

Reporter

Volume 29.2

 **SUPELCO**
Analytical

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



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

Liquid Chromatography

Sample Preparation

Gas Chromatography

Standards

Accessories

Chiral Chromatography

Reporter

Volume 29.2

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Multimedia Communication



Wayne Way

Market Segment Manager
HPLC/GC

Dear Colleague:

Communicating with customers is the most important aspect of a marketing program. 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 program 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

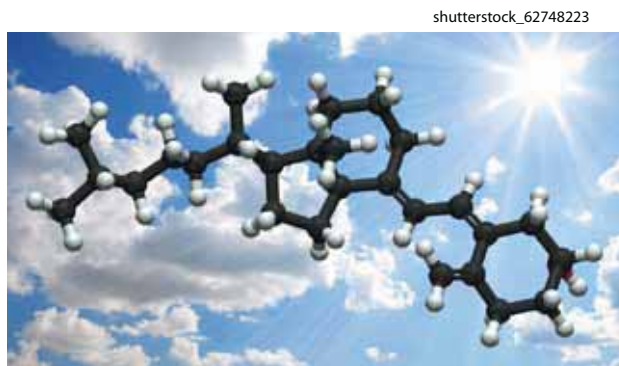
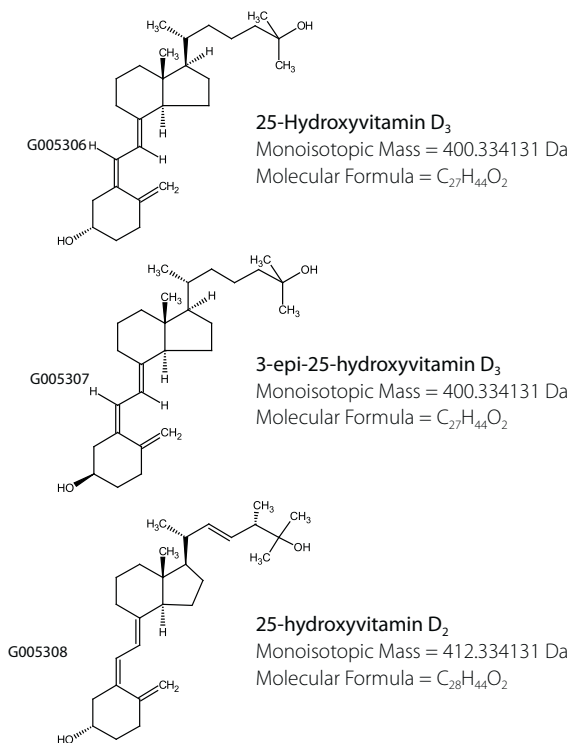
Fast and Accurate LC-MS Analysis of Vitamin D Metabolites Using Ascentis® Express F5 HPLC Columns

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wayne.way@sial.com

Introduction

Vitamin D deficiency has become a topic of interest in recent publications (1-3). Vitamin D, along with calcium, promote 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. D₃ is produced after ultraviolet light-stimulated conversion of 7-dehydrocholesterol in the skin (3). Vitamin D₂ is derived from plant sources. Both D₂ and D₃ are metabolized 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 analogs 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 analogs 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 analogs may become essential for diagnosis and monitoring of patients with vitamin D disorders.

Figure 1. Vitamin D 25-hydroxy Metabolite Structures



Vitamin D₃ is produced in the skin after exposure to sunlight

HPLC analysis of 25-OH D₂ and 25-OH D₃ is classically performed using C18 stationary phases. Under such conditions, the 3-epi analogs 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 and the 3-epi forms for use in reference measurement procedures (1). Although effective, the conditions necessitate a run time of better than 40 minutes limiting its utility for routine high-throughput analyses.

As an outcome of some recent application development efforts, it was observed that a pentafluorophenyl (PFP, Ascentis Express F5) stationary phase provided increased selectivity toward 25-OH D₃ and the corresponding 3-epi analog relative to reported methods. This report provides a brief synopsis of continuing efforts to assess the potential impact of this additional selectivity on routine clinical vitamin D diagnostics.

Discussion

The structures of the vitamin D analytes are shown in **Figure 1**, while the initial separation of 25-OH D₃ and 3-epi-25-OH D₃ using the fluorinated phase is presented in **Figure 2**. The separation demonstrates that selectivity between the analogs can be achieved in under 10 minutes, whereas separation using a cyano column required nearly 40 minutes.

The ultimate goal for this separation is likely to entail the use of mass spectrometry to reach the desired levels of quantification and specificity. With this in mind the initial conditions were adopted for fast LC-MS methodology. **Figure 3** shows some preliminary results indicating that 25-OH D₃ and 3-epi-25-OH D₃ can be rapidly resolved. 25-OH D₂ and 3-epi-25-OH D₃ coelute under these high throughput conditions, however they are easily resolved by mass response. The methodology thus enables quantification of all three components in one analysis.

(continued on page 4)

Figure 2. Separation of 25-Hydroxyvitamin D₃ and 3-epi-25-Hydroxyvitamin D₃ Using Ascentis Express F5

column: Ascentis Express F5, 10 cm x 3.0 mm I.D., 2.7 μm (53578-U)
 mobile phase: water:methanol (28:78, v/v)
 temp: 30 °C
 flow rate: 0.5 mL/min.
 backpressure: 3100 psi
 detection: MS, APCI (+), SIR mode, m/z 401
 sample: 20 μg/mL each in methanol
 inj vol: 2 μL

1. 25-Hydroxyvitamin D₃
2. 3-epi-25-Hydroxyvitamin D₃

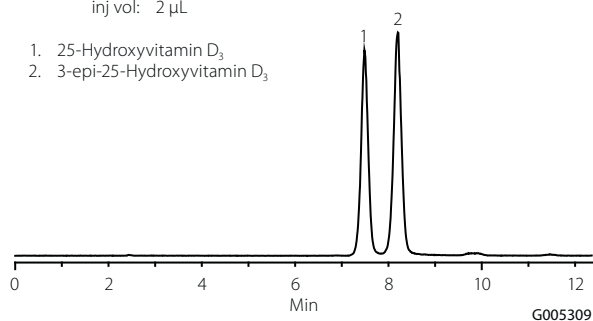
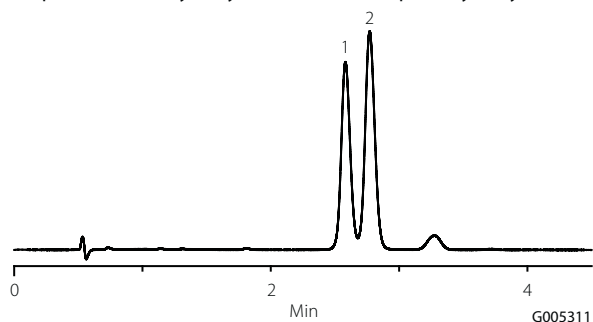


