

Reporter

Volume 26.3



HybridSPE™ - Precipitation Technology Bridging the Gap between Simplicity and Selectivity in Pharma Bioanalytical Sample Preparation



A new sample prep platform that combines the simplicity of protein precipitation and the selectivity of SPE for the targeted removal of proteins and phospholipids

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



Wayne K. Way

Market Segment Manager

NEW Feature!



Contributed Article

"Contributed Articles" are submitted by our customers based upon their work with Sigma-Aldrich products.

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Dear Colleague,

Improving speed and throughput of analysis has driven recent advances in HPLC instrumentation, columns, and accessories. These advances include instrumentation capable of higher pressure operation, smaller particle columns for improved efficiency, and sample preparation devices to simplify analysis. While many of these advances have been accepted into an R&D setting, some changes are still too costly from a capital expenditure as well as a training perspective to implement and transfer to a production or manufacturing environment.

Supelco has been applying a customer-centric approach to its innovative new products over the last several years. Our goal is to provide products and services that meet the challenging needs of the HPLC lab, without requiring the laboratory to be re-equipped from the ground up.

Some recent innovative products and services introduced to the HPLC market include:

- Ascentis® Express with Fused-Core™ particle technology that delivers high speed and resolution to any HPLC system
- High Performance HPLC Fittings/Interconnects that offer low dispersion and a high pressure rating with ease-of-use
- HybridSPE Precipitation technology that bridges the gap between simplicity and selectivity in bioanalytical sample prep
- Chiral Screening service that offers an efficient means of determining the best method for chiral separation
- HPLC seminars hosted on the web and available anytime, just click to start

While we are excited about the recent advances in HPLC, we continue to explore new ways of improving the products and services offered by Supelco.

Regards,

Wayne K. Way

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Introducing HybridSPE™-Precipitation Technology for Pharmaceutical Bioanalytical Sample Preparation

Craig Aurand, An Trinh, Michael Ye and Charles Mi
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In pharmaceutical bioanalysis, researchers develop and run various assays to quantitate drugs, pharmaceutical candidates, and their metabolites in biological fluids such as serum and plasma. The data resulting from these assays are used to help determine the pharmacodynamic and pharmacokinetic properties as well as the toxic and therapeutic concentrations of existing and emerging

Features & Benefits:

- Merges both Protein PPT & SPE
- Offers simplicity & generic nature of protein PPT

PLUS

- Selectivity approaching SPE via the targeted removal of phospholipids
- 2-3 step generic procedure
- 100% removal of phospholipids & precipitated proteins
- Minimal to no method development
- Available in 96-well and 1 mL cartridge dimensions
- Patent pending technology

pharmaceutical compounds in living cells, tissues, and animals. Although advances in Liquid Chromatography-Mass Spectrometry (LC-MS) technology have reaped overwhelming benefits in terms of increased throughput and sensitivity, good sample preparation

continues to be a critical component of bioanalysis.

The three most common sample prep techniques used in bioanalytical sample prep are protein precipitation (protein PPT), liquid-liquid extraction (LLE), and solid phase extraction (SPE). Each technique offers unique advantages and disadvantages that are considered during the method development process. For example, protein precipitation methods are simple (2-3 steps), fast, and often require minimal method development. However, the technique offers minimal selectivity as it only removes gross levels of protein from a sample prior to analysis. In contrast, SPE offers significant benefits in terms of selectivity/sample cleanup, but the technique often requires moderate to extensive levels of expertise and time for adequate method development. In addition, SPE often requires multiple steps (5-8), resulting in increased assay time.

In this report, we introduce a new sample prep platform trademarked HybridSPE-Precipitation (HybridSPE-PPT) in which we merge two predominate techniques in bioanalytical sample prep: protein precipitation and SPE. The end result is a technique that offers the advantages of both approaches while minimizing their disadvantages.

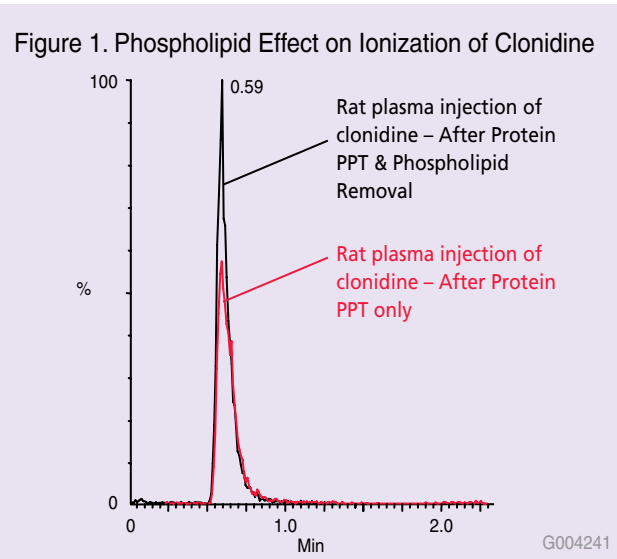
Ion-Suppression & Phospholipid Contamination

Excessive background from endogenous matrix components has always been a great concern in quantitative bioanalysis, and has become paramount with decreasing analytical run times. In bioanalytical mass spectrometry, the issue of excessive background contributes to the growing problem of ion-suppression.

Ion-suppression is caused by one or more interfering components or species that co-elute with the analyte(s) of interest during LC-MS analysis and manifests itself as a loss of analyte response. These co-eluting species can affect droplet formation or ionize concurrently resulting in an erroneous decrease (suppression) or increase (enhancement) in signal response. Ion-suppression often leads to poor assay reproducibility, accuracy, and sensitivity, and such deleterious effects are often most notable at the lower limits of quantitation (LLOQ) (1).

One of the major causes of ion-suppression in bioanalysis is the presence of phospholipids during LC-MS or LC-MS-MS analysis in the positive ion electrospray mode (+ESI) (2). Phospholipids are the second largest lipid component in biological matrices after triglycerides, and are typically present in extremely high concentrations in biological plasma samples. Figure 1 compares the LC-MS chromatograms of two clonidine spiked rat plasma samples processed by protein precipitation (100 µL spiked plasma + 300 µL 1% formic acid in acetonitrile) alone and protein precipitation followed by phospholipid removal. The black trace chromatogram shows the response of clenbuterol

(continued on page 4)



(continued from page 3)

after protein precipitation and phospholipid removal. The red trace chromatogram was subjected to protein precipitation only. By removing phospholipid interferences prior to analysis, response for clonidine was nearly doubled.

How Does HybridSPE-PPT Technology Work?

HybridSPE-PPT technology is a simple and generic sample prep platform designed for the gross level removal of endogenous protein and phospholipid interferences from biological plasma and serum prior to LC-MS or LC-MS-MS analysis. Biological plasma or serum is first subjected to protein precipitation via the addition and mixing of acidified (with formic acid) acetonitrile. Precipitated proteins are then removed by centrifugation and the resulting supernatant is loaded on the HybridSPE-PPT 96-well plate or cartridge which acts as a chemical filter that specifically targets the removal of endogenous sample phospholipids. The 96-well version contains a series of low porosity hydrophobic filters/frits, the packed-bed filter/frit assembly acts as a depth filter facilitating the concurrent removal of both phospholipids and precipitated proteins during the extraction process. The phospholipid retention mechanism is based on a highly selective Lewis acid-base interaction between the proprietary zirconia ions functionally bonded to the HybridSPE-PPT stationary phase and the phosphonate moiety consistent with all phospholipids. The resulting eluent is ready for immediate LC-MS or LC-MS-MS analysis.

An alternative “In-Well Precipitation” method is available for the HybridSPE-PPT 96-well version in which biological plasma/serum is first added to the 96-well plate followed by acidified acetonitrile (precipitation agent). After a brief mixing/vortexing step, vacuum is applied to the 96-well plate. Figure 2 visually depicts the HybridSPE-PPT process (“In-Well Precipitation”) and describes how phospholipids are removed.

How are Phospholipids Selectively Removed?

Once the plasma/serum sample is subjected to protein precipitation via the addition of 1% formic acid diluted in acetonitrile, it is passed through the HybridSPE-PPT packed bed. The packed bed consists of a proprietary zirconia coated silica particle. The zirconia sites exhibit Lewis acid (electron acceptor) properties that will interact strongly with Lewis bases (electron donor). Phospholipids structurally consist of a polar head group (zwitterionic phosphonate moiety) and a large hydrophobic tail (two fatty acyl groups that are hydrophobic). The phosphate group inherent with all phospholipids acts as a very strong Lewis base that will interact strongly with zirconia atoms functionalized on the particle surface (Figure 3).

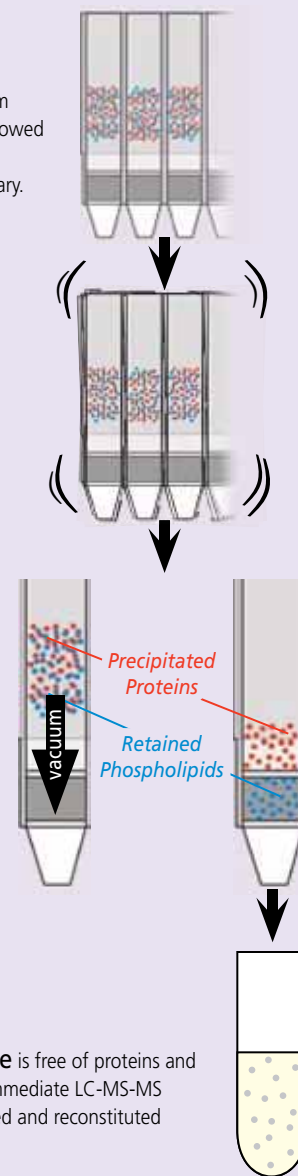
Figure 2. HybridSPE-PPT “In-Well” 96-well Precipitation Method and Phospholipid Removal

1) **Precipitate Proteins** by adding 100 μ L plasma or serum to the HybridSPE-PPT plate followed by 300 μ L 1% formic acid in acetonitrile. Add I.S. as necessary.

2) **Mix** by vortexing/shaking HybridSPE-PPT plate or by aspirating/dispensing with 0.5-1 mL pipette tip (e.g., TOMTEC Quadra liquid handler)

3) **Apply vacuum.** The packed-bed filter/frit assembly acts as a depth filter for the concurrent physical removal of precipitated proteins and chemical removal of phospholipids. Small molecules (e.g., pharma compounds and metabolites) pass through unretained.

4) **Resulting filtrate/eluate** is free of proteins and phospholipids and ready for immediate LC-MS-MS analysis; or it can be evaporated and reconstituted as necessary prior to analysis



Note: The presence of $\geq 1\%$ formic acid in the acetonitrile precipitation agent is critical because:

- 1) Formic acid is a stronger Lewis base than most carboxyl (-COOH) groups found in acidic pharmaceutical compounds. As a result, formate ions will tie up the phase's zirconia ions minimizing retention of acidic analytes of interest. Because formate is not a strong enough Lewis base to displace the phosphate moiety found in phospholipids, phospholipids preferentially retain on the HybridSPE-PPT phase.
- 2) The low pH environment induced by formic acid neutralizes residual silanol activity on the silica surface thereby eliminating secondary cation-exchange interaction with basic compounds of interest.

