Scientia, Graz, AUT www.invino2011.at
21–25 Aug. 2011	Dioxin 2011, Brussels, BEL www.dioxin2011.org
11–15 Sept. 2011	EuroAnalysis, Belgrade, SER www.euroanalysis2011.rs

10–12 Oct. 2011 QC of Botanicals, TCM, Herbal Food Supplements and Medicinal Products, Erlangen, GER
www.aoaceurope.com

1–4 Nov. 2011 Recent Advances in Food Analysis, RAFA 2011, Prague, CZE
www.rafa2011.eu

16–18 Nov. 2011 European Bioanalytical Forum, Barcelona, ESP
www.europeanbioanalysisforum.eu

Analytical and Chromatography Seminars provide technically advanced presentations for end users, given by experienced scientists from R&D, Product Management and by external guest speakers to provide an overview on recent developments plus useful guidance, tips and tricks for the lab. For an updated list of events and an overview on scientific Supelco and Fluka seminars, visit: sigma-aldrich.com/events

High-Purity Solvents for Sensitive Analysis

Shyam Verma
shyam.verma@sial.com

Solvent impurities are the most common cause of extraneous peaks and unstable baselines. Solvent-derived impurities do not condition out over time and can interfere in the analysis in multiple ways, such as: a) collect on head of the column and elute as a distinct peak or as baseline rise, b) cause general elevation in baseline, lowering sensitivity of analysis, c) foul or damage sensitive instrument components and d) cause cluster ion formation that prevents reliable identification and quantification. For minimising or eliminating these issues, sensitive tests such as LC-MS and GC Headspace require the use of highly pure solvents and additives.

Most common contaminants include inorganic ions, decomposition products, microbes and their excretion products and particulate matter. High-purity solvents designed for use in sensitive analyses such as LC-MS and GC headspace are manufactured with utmost precision and are tested under strict quality control requirements.

LC-MS CHROMASOLV® Solvents and Blends

The LC-MS CHROMASOLV solvents undergo 34 distinct and relevant tests to ensure solvent requirements of sensitive LC-MS analyses. Some of the most important features are:

- Application-tested for LC-MS using the reserpine test
- Low-level inorganic and metal ions for high-sensitivity spectra
- Particle/non-volatile compound-free for system integrity
- Low-gradient baseline, also with your own optimised protocols
- Significantly reduced level of phthalate contaminants

Pre-Blended LC-MS Solvents

Sigma-Aldrich offers pre-blended solutions of most commonly used LC-MS mobile phases prepared with precision and unsurpassed attention to quality. Using the precisely blended solvents eliminates time-consuming mobile phase preparation, and can eliminate lost sample information and instrument downtime caused by impure mobile phase. A special formulation assures that no precipitation or decomposition of the additive occurs under normal laboratory conditions. These pre-blended solvents offer: 1) time savings, 2) accurate composition, 3) minimised baseline and artefacts, and 4) high quality.

GC Headspace Solvents

An important application of GC Headspace (GC-HS) is for the determination of residual volatile organic impurities (OVIs) in active drug substances or excipients in drug formulations. The allowable limits for these OVIs are listed by the United States Pharmacopeia (USP), European Pharmacopoeia (Ph. Eur.) and in the International Conference on Harmonization (ICH) guidelines. Other consumer-oriented applications include the detection of residual solvents in foods, dietary supplements and packaging materials.

In the GC-HS method, the composition and purity of the sample solvent have significant effects on the recovery and quality of the chromatogram. Sigma-Aldrich/Fluka® have developed solvents specifically for

GC-HS applications. These solvents, microfiltered at 0.2 µm and packed under inert gas in 1 L bottles, offer the following benefits:

- High purity and longer shelf life
- Cleaner blanks and improved analyte recoveries
- No major interference peaks in elution range
- Specifications matching USP, Ph Eur. & ICH guidelines

An earlier article (1) presented the results of tests done on two grades of DMSO, Fluka's high-purity headspace grade and an organic synthetic grade, using GC-MS. The analysis of impurities in these solvents was performed using solid phase microextraction (SPME) in the headspace.

The organic synthesis grade was found to contain many impurities. The GC-HS grade produced a cleaner headspace blank and did not show any major interference peaks in the elution range of the target analytes.