Figure 3. Fast, LC-MS Analysis of Vitamin D Metabolites Using Ascentis Express F5

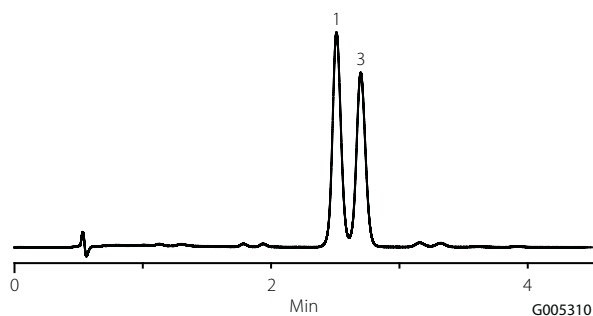
column: Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 μm (53569-U)
 mobile phase: 25% 5mM ammonium formate water,
 75% 5mM ammonium formate methanol
 flow rate: 0.4 mL/min
 temp: 40 °C
 inj vol: 1 μL
 UV detection: 265 nm
 MS detection: m/z 100-1000

1. 25-Hydroxyvitamin D₃ 2.57 min
2. 3-epi-25-Hydroxyvitamin D₃ 2.76 min
3. 25-hydroxyvitamin D₂ 2.77 min

3a. Separation of 25-Hydroxyvitamin D₃ and 3-epi-25-Hydroxyvitamin D₃



3b. Analysis of 25-Hydroxyvitamin D₃ and 25-Hydroxyvitamin D₂



(continued from page 3)

Conclusions

Separation of the biologically inactive 3-epi analog may serve 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 as compared to methods reported in the literature. Initial efforts to show selectivity in a fast, LC-MS system provides promising evidence for implementation in real-world situations. As low analyte mass response and interferences from sample matrices may pose additional analysis problems, work is currently underway to further explore both chromatographic and sample preparation procedures in an attempt to optimize both speed and sensitivity.

References

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3. Higashi, T.; Shimada, K.; Toyo'oka, T. *Journal of Chromatography B* 2010, 878, 1654-1661.

+ Featured Products

Ascentis Express F5 HPLC Columns (2.7 μm)

I.D. (mm)	Length (cm)	Cat. No.
2.1	2	53592-U
2.1	3	53566-U
2.1	5	53567-U
2.1	7.5	53568-U
2.1	10	53569-U
2.1	15	53571-U
3.0	3	53574-U
3.0	5	53576-U
3.0	7.5	53577-U
3.0	10	53578-U
3.0	15	53579-U
4.6	3	53581-U
4.6	5	53583-U
4.6	7.5	53584-U
4.6	10	53590-U
4.6	15	53591-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

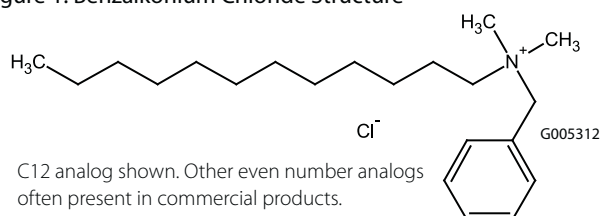
LC-MS Analysis of Benzalkonium Chloride using Hydrophilic Interaction Chromatography (HILIC)

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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 mold, algae and moss remover. It is used in:

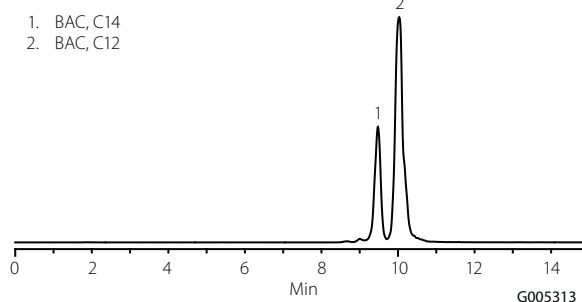
- Pharmaceuticals such as leave-on skin antiseptics
- Antiseptic to safely treat childhood scrapes and cuts
- Advanced, next generation hand sanitizers
- Hygienic towelettes and wet wipes
- Cosmetics such as eye and nasal drops, as a preservative
- Cleaners for floor and hard surfaces as a disinfectant
- High-level surgical instrument sterilizing 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 (herein referred to as BAC), the objective of the study was to develop an LC-MS compatible set of conditions to analyze BAC in various pharmaceutical formulations and household products. Mass spectrometric detection would allow for an additional separation mode in more complex matrices as well as provide supportive identification information. A literature search revealed several methods for the analysis of BAC,

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.



however, all those found utilized nonvolatile buffers and/or strong ion-suppressing mobile phase modifiers (2-5). Initial attempts to produce quality chromatography through simple alteration of the literature methods utilizing 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 μ m (53981-U)
Mobile Phase: 2, 5 or 10 mM ammonium acetate in 10:90 water:acetonitrile
Temperature: 35 $^{\circ}$ C
Flow Rate: 1 mL/min
Backpressure: ~1200 psi
Detection: ESI(+), scan range m/z 150-500, uv at 263 nm
Injection: 2 μ L
Sample: various

Results and Discussion

Initial method development commenced using a commercially available benzalkonium chloride material (Fluka, catalog number 09621, 50% benzalkonium chloride in water) that consists mainly of the C12 and C14 analogs. The two analogs exhibit a mass/charge (m/z) of 304 and 332, respectively. Figure 2 shows the chromatographic separation of this standard using the conditions stated in the Experimental section (2 mM ammonium acetate). Excellent peak shape and selectivity are observed. The elution order of the analogs is the opposite of what is found using reversed-phase systems owing to the HILIC mode of operation.

(continued on page 6)



(continued from page 5)

It is often desired to analyze for BAC in ophthalmic formulations. The suitability of the developed conditions is thus dependent on selective separation from other active and inactive components that may be present. To examine this, several antiglaucoma 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 Antiglucoma 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, representative of a polar, strong base, was shown to separate well from the BAC responses and was relatively insensitive to buffer concentration. Pilocarpine, a polar, weak base, is also shown to be well separated from BAC and to be insensitive to buffer concentration. Epinastine, representing a nonpolar, strong base, shows excellent selectivity from BAC, but retention is highly dependent on buffer concentration. In a similar fashion the moderately polar, strong base, betaxolol exhibits good separation and strong retention dependence on buffer concentration. It is apparent that the polar analytes retain primarily by a HILIC partitioning mechanism and that the retention of the nonpolar bases is dominated by ionic interactions. Retention of the BAC components is easily manipulated using buffer concentration, where increasing buffer concentration results in earlier elution. Latanoprost, a nonpolar, nonionic analyte is unretained under the present conditions. Representative chromatograms from the study are presented in Figures 3-4.