Figure 3. Lewis Acid Base Interaction Between Hybrid SPE Zirconia Ions and Phospholipids

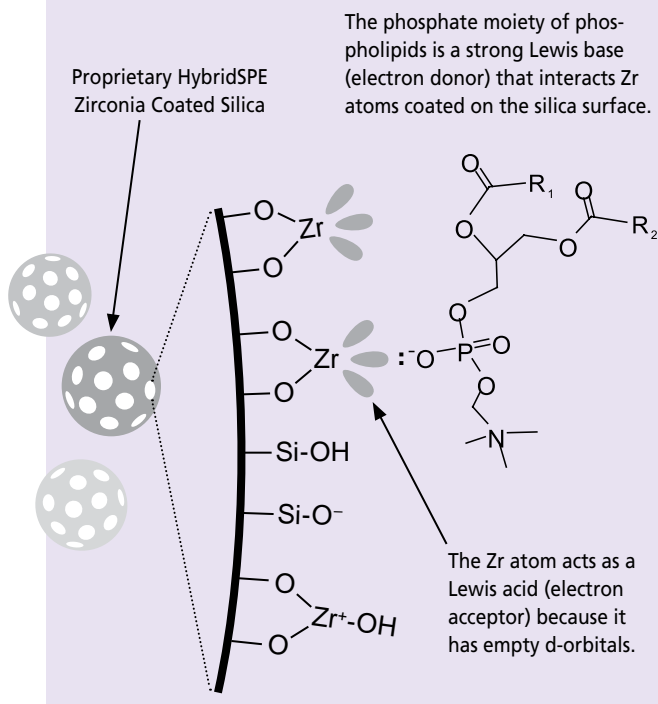
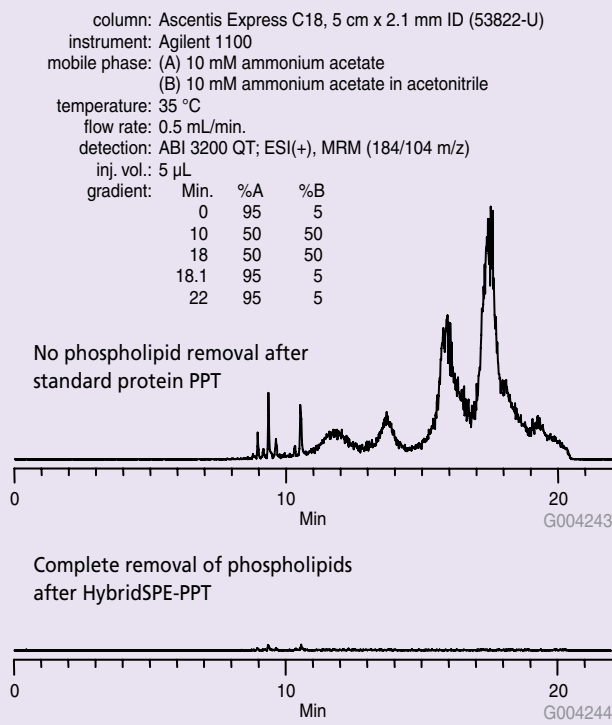


Figure 4. Efficiency of Phospholipid Removal of HybridSPE-PPT Technology (575656-U) - MRM 184 and 104 m/z



To demonstrate the efficiency of phospholipid removal using HybridSPE-PPT technology, 100 µL of blank rat plasma was subjected to protein precipitation via the addition of 1% formic acid in acetonitrile followed by 1 min. of vortex and centrifugation. A second set of rat plasma samples were processed using the HybridSPE-PPT procedure described in Figure 2. The resulting supernatant (standard protein PPT) and filtrate/eluent (HybridSPE-PPT) was analyzed via LC-MS specifically monitoring for phospholipids (184 /104 m/z). These transition ions represent trimethylammonium-ethyl phosphate MS fragment consistent between the major phospholipids (e.g., phosphatidylcholine) found in plasma (2). In Figure 4, samples processed using HybridSPE-PPT resulted in 100% removal of phospholipids from 100 µL of rat plasma. In contrast, standard protein precipitation yielded high levels of phospholipid contamination which can potentially co-elute with analytes of interest or build up on the column and elute uncontrollably during a given injection sequence. This is especially problematic as analysts strive for shorter analytical run times through the use of smaller column dimensions and particle sizes.

Comparison of HybridSPE-PPT, Protein Precipitation, and SPE

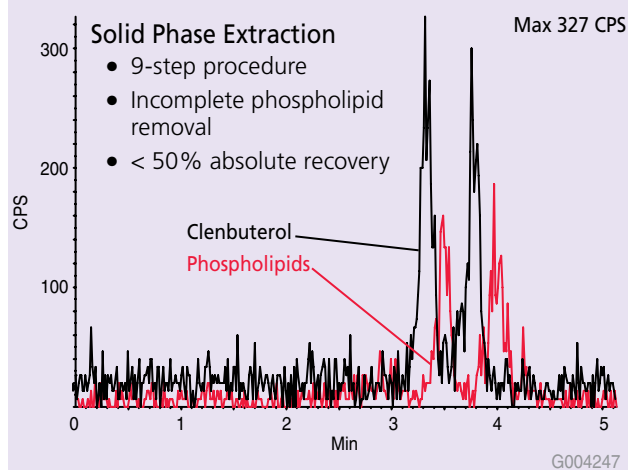
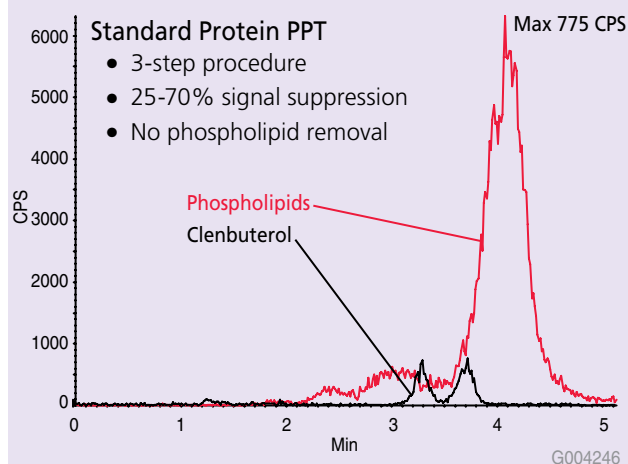
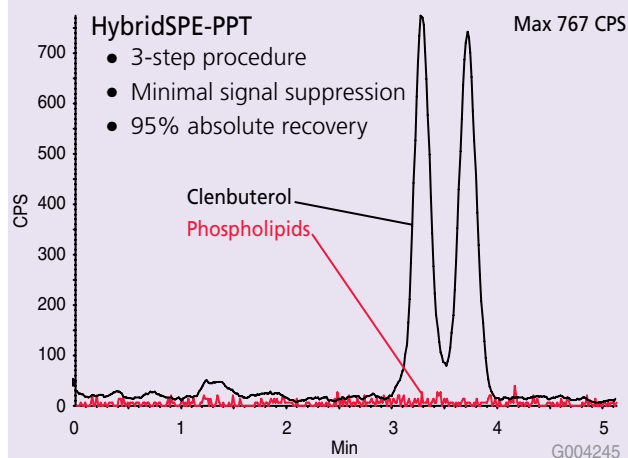
In this application example, rat plasma samples were spiked with clenbuterol (R(-) and S(+)) enantiomers at the level of 10 ng/mL and extracted using three different procedures: HybridSPE-PPT, Protein PPT, and a 9-step SPE procedure optimized for trace level clenbuterol analysis. The analysis was performed using a chiral stationary phase containing a macrocyclic glycopeptide covalently bound to silica and detection via MS-MS. Comparisons of sample preparation methods were made in terms of the amount of phospholipids in the sample extract and the overall effect on signal response of clenbuterol enantiomers. Absolute recovery was determined against an external standard.

Representative chromatograms of each the sample prep techniques are depicted in Figure 5. From the results indicated in Figure 5, phospholipid contamination levels were highest for protein precipitation resulting in signal suppression levels 70 and 25% for the R(-) and S(+) enantiomers of clenbuterol, respectively. For the SPE procedure, phospholipid contamination was still evident after multiple wash steps, and overall absolute recovery was less than 50%. In contrast, HybridSPE-PPT offered 100% removal of phospholipids resulting in absolute recovery levels of 95%.

(continued on page 6)

Figure 5. Comparative Extraction and LC-MS-MS of 10 ng/mL Clenbuterol (R-) and S(+) enantiomers) in Rat Plasma

column: Chirobiotic T, 10 cm x 2.1 mm, 5 μ m (12018AST)
 instrument: Agilent 1100
 mobile phase: 10 mM ammonium formate in methanol
 temperature: 30 $^{\circ}$ C
 flow rate: 0.3 mL/min.
 detection: ABI 3200 QT; ESI(+), MRM: 184/104 m/z (phospholipids)
 and 277.2/203.1 (clonidine)
 inj. vol.: 10 μ L



HybridSPE-PPT Plate and Vacuum Manifold



(continued from page 5)

Conclusion

In this report, a new sample prep platform specifically designed for pharmaceutical bioanalysis was introduced. The technique, trademarked HybridSPE-Precipitation or HybridSPE-PPT, merges the simplicity of protein precipitation with the selectivity of SPE for the targeted removal of endogenous proteins and phospholipids from biological plasma for subsequent LC-MS analysis. Examples applications demonstrate the chromatographic impact of phospholipids and how its presence can result in signal suppression during MS quantitation. When compared with traditional sample prep techniques such as protein precipitation and solid phase extraction for the extraction of clenbuterol enantiomers from rat plasma, HybridSPE-PPT offered complete phospholipid removal resulting in excellent recovery, minimal signal suppression, and improved S/N ratios. In contrast, lower recovery and higher signal suppression was evident using the traditional sample prep techniques such as protein precipitation and solid phase extraction.

References

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2. Little et al., Journal of Chrom B, 833 (2006), 219-230

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Description

HybridSPE-Precipitation
 96-well Plate, 50 mg/well, pk. 1
 Cartridge, 30 mg/1 mL, pk. 100

Cat. No.

575656-U
 55261-U

! Related Information

For more information on HybridSPE-Precipitation technology, please visit sigma-aldrich.com/hybridspe-ppt

Class-Selective Enantiomeric Separation of β -Androgenic Receptors using CHIROBIOTIC™ T Stationary Phase

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Introduction

The inherent chirality of bio-organic molecules and its affect on biological activity creates a growing need for chiral separations (1). The environmental, food and beverage, and pharmaceutical industries have a need for enantiomerically pure compounds to determine safety, efficacy, and toxicology of products and contaminants. Understanding the role of chirality in pharmacology is extremely important in light of the tragedy of thalidomide use in the 1960s. While one enantiomer of the racemate treated morning sickness, the other caused birth defects and malformity. This event as well as other studies has increased the understanding of chirality in the life sciences. In 1992, the FDA formalized its position on the development of new chiral drugs, now requiring racemate studies to be performed.

Pharmacokinetic, metabolic, and racemic inversion studies are important in the drug development process. Furthermore, with increased pressure for more comprehensive clinical studies, methods that are selective for a class of compounds become attractive. The combination of a class-selective separation with mass spectroscopy provides an informative, high throughput method with the sensitivity necessary for clinical applications. It also has applications in toxicological screenings.

For this study, we chose β -androgenic receptors as a class of pharmaceutically active compounds. β -androgenic receptors mediate metabolic processes and are important in cardio and pulmonary functions. β -blockers have shown beneficial effects in treating cardiac disorders and tremor, while β -agonists have been used to treat asthma and pulmonary disorders (2). Some β -agonists, like clenbuterol, have been shown to increase muscle mass and decrease adipose tissue. This has made β -agonists popular as a performance enhancer. Moreover, the controversial use in raising livestock has increased concern over contaminated food sources (3).

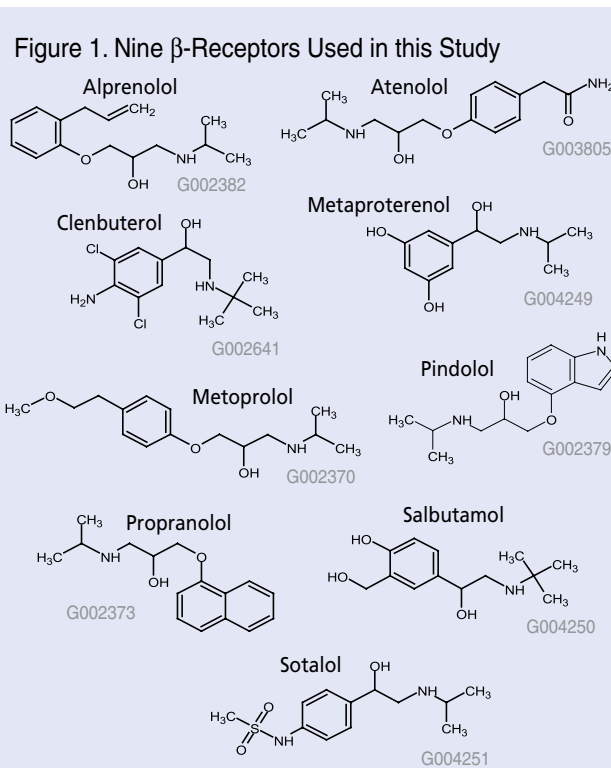
β -androgenic receptors have in common an alcohol functionality in the beta position relative to a secondary amine (Figure 1). The chiral center of these molecules is located at this site, which is the key interaction for selectivity on a chiral stationary phase. The CHIROBIOTIC T stationary phase was chosen for this study. The CHIROBIOTIC T stationary phase is a teicoplanin macrocyclic

glycopeptide bonded silica phase that is capable of ionic, π - π , and hydrogen bonding interactions for the separation of enantiomers. CHIROBIOTIC T has unique selectivity for a number of classes of molecules including carboxylic acids, phenols, neutral aromatic analytes, and cyclic aromatic and aliphatic amines. Moreover, the CHIROBIOTIC T provides chiral separation using reversed-phase solvent mobile phases that are preferred by analytical chemists. Such mobile phases allow for easy transfer to MS (mass spectrometric) as well as ELSD (evaporative light scattering detectors).