Fluka Brand headspace solvents ensure that the demands of headspace analysis are met. These products are listed on the next page.

Reference

1. Stenerson, K.K. and Verma, S., Reporter, Vol. 28.5, 2010.

+ Featured Products

Description	Cat. no.
LC-MS CHROMASOLV Solvents	
Water	39253
Acetonitrile	34967
Methanol	34966
2-Propanol	34965
Ethyl acetate	34972
LC-MS CHROMASOLV Solvent Blends	
Acetonitrile with 0.1% TFA	34976
Methanol with 0.1% TFA	34974
Acetonitrile with 0.1% formic acid	34668
Acetonitrile with 0.1% ammonium acetate	34669
Acetonitrile with 0.1% formic acid and 0.01% TFA	34676
Water with 0.1% TFA	34978
GC Headspace Solvents	
1,3-Dimethyl-2-imidazolidinone	67484
Cyclohexanone	68809
1-Methyl-2-pyrrolidinone	69337
Dimethyl sulphoxide	51779
N,N-Dimethylformamide	51781
Water	53463

+ Related Information

For more information visit our websites:

LC-MS solvents mobile phase additives sigma-aldrich.com/lc-ms

GC-HS solvents sigma-aldrich.com/gc-hs

Monthly Savings Programme

SAVE 30%



Get 30% Discount for LC-MS Solvents

LC-MS solvents are specifically formulated to have a low content of alkaline impurities, such as calcium, magnesium, potassium and sodium, which can interfere in the analysis by forming artefacts with the analyte.

The CHROMASOLV® LC-MS solvents are also run through specific UV-spectroscopic quality control tests to guarantee the traditional CHROMASOLV specification.

More information is available on our website sigma-aldrich.com/lc-ms
More valuable offers are available at sigma-aldrich.com/savings

The following LC-MS solvents are available with a 30% OFF discount.

Part no..	Brand	Description
34965-1L	Fluka®	2-Propanol LC-MS CHROMASOLV
34965-2.5L		2-Propanol LC-MS CHROMASOLV
34966-1L	Fluka	Methanol LC-MS CHROMASOLV, ≥ 99.9%
34966-2.5L		Methanol LC-MS CHROMASOLV, ≥ 99.9%
34967-1L	Fluka	Acetonile LC-MS CHROMASOLV
34967-2.5L		Acetonile LC-MS CHROMASOLV
34967-250ML		Acetonile LC-MS CHROMASOLV
39253-1L-R	Fluka	Water, LC-MS CHROMASOLV

To take advantage of this monthly savings offer, please use [promotion code 993](#).
Offer is valid until 31 August, 2011.

Innovations in Chiral Chromatography

Overview of Modern Chiral Stationary Phases

Tracy Ascah

tracy.ascah@sial.com

Through our own Astec line and partnerships with other innovative companies, Supelco offers the widest range of chiral stationary phase (CSP) classes for HPLC, GC and SFC. They form part of Sigma-Aldrich's "universal" chiral offering that also includes reagents, chiral catalysts, reagents for enantioselective crystallisation, services and more. This article will describe the major CSPs for HPLC and SFC in use today. Subsequent articles in this series will focus on putting them to practical use.

Chromatography has become an important tool for the separation of enantiomers, and analysts today have many CSPs from which to choose. The dynamic abundance of CSPs is necessary; each enantiomer separation is unique and requires specific differentiating interactions.

Common Features of Modern CSPs

The chiral selectors of today's successful CSPs are based on or mimic complex biomolecules, such as proteins, peptides and carbohydrates. This is no coincidence: it is because biomolecules can distinguish enantiomers that biological systems recognise chirality. Biomolecules are also rich in the number and diversity of chiral recognition sites, both structural and chemical. This helps both enantioselectivity and capacity.

- **Structural:** Pockets or other 3-dimensional regions distinguish molecular shape
- **Chemical:** Functional groups provide specific and differentiating interactions

Modern CSPs generally rely on spherical, porous silica gel as the underlying support particle. Silica has advantages of efficiency, stability and ease of modification over synthetic polymer particles. So, although there are exceptions, CSPs for HPLC and SFC typically utilise silica particles bonded or coated with native, modified or mimetic biomolecules.