The developed conditions were also applied to a commercial disinfecting towelette that reportedly includes various n-alkyl dimethylbenzylammonium chloride active ingredients. The analytical trace shown in Figure 5 is an overlay (total ion chromatogram) of many BAC related compounds. In this case, the added separation dimension afforded by the mass spectrometric detection is required for resolution of the complex mixture. The application provides evidence for the broad relevance for this analytical approach.

Conclusions

HILIC has been shown to provide an 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 provide a means for simultaneous analysis of the active components as well as BAC in the same run. Retention of both the BAC components and the

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.

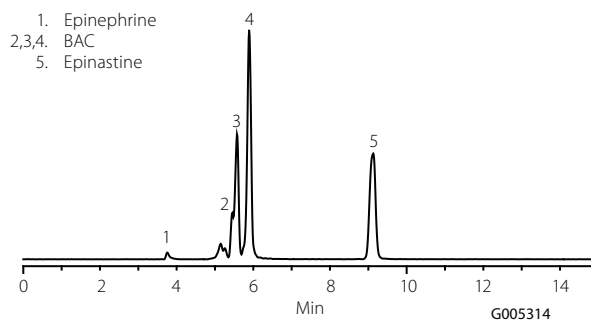


Figure 4. Separation of Pilocarpine and Betaxolol from Benzalkonium Chlorides Using Ascentis Express HILIC

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

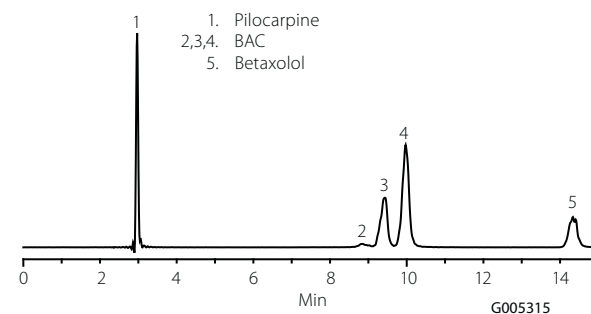
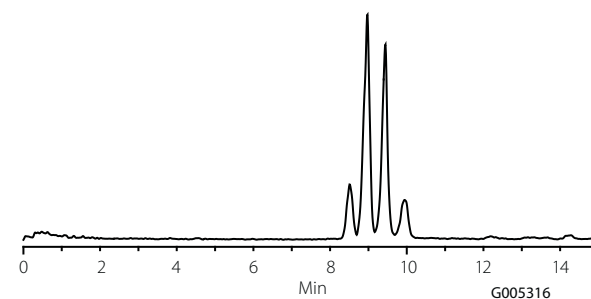


Figure 5. Separation of Benzalkonium Chloride Active Ingredients in Disinfecting Towelettes Using Ascentis Express HILIC

Total ion chromatogram (TIC) of a commercially available disinfecting towelette extract. Conditions as per Experimental Section, 2 mM ammonium acetate in 90% acetonitrile. Sample preparation: a single towelette moistened with 10 mL methanol was placed in a 25 mL syringe barrel. Liquid was then "squeezed out" and simultaneously filtered through a 0.45 μ m syringe filter. The resulting fluid was diluted 1 to 10 with methanol prior to analysis. Primary mass responses: m/z 304, 332, 360 and 388.



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. Relevance of the methodology toward other chemical and industrial uses, such as the analysis of BAC related compounds in disinfecting wipes, has also been demonstrated.

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Ascentis Express HILIC

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

Ascentis Express Guard Cartridges

Description	I.D. (mm)	Pkg. Size	Cat. No.
HILIC	2.1	3	53520-U
HILIC	3.0	3	53521-U
HILIC	4.6	3	53523-U

Analysis of Aromatic and Aliphatic Analytes in Gasoline on the Extremely Polar SLB-IL11

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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 leukemia. (1) On January 1st of 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%. (3)

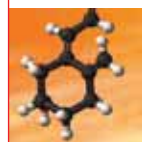
Reformulated Gasoline and Ethanol

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 nonpolar polydimethylsiloxane column, and the two are connected using a switching valve. Sample enters the polydimethylsiloxane column first, where compounds retain based on their boiling points. After the elution of n-octane, flow through this column is reversed and the 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. Traditional methodology for this application employs the use of packed GC column versions of the polydimethylsiloxane and TCEP columns. An alternate approach would be to take advantage of the higher efficiency, greater stability, and better column-to-column reproducibility offered by capillary columns. However, if TCEP is used as the highly polar column, the same issues related to temperature will still exist, and require the use of a two column system with back-flush.

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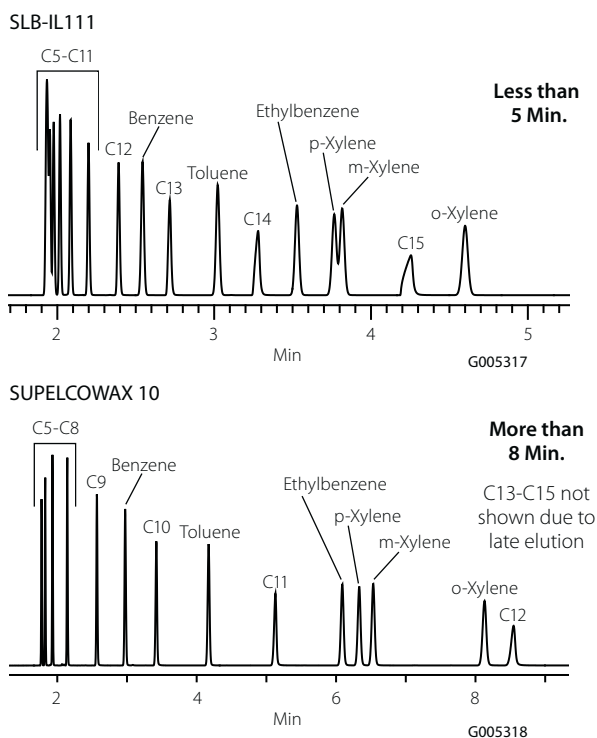
The SLB-IL111

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. The ionic liquid phase used in the SLB-IL111 is inherently more stable than TCEP, and can be used to a substantially higher temperature of 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 nonpolar 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. 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 program is used. However,

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



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 analyzed 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.

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 the 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.