Experimental

This application was developed on a Hitachi® L-2300 chromatograph with UV detection using a CHIROBIOTIC T, 25 cm x 4.6 mm I.D. column, 5 μ m silica. The mobile phase was 15 mM ammonium formate in methanol with a column temperature of 25 °C. Atenolol, clenbuterol, metoprolol, and sotalol at a concentration of 1.0 mg/mL were separated under these conditions (Figure 2). The method was then transferred to an Agilent® 1100 equipped with an Applied Biosystems 3200 Q-Trap mass spectrometer run in multiple reaction-monitoring (MRM) mode. The nine β -agonists and blockers from Figure 1 were analyzed using the same chromatographic conditions.

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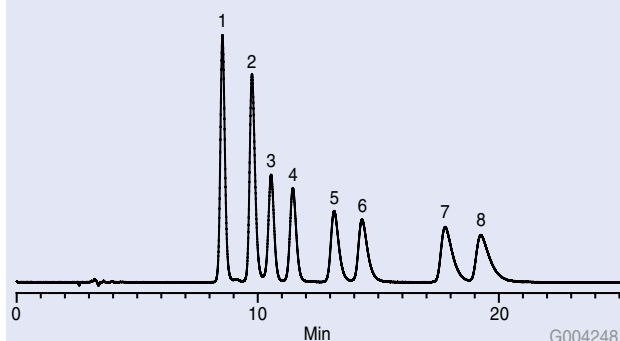
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Liquid Chromatography

SUPELCO
Analytical

Figure 2. Analysis of Four β -Receptor Compounds using CHIROBIOTIC T in Polar Ionic Mode

column: CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 μ m particles
 mobile phase: 15 mM ammonium formate in methanol
 flow rate: 1.0 mL/min
 temp.: 25 $^{\circ}$ C
 det.: UV at 220 nm
 injection: 3 μ L
 sample: 1.0 mg/mL in methanol



(continued from page 7)

Figure 2 shows the UV chromatogram of the separation of four beta-receptor compounds. Clenbuterol elutes first followed by the metoprolol, sotalol, and atenolol. These compounds demonstrate the potential for class selective enantiomeric separation with baseline resolution. The specific conformation of each enantiomer peak was not determined. Table 1 provides chromatographic data including retention times, capacity factor, and selectivity for enantiomer pairs.

Adjustments can be made to flow rate, buffer strength, temperature, and mobile phase composition based on the requirements of the analysis. Typically in this mode, decreased flow rate enhances residence time between the analyte and stationary phase thereby increasing resolution. A decrease in temperature seems to provide interaction, leading to increased retention and resolution. Changes in buffer strength affect counter ion interaction with the stationary phase. Higher buffer strength decreases retention, while lower buffer strength increases retention. The addition of water to the mobile phase affects polarity of the mobile phase and changes the solvation of analytes and stationary phase. In some cases, this has shown a decrease in retention, but an increase in enantiomeric resolution. Decreased injection volume and loading often show better resolution.

The capability of class selective enantiomeric separation was further explored by utilizing mass spectrometric detection. LC-MS provides enhanced sensitivity as well as MS selectivity. Table 2 provides a summary of results from the analysis of nine β -receptor compounds by mass spectrometry. The extracted ion chromatograms for each mass transition are shown in Table 2. The retention times

Table 1. Peak Descriptions from UV Spectrum of Four Beta Receptor Compounds

No.	Name	t_R	k'	Selectivity
1	clenbuterol	8.5	2.4	1.20
2	clenbuterol	9.8	2.9	
3	metoprolol	10.6	3.2	1.13
4	metoprolol	11.5	3.6	
5	sotalol	13.2	4.3	1.10
6	sotalol	14.3	4.7	
7	atenolol	17.8	6.1	1.10
8	atenolol	19.3	6.7	

for each peak, the capacity factor, and selectivity between enantiomer peaks are also given. All of the compounds have been identified and each enantiomeric pair separated with good selectivity. The chromatographic conditions are identical to those in Figure 2, with only the sample concentrations and injection volumes varying based on the detector.

The column used in both analyses is a 25 cm x 4.6 mm I.D. at a flow of 1 mL/min. This is a much larger column than is typically used in an LC-MS analysis. Source gas and capillary adjustments were optimized for this higher flow rate. This method shows the direct transfer of chromatographic conditions between detection techniques, but the LC-MS analysis could easily have been transferred to a smaller column length and internal diameter at a lower flow rate. While a direct transfer may not always be possible, the use of UV detection for chromatographic method development is useful before optimization of LC-MS conditions for trace analysis.

Conclusion

This application demonstrates a chiral separation in a mobile phase system preferred with LC-MS analysis. The separation of compounds with similar functionality and pharmacological properties by one method has many benefits for clinical and toxicological applications. It increases sample throughput and has increased sensitivity with MS detection. Future experiments with column dimension may lead to faster LC-MS separations for chiral analytes.

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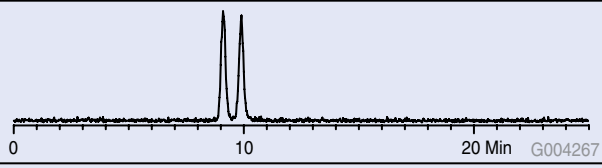
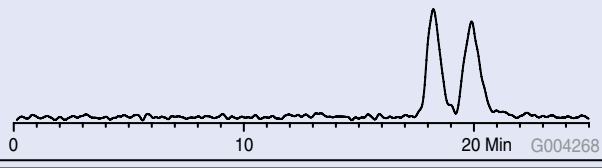
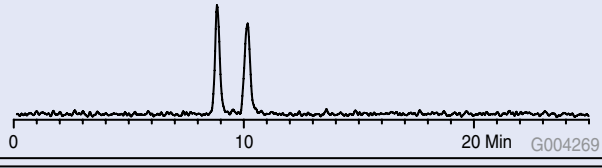
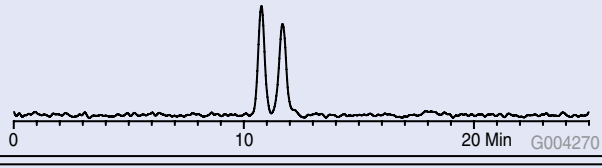
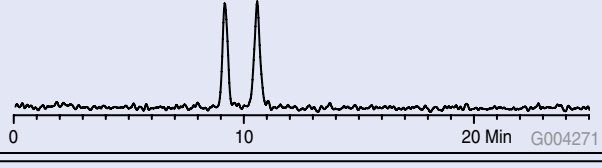
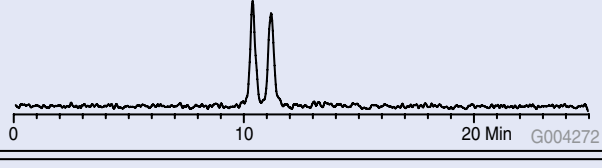
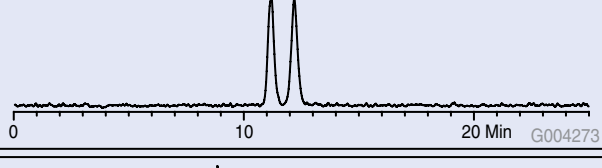
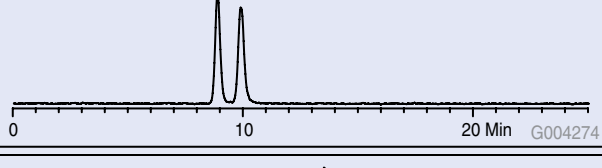
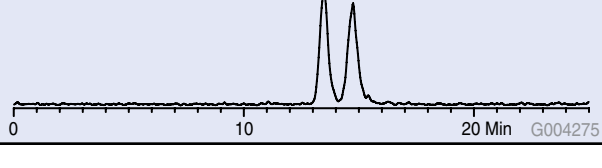
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Description	Cat. No.
Chirobiotic T HPLC Column 25 cm x 4.6 mm I.D.	12024AST

Table 2. Individual Extracted Ion Chromatograms from Analysis of Nine β -Receptor Compounds by LC-MS on CHIROBIOTIC T

column: CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 μ m particles
 mobile phase: 15 mM ammonium formate in methanol
 flow rate: 1 mL/min
 temp.: 25 $^{\circ}$ C
 det.: mass spectrometer in multiple MRM mode
 injection: 5 μ L
 sample: 100 ng/mL in methanol

Analyte	Extracted Ion Chromatogram	MRM	t_r	k'	Selectivity
Alprenolol		250/91	9.12 9.92	2.04 2.31	1.13
Atenolol		267/145	18.22 19.87	5.07 5.62	1.11
Clenbuterol		278/204	8.76 10.10	1.92 2.37	1.23
Metoprolol		268/56	10.77 11.68	2.59 2.89	1.12
Metaproterenol		212/152	9.12 10.54	2.04 2.51	1.23
Pindolol		249/116	10.36 11.16	2.45 2.72	1.11
Propranolol		260/116	11.19 12.17	2.73 3.06	1.12
Salbutamol		240/148	8.86 9.87	1.95 2.29	1.17
Sotalol		273/133	13.49 14.75	3.50 3.92	1.12

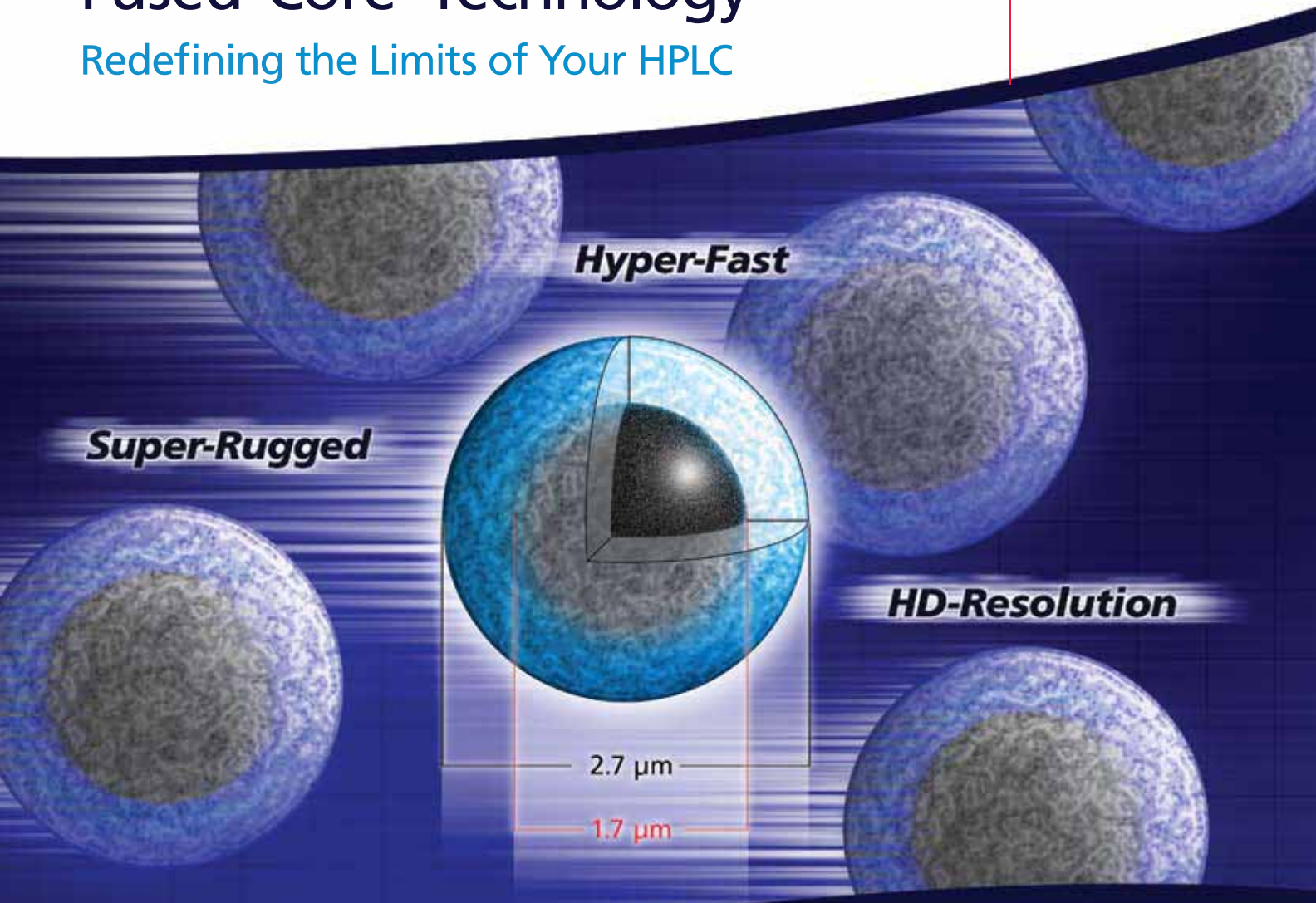
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Volume	Needle OD/ID (inches)	Pk. Size	Cat. No.
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100 µL	0.029/0.005	1	22270-U
250 µL	0.029/0.012	1	22271
500 µL	0.029/0.012	1	22272
1 mL	0.029/0.012	1	22273
2 mL	0.029/0.012	1	22274
5 mL	0.029/0.012	1	22275
10 mL	0.029/0.012	1	22276

