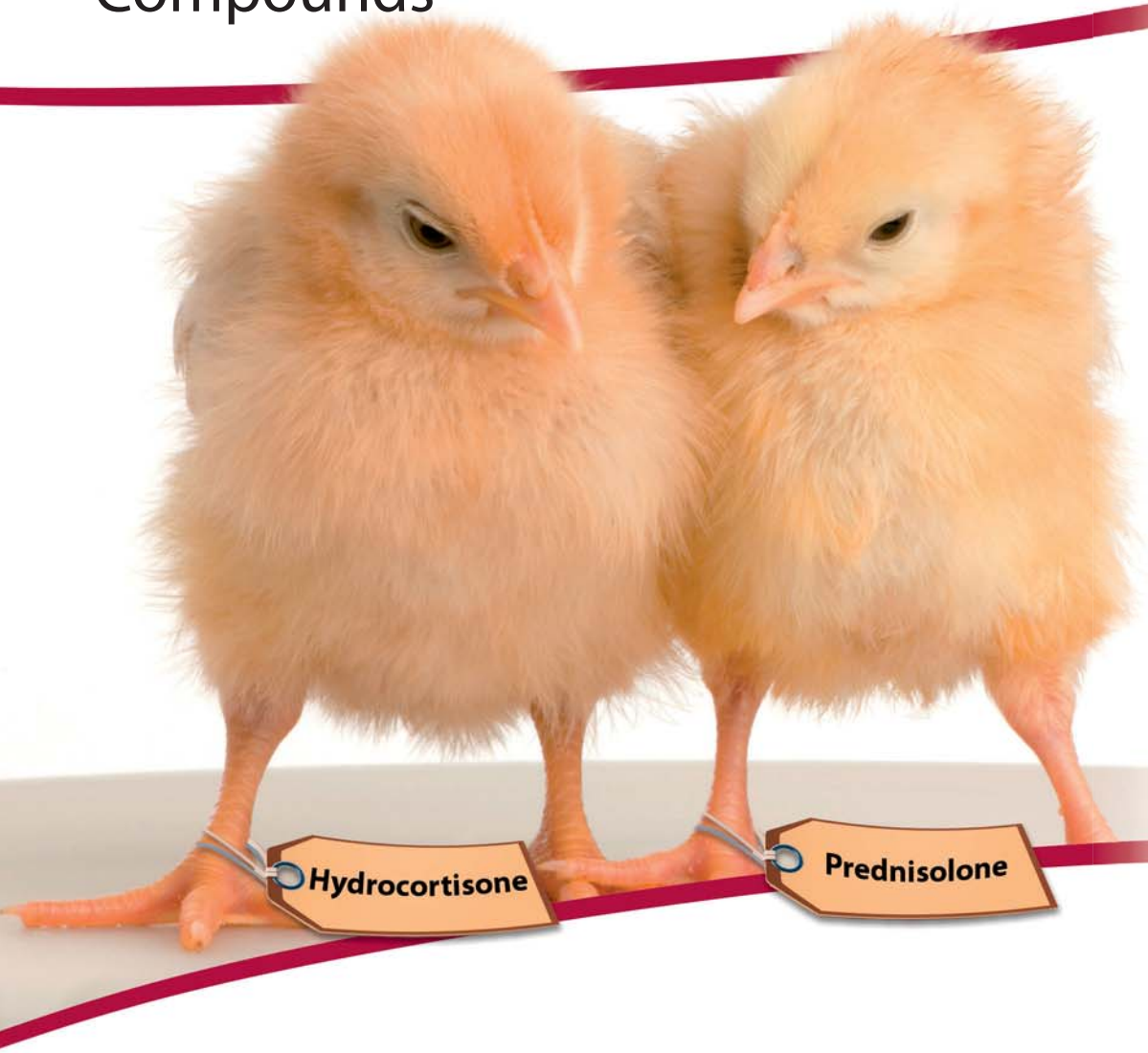
Reporter

Volume 45, March 2011, International

SUPELCO Analytical

Separation of Closely Related Compounds



Ascentis® Express F5 HPLC columns, due to enhanced shape selectivity, provide the separation of closely related compounds.