Polysaccharides (cellulose, amylose)



The most popular class of CSPs for HPLC and SFC, the polysaccharides amylose and cellulose are naturally occurring, optically active, linear (cellulose) and helical (amylose) polymers, comprising hundreds to thousands of D-(+)-glucose units joined by $\alpha(1 \rightarrow 4)$ glycosidic (amylose) bonds or $\beta(1 \rightarrow 4)$ glycosidic (cellulose) bonds. The long polysaccharide chains form rope-like bundles held together via multiple hydrogen bonds between proximate hydroxyl groups. Derivatised cellulose- and amylose-based CSPs owe their high enantioselectivity to the large number of chiral centres in the polysaccharide backbone and to its highly ordered structure. The shape of the pockets formed by the intertwined chains provides chiral discrimination based on molecular shape. Derivatives at the 2, 3 and 6-position hydroxyls confer additional enantioselectivity. An example chromatogram is shown in Figure 1.(1, 2)

Figure 1. Demonstration of cellulose used as a CSP (etodolac enantiomers)

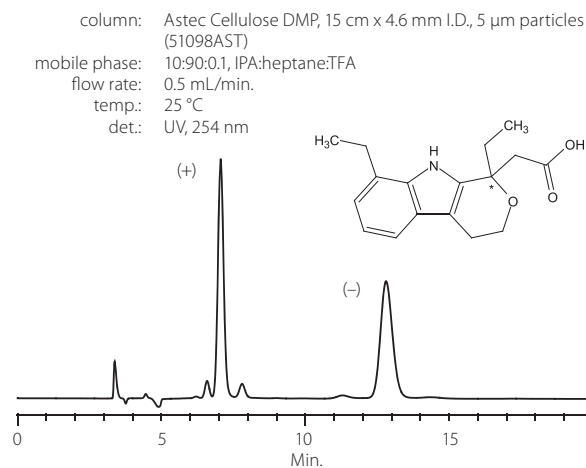
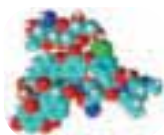


Table 1. Selection of Chiral HPLC and SFC phases from Sigma-Aldrich

Class	Chiral selectors (phases)	Product line
Polysaccharide	tris-(3,5-dimethylphenyl) carbamoyl cellulose	Astec Cellulose DMP, Kromasil®
Macrocyclic glycopeptide	teicoplanin, teicoplanin aglycone, vancomycin, ristocetin A	Astec CHIROBIOTIC®
Cyclodextrin	β - and γ -cyclodextrins, native and derivatised Astec CYCLOBOND™	
Protein	α ,-acid glycoprotein, cellobiohydrolase, albumin (human serum)	Chiral-AGP, Chiral-CBH, Chiral-HSA
Chiral synthetic polymer	poly(trans-1,2-cyclohexanediyl-bis-acrylamide) poly(diphenylethylenediamine-bis-acryloyl)	Astec P-CAP™ Astec P-CAP-DP
Chiral ligand exchange	chiral bidentate ligand	Astec CLC-L, Astec CLC-D
Cyclofructan	derivatised cyclofructan 6	LARIHC™

Macrocyclic Glycopeptides

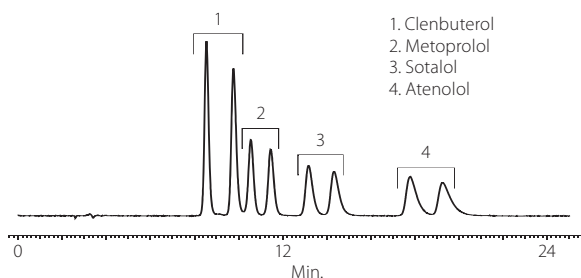


This successful class of CSPs uses naturally occurring macrocyclic glycopeptides as the chiral selector. They offer five different types of molecular interactions: ionic, H-bond, π - π , dipole and hydrophobic, and multiple inclusion sites that