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

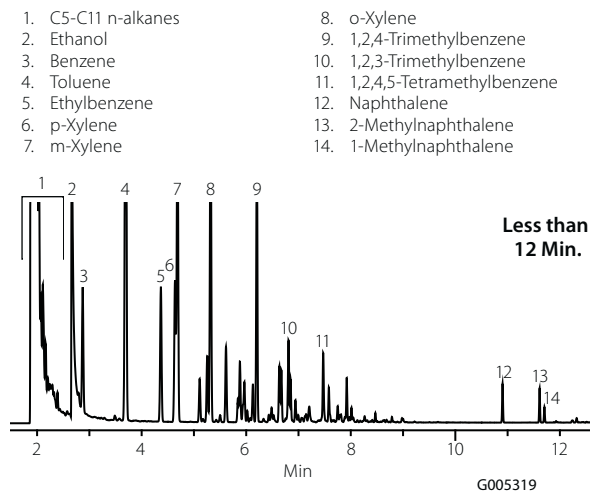
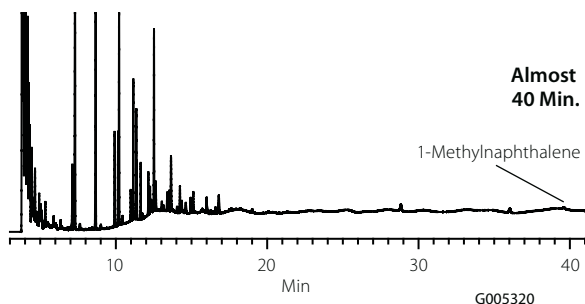




Figure 3. Reformulated Gasoline on the TCEP

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



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 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 $^{\circ}$ 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

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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 μ m	28927-U
SUPELLOWAX 10, 30 m x 0.25 mm I.D., 0.25 μ m	24079
TCEP, 60 m x 0.25 mm I.D., 0.44 μ m	24153

+ Related Products

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

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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 program 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 called 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 inquired 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 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 phenomenon create adsorption sites that interact with the sample as it passes through the inlet liner.

Figure 1. Poor Chromatography Before Liner & Septum are Changed

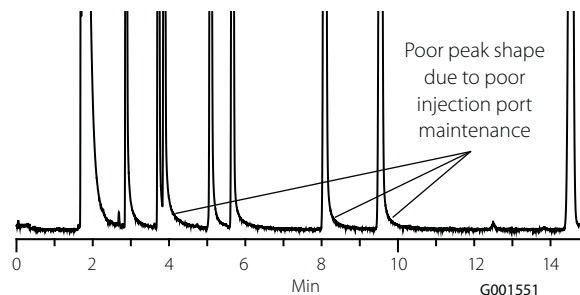
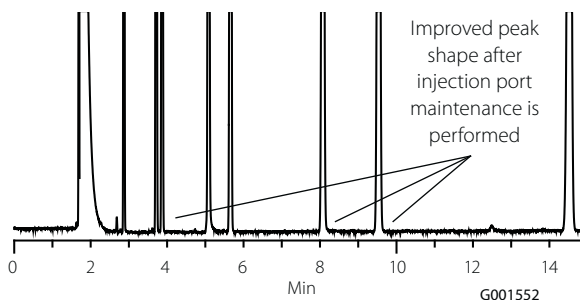


Figure 2. Normal Chromatography After Liner & Septum are Changed



Case Study 1 Solution

The Technical Service Chemist suggested that the current septum and inlet liner should be changed. A molded Thermogreen™ LB-2 septum was recommended due to its bleed-temperature-puncturability-optimized 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 septa can accumulate at the head of the column during the cool-down portion of the program. 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 Liner Type and Selection

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 vaporization, and/or to keep non-volatile material from entering the column.

Splitless Injection: Similar to split inlet liners but, without cups, baffles, and 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 vaporization, 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, minimizing band broadening. All of the sample is transferred to the column. Also known as flash vaporization.

Figure 3. Customer's First Chromatogram – Low Response

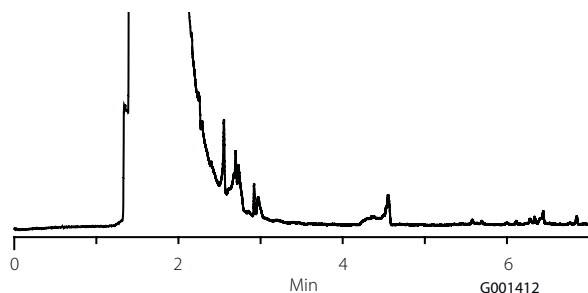
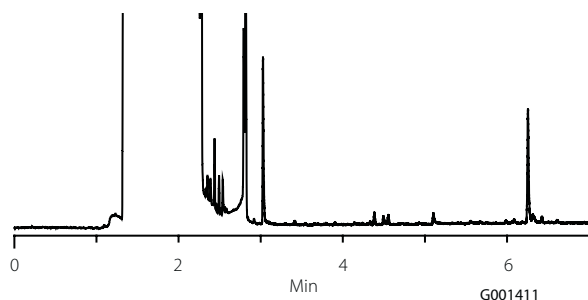


Figure 4. Result of Installing the Correct Liner



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 be 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 catalog 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 catalog numbers, request "Molded Thermogreen LB-2 Septa" (T407082, JQV) or visit sigma-aldrich.com/moldedsepta

TRADEMARKS: Ascentis, Carbotrap, CHIROBIOTIC, CHROMASOLV, CYCLOBOND, P-CAP, PESTANAL, SLB, Supel, SUPELCOWAX, Thermogreen – Sigma-Aldrich Biotechnology LP; BrightTALK – BrightTALK, Inc.; EVol, FocusLiner, XCHANGE – SGE Analytical Science Pty Ltd; Kromasil, CelluCoat, AmyCoat – Eka Chemicals AB; LARIHC – AZYP, LLC; Microsoft – Microsoft Corporation; Tedlar – E.I. duPont de Nemours; YouTube – Google, Inc.



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

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



Model 1062 E001175

Introduction

Gas sampling bags are devices for whole air sampling and are recognized as economical alternatives to canisters for sampling VOC's and other gases. Until recently, Tedlar® was the most recognized film used to manufacture sampling bags. Recently, DuPont informed its customers that they will 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. Our Supel-Inert Gas Sampling bags are supplied with our exclusive Thermogreen LB-2 polymer has the industry's lowest bleed preventing sample contamination from the septum. **Table 1** provides a physical 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 °C)
Specific Gravity	1.78	1.70
Oxygen Permeability	58 mL/(m ² x d)	50 mL/(m ² x d)
Water Vapor 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 your 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.