What Supelco® Brings to the Table

Wayne Way
Market Segment Manager
HPLC

Seminars on Food Analysis in Europe

These seminars provide an overview on current topics from food analysis such as determination of pesticides, fatty acids, and flavors. Presentations on the most recent developments in GC, HPLC and sample handling will complete the program.

The seminars will take place in different locations in Austria, Czech Republic, Germany, Hungary, Poland and Switzerland during May and June.

Please find more details on the seminars at:
sigma-aldrich.com/events

Dear Colleague,

In my role as Market Segment Manager for HPLC, I have the privilege of meeting fellow chromatographers. Often I am asked to tell them about Supelco, who we are and what we represent, compared to the other chromatography companies. In the few words I have for this editorial, I'd like to share my thoughts on this.

The Sigma-Aldrich Mission is *Enabling Science to Improve the Quality of Life.*
That sounds good, but from a practical standpoint, what does it mean to our customers?

How do we Enable Science, within the Supelco Analytical HPLC Offering?
It's through a combination of internal product development, partnering with companies to be their innovation outlet, and providing the supply chain power of our parent company, Sigma-Aldrich, to leading chromatography products from many manufacturers. No other chromatography company, whether it is a major distributor or a world-class innovator, can offer this breadth of products. As an example of this breadth, let's take a look at a sampling of what Supelco can provide to the HPLC chromatographer.

Small Molecule HPLC Columns

- Ascentis® Express with Fused-Core® Particle Technology – Delivers twice the speed and performance of traditional columns at half of the backpressure of sub-2 micron columns.
- SUPELCOSIL™, Discovery® and Ascentis – Three generations of HPLC columns – well known from numerous citations in Scientific Journals.
- apHera – High performance 300 Å polyvinyl alcohol phases for the separation of sugars and peptides over a wide pH range.
- Other Important HPLC Column Brands – Nucleosil®, Spherisorb®, LiChrosorb® and Hamilton®, just to name a few, historically important products written into many HPLC methods.

Large Molecule HPLC Columns

- TSK-GEL® Columns – World-class leader in GFC/SEC, IEX, HIC and HILIC biochromatography technology.
- Discovery HPLC Columns – Supelco brand HPLC columns designed for protein and peptide separations.

Chiral HPLC Columns

- Astec Chiral Columns – CHIROBIOTIC®, CYCLOBOND™ and Astec CLC columns for reversed-phase and LC-MS chiral applications, developed in collaboration with Professor Dan Armstrong.

HPLC Accessories

- Supel™-Fit connections, Upchurch Fittings, Rheodyne® Valves, Optimize® Technologies Guards and Pre-column Filters – High-quality HPLC consumables. Available directly from Supelco/Sigma-Aldrich through our partnership with IDEX® and Optimize Technologies.

I hope Sigma-Aldrich will continue to be your supplier of innovative and reliable chromatography products to solve your analytical challenges, supported by the service you expect from Sigma-Aldrich. Please tell me how we can help you!

Kind regards,

Wayne K. Way

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wayne.way@sial.com

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Using Fluorinated Stationary Phases for Alternative Selectivity

Separation of Closely Related Compounds

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Mechanisms of Retention on Fluorinated Phases

The use of fluorinated stationary phases in liquid chromatography and hyphenated techniques has become significant in recent years. Early applications in the effective separation of paclitaxel provided such phases much notoriety. However, more recent studies focusing on their orthogonality to traditional alkyl phases has invited even broader attention. Due to the different retention mechanisms fluorinated stationary phases provide, they are often employed for the separations not easily obtained using common C18 phases. Applications in arenas such as biopharmaceutical, pharmaceutical, natural product and environmental analyses are increasingly being reported.

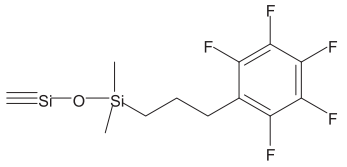
Fluorinated phases have been shown to exhibit greater ion-exchange character than their alkyl counterparts. Fluorinated phases often provide excellent chromatographic results when analytes to be separated differ in their ionisation constants or where some ion-exchange is necessary for the retention of polar metabolites or degradation products. A second important attribute of the fluorinated phases lies in their apparent increased shape selectivity relative to common stationary phase chemistries. Fluorinated phases, therefore, are often superior to their alkyl counterparts for the separation of closely related compounds that differ in size and shape.