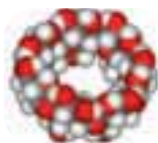
influence selectivity based on the molecular shape of the analyte. Ionic interactions are unique to these CSPs, and are responsible for their success with polar and ionisable analytes, and their utility in reversed-phase and LC-MS mobile phases. An example chromatogram is shown in Figure 2.(3)

Figure 2. Macrocyclic glycopeptide used as a CSP (enantiomers of β -blockers)

column: Astec CHIROBIOTIC® T, 25 cm x 4.6 mm I.D., 5 μ m particles (12024AST)
mobile phase: 15 mM ammonium formate in methanol
flow rate: 1 mL/min.
temp.: 25 °C
det.: UV, 220 nm



Cyclodextrins

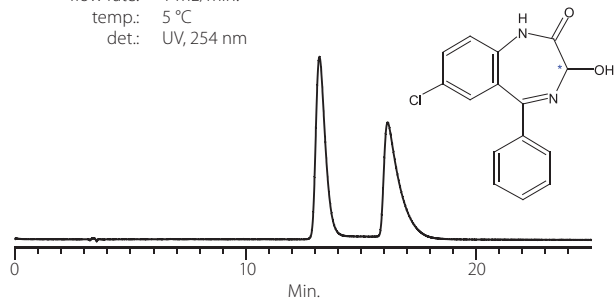


Cyclodextrins (CDs) comprise D-(+)-glucose residues bonded through $\alpha(1 \rightarrow 4)$ glycosidic linkages. The chair configuration of glucose makes the toroid bucket narrower at one end. Derivatisation of the 2- and 3-position hydroxyl groups affects selectivity. Enantioseparations

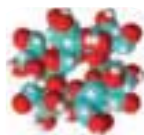
occur on the inside (inclusion complexing) and outside surfaces (surface interactions). The most important consideration for retention and chiral recognition in reversed phase is proper fit of the analyte into the CD cavity. This fit is a function of both molecular size and shape of the analyte relative to the cavity. An example chromatogram is shown in Figure 3.(4)

Figure 3. β -Cyclodextrin used as a CSP (oxazepam enantiomers)

column: Astec CYCLOBOND™ I 2000 DNP, 25 cm x 4.6 mm I.D., 5 μ m particles (25024AST)
mobile phase: 20:80, acetonitrile:20 mM ammonium phosphate, pH 2.9
flow rate: 1 mL/min.
temp.: 5 °C
det.: UV, 254 nm



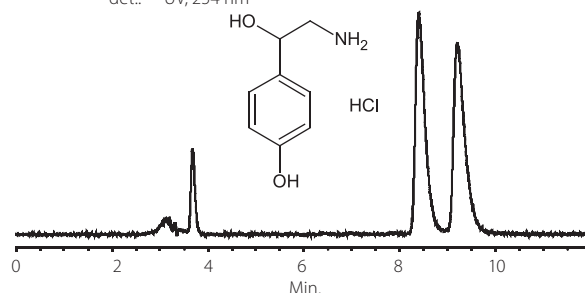
Cyclofructans



Cyclofructans are the newest class of CSPs. They comprise six or more $\beta(2 \rightarrow 1)$ linked D-fructofuranose units. Although structurally similar to cyclodextrins, they have very different selectivity. The propyl derivative is particularly adept at separating chiral primary amines (Figure 4).(5)

Figure 4. Cyclofructan-6 used as a CSP (octopamine enantiomers)

column: LARIHC™ CF6-P, 25 cm x 4.6 mm I.D., 5 μ m particles (AZYF Part No. L1001, available from Supelco®/Sigma-Aldrich as a custom item)
mobile phase: 70:30:0.3:0.2, methanol:acetonitrile:acetic acid:triethylamine
flow rate: 1 mL/min.
temp.: 20 °C
det.: UV, 254 nm



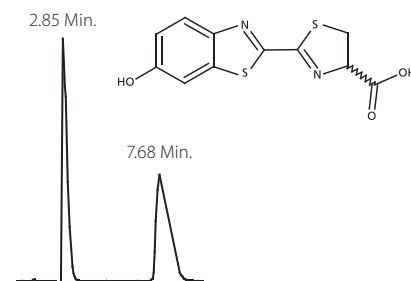
Proteins



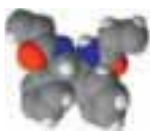
Proteins contain a large number of chiral centres and many other sites that contribute to the general retention process. Three proteins that have been particularly successful as CSPs are α_1 -acid glycoprotein (AGP, shown in Figure 5), cellobiohydrolase (CBH), and human serum albumin (HSA).(6)