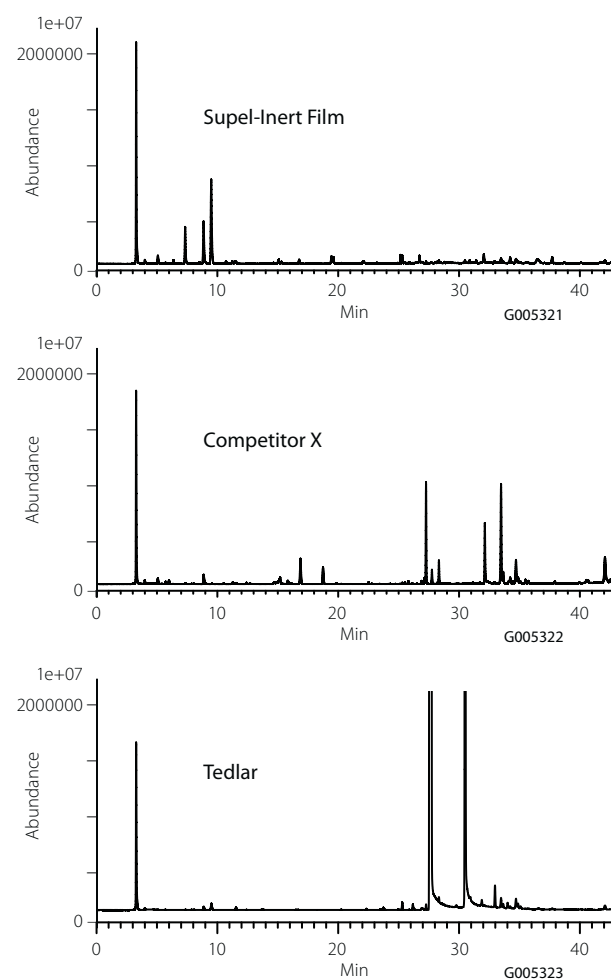
It is well-known that Tedlar film contains the contaminants DMAC and phenol as part of the film composition. Supel-Inert film does not contain these compounds. Typically gas sampling bags are used to sample atmospheres in the ppmv range. Our Supel-Inert bags can be used to sample in the ppbv range, but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

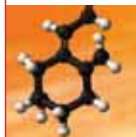
you to flush the bag with clean nitrogen or air to further reduce background levels to meet ppbv analysis requirements.

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 5-Liter gas sampling bags. The bags were filled with clean nitrogen and stored at ambient temperatures for 24 hours; 1-Liter was extracted from each bag and concentrated on a multi-bed thermal desorption tube (Carbotrap-300). The top chromatogram shows the background levels from our new Supel-Inert bags. The middle chromatogram shows background levels from a competitor's bag, and the bottom chromatogram is from the current line of Tedlar bags. The two large peaks are dimethylacetamide and phenol.

Figure 1. Comparison of Background Levels on Supel-Inert Film, Competitor X, and Tedlar





Key Features and Benefits:

- Low VOC and sulfur background levels, there are no detectable background levels of DMAC (Dimethylacetamide) and phenol, common with Tedlar bags
- Inertness properties similar to Tedlar for a wide range of compounds.
 - Suitable for sampling and analysis for most VOC's within 2 days and many sulfur 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 are 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



Sampling Valves



Push-Lock Valve (PLV)
E001171

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 and our Thermogreen LB-2 septum is incorporated into each valve type. Our original valve, the Push-Lock Valve (PLV) is designed with a septum sandwiched between the film and the valve body, so the sample in the bag is not exposed to

the septa, until the septa is punctured to remove the sample. This valve design will not deadhead the sampling pump, causing 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 then 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 center of the bag, and the stem of the valve is perpendicular to the bag surface.



Screw-Cap Valve (SCV)
E001172

Our new valve is a screw cap design. The valve is opened and closed by only turning the cap (not the body) a half turn. Our inert Thermogreen LB-2 septum is in contact with the sample with this valve design. When the valve is closed our septa makes the seal to maintain the integrity of your sample. This screw-cap Valve is securely fastened in the upper 2/3 of the bag, and the stem of the valve is parallel to the bag. This valve is an excellent choice when your application requires replacing septa.

Method Suitability

Like Tedlar, Supelco Inert gas sampling bags are suitable for use for the following methods and applications. When you need to draw a sample with a syringe or make a calibration mix, Supelco Inert is the most suitable option for this purpose due 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)
Vapor Intrusion	DCE, TCE, 1,1,1-TCA, PCE, Benzene, Toluene
Calibration Mixes	Preparation of Gas Phase Standards/ Gas Mixtures

* Supelco Inert film is not recommended for storing hydrogen sulfide

Summary

Supelco Inert Gas Sampling Bags with our exclusive Thermogreen LB-2 septa are an ideal replacement for Tedlar film due 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 and easy access to the product.

+ Related Information

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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 bioanalysis opportunities (**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

Figure 1. Design and Enlarged View of the First Commercial SPME Device Made by Supelco

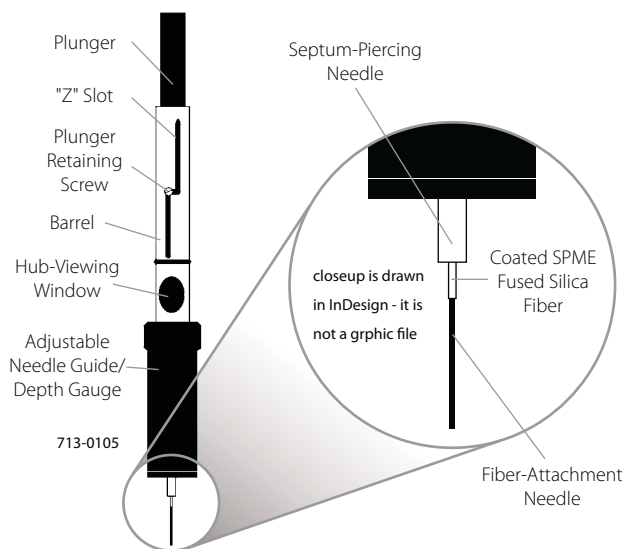
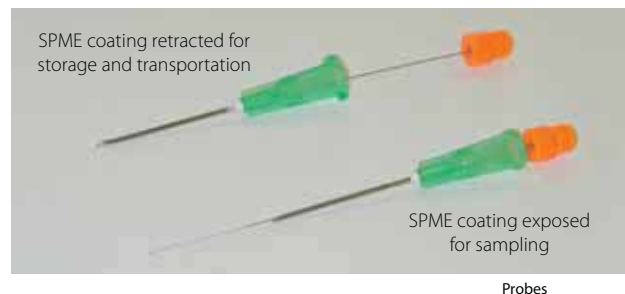


Figure 2. New Biocompatible SPME Devices for Bioanalysis and in vivo Sampling (45 μm thickness, 15 mm length of the coating, Cat. No. 57281-U)



diameter), offering both robustness and an inert support, and the coatings are 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 the conventional SPME devices.