In order to effectively utilise this interesting and useful tool, it is important to have a basic understanding of the underlying mechanisms that govern retention and selectivity. This report will focus on two main mechanistic features of fluorinated phases that differentiate them from common alkyl phases; increased ionic interactions relative to alkyl phases and shape selectivity.

Practical Implications of Alternative Retention Mechanisms

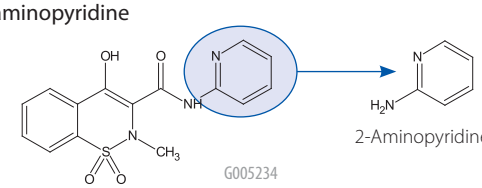
The structure of a popular form of fluorinated phases, penta-fluorophenylpropyl (PFP or F5), is shown in **Figure 1**. The F5 bonded phase exhibits strong dipole potential (polar interaction) from the carbon-fluorine bonds, pi-pi interaction potential and the ability to interact via charge-transfer interactions due to the electronegativity of the fluorine atoms. The relative rigidity of the bonded phase is also believed to provide enhanced shape selectivity of analytes differing in size and spatial attributes.

Figure 1. Chemical Structure of Pentafluorophenylpropyl Bonded Phase



G005233

Figure 2. Structures of Piroxicam and its Potential Impurity 2-aminopyridine



G005234

Piroxicam

G005235

2-Aminopyridine

A common shortcoming of traditional alkyl phases such as C18 (ODS) and C8 (octyl) is their inability to retain polar compounds. Because the F5 phase exhibits ion-exchange and polar interactions, retention of polar compounds are often achieved. **Figure 2** shows the structures of the anti-inflammatory drug piroxicam and a potential synthetic impurity, 2-aminopyridine (2-AMP). 2-AMP is relatively polar (log P = 0.53±0.27) and thus difficult to retain on a conventional alkyl column. **Figure 3** (page 4) shows the separation of the two analytes using a C18 phase. Retention of piroxicam is easily achieved; however, 2-AMP is unretained and thus not quantifiable. Efforts to lower mobile phase organic content and raise pH to improve retention were ineffective using the alkyl phase. It is possible that retention of 2-AMP may be accomplished through the addition of ion-pair reagents; however, such methods are often difficult to validate and suffer from robustness and ruggedness issues. **Figure 4** (page 4) shows the separation of 2-AMP and piroxicam using a fluorinated phase. In this case, 2-AMP is well retained and separated from the parent molecule using a simple mobile phase. The retention of 2-AMP demonstrates the availability and utility of the polar and ionic interactions the F5 phase exhibits.

(continued on page 4)

Figure 3. Separation of Piroxicam and 2-Aminopyridine on a C18 Column

column: Ascentis® Express C18, 10 cm x 4.6 mm, I.D. 2.7 µm particle size (53827-U)
mobile phase A: 10 mM ammonium formate (aq), pH 3 with concentrated formic acid
mobile phase B: acetonitrile
mobile phase ratio: A:B 75:25, v/v
flow rate: 0.8 mL/min
temp.: 35 °C
pressure: 1635 psi
det.: UV PDA at 230 nm and MS in ESI (+), SIR (single ion recording mode)
injection: 5 µL
sample: 10 µg/mL 2-AMP and 100 µg/mL piroxicam in 90:10 10 mM ammonium formate (aq), pH 3 with concentrated formic acid:acetonitrile

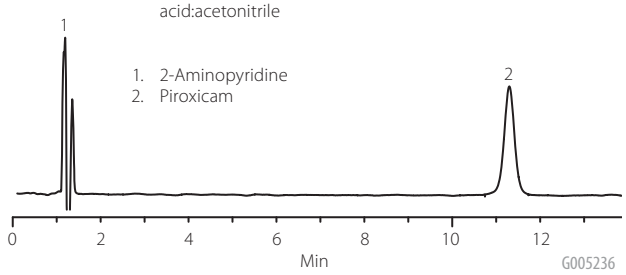
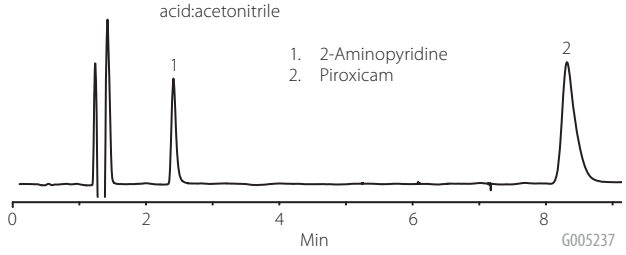