Replacement Needles

Volume	Needle Style	Pk. Size	Cat. No.
25-100 µL	Bevel, open end	3	22298-U
250 µL-10 mL	Bevel, open end	3	22299
250 µL-10 mL	Side port, taper	3	22289

Replacement Plunger Tips

Volume	Pkg Size	Cat. No.
500 µL	2	21950
1 mL	2	21951
2 mL	2	21952-U
5 mL	2	21953-U

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Accessories

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Supelco SLB-IL100 Ionic Liquid Capillary GC Column

Polarity/Selectivity Benefits Without the Penalty of Low Maximum Temperature

Katherine K. Stenerson
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Introduction

In our last Reporter (1), we introduced an exciting new phase technology using ionic liquids. Our first column in this line, the SLB-IL100, is similar in polarity to 1,2,3-tris(2-cyanoethoxypropane) (TCEP), one of the most polar stationary phases currently available. The benefits the SLB-IL100 offers is its significantly higher maximum temperature of 230 °C vs. 140 °C for the TCEP, and improved phase stability. These characteristics allow “TCEP-like” polarity to be utilized in applications requiring a column with a maximum temperature higher than 140 °C, while providing an alternative selectivity to currently available polar columns. In this article, we will compare the selectivity of the SLB-IL100 to “traditional” polar capillary GC columns for applications, fatty acid methyl esters (FAMES), and PCB congeners.

Fatty Acid Methyl Esters on the SLB-IL100 Column

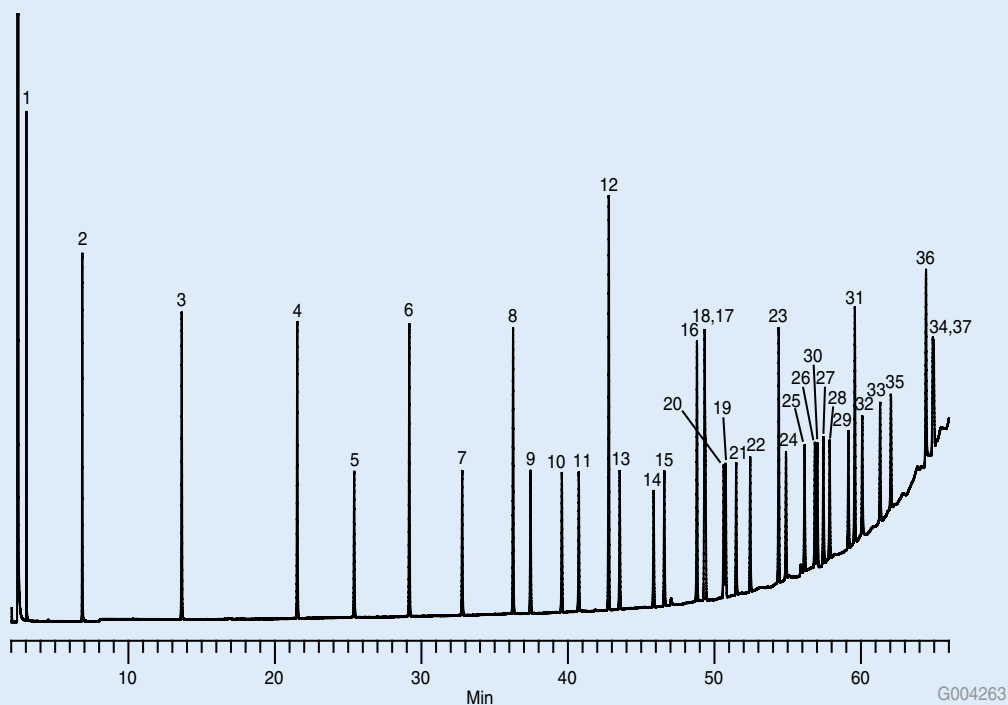
TCEP is not typically used for the analysis of FAMES due to its low temperature limit of 140 °C. There are a variety of columns less polar than TCEP available for the analysis of FAMES, each with distinctive characteristics for this application. For example, the Omegawax™ is a polyethylene glycol (PEG) phase capillary column, which elutes FAMES primarily by chain length and degree of unsaturation (Figure 1). However, this phase does not have the selectivity to resolve all groups of geometric isomers. For example, in Figure 1, C18:1n9 cis and trans coelute. For resolving geometric isomers, phases with higher polarity than PEG-based are used. The SLB-IL100 falls under this classification, and exhibits different selectivity towards FAMES than the PEG-based Omegawax. The same FAME mixture shown on the Omegawax in Figure 1 was run under similar conditions on an SLB-IL100 column (Figure 2). The C18:1n9 geometric isomers were resolved, and differences in elution order were

Peak IDs for Methyl Esters in Figures 1 and 2

1. C4:0 at 4 wt %
2. C6:0 at 4 wt %
3. C8:0 at 4 wt %
4. C10:0 at 4 wt %
5. C11:0 at 2 wt %
6. C12:0 at 4 wt %
7. C13:0 at 2 wt %
8. C14:0 at 4 wt %
9. C14:1 at 2 wt %
10. C15:0 at 2 wt %
11. C15:1 at 2 wt %
12. C16:0 at 6 wt %
13. C16:1 at 2 wt %
14. C17:0 at 2 wt %
15. C17:1 at 2 wt %
16. C18:0 at 4 wt %
17. C18:1n9c at 4 wt %
18. C18:1n9t at 2 wt %
19. C18:2n6c at 2 wt %
20. C18:2n6t at 2 wt %
21. C18:3n6 at 2 wt %
22. C18:3n3 at 2 wt %
23. C20:0 at 4 wt %
24. C20:1n9 at 2 wt %
25. C20:2 at 2 wt %
26. C20:3n6 at 2 wt %
27. C20:3n3 at 2 wt %
28. C20:4n6 at 2 wt %
29. C20:5n3 at 2 wt %
30. C21:0 at 2 wt %
31. C22:0 at 4 wt %
32. C22:1n9 at 2 wt %
33. C22:2 at 2 wt %
34. C22:6n3 at 2 wt %
35. C23:0 at 2 wt %
36. C24:0 at 4 wt %
37. C24:1n9 at 2 wt %

Figure 1. 37-Component FAME Mix on the Omegawax 250

column: Omegawax 250, 30 m x 0.25 mm I.D., 0.25 µm (24136)
oven: 50 °C, 3.0 °C/min. to 280 °C
inj.: 240 °C
det.: FID, 280 °C
carrier gas: helium, 40 cm/sec constant
injection: 1 µL, 50:1 split
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride



observed between the two columns, as noted in Table 1. Due to its higher polarity, retention was less on the SLB-IL100 compared to the Omegawax, and elution of the saturated FAMES was generally shifted further in ahead of the unsaturates. For example, C21:0 elutes prior to C20:2 and C20:3n6, and C22:0 elutes prior to C20:3n3 and C20:5n3 on the SLB-IL100, while on the Omegawax these saturates elute after.

PCB Congeners on the SLB-IL100

PCB congeners are hydrophobic, and when analyzed on a non-polar capillary column, such as a poly(5% diphenyl/95% dimethylsiloxane), retention increases with the degree of chlorination, with more overlap between the elution ranges as chlorination increases. The deca-chlorinated congener (decachlorobiphenyl), being the heaviest and most hydrophobic, elutes last. By contrast, on a highly polar column such as the SP-2331, the elution pattern is quite different. The analysis of a mixture of mono-chlorinated through deca-chlorinated congeners on the SP-2331 is presented in Figure 3. While dispersive interaction is the primary mechanism exhibited by non-polar columns, separation using a polysiloxane phase with a

high biscyanopropyl content, such as the SP-2331, will be governed by additional mechanisms such as strong dipole-induced dipole interactions. As a result, the deca-chlorinated congener is not as retained on this phase. The deca-chlorinated congener, #209, actually elutes prior to several other congeners. The same mixture on the SLB-IL100, under the same analysis conditions, is presented in Figure 4. Notice that, similar to the SP-2331, #209 elutes prior to several other congeners. However, the higher polarity of the SLB-IL100 has resulted in shorter elution time than the SP-2331 for all congeners. In addition to a shorter elution time, some elution order differences were observed between the two columns, as summarized in Table 2. The structure of PCB congeners suggests that they are capable of dipole and π interactions. While dipole-induced dipole interactions are likely the predominant contributor to selectivity for the SP-2331, the ionic liquid used in the SLB-IL100 has the additional capability of interacting with the congeners' π electrons, thus providing it with different selectivity than the SP-2331.

(continued on page 14)

Figure 2. 37-Component FAME Mix on the SLB-IL100

column: SLB-IL100, 30 m x 0.25 mm I.D., 0.20 μ m (28884-U)
oven: 50 °C, 3.0 °C/min. to 240 °C
inj.: 240 °C
det.: FID, 240 °C
carrier gas: helium, 40 cm/sec constant
injection: 1 μ L, 50:1 split
sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

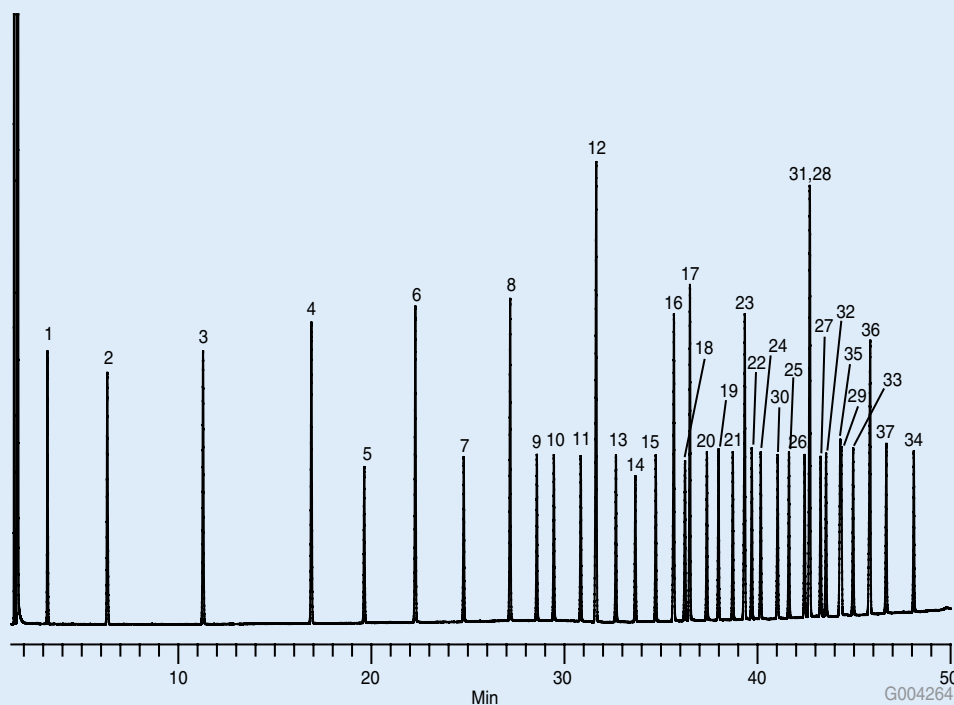


Table 1. Elution Order Differences of FAMES on the Omegawax and the SLB-IL100

Omegawax	SLB-IL100
C4:0	C4:0
C6:0	C6:0
C8:0	C8:0
C10:0	C10:0
C11:0	C11:0
C12:0	C12:0
C13:0	C13:0
C14:0	C14:0
C14:1	C14:1
C15:0	C15:0
C15:1	C15:1
C16:0	C16:0
C16:1	C16:1
C17:0	C17:0
C17:1	C17:1
C18:0	C18:0
C18:1n9t (1)	C18:1n9t
C18:1n9c (1)	C18:1n9c
C18:2n6t (2)	C18:2n6t
C18:2n6c (2)	C18:2n6c
C18:3n6	C18:3n6
C18:3n3	C20:0
C20:0	C18:3n3
C20:1n9	C20:1n9
C20:2	C21:0
C20:3n6 (2)	C20:2
C21:0 (2)	C20:3n6
C20:3n3	C22:0 (1)
C20:4n6	C20:4n6 (1)
C20:5n3	C20:3n3
C22:0	C22:1n9
C22:1n9	C23:0 (2)
C22:2	C20:5n3 (2)
C23:0	C22:2
C24:0	C24:0
C22:6n3 (1)	C24:1n9
C24:1n9 (1)	C22:6n3