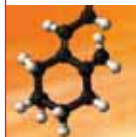
An important advantage of SPME, particularly for on-site sampling, is the possibility of performing analyses without pre-defining a specific sample size. For SPME from small volumes of sample, the amount of an analyte extracted from a sample is given by Equation 1 (1).

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

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 fiber volume and K_{fs} is the analyte distribution constant between the fiber and sample matrix. However, when sample size is large relative to the fiber 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 standardization by pre-loading standards on the SPME device allow rapid pre-equilibrium sampling and control of variability in complex matrices.



Initially, *in vivo* SPME was used to study the pharmacokinetics (PK) of various drugs in directly from the veins of animals. A specialized 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 stabilization 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 minimized, 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. J. Pawliszyn, *Handbook of SPME*, Chemical Industry Press, Beijing, 2009.
2. D Vuckovic, J Pawliszyn, *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 Fiber Probes, pk. of 5	57281-U

Solid Phase Microextraction (SPME) Training Courses

Organized by the inventor of SPME, Prof. Janusz Pawliszyn, and held several times per year, this two-day course covers:

- Introduction to SPME
- Theoretical principles of SPME
- Method development
- Selected aspects of GC analysis for SPME
- Examples of applications
- Future directions

In addition to the lectures, the University of Waterloo course includes 8 hours of laboratory hands-on experiments devoted to method development, analysis of semi-volatile compounds in liquid matrices and the affect of sample volume on analysis results. Advanced experiments can also be arranged for more experienced users.

Upcoming Courses for 2011:

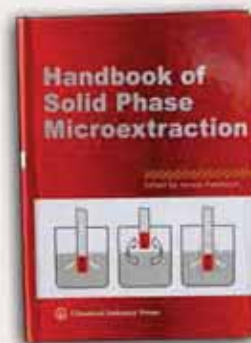
- April 28-29 (Waterloo, ON, Canada) hands-on training
- December 8-9 (Waterloo, ON, Canada) hands-on training
- March, 2012 (Pittcon 2012, Orlando, FL, USA) Lecture component only

For more information contact:

SPME Course, c/o Dr. Barbara Bojko
Department of Chemistry, University of Waterloo
200 University Avenue West
Waterloo, ON N2L 3G1 Canada
Phone: (519) 888-4567 ext.37288
Fax: (519) 746-0435
E-mail: bbojko@uwaterloo.ca
<http://www.science.uwaterloo.ca/chemistry/pawliszyn>

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. The handbook contains 13 chapters with topics including: Theory of SPME, SPME devices and fiber 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.



SPME book

Description	Cat. No.
Handbook of SPME	Z569046



High-Purity Solvents for Sensitive Analysis

Shyam Verma
shyam.verma@sial.com

Solvent impurities are the most common cause of extraneous peaks and unstable baseline. 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 minimizing or eliminating these issues, sensitive tests like LC-MS and GC Headspace require the use of highly pure solvents and additives.

Solvent-derived impurities do not condition out over time. Most common contaminants include inorganic ions, decomposition products, microbes and their excretion products and particulate matter. The solvents designed for use in the sensitive analysis like 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 with your own optimized 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) minimized baseline and artifacts, 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 developed solvents specifically for GC-HS applications. These solvents, microfiltered at 0.2 µm and packed under inert gas, 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 gas chromatography-mass spectrometry (GC-MS). The analysis of impurities in these solvents was performed using solid phase microextraction (SPME) to do a headspace extraction.

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 are manufactured under strictly controlled processes, including micro-filtration and packaging under an inert atmosphere. This ensures their suitability for meeting the demands of headspace analysis. These products are listed on the next page.

Reference

1. K.K.Stenerson and S. Verma, 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
LC-MS CHROMASOLV Mobile Phase Additives	
Trifluoroacetic acid, puriss p.a.	40967
Formic acid, puriss p.a.	56302
Acetic acid, puriss p.a.	49199
Ammonium formate, puriss p.a.	55674

Comparison of Two N,N-Dimethylacetamide Materials for GC Headspace Analysis

Contributed Article

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

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PharmaCore, Inc. High Point, NC

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Introduction

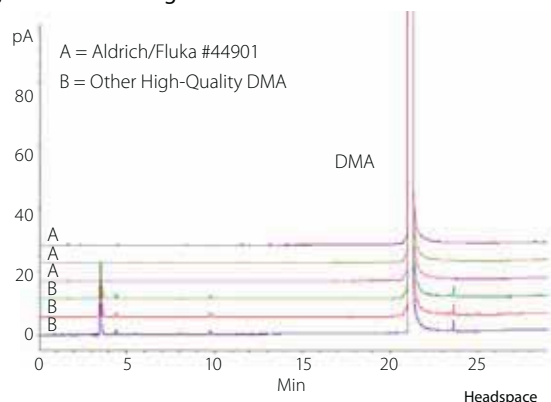
GC headspace analysis for residual solvents in pharmaceuticals is an established practice. ICH guidelines for Class 3 solvents, where permissible levels and thus responses are rather on the larger side, tend to be the most forgiving when it comes to interfering peaks in the baseline. However, when analyses are performed on low responding Class 2 solvents like dimethylformamide, chloroform, or dichloromethane, or on Class 1 solvents, any peaks in the baseline may present analytical issues. For these reasons, many chromatographers prefer to employ the most pure diluents for developing and validating their GC headspace residual solvents methods. One of the most commonly used diluents for GC headspace residual solvent analysis is N,N-Dimethylacetamide (DMA). We undertook to compare in our laboratory two different sources of DMA: Aldrich/Fluka #44901 (GC-HS Grade) and another high-quality DMA from a leading supplier.

Experimental

The following chromatographic conditions were employed for this study. Three separate determinations of each DMA material were made.

Column	DB-624, 30 m, 0.25 mm I.D., 1.4 µm film thickness				
Oven	Initial Temp (°C)	Rate (°C/min)	Final Temp (°C)	Hold Time	Total Time (min)
	35	---	35	6.00	---
	35	5.00	150	0.00	29.00
	35	---	35	3.00	---
Inlet	Mode:		Split		
	Split Flow:		10.0 mL/min		
	Split Ratio:		6.5:1		
	Total Flow:		14.2 mL/min		
	Inlet/Column Pressure:		16 psi		
	Temperature:		250 °C		
Headspace Autosampler	Run Time:		29.00 min		
	Carrier Gas:		Helium		
	Oven Temperature:		90 °C		
	Equilibration Time:		25 min – low shake speed		
	Loop Temperature:		130 °C		
	Transfer Line Temperature:		155 °C		
	GC Cycle Time:		35 min		
	Injection Time:		1.0 min		
	Injection Volume:		1.0 mL		
	Pressurization Time:		0.2 min		
	Vial Pressure:		14 psi		
	Loop Fill Time:		0.2 min		
Loop Equilibration Time:		0.05 min			
Detector	Type:		FID		
	Temperature:		300 °C		

Figure 1. Chromatograms of Two DMA Materials



Results

The chromatograms in **Figure 1** clearly demonstrate the superior quality of the Aldrich/Fluka #44901 DMA. The other high-quality DMA contains several peaks that could interfere with residual solvent peaks of interest. These potentially troublesome peaks at RTs ca. 3.5 min, 4.4 min, 5.4 min, 8.0 min, and 9.8 min are absent in the Aldrich/Fluka DMA #44901.