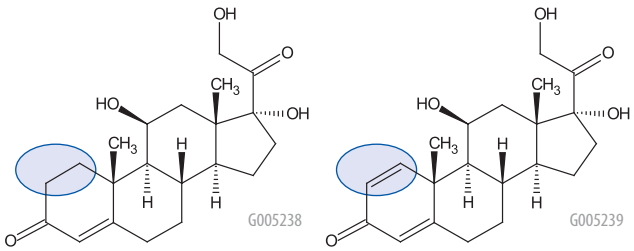
Figure 4. Separation of Piroxicam and 2-Aminopyridine on an F5 Column

column: Ascentis Express F5, 10 cm x 4.6 mm, I.D. 2.7 µm particle size (53590-U)
mobile phase A: 10 mM ammonium formate (aq), pH 3 with concentrated formic acid
mobile phase B: acetonitrile
mobile phase ratio: A:B 75:25, v/v
flow rate: 0.8 mL/min
temp.: 35 °C
pressure: 1635 psi
det.: UV PDA at 230 nm and MS in ESI (+), SIR (single ion recording mode)
injection: 5 µL
sample: 10 µg/mL 2-AMP and 100 µg/mL piroxicam in 90:10 10 mM ammonium formate (aq), pH 3 with concentrated formic acid:acetonitrile



Chromatographers are often faced with the challenge of separating compounds that are very similar in their solubilities. Separation on non-polar phases such as C18 is driven by differential partitioning of analytes, therefore, the alkyl phases are often ineffective in meeting this challenge. Hydrocortisone and prednisolone (see **Figure 5**) differ by a single double bond. Their solubilities are very similar; however, their shapes differ significantly. **Figure 6** shows a comparison of their separation, along with prednisone internal standard (IS), using both a C18 and an F5 stationary phase. The fluorinated phase, apparently due to the enhanced shape selectivity, is shown to provide the separation of these closely related compounds.

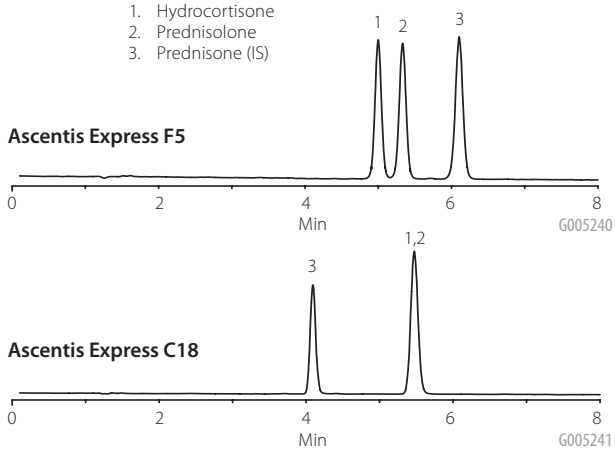
Figure 5. Structures of Hydrocortisone and Prednisolone



Hydrocortisone Prednisolone

Figure 6. Comparison of C18 and F5 for the Separation of Closely Related Steroids

column(s): Ascentis Express F5 (53590-U) and Ascentis Express C18, (53827-U), 10 cm x 4.6 mm, I.D., 2.7 µm particle size
mobile phase A: water
mobile phase B: methanol
mobile phase ratio: A:B 50:50, v/v
flow rate: 0.8 mL/min
temp.: 35 °C
pressure: ~2400 psi
det.: UV at 240 nm
injection: 5 µL
sample: 10 µg/mL each in 90:10 water:methanol