Figure 5. Protein (HSA) used as a CSP (luciferin enantiomers)

column: Chiral-AGP, 10 cm x 4 mm I.D., 5 μ m particles (58150AST)
mobile phase: 10 mM sodium phosphate, pH 6.0
flow rate: 0.9 mL/min.
temp.: 25 °C
det.: UV, 225 nm



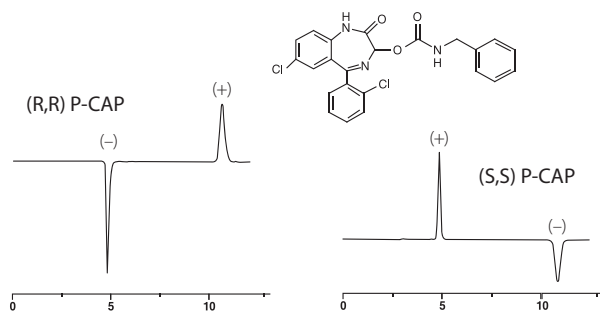
Chiral Synthetic Polymers



Synthetic CSPs have a defined structure and controlled degree of polymerisation, and mitigate certain drawbacks associated with natural compounds. Most synthetic CSPs comprise a thin, ordered layer of chiral polymer covalently bonded to the silica surface. Because they are synthetic, they can be identically manufactured in both R,R and S,S forms, providing a predictable reversal of elution order. An example is shown in Figure 6.(7–9)

Figure 6. Chiral synthetic polymer used as a CSP (fuoin enantiomers)

columns: 25 cm x 4.6 mm I.D., 5 μ m
mobile phase: 95:5, methylene chloride:methanol
flow rate: 1 mL/min.



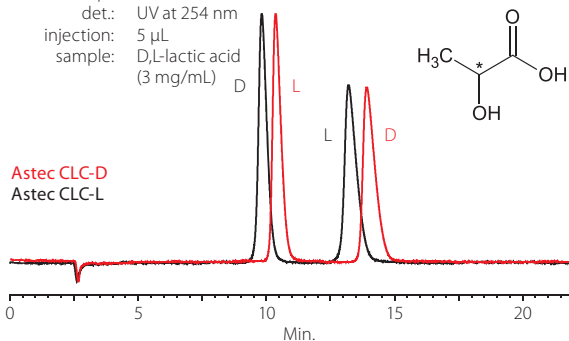
Chiral Ligand Exchange



Copper ions in the mobile phase coordinate with the chiral selector on the stationary phase (a small, chiral bidentate ligand) and carboxylic acid functional groups on the analytes to form transient diastereomeric complexes in solution. Analytes include alpha-hydroxy acids, such as lactic, malic, tartaric and mandelic acids, amino acids, other amines and bifunctional racemates, such as amino alcohols. The technique also gives analytes a strong 254 nm signal. Two versions (D and L, Figure 7) provide elution order reversal.(10)

Figure 7. Chiral ligand exchange chromatogram (lactic acid enantiomers)

columns: Astec CLC-D (53023AST) and Astec CLC-L (53123AST), both 15 cm x 4.6 mm I.D., 5 μ m particles
mobile phase: 5 mM CuSO₄
flow rate: 1.0 mL/min.
temp.: ambient
det.: UV at 254 nm
injection: 5 μ L
sample: D,L-lactic acid (3 mg/mL)



Conclusion

Irrespective of the success of the CSPs discussed in this article, there is plenty of room in the field for other types. Continue to look to Supelco® for innovative, practical solutions for chiral separations.

Visit our chiral web portal sigma-aldrich.com/chiral to learn more about Sigma-Aldrich's wide range of products and services for chiral chemistry and separations, and for method development protocols.