(1) Co-elution
(2) Partial co-elution

(continued from page 13)

SLB-IL100: A Superior Highly Polar Column

The SLB-IL100 shows promise as a superior highly polar column. Its higher maximum temperature and phase stability make it capable of analyzing higher molecular weight compounds than those possible with the highly polar TCEP phase. In comparison with other current polar and highly polar GC phases, such as PEG and biscyanopropylsiloxane, it offers alternative selectivity that can be utilized to "fine tune" specific separations. Future Reporter issues will highlight the applicability of the SLB-IL100 for additional uses.

Outlook for Future Supelco Ionic Liquid GC Columns

The patented and successful use of ionic liquids as viable GC stationary phases heralds in a new and exciting chapter in GC phase technology. A full family of columns that utilize ionic liquid phase chemistry is planned. For example, an ionic liquid column with a polarity and selectivity similar to that of Carbowax® 20M, but with a maximum temperature

over 300 °C, is just one of many possibilities currently being investigated. Look for additional Supelco ionic liquid GC columns to be introduced in the coming months. This is truly an exciting time in capillary GC column technology!

Reference

1. L.M. Sidisky and M.D. Buchanan, Supelco Patented Ionic Liquid GC Phase Technology, Supelco Reporter, April 2008; Vol. 26.2: 3-4.

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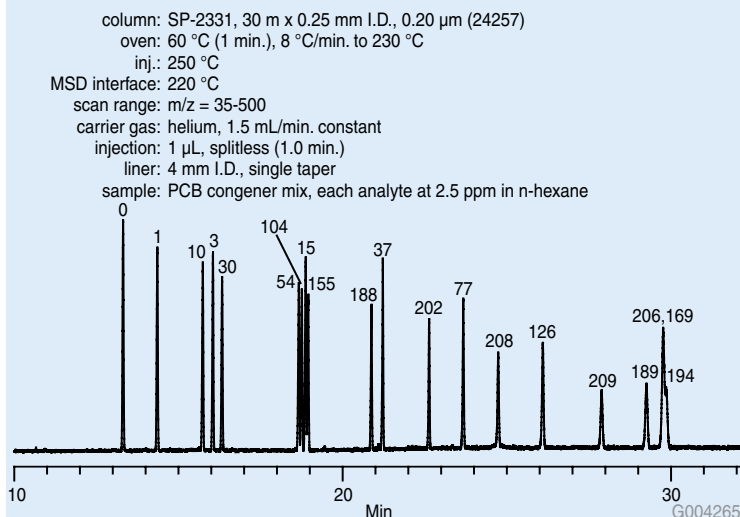
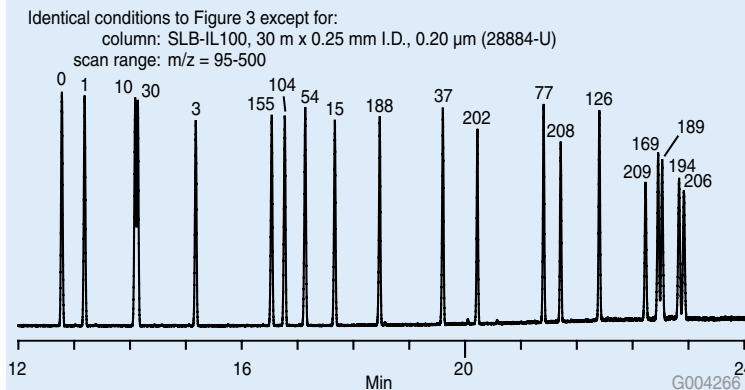
Description	Cat. No.
SLB-IL100, 30 m x 0.25 mm I.D., 0.20 µm	28884-U
SP-2331, 30 m x 0.25 mm I.D., 0.20 µm	24257
Omegawax 250, 30 m x 0.25 mm I.D., 0.25 µm	24136
Supelco 37-Component FAME Mix (listed on Figure 1) 10 mg/mL (total wt.) in methylene chloride, 1 mL	47885-U

+ Related Products

Description	Cat. No.
SLB-IL100, 60 m x 0.25 mm I.D., 0.20 µm	28886-U
SLB-IL100, 30 m x 0.32 mm I.D., 0.26 µm	28887-U
SLB-IL100, 60 m x 0.32 mm I.D., 0.26 µm	28888-U

Peak IDs for Figures 3 and 4

- 0: Biphenyl
- 1: 2-Monochlorobiphenyl
- 3: 4-Monochlorobiphenyl
- 10: 2,6-Dichlorobiphenyl
- 15: 4,4'-Dichlorobiphenyl
- 30: 2,4,6-Trichlorobiphenyl
- 37: 3,4,4'-Trichlorobiphenyl
- 54: 2,2',6,6'-Tetrachlorobiphenyl
- 77: 3,3',4,4'-Tetrachlorobiphenyl
- 104: 2,2',4,6,6'-Pentachlorobiphenyl
- 126: 3,3',4,4',5-Pentachlorobiphenyl
- 155: 2,2',4,4',6,6'-Hexachlorobiphenyl
- 169: 3,3',4,4',5,5'-Hexachlorobiphenyl
- 188: 2,2',3,4',5,6,6'-Heptachlorobiphenyl
- 189: 2,3,3',4,4',5,5'-Heptachlorobiphenyl
- 194: 2,2',3,3',4,4',5,5'-Octachlorobiphenyl
- 202: 2,2',3,3',5,5',6,6'-Octachlorobiphenyl
- 206: 2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl
- 208: 2,2',3,3',4,5,5',6-Nonachlorobiphenyl
- 209: Decachlorobiphenyl

Figure 3. Mono- thru Deca-chlorobiphenyl PCB Congener Standard on the SP-2331**Figure 4. Mono- thru Deca-chlorobiphenyl PCB Congener Standard on the SLB-IL100****Table 2. Elution Order Differences of PCB Congeners on SP-2331 and SLB-IL100**

SP-2331	SLB-IL100
0	0
1	1
10	10 (2)
3	30 (2)
30	3
54 (2)	155
104 (2)	104
15 (2)	54
155 (2)	15
188	188
37	37
202	202
77	77
208	208
126	126
209	209
189	169 (2)
206 (1)(2)	189 (2)
169 (1)(2)	194 (2)
194 (2)	206 (2)

- (1) Co-elution
 (2) Partial co-elution

Hydrogen: A Carrier Gas Alternative to Helium

Robert F. Wallace and Leonard M. Sidisky
bob.wallace@sial.com

Introduction

Carrier gas for gas chromatography (GC) should be an inert gas that does not react with the sample component. Its main role is to transport the vaporized solute molecules through the column. The selection of the carrier gas and the linear velocity it uses both affect resolution and retention times.

Nitrogen, hydrogen, and helium are the most widely used gases by today's chromatographer. Each has unique benefits as well as drawbacks. Nitrogen shows the best efficiency, but over a low and narrow linear velocity range. Therefore, it is extremely slow as a carrier gas, and not a great choice for temperature-programmed use. Hydrogen provides the fastest analysis time over a broad linear velocity range. However, safety concerns must be addressed. Helium is a compromise between nitrogen and hydrogen, with regards to efficiency and analysis times. However, it is becoming an expensive choice for a carrier gas.

As long as the proper safety controls are in place, hydrogen, with its broad working range, may be a more suitable carrier gas choice for capillary GC

Let's take a look and compare the advantages of using hydrogen over helium.

Benefits of Hydrogen as a Carrier Gas: Speed 1

The Golay Theory for open tubular columns predicts that optimum gas velocity is proportional to diffusivity. Hydrogen has a higher diffusivity than helium, thus its optimum linear velocity is higher and can be used at a higher flow rate without adversely affecting efficiency. Specifically, under isothermal analysis conditions, the typical linear velocity for hydrogen is 40 cm/sec, which is twice the optimum linear velocity of helium at 20 cm/sec. Therefore, simply switching to hydrogen carrier gas, even while keeping column dimensions and oven conditions constant, can be expected to decrease analysis time.

Figures 1 and 2 illustrate this benefit of hydrogen with the separation of 16 commonly analyzed polynuclear aromatic hydrocarbons. The analysis was performed on a 15 m x 0.10 mm I.D., 0.10 μ m Equity[®]-5 column. A fast temperature-programming rate was used to decrease the

Fast Analysis of Polynuclear Aromatic Hydrocarbons; Helium vs. Hydrogen Carrier Gas

Figure 1. Helium

column: Equity-5, 15 m x 0.10 mm I.D., 0.10 μ m (28083-U)
oven: 100 °C (1 min.), 35 °C/min. to 325 °C (5 min.)
inj.: 250 °C
det.: FID, 350 °C
carrier gas: helium, 20 cm/sec constant
injection: 1 μ L, 200:1 split
liner: 4 mm I.D., split, cup design
sample: each analyte at 200 ppm in methylene chloride

- | | | |
|-------------------|--------------------------|----------------------------|
| 1. Naphthalene | 7. Fluoranthene | 13. Benzo(a)pyrene |
| 2. Acenaphthylene | 8. Pyrene | 14. Indeno(1,2,3-cd)pyrene |
| 3. Acenaphthene | 9. Benzo(a)anthracene | 15. Dibenzo(a,h)anthracene |
| 4. Fluorene | 10. Chrysene | 16. Benzo(g,h,i)perylene |
| 5. Phenanthrene | 11. Benzo(b)fluoranthene | |
| 6. Anthracene | 12. Benzo(k)fluoranthene | |

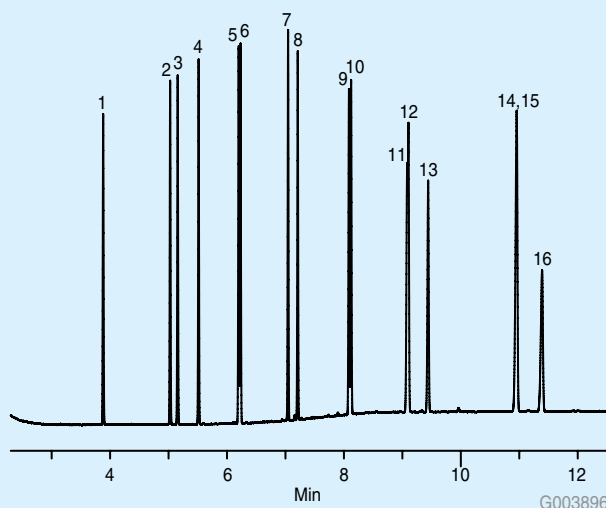
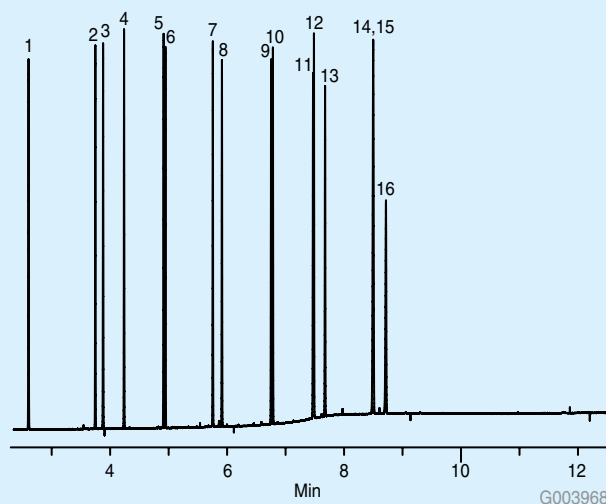


Figure 2. Hydrogen

column: Equity-5, 15 m x 0.10 mm I.D., 0.10 μ m (28083-U)
oven: 100 °C (1 min.), 35 °C/min. to 325 °C (5 min.)
inj.: 250 °C
det.: FID, 350 °C
carrier gas: hydrogen, 45 cm/sec constant
injection: 1 μ L, 200:1 split
liner: 4 mm I.D., split, cup design
sample: Each analyte at 200 ppm in methylene chloride

Same Peak IDs as Figure 1



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run time to less than 12 minutes using helium at its optimal linear velocity. Switching to hydrogen carrier gas (at its optimal linear velocity) under the same conditions resulted in a 25% decrease in run time.