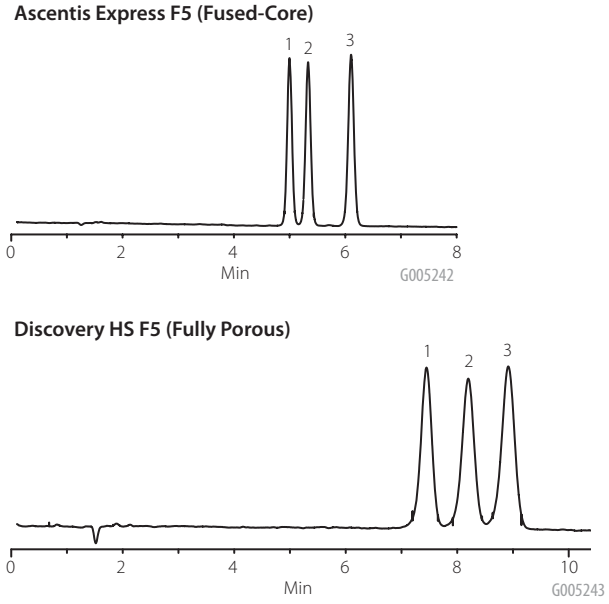


Alternative Selectivity with High Efficiency

The F5 bonded phase has recently been introduced on the highly efficient Fused-Core® technology platform with the brand name Ascentis® Express F5. **Figure 7** shows a comparison of the fully-porous Discovery® HS F5 and the new Ascentis Express F5 using the same conditions as **Figure 6**. Similar overall selectivity is observed due to the similar interactions provided by the F5 moiety. A dramatic increase in efficiency due to the Fused-Core particle support is demonstrated when using the Ascentis Express F5.

Figure 7. Comparison of Fluorinated Phases Based on Totally Porous and Fused-Core Particle Technologies

Conditions same as Figure 6.



Did you know . . .

Ascentis Express HPLC Columns with Fused-Core particle technology were first introduced at PITTCON 2007. Columns are available in nano, capillary, analytical, and semiprep dimensions including guard cartridges. Seven phases provide a wide range of selectivities for your small molecule and peptide applications. For more information on these products, visit ***sigma-aldrich.com/express***

Conclusions

Fluorinated stationary phases exhibit increased ionic and polar interactions relative to common alkyl phases. The rigidity of the F5 bonded phase is also believed to provide increased shape selectivity over commonly used alkyl phases. These alternative mechanisms of retention often provide selectivity not readily achieved on the more traditional phases. Retention and selectivity of highly polar and ionic species as well as separation of closely related neutral compounds have been used to demonstrate the power of the bonded phase chemistry. The recent combination of the selectivity provided by the fluorinated bonded phase and the efficiency of Fused-Core particle technology provides even greater resolution power.

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

Related Products

Description	Qty.	Cat. No.
Hydrocortisone standard	250 mg	31719
Prednisolone standard	250 mg	46656
2-Aminopyridine standard	1 g	36685

Monthly Savings Programme

SAVE 70%

Fluka® Analytical

Your Day-to-Day Demand for Silica at 70% Off

Sigma-Aldrich offers a broad range of silica gels and other separation and purification media – from high-efficiency spherical silica gel to standard irregular silica gel. Request a quote today for silica gel that combines Sigma-Aldrich service and quality with competitive pricing. Visit us at: ***sigma-aldrich.com/savings*** or ***sigma-aldrich.com/silicagel***

Part No.	Brand	Description
60752 -1KG, -5KG, -25KG	**Fluka®**	Silica gel 60 for prep.chrom. (40–63 µm) Act. II–III
60741 -1KG, -5KG, -25KG	**Fluka**	Silica gel 60 for flash chrom. (63–200 µm) Act. II–III
60738 -1KG, -5KG, -25KG	**Fluka**	Silica gel 60 for flash chrom. (35–70 µm) Act. II–III
60737 -1KG, -5KG, -25KG	**Fluka**	Silica gel 60 for flash chrom. (40–63 µm) 4–6% H_20

To take advantage of this monthly savings offer, please use promotion code 958.
Offer is valid until 30 April 2011.