References

- Hesse, G., Hagel, R. *Chromatographia* 1973, 6(6), 277–280.
- Okamoto, Y., Kawashima, M., Hadata, K. *J. Amer. Chem. Soc.* 1984, 106, 5357–5359.
- Armstrong, D. W., Tang, Y., Chen, S., Zhou, Y., Bagwill, C., Chen, J. *Anal. Chem.* 1994, 66, 1473–1484.
- Armstrong, D. W., DeMond, W. *J. Chrom. Sci.* 1984, 22(9), 411–415.
- Sun, P., Wang, C., Breitbach Z. S., Zhang, Y., Armstrong, D. W. *Anal. Chem.* 2009, 81, 10215–10226.
- Hermansson, J. *J. Chromatogr. A* 1983, 269, 71–80.
- Gasparrini, F., Misiti, D., Rompietti, R., Villani, C. *J. Chromatogr. A* 2005, 1064(1), 25–38.
- Zhong, Q., Han, X., He, L., Beesley, T. E., Trahanovsky, W. S., Armstrong, D. W. *J. Chromatogr. A* 2005, 1066(1-2), 55–70.
- Allenmark, S. G., Andersson, S., Möller, P., Sanchez, D. *Chirality* 1995, 7(4), 248–256.
- Davankov, V. A., Rogozhin, S. V. *J. Chromatogr.* 280–283 1971, 60.

+ Related Information

For further reading:

Chiral Liquid Chromatography; Lough, W. J., Ed.; Blackie and Son, Ltd., Glasgow

Chiral Chromatography; Beesley, T. E., and Scott, R. P. W.; John Wiley & Sons, New York

New! USP Residual Solvent Standards

Sigma-Aldrich now offers four mixes that cover all USP Monograph 467 Class 1, Class 2 and Class 3 solvents. These standards, produced according to ISO 9001, are prepared with high-purity headspace grade dimethylsulphoxide (DMSO). Headspace grade DMSO is used because it produces cleaner blanks and does not introduce any major interference peaks in the chromatographic elution range of the target analytes.

+ Featured Products

Description	Pack size	Cat. no.	
USP 467 Class 1 Residual Solvents Mix A Varied concentration, DMSO	1 x 1 mL	40131-U	
Benzene.....	10,000 µg/mL	1,1-Dichloroethane.....	40,000 µg/mL
Carbon tetrachloride.....	20,000 µg/mL	1,1,1-Trichloroethane.....	50,000 µg/mL
1,2-Dichloroethane.....	25,000 µg/mL		
USP 467 Class 2 Residual Solvents Mix A Varied concentration, DMSO	1 x 1 mL	40132-U	
Acetonitrile.....	2050 µg/mL	Methylcyclohexane.....	5900 µg/mL
Chlorobenzene.....	1800 µg/mL	Methylene chloride.....	3000 µg/mL
Cyclohexane.....	1940 µg/mL	Tetrahydrofuran.....	36,600 µg/mL
cis-1,2-Dichloroethene.....	4700 µg/mL	Toluene.....	4450 µg/mL
trans-1,2-Dichloroethene.....	4700 µg/mL	m-Xylene.....	980 µg/mL
1,4-Dioxane.....	1900 µg/mL	o-Xylene.....	6510 µg/mL
Ethylbenzene.....	18,400 µg/mL	p-Xylene.....	1520 µg/mL
Methanol.....	1500 µg/mL		

Supelco® residual solvent standards, offered at 5x the monograph concentrations, are prepared using Class A volumetric glassware and NIST traceable calibrated balances. The concentration of each component is within +/- 0.5% of the stated value. The certificate of analysis accompanying each product indicates CAS numbers, % purity, raw material lot number (for traceability), purity determination method, stated concentration, and analytical concentration for all solution components. Additionally, manufacture/expiration dates are included.