Benefits of Hydrogen as a Carrier Gas: Speed 2

What if carrier gas is run at higher than optimal linear velocity to decrease analysis times? Hydrogen, having a flatter Golay curve, may be operated over a wide range of linear velocities while maintaining a low height equivalent to a theoretical plate (HETP). This allows the use of a linear velocity higher than optimal with little decrease in efficiency, resulting in shorter analysis times.

Benefits of Hydrogen as a Carrier Gas: Cost 1

A shortage of helium began surfacing in 2006. Helium must be extracted from the ground and then refined. However, there are not enough refineries to keep up with the increased demand, such as for applications in the medical, scientific, and industrial fields. As a result, helium is becoming a rare commodity with rising prices. Hydrogen has not seen a drastic rise in price because its production is not dependent on the same factors.

Benefits of Hydrogen as a Carrier Gas: Cost 2/Safety

Because helium generators are not available, it can only be obtained in expensive cylinders. Hydrogen is also available in cylinders, but can also be generated on-demand on-site using a gas generator. In addition to being a much more sensible source of gas from a cost standpoint, generators are safer, more aesthetically pleasing, take up less space, and do not require the labor needed to move bulky cylinders around the lab. The safety benefit of a hydrogen generator over a cylinder is due to the fact that a minimal volume of hydrogen gas is stored at a relatively low pressure.

Conclusion

Hydrogen has several features (higher optimal linear velocity, and flatter Golay curve) that result in desirable benefits (decreased analysis times) when compared to other GC carrier gas choices. If operating exactly at optimal linear velocities, hydrogen results in a faster analysis time. Because hydrogen has the flattest Golay curve, the GC can be operated with an even higher linear velocity without a significant loss in efficiency. As long as the proper safety controls are in place, hydrogen, with its broad working range, may be a more suitable carrier gas choice for capillary GC.

References

1. L. Sidisky, Pittsburgh Conference Seminar 2008, Carrier Gas Selection: Helium versus Hydrogen
2. K. Stenerson, L. Sidisky, and G. Baney, Poster, T406106, Fast GC in Environmental Analysis
3. Supelco, Fast GC Brochure, T407096, 2007
4. R. F. Wallace and M. D. Buchanan, Generate High Purity Gas with Reliability and Safety, Supelco The Reporter, October 2007; Vol. 25.5:11

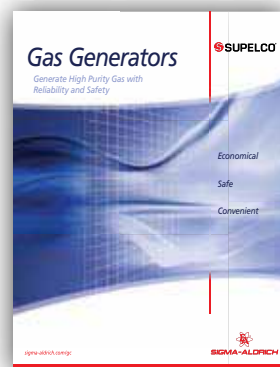
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Description	Cat. No.
Equity-5, 15 m x 0.10 mm I.D., 0.10 μ m	28083-U

! Related Information



For more information on the principles of Fast GC, including practical considerations, theoretical discussions, a listing of columns in Fast GC dimensions, twenty-six chromatograms, plus a list of literature for additional reading, request the "Fast GC: A Practical Guide for Increasing Sample Throughput without Sacrificing Quality" brochure, T407096 (JTW)



For more information on gas generators, request "Gas Generators: Generate High Purity Gas with Reliability and Safety", T407110 (JXP). Alternatively, visit our website sigma-aldrich.com/gc

Did you know...?

The biggest concern with a high-pressure cylinder is that a full cylinder may be accidentally knocked over, causing the valve to break off, and the cylinder to become airborne, damaging anything and everything in its path. Each time a cylinder is handled, extreme caution must be used.

Also, if a high-pressure cylinder regulator fails, the full pressure of the cylinder will be released into the system. Very few plumbing systems can withstand pressure of 2000-3000 psi. Such pressures are never encountered with gas generators – as pressures in these systems rarely exceed 125 psi.

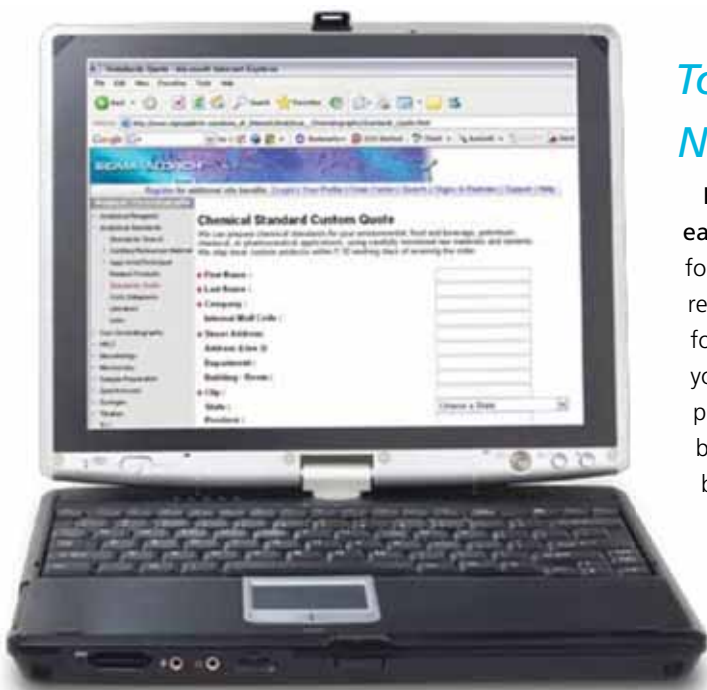
New Environmental Drinking Water and Solid Waste Reference Materials

Sigma-Aldrich is continuously adding new reference materials to meet the needs of analysts performing environmental monitoring as described by the U.S. Environmental Protection Agency's Series 500, 600, and 8000. Below are three recent product additions to the Supelco line of environmental standards.

All raw materials and solvents used in the preparation of these calibration standards have been screened for identity and purity. The mixtures are gravimetrically prepared and quantitatively analyzed by gas chromatography. A certificate of analysis accompanies each standard. As always, free data packets are available upon request.

Description	Concentration	Pkg. Size	Cat. No.
EPA Method 506: Determination of Phthalate and Adipate Esters in Drinking Water			
EPA 506 Phthalate Mix	1000 µg/mL each component in isooctane	1 x 1 mL	40077-U
<i>Dimethyl phthalate</i>	<i>Bis-(2-ethylhexyl) phthalate</i>		
<i>Diethylphthalate</i>	<i>Di-n-octyl phthalate</i>		
<i>Di-n-butyl phthalate</i>	<i>Bis-2-ethylhexyl adipate</i>		
<i>Benzyl butyl phthalate</i>			
EPA Method 521: Determination of Nitrosamines in Drinking Water			
EPA 521 Nitrosamines Mix	2000 µg/mL each component in methylene chloride	1 mL	40035-U
<i>N-Nitrosodimethylamine</i>	<i>N-Nitrosodibutylamine</i>		
<i>N-Nitrosodi-n-propylamine</i>	<i>N-Nitrosomethylethylamine</i>		
<i>1-Nitrosopyrrolidine</i>	<i>N-Nitrosodiethylamine</i>		
<i>1-Nitrosopiperidine</i>			
EPA Method 8100: Polynuclear Aromatic Hydrocarbons in Solid Waste			
EPA 8100 PAH Additional Components Mix	1000 µg/mL methylene chloride	1 x 1 mL	44694-U
<i>Dibenzo(a,e)pyrene</i>	<i>Dibenzo(a,h)acridine</i>		
<i>Dibenzo(a,h)pyrene</i>	<i>Benzo(j)fluoranthene</i>		
<i>Dibenzo(a,l)pyrene</i>	<i>Dibenzo(a,j)acridine</i>		
<i>3-Methylcholanthrene</i>	<i>7H Dibenzo (c,g) carbazole</i>		

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Standards

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Fuel Ethanol: Fermentation Analysis by HPLC

One Source Solution of Column and Quantitative Calibration Standard

Steve Cecil

techservice@sial.com

Fuel ethanol continues to be the mainstay in the biofuel arena, with increasing production yield and higher conversion percentages of corn-to-ethanol driving discussion of both economic and environmental viability of the product.

Ethanol is traditionally produced by the fermentation of sugar by yeast. Typically, commercial production of fuel ethanol involves breakdown of the starch into simple sugars, yeast fermentation of these sugars, and finally recovery of the main ethanol product and byproducts (e.g., animal feed).

Many areas of the process are important to ensure a quality end product, such as the breakdown of the corn substrate to fermentable sugars and distillation. However, none are more critical than the ethanol-producing step of fermentation.

Optimized fermentation leads to increased ethanol yield and profitability of the biofuel facility.

Residual sugars left unfermented lower ethanol concentrations, increase plant water usage and often require additional fermentation equipment cleaning and maintenance. Consequently, fuel ethanol producers continually look for more efficient processing techniques.

Importance of HPLC Analysis of Residual Saccharides

A key measurement is the residual sugar and ethanol concentrations in the fermentation broth. Fuel ethanol facilities use High-Performance Liquid Chromatography (HPLC) as the technique of choice to

monitor the ethanol fermentation process. HPLC permits detailed monitoring of the complete cycle, including conversion of the sugars to ethanol and ethanol breakdown to acetic acid.

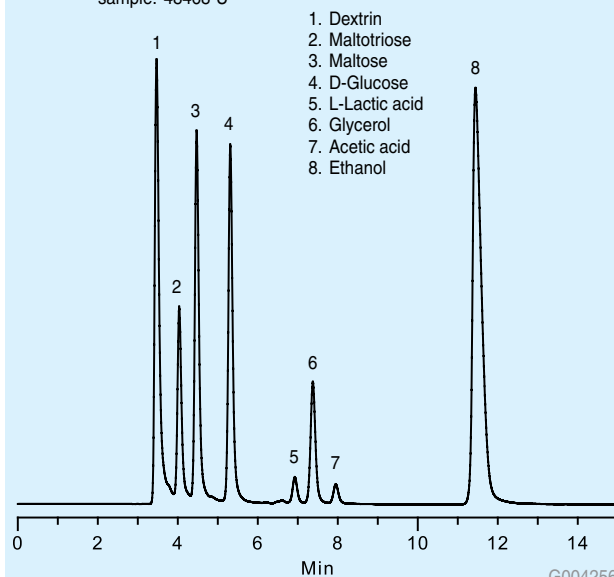
The HPLC analysis utilizes a crosslinked polystyrene / divinylbenzene resin ion exchange column. Traditional methods suffer from a) poor resolution or b) long run times.

- A) Methods with fast run times (< 12 minutes) sacrifice resolution of the early eluting saccharides. These fast methods often show co-elution of the dextrin, maltotriose and maltose peaks.
- B) Methods focused on improved resolution of the simple sugars suffer from long run times. To achieve improved resolution for the early eluting saccharides, these methods have run times exceeding 22 minutes.

Neither of these is an acceptable compromise for your lab. The SUPELCOGEL™ C-610H column has proven to be an excellent choice for this analysis, yielding a shorter run time as well as resolution of all eight key components. Figure 1 illustrates the improved separation of components in the Supelco Fuel Ethanol Residual Saccharides Mix.

Figure 1. Fuel Ethanol Residual Saccharides Mix Run on the SUPELCOGEL C-610H HPLC Column

column: SUPELCOGEL C-610H, 30 cm x 7.8 mm, 9 µm (59320-U)
mobile phase: 5 mM Sulfuric acid (pH 2.2 unadjusted)
flow rate: 1.2 mL/min
temp.: 80 °C
det.: R.I. 30 °C
injection: 25 µL
sample: 48468-U



Importance of Fuel Ethanol Residual Saccharides Mix

It is critical to ensure the analysis is calibrated through a commercially available quantitative calibration standard. The Supelco Fuel Ethanol Residual Saccharides Mix contains key components used to monitor the fermentation process. These components include dextrin (DP4+), maltotriose (DP3), maltose (DP2), D-glucose, and ethanol.