Conclusion

Our laboratory has chosen to use Aldrich/Fluka #44901 DMA for our GC Headspace residual solvent methods development/validation exercises and routine release methodology where DMA is the preferred sample diluent.

+ Featured Products

Description	Pkg. Size	Cat. No.
N,N-Dimethylacetamide	1 L	44901

+ Related Products

Description	Pkg. Size	Cat. No.
1,3-Dimethyl-2-imidazolidinone	1 L	67484
Cyclohexanone	1 L	68809
1-Methyl-2-pyrrolidinone	1 L	69337
Dimethyl sulfoxide	1 L	51779
N,N-Dimethylformamide	1 L	51781
Water	1 L	53463

For USP residual solvent standards, please refer to page 21.





Innovations in Chiral Chromatography

Overview of Modern Chiral Stationary Phases

Tracy Ascah

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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, cocrystallization, 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.

One of the most specialized areas of chromatography deals with the separation of enantiomers. Since the discovery of optical activity in the early 19th century, materials and techniques have evolved to separate and purify enantiomers. Chromatography has become an important tool for this purpose, 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, like proteins, peptides, and carbohydrates. This is no coincidence. It is because biomolecules can distinguish enantiomers that biological systems recognize 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 are silica particles bonded or coated with native, modified, or mimetic biomolecules.

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® CelluCoat
	tris-(3,5-dimethylphenyl)carbamoyl amylose	Kromasil AmyCoat
Macrocyclic glycopeptide	teicoplanin, teicoplanin aglycone, vancomycin, ristocetin A	Astec CHIROBIOTIC®
Cyclodextrin	β- and γ-cyclodextrins, native and derivatized	Astec CYCLOBOND®
Protein	α1-acid glycoprotein, cellobiohydrolase, albumin (human serum)	Chiral-AGP, Chiral-CBH, Chiral-HSA
Chiral synthetic polymer	poly(trans-1,2-cyclohexanediyl-bis-acrylamide)	Astec P-CAP™
	poly(diphenylethylenediamine-bis-acryloyl)	Astec P-CAP-DP
	O,O'-bis (3,5-dimethylbenzoyl)-N,N'-diallyl-L-tartar diamide	Kromasil Chiral DMB
	O,O'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-L-tartar diamide	Kromasil Chiral TBB
Chiral ligand exchange	chiral bidentate ligand	Astec CLC-L, Astec CLC-D
Cyclofructan	derivatized cyclofructan 6	LARIHC™

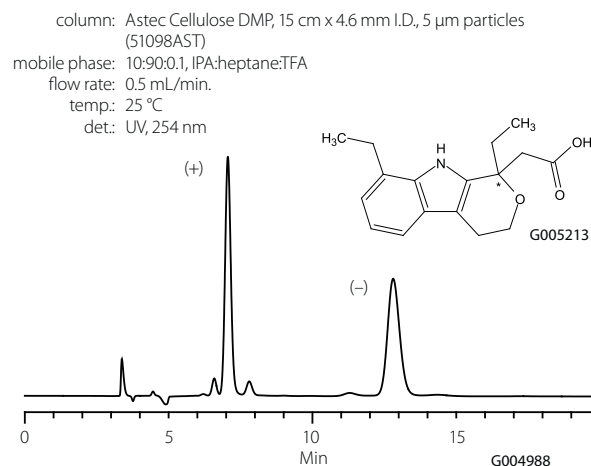
Polysaccharides (cellulose, amylose)



G005297

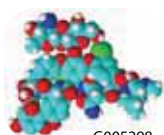
The most popular class of CSPs for HPLC and SFC, the polysaccharides amylose and cellulose are naturally-occurring, optically-active, linear (cellulose) and helical (amylose) polymers comprising hundreds to thousands of D-(+)-glucose units joined by α(1→4) glycosidic (amylose) bonds or β(1→4) glycosidic (cellulose) bonds. The long polysaccharide chains form rope-like bundles held together via multiple hydrogen bonds between proximate hydroxyl groups. Derivatized cellulose- and amylose-based CSPs owe their high enantioselectivity to the large number of chiral centers 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)





Macrocylic Glycopeptides

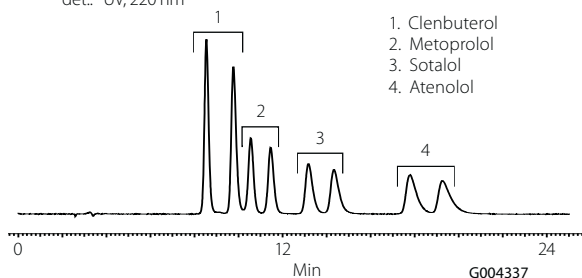


G005298

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 ionizable analytes, and their utility in reversed-phase and LC-MS mobile phases. An example chromatogram is shown in Figure 2. (3)

Figure 2. Macrocylic Glycopeptide Used as a CSP (Enantiomers of β -Blockers)

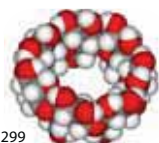
column: Astec CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 μ m particles (12024AST)
mobile phase: 15 mM ammonium formate in methanol
flow rate: 1 mL/min.
temp.: 25 $^{\circ}$ C
det.: UV, 220 nm



- 1. Clenbuterol
- 2. Metoprolol
- 3. Sotalol
- 4. Atenolol

G004337

Cyclodextrins

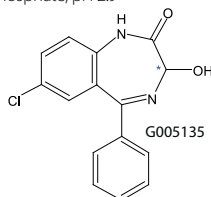
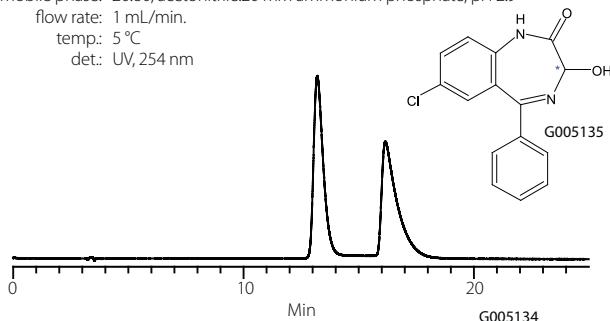