Description	Pack size	Cat. no.	
USP 467 Class 2 Residual Solvents Mix B Varied concentration, DMSO	1 x 1 mL	40133-U	
Chloroform.....	300 µg/mL	Nitromethane.....	250 µg/mL
1,2-Dimethoxyethane.....	500 µg/mL	Pyridine.....	1000 µg/mL
n-Hexane.....	1450 µg/mL	Tetralin.....	500 µg/mL
2-Hexanone.....	250 µg/mL	Trichloroethene.....	400 µg/mL
USP 467 Class 2 Residual Solvents Mix C Varied concentration, DMSO	1 x 1 mL	40134-U	
2-Ethoxyethanol.....	5450 µg/mL	N,N-Dimethylformamide.....	1100 µg/mL
Ethylene glycol.....	4400 µg/mL	2-Methoxyethanol.....	250 µg/mL
Formamide.....	800 µg/mL	N-Methylpyrrolidine.....	2650 µg/mL
N,N-Dimethylacetamide.....	3100 µg/mL	Sulfolane.....	800 µg/mL

High-Purity PESTANAL® Standards



Sigma-Aldrich routinely stocks more than 1,200 high-purity pesticide and metabolite reference materials through our PESTANAL product line. The standards are formulated for single use and packaged in glass ampuls. Each reference material is supplied with a certificate of analysis. For the convenience of our customers, the purity is also noted on the product label. Most PESTANAL standards have a minimum purity >99%.

Because this portfolio is continually growing to meet the changing needs of environmental and food safety analysts, we recommend visiting our website for the most current offering. Listed to the right is a sample of the many PESTANAL pesticide standards you will find.

+ Featured Products

Description	Quantity	Cat. no.
Abamectin	100 mg	31732
Aldicarb	100 mg	33386
Carbendazim-d ₃	10 mg	32413
Cyflufenamid	25 mg	32403
Etoazole	50 mg	32506
Isofenphos-methyl	50 mg	33436
Methoxychlor	100 mg	36161
TEPP	50 mg	32434

Did you know . . .

Sigma-Aldrich also offers high-purity solvents for pesticide analysis? Our PESTANAL product line of solvents has been developed specifically for trace residue analysis of pesticides using GC/ECD and GC/NPD.

These high-purity solvents are manufactured in large homogenous lots involving multiple purification procedures. The solvents are then packaged under clean room conditions.

For more information, please visit sigma-aldrich.com/pr

eVol® Hand-Held Automated Analytical Syringe



The first step in most analytical methods is making analytical calibration standards. This typically involves making serial dilutions from a stock standard solution. The precision and accuracy demanded for these dilutions has traditionally required the use of manual pipettes, which is time-consuming and results in lost productive time washing glassware. Additionally, manual pipette use is prone to errors introduced by variability in user technique.

The eVol Hand-held Automated Analytical Syringe combines a digitally controlled electronic drive with precision analytical syringes using the patent pending XCHANGE® interface. The result is a positive-displacement dispensing system that is easily programmed to perform a variety of liquid-handling procedures, both accurately and reproducibly.

Key Benefits:

- User-independent precision and accuracy
- Intuitive user interface
- Dedicated syringes prevent cross contamination
- Gravimetric calibration by user

Everyone is an Expert

The ease of use and programmability of the eVol Hand-held Automated Analytical Syringe makes anyone using it an expert in fluid handling. All aspects of volumetric fluid transfer, including: aspiration rate, dispensing rate and sample volume are controlled by the digital drive. This decreases the possibility of variation from one user to another and eliminates concern over pipetting technique when making dilutions. Workflow scheduling issues related to operator expertise are eliminated. Additionally, fewer errors in sample processing reduce the number of samples that must be re-analysed.

Touch Wheel Control

A full-colour display and a convenient touch wheel controller make using the eVol easy. The touch wheel uses a menu-driven approach similar to popular music devices. Intuitive functions include help screens, while prompts make programming and use effortless.






XCHANGE Analytical Syringes

XCHANGE analytical syringes can be easily and quickly changed. This allows the user to choose the best syringe for the volume being measured. It also allows the user to dedicate individual syringes to specific liquids or methods, reducing the possibility of cross-contamination. Only three XCHANGE syringes are required to dispense liquid volumes from 0.2 μL up to 500 μL .