In addition to the saccharides and ethanol components, acetic acid, lactic acid and glycerol are included in the quantitative standard. Lactic acid and acetic acid are breakdown products produced during fermentation. Glycerol is also added to measure the stress being placed on the yeast during fermentation. Figure 1 illustrates the Fuel Ethanol Residual Saccharides Mix run on the SUPELCOGEL C-610H HPLC column.

Utilizing Supelco's Fuel Ethanol Residual Saccharides Mix in conjunction with the SUPELCOGEL C-610H column can lead to improved fermentation and higher ethanol yields. Both of these products are backed by strong technical support from the people you trust at Sigma-Aldrich.

+ Featured Products

Description	Cat. No.
Fuel Ethanol Residual Saccharides Mix w/v% varied conc., deionized water, 10 x 2 mL	48468-U
Glycerol, 1.0%	L-(+)-Lactic acid, 0.3%
D-(+)-Glucose, 2.0%	Acetic acid, 0.3%
Maltotriose (DP3), 1.0%	Dextrin (DP4+), 3.25%
Maltose (DP2), 2.0%	Ethanol, 12.0%
SUPELCOGEL C-610H Column 30 cm x 7.8 mm I.D., 9 µm particle size	59320-U

+ Related Products

Description	Cat. No.
Empty Durapore® PVDF Disposable Millex® Syringe Filter Units 0.22 µm pore size, box of 100	Z227498
Polypropylene Syringe with Luer-Lok™ Tip, 1 ea	Z192104

Did you know...?

To prevent column fouling, pass the Fuel Ethanol Residual Saccharide Mix and fermentation samples through a filter prior to injection.

Nutritive and Non-nutritive Sweetener Standards

The increased use of non-caloric, non-nutritive or low caloric sweeteners in recent years has coincided with growing public awareness of the adverse health effects of excess consumption of simple carbohydrates. More products containing non-nutritive sweeteners are made available to consumers each year as the supply of sweeteners increases.

The availability of high quality analytical standards is essential for their monitoring in food products, and for applied and basic research aimed at developing new sweeteners and understanding their biological effects.

Sigma-Aldrich offers Supelco brand individual standards and multi-component kits for both nutritive and non-nutritive sweeteners. The standards have been tested for identity and purity. The small package sizes eliminates the need to buy bulk material standards.

Description	Pkg. Size	Cat. No.
Neats		
Acesulfame K	1 g	47134
Aspartame	500 mg	47135
D-(+)-Glucose	1 g	47829
Saccharin, hemicalcium	1 g	47840
Sodium cyclamate	1 g	47827
Sodium saccharin	1 g	47839
D-Sorbitol	1 g	47841
Xylitol	1 g	47844
Kits		
Monosaccharides Kit		47267
7 components individually packaged, 500 mg each		
D-(-)-Arabinose	D-(+)-Mannose (mixed anomers)	
Fructose	D-(-)-Ribose	
D-(+)-Galactose	D-(+)-Xylose	
D-(+)-Glucose (mixed anomers)		
Disaccharides Kit		47268-U
4 components individually packaged		
Isomaltose (mixed anomers) (100 mg)		
α-Lactose (500 mg)		
Maltose (500 mg)		
Sucrose (500 mg)		
Sugar Alcohols Kit		47266
8 components individually packaged, 500 mg each		
Ribitol	Dulcitol	
Maltitol	D-Sorbitol	
iso-Erythritol	D-Mannitol	
Glycerol	D-(+)-Arabitol	

ordering: 800-247-6628 (us only) / 814-359-3441 technical service: 800-359-3041 (us and Canada only) / 814-359-3041

Standards

SUPELCO
Analytical

BPE-DNPH Cartridge for Simultaneous Determination of Ozone and Carbonyls



Contributed Article

The following was generated by an outside source using Sigma-Aldrich products. Technical content provided by:

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The BPE-DNPH cartridge is a patent pending sampler for the simultaneous determination of ozone and carbonyls in air. This sampler has a two-bed cartridge system and each bed consists of reagent-impregnated silica particles. The first contains *trans*-1,2-bis-(4-pyridyl) ethylene (BPE) while the second contains 2,4-dinitrophenylhydrazine (DNPH). Air samples are drawn through the cartridge first through the BPE and then through the DNPH. Ozone in the air sample is trapped in the first bed by the BPE-coated silica particles and produce pyridine-4-aldehyde. Airborne carbonyls pass unimpeded through the BPE and are trapped in the second bed by the DNPH-coated silica particles. They produce carbonyl 2,4-DNPhydrazones. DNPH and carbonyl 2,4-DNPhydrazones are not influenced by ozone because of effective trapping by the BPE. All of the hydrazones derived from airborne carbonyls and pyridine-4-aldehyde (derived from ozone) are completely separated and measured using high-performance liquid chromatography.

The use of a BPE-DNPH cartridge has made possible the simultaneous determination of ozone and carbonyls. A separate ozone scrubber is not necessary with the BPE-DNPH cartridge because the BPE portion of the sampler serves this function.

Construction of BPE-DNPH and Measuring Procedure

The air sample is drawn through the cartridge from the BPE bed to the DNPH bed at a flow rate of 100 mL/min for 24 hours or 1000 mL/min for 1 hour. Ozone in the air sample is trapped in the BPE-coated silica bed and produces pyridine-4-aldehyde. Carbonyls in the air sample are trapped in the DNPH-coated silica bed and produce carbonyl 2,4-dinitrophenylhydrazones.

DNPH and carbonyl 2,4-DNPhydrazones in the second bed are not influenced by ozone because it is effectively trapped by the reaction with BPE in the first bed.

Extraction is performed in the reverse direction to air sampling. Solvent passing through the BPE-DNPH cartridge washes DNPH into the BPE bed where it reacts with pyridine-4-aldehyde and forms the corresponding hydrazone derivative. The eluate from the BPE-DNPH cartridge contains hydrazones derivatized with various carbonyls including pyridine-4-aldehyde formed from ozone.

HPLC Analysis

Analytical conditions for pyridine-4-aldehyde and C1-C3 carbonyl-DNPH derivatives were examined using an Ascentis RP-Amide column (565322-U; 15 cm × 4.6 mm, 3 μm). When the mobile phase contained no buffer solution, the pyridine-4-aldehyde 2,4-DNPhydrazone peak was extremely broad. The mobile phase consisting of acetonitrile/water (40:60 v/v) containing ammonium acetate (0, 0.1, 1, 2, 5, 10 or 20 mmol/L) was tested. Figure 1 shows the chromatographic profiles of pyridine-4-aldehyde (PA), formaldehyde (FA), acetaldehyde (AA), and acetone (AC) 2,4-DNPhydrazones in the solutions extracted from air samplers and standard solutions (20 μmol/L). The mobile phase mixture

Reaction Principle

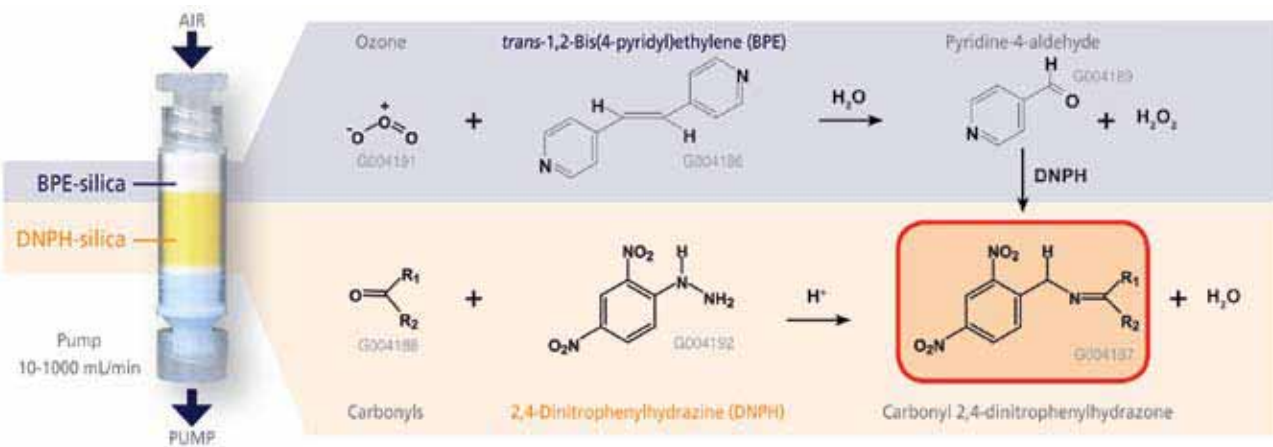
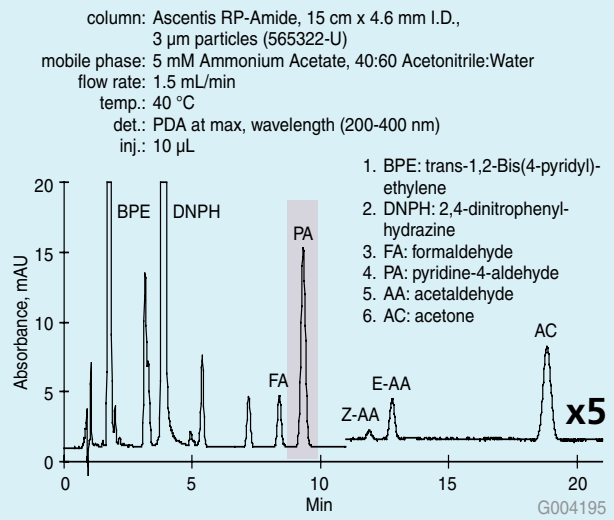


Figure 1. Pyridine-4-aldehyde and Other Carbonyl 2,4-DNPhydrazones from Ambient Air Sample



was acetonitrile / water (40:60 v/v) and acetonitrile/water (40:60 v/v) containing 2 μ mol/L ammonium acetate with a phase flow rate of 1.5 mL/min.

When the mobile phase contained no ammonium acetate, the pyridine-4-aldehyde 2,4-DNPhydrazone peak was extremely broad. When ammonium acetate was added to the mobile phase, the peak sharpened significantly. The addition of ammonium acetate in excess of 2 μ mol/L to the mobile phase provides no further reduction in peak width. In the case of samples derived from standard solutions, the peak width did not change with the addition of ammonium acetate.

Accuracy and Precision

Both the limit of detection (LOD) and the limit of quantitation (LOQ) for the BPE-DNPH-method were calculated using the linear regression theory. A mixture of pyridine-4-aldehyde-DNPhydrazone, formaldehyde-DNPhydrazone, acetaldehyde-DNPhydrazone and acetone-DNPhydrazone

(0.5 μ mol/L each) was analyzed. The LOD and LOQ for the BPE-DNPH cartridge method was calculated as three and ten times the standard deviations, respectively, obtained from the data of 10 replicate measurements (Table 1). The reproducibility of the BPE-DNPH cartridge method was estimated from data of the six parallel air samplings.

Table 1. Limit of Detection, Limit of Quantification and reproducibility of BPE-DNPH Cartridge Method

	LOD, μ g/m ³	LOQ, μ g/m ³	RSD, μ g/m ³
ozone (pyridine-4-aldehyde)	0.03	0.10	5.0
formaldehyde	0.05	0.17	3.4
acetaldehyde	0.15	0.50	4.0
acetone	0.33	1.1	5.6

Concentrations of ozone and carbonyls were measured in ambient air collected by a DNPH cartridge, a DNPH cartridge coupled with a KI ozone scrubber, and a BPE-DNPH cartridge. Air collections were performed simultaneously in Chiba city, Japan, June-July 2007.

The KI scrubber adsorbed a considerable amount of moisture and some mist moved into the DNPH cartridge. As a result, aldehyde and ketone concentrations show lower results compared to DNPH alone and BPE-DNPH cartridge (Table 2).