G005299

Cyclodextrins (CDs) comprise D-(+)-glucose residues bonded through α (1 \rightarrow 4) glycosidic linkages. The chair configuration of glucose makes the toroid bucket narrower at one end. Derivatization 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 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)

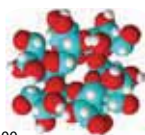
column: Astec CYCLOBOND I 2000 DNP, 25 cm x 4.6 mm I.D., 5 μ m particles (25024AST)
mobile phase: 20:80, acetonitrile:20 mM ammonium phosphate, pH 2.9
flow rate: 1 mL/min.
temp.: 5 $^{\circ}$ C
det.: UV, 254 nm



G005135

G005134

Cyclofructans

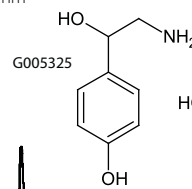
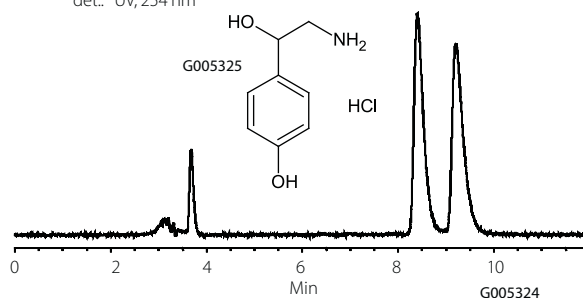


G005300

Cyclofructans are the newest class of CSPs. They comprise six or more β (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 (AZYP 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 $^{\circ}$ C
det.: UV, 254 nm

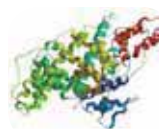


G005325

HCl

G005324

Proteins

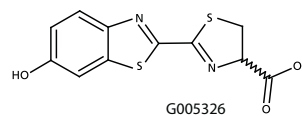
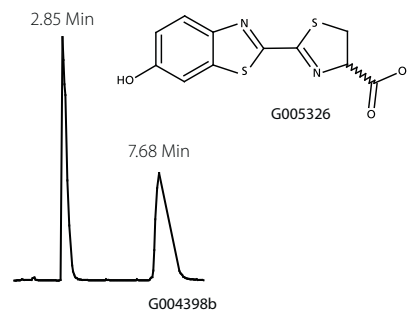


G005301

Proteins contain a large number of chiral centers 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 (AGP) Used as a CSP (Luciferin Enantiomers)

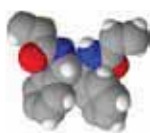
column: Chiral-AGP, 10 cm x 4 mm I.D., 5 μ m particles (58150AST)
mobile phase: 10 mM sodium phosphate, pH 6.0
flow rate: 0.9 mL/min.
temp.: 25 $^{\circ}$ C
det.: UV, 225 nm



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G004398b

Chiral Synthetic Polymers

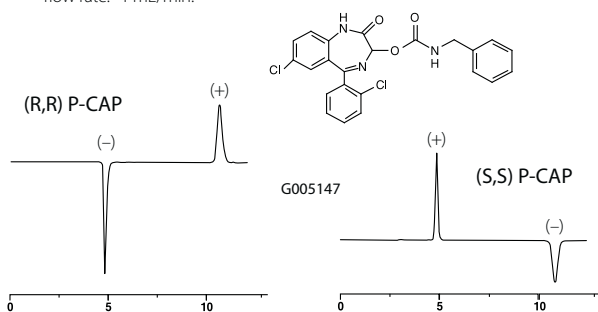


Synthetic CSPs have a defined structure and controlled degree of polymerization, and mitigate certain drawbacks associated with natural compounds. Most comprise a thin, ordered layer of chiral polymer covalently

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 (Furoin 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

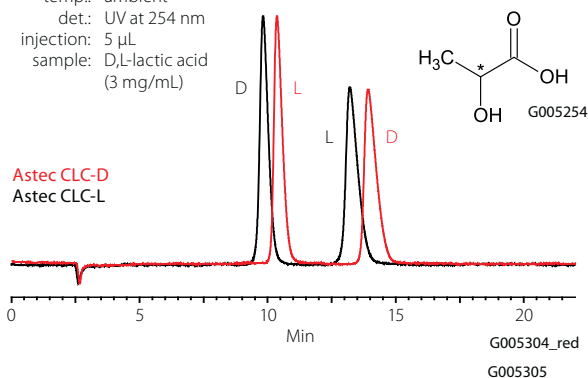


Copper ions in the mobile phase coordinate with the chiral selector on the stationary phase (a small, chiral bidentate ligand)

and carboxylic acid functional groups on the analytes to form transient diastereomeric complexes in solution. Analytes include alpha-hydroxy acids, like lactic, malic, tartaric, and mandelic acids, amino acids, other amines and bifunctional racemates, like 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.

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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



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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.

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Description	Pkg Size	Cat. No.
USP 467 Class 1 Residual Solvents Mix 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		

Description	Pkg Size	Cat. No.
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.....	36600 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.....	18400 µg/mL	p-Xylene.....	1520 µg/mL
Methanol.....	1500 µg/mL		

Description	Pkg 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

Description	Pkg Size	Cat. No.
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

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+ Featured Products

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Aldicarb	100 mg	33386
Carbendazim-d ₃	10 mg	32413
Cyflufenamid	25 mg	32403
Etozazole	50 mg	32506
Isofenphos-methyl	50 mg	33436
Methoxychlor	100 mg	36161
TEPP	50 mg	32434

eVol® Hand-Held Automated Analytical Syringe



E001177

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:

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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-analyzed.

Touch Wheel Control

A full-color 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 and prompts make programming and use effortless.



Full-Color Display

Menu-driven
Touch Wheel
Control

IM-2910005...




XCHANGE Analytical Syringes

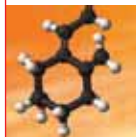
XCHANGE analytical syringes can be easily and quickly changed. This allows the user to choose the best syringe for the volume being measured. It also allows the user to dedicate individual syringes to specific liquids or methods, reducing the possibility of cross-contamination. Only three XCHANGE syringes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



3 Syringes

Table 1. Syringe Capacity Chart

Syringe Capacity (μL)	5	50	100
Color 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%



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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
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- Precise dispensing of liquids
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eVol Electronic Syringe Starter Kit	1	29841-U
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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

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Replacement Needles for 5 µL Syringes					
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Needle	25	70	bevel tip (#2)	5	29862-U
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Replacement Needles for 50 µL Syringes					
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Needle	23	50	bevel (#2)	5	29864-U
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