Table 1. Syringe capacity chart

Syringe capacity (μL)	5	50	100
Colour Code			
Volume Range (μL)	0.2–5	2–50	20–500
Accuracy			
Calibrated Syringe at Full Scale	$\pm 0.2\%$	$\pm 0.2\%$	$\pm 0.2\%$
Uncalibrated Syringe at Full Scale	$\pm 1.0\%$	$\pm 1.0\%$	$\pm 0.5\%$
Precision RSD at Full Scale	0.5%	0.4%	0.3%

World's First User-Calibrated Analytical Syringe

Compliance with laboratory standards such as GLP, GMP and FDA, requires regular calibration of liquid-measuring devices. Calibration is typically done outside the laboratory, resulting in additional cost and a loss in productivity. The eVol Hand-held Automated Analytical Syringe can be calibrated using only a liquid of known density and an analytical balance. Microsoft® Excel worksheets provide a mechanism for calculating the required calibration factor and recording calibration records to document compliance. Calibration factors can be stored for up to 10 XCHANGE syringes and can be quickly loaded when the syringe is changed.

Typical Applications for eVol®

- Preparation of calibration standards
- Addition of internal standards
- Precise dispensing of liquids
- Sample dilution

Technical Support & Warranty

Each eVol syringe unit is shipped with a user's manual. The manual provides step-by-step instructions for getting started, storing syringe methods, calibrating syringes, and custom programming the eVol unit.

In addition, each eVol syringe is warranted by SGE to be free of defects in material or workmanship for a period of one year from the date of purchase. To learn more, please contact EurTechServ@sial.com

+ Featured Products

Description	Pack size	Cat. no.
Kit		
eVol Electronic Syringe Starter Kit	1	29841-U
Kit includes unit, charger, stand, 3 syringes: 5 µL, 50 µL, and 500 µL		
Individual components		
eVol Electronic Syringe	1	29842-U
eVol Stand	1	29843-U
eVol Charger with Adapters	1	29844-U
eVol Single Charging Stand with Adapters	1	29845-U
eVol Replacement Battery	1	29846-U
5 µL eVol Syringe	1	29847-U
5 µL eVol Syringe	3	29853-U
5 µL eVol Syringe without needle	1	29848-U
50 µL eVol Syringe	1	29849-U
50 µL eVol Syringe	3	29854-U
50 µL eVol Syringe without needle	1	29850-U
500 µL eVol Syringe	1	29851-U
500 µL eVol Syringe	3	29855-U
500 µL eVol Syringe without needle	1	29852-U

You can find a complete list of our syringe offers at:

sigma-aldrich.com/syringes

+ Related Products

Description	Gauge	Needle length (mm)	Point style	Pack size	Cat. no.
Replacement Needles for 5 µL Syringes					
Needle	25	50	bevel tip (#2)	5	29859-U
Needle	22	51	blunt tip (#3)	5	29860-U
Needle	23	50	cone tip (#1)	5	29861-U
Needle	25	70	bevel tip (#2)	5	29862-U
Needle	26	70	cone tip (#1)	5	29863-U
Replacement Needles for 50 µL Syringes					
Needle	25	50	bevel (#2)	5	24447
Replacement Needles for 500 µL Syringes					
Needle	23	50	bevel (#2)	5	29864-U
Replacement Plungers					
for 5 µL syringe				1	29856-U
for 50 µL syringe				1	29857-U
for 500 µL syringe				1	29858-U



Faster HPLC on **ANY** System

Go to sigma-aldrich.com/express

1. Learn about the Fused-Core® Advantage
2. Find the phases you need (7 choices) →
3. Request application support

- C18
- RP-Amide
- C8
- Phenyl
- F5 (pentafluorophenyl) **NEW!**
- HILIC (Si)
- Peptide ES-C18



Ascentis® is a registered trademark of Sigma-Aldrich Biotechnology LP.
Fused-Core is a registered trademark of Advanced Materials Technology, Inc.

Sigma-Aldrich Offices

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

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

Ireland

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

Italy

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

The Netherlands

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

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 5923

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

Internet

sigma-aldrich.com



MIX
Paper from
responsible sources
FSC® C002727

*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 EurTechServ@sial.com • sigma-aldrich.com/techservice
Safety-related Information sigma-aldrich.com/safetycenter

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

©2011 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA, SAFIC, SIGMA-ALDRICH, ALDRICH, and SUPELCO are trademarks of Sigma-Aldrich Co. LLC, registered in the US and other countries. FLUKA is a trademark of Sigma-Aldrich GmbH, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.

Date: 08/2011;
SAMS Code: NLX