Reference

Uchiyama, Shigehisa, Anal. Chem. 2008, 80, 3285-3290 to Uchiyama, S. Anal. Chem. 2008, 80, 3285-3290

Table 2. Aldehyde and Ketone Concentrations in Different Ambient Conditions

Analyte:	Ozone		Formaldehyde		Acetaldehyde		Acetone	
	Sunny	Rainy	Sunny	Rainy	Sunny	Rainy	Sunny	Rainy
DNPH	n.a.	n.a.	4.1	3.0	2.2	2.9	2.6	3.1
KI+DNPH	n.a.	n.a.	5.0	1.8	2.8	1.2	3.4	1.6
BPE-DNPH	41.9	15.6	5.2	3.8	2.9	3.2	3.5	3.3

Conc. units are in μ g/m³

Featured Products

Description	Qty.	Cat. No.
Cartridges		
BPE-DNPH 90/260 mg Rezorian	10	54269-U
BPE-DNPH 90/260 mg Rezorian	50	54270-U
Calibration Standards		
TO11/IP-6A Aldehyde/Ketone DNPH Mix (contains 15 components)	1	47285-U
Formaldehyde-DNPH, 1 mL	1	47177
Formaldehyde-DNPH, 1 mL (100 μ g/mL as aldehyde)	5	4M7177
Acetaldehyde-DNPH, 1 mL	1	47340-U
Acetaldehyde-DNPH, 1 mL (1000 μ g/mL as aldehyde)	5	4M7340-U
Analytical Columns		
Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 3 μ m	1	565322-U
Ascentis C18, 15 cm x 4.6 mm I.D., 3 μ m	1	581322-U

Description	Qty.	Cat. No.
Accessories		
LpDNPH Cartridge Adapter	10	21018-U
Male Luer Fitting to 1/8"	20	21016
Male Luer Fitting to 3/16"	20	23364
Male Luer Fitting to 1/4"	10	24856
Female Luer Fitting	20	21017
Female Luer Coupler	20	21015
Male Luer Coupler	20	25064-U
Male Luer Plug	12	504351
Visidry™ Female Luer Plug	12	57098
LpDNPH Label Clips	6	21019-U
Filtration Column w/o Frit, 6 mL	30	57242
Elution Rack	1	21043-U

Proline Derivatization and Enantioresolution by Chiral GC

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Introduction

Amino acids are building blocks of peptides and proteins. Enantiomeric separation of these molecules can be performed through chromatography by chiral stationary phases. This article describes the chiral GC analysis of one amino acid, proline, after achiral derivatization. The benefits of a chiral GC analysis method include:

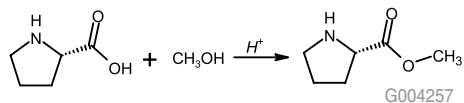
1. better sensitivity than standard HPLC methods
2. shorter analysis times, and
3. simple method development as chiral selectivity can be increased by decreasing the analysis temperature. In addition, the elution order of enantiomers can be reversed, a phenomenon called enantioreversal, using various techniques, including derivatization. Enantioreversal can be utilized to analyze trace levels of one enantiomer in the presence of another.

An amino acid molecule contains both amine and carboxyl functional groups. Before GC analysis, the carboxyl group of the analyte must be esterified, and subsequently the amino group needs to be blocked in order to obtain good peak shape and selectivity. The carboxyl group can be esterified using methanolic HCl (methylated), trimethyl chlorosilane (TMCS), or N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA). The reagents used for the amino group's derivatization typically are trifluoroacetic anhydride (TFAA), acetic anhydride and chloroacetic anhydride.

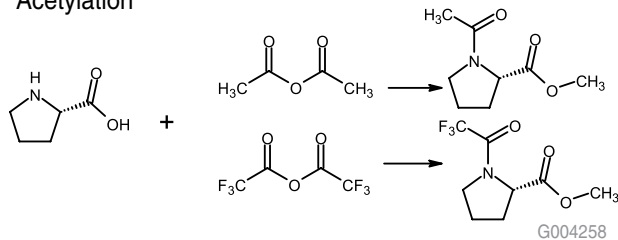
Experimental

A sample mixture of D and L proline was methylated and then acetylated using a two step reaction (1):

Methylation



Acetylation



1. **Methylation:** 1 mL of 3 N methanolic HCl was added to 1 mg of sample. The sample vial was then capped and heated at 100 °C for 30 minutes. After this treatment, the cap was removed and the mixture was allowed to cool down. The mixture was dry at this point (gentle heating can be used for drying if any liquid remains behind).

2. **Acetylation:** The remaining sample residue from step 1 was dissolved in 1 mL of methylene chloride, and 100 μ L of TFAA or acetic anhydride was added. The vial was capped and heated at 60 °C for 20 minutes. The sample was then cooled and the cap carefully removed from the vial. The remaining liquid was evaporated at room temperature under a gentle nitrogen stream (this procedure allowed removal of volatile acids formed during the reaction). The remaining residue was reconstituted in methylene chloride for GC analysis.

Results and Discussion

Chromatograms showing the GC analyses of the proline mixture are presented in Figures 1 and 2. An Astec CHIRALDEX™ G-TA (2), which is a unique chiral GC column made using a trifluoroacetyl derivatized cyclodextrin, was used. The achiral derivatization did not affect the chiral center of the molecule, as evidenced by the presence of two peaks in the chromatograms. However, the two different acetylation reagents resulted in an elution order reversal of the D and L enantiomers (enantioreversal). Trifluoroacetic anhydride also produced more volatile derivatives than the acetic anhydride, resulting in a shorter analysis.

Run Conditions for Figures 1-4

column: CHIRALDEX G-TA, 30 m x 0.25 mm I.D. x 0.12 μ m (73033AST)
oven: 160 °C
inj.: 200 °C
det.: FID, 200 °C
carrier gas: helium, 50 cm/sec
injection: 1 μ L, 50:1 split

Figure 1. Chiral GC Analysis of D and L Proline on the CHIRALDEX G-TA after Methylation with Methanolic HCl and Acetylation with Acetic Anhydride

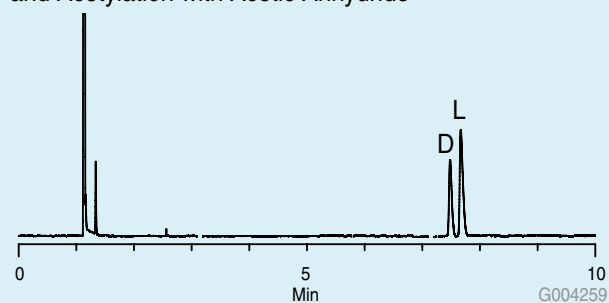
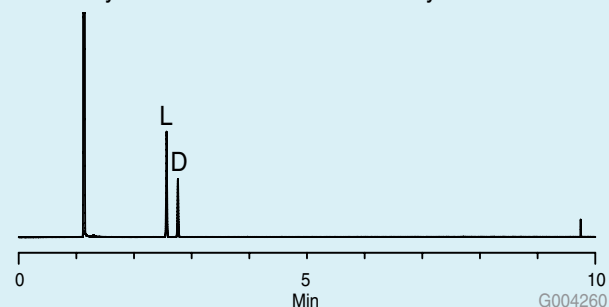


Figure 2. Chiral GC Analysis of D and L Proline on the CHIRALDEX G-TA after Methylation with Methanolic HCl and Acetylation with Trifluoroacetic Anhydride



The derivatization scheme used in this study enabled the replacement of both active hydrogens in the proline molecule thus improving its chromatographic properties for GC analysis. If just the methylation step is done, the remaining underivatized amine group will result in peak tailing on the CHIRALDEX G-TA. This is demonstrated in Figure 3. However, after the acetylation step, protection of the amine group resulted in a significant improvement in peak shape (Figure 4) as well as a more volatile derivative resulting in a shorter retention time.

Conclusions

A two-step derivatization process (methylation followed by acetylation) can be used to selectively replace active hydrogens on the amino acid proline. This process will not cause racemization of the compound and, therefore, allows a successful separation of the enantiomers on the CHIRALDEX G-TA column. Using different acetylation reagents, such as acetic anhydride and trifluoroacetic anhydride, enantioreversal of the D and L enantiomers can be achieved.

References

1. Derivatization Reagents – For Selective Response and Detection in Complex Matrices, Sigma-Aldrich publication, T407138, KDI.
2. Astec CHIRALDEX GC Columns, Sigma-Aldrich publication, T407123, JCH.

Figure 3. L-Proline on the CHIRALDEX G-TA Column after Methylation with Methanolic HCl only

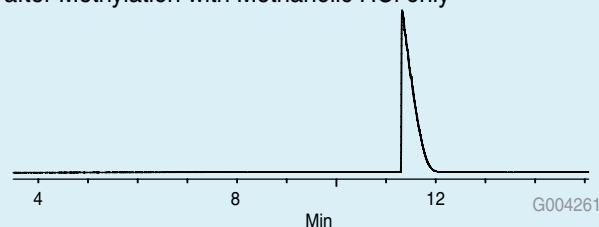
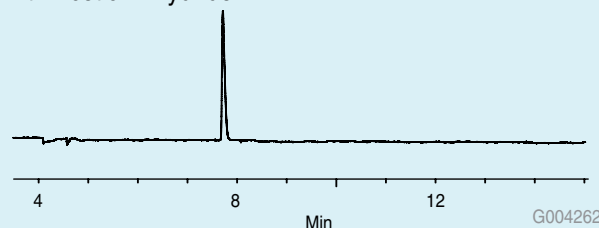


Figure 4. L-Proline on the CHIRALDEX G-TA Column after Methylation with Methanolic HCl and Acetylation with Acetic Anhydride



! Related Information

For more information, request T407138 (KDI), *Derivatization Reagents – For Selective Response and Detection in Complex Matrices* and T407123 (JCH), *Astec CHIRALDEX GC Columns*.

New Solvents Optimized for Static Headspace GC Applications

Michael Jeitziner

michael.jeitziner@sial.com

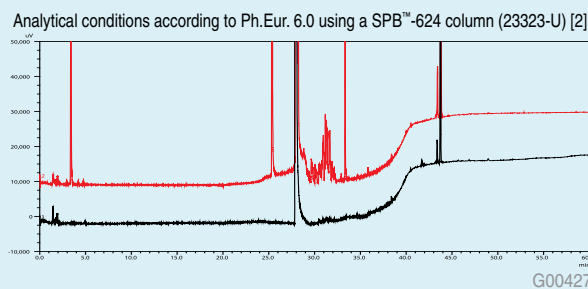
Static headspace GC (GC-SH) is a technique used to concentrate volatile analytes prior to analysis. It improves detection of low-level volatile analytes, and minimizes matrix interference by eliminating the need to inject the sample directly. An important application of GC-SH is for the determination of residual volatile organic impurities in active drug substances or excipients in drug formulations. Other consumer-oriented applications include the detection of residual solvents in foods, dietary supplements and packaging materials.

GC-SH application to residual solvents in pharmaceuticals is described and validated in specific monographs [1-3].

When developing a GC-SH method, such parameters as sample solvent, extraction temperature, extraction time, sample volume and headspace volume are optimized [4, 5].

Because the composition of the sample solvent and its purity have significant effects on the recovery and quality of the chromatogram (see Figure 1), we have developed solvents specifically for GC-HS applications. Their purity and handling specifications meet the requirements of Ph.Eur., USP and ICH guidelines.

Figure 1. Headspace Gas Chromatogram of GC-HS Grade (Black) and Conventional Grade (Red)



References

1. United States Pharmacopeia, 31st Edition (2008), <467> Residual Solvents.
2. Ph Eur. 6.0 (2008) Method 2.4.24, Identification and control of residual solvents.
3. ICH Guideline Q3C, Impurities: Guideline for Residual Solvents, The Fourth International Conference on Harmonization, July 17, 1997.
4. Camarasu, C. C. Residual Solvents Determination in Drug Products by Static Headspace-Gas Chromatography. *Chromatographia* 2002, 56, S137 – S143.
5. Lee, C. R.; Nguyen van Dau, C.; Krstulovic, A. M. Artefact formation in the determination of residual solvents according to a method of the European Pharmacopeia. *Int. J. Pharm.* 2000, 195, 159 – 169.

+ Featured Products

Description	Boiling Point	Pkg. Sz.	Cat. No.
N,N-Dimethylacetamide, puriss. p.a. for GC-HS	166 °C	1 L	44901
Dimethyl sulfoxide, puriss. P.a. for GC-HS	189 °C	1 L	51779
N,N-Dimethylformamide, puriss. p.a. for GC-HS	153 °C	1 L	51781
Water, puriss. p.a. for GC-HS	100 °C	1 L	53463

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