Fast, fully resolved separation of 25-Hydroxyvitamin D_3 and 3-epi-25-Hydroxyvitamin D_3 using Ascentis® Express F5

Introduction

The importance of vitamin D in deficiency symptoms such as bone deformity due to rickets in children has been known for a long time. Vitamin D has also been reported as an important preventive agent for cancer. Epidemiological studies imply that several hundred thousand cases of cancer could be avoided with generally higher levels of vitamin D in the human population.

Important biomarkers for Vitamin D often measured in clinical laboratories are the 25-hydroxy metabolites of vitamin D_2 and D_3. Recently more interest has arisen on the importance of the epimers of 25-hydroxyvitamin D_3 and D_2 in infants due to immature vitamin D metabolism.

Challenges for the lab result from the similarity in structure and the fact that epimers have the same parent ion in MS detection. Several different methods have been suggested in the literature but up to now, long chromatographic run times have been a limiting factor for many labs working in research on the importance of the effects of vitamin D_3. A new method recently developed (**Figure 1**) significantly improves the speed of this assay.

The Ascentis Express F5 Fused-Core® particle column combines two features that make it extremely useful for this kind of separation and a necessary tool in every method developer's lab.

- 2.7 µm Fused-Core particle technology for increased efficiency at reasonable backpressure in HPLC
- The multi-functional character of the F5 Phase which gives possibilities to separate by RP, Normal, HILIC, π–π and steric interactions

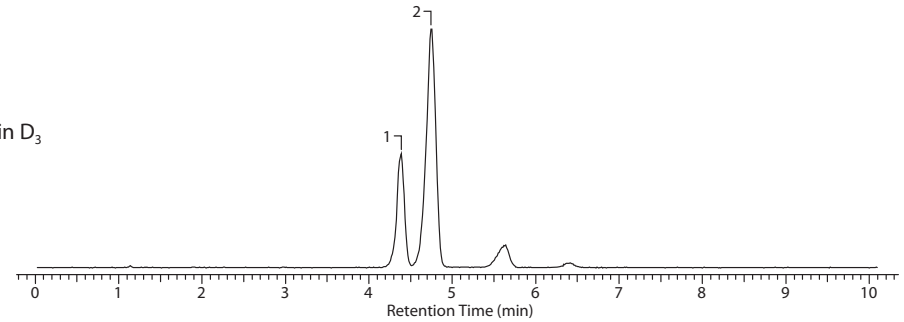
References

1. Vitamin D at Merck Manual of Diagnosis and Therapy Professional Edition.
2. Measurement of 25-OH-vitamin D in human serum using liquid chromatography tandem-mass spectrometry with comparison to radioimmunoassay and automated immunoassay, Johannes M. W. van den Ouweland*, Antonius M. Beijers, Pierre N. M. Demacker, Henny van Daal, Department of Clinical Chemistry, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands, Journal of Chromatography B, 878 (2010) 1163–1168.
3. Vitamin D for Cancer Prevention: Global Perspective, Cedric F. Garland, Dr PH, Face, Edward D. Gorham, MPH, PHD, Sharif B. Mohr, MPH, and Frank C. Garland, PHD.
4. Vitamin D and Prevention of Human Disease, Sigma-Aldrich Biofiles, Annals of Epidemiology, Volume 19, Issue 7, Pages 468–483 (July 2009).
5. C-3 Epimers Can Account for a Significant Proportion of Total Circulating 25-Hydroxyvitamin D in Infants, Complicating Accurate Measurement and Interpretation of Vitamin D Status, Ravinder J. Singh, Robert L. Taylor, G. Satyanarayana Reddy and Stefan K. G. Grebe. The Journal of Clinical Endocrinology & Metabolism Vol. 91, No. 8 3055–3061

Related products

Description	Cat. No.
Ascentis Express F5, 10 cm x 3.0 mm I.D., 2.7 µm particles	53578-U
3-epi-25-Hydroxyvitamin D_3	705993
25-Hydroxyvitamin D_3	17938
25-Hydroxyvitamin D_2 (6,19,19-d3) 97 atom % D, 98% (CP)	705497
25-Hydroxyvitamin D_3 (6,19,19-d3) 97 atom % D, 98% (CP)	705888
Vitamin D_3(6,19,19-d3) solution 97 atom % D, 1.33 mg/mL in ethanol	731285
Vitamin D_2 (6,19,19-d3) 97 atom % D, 98% (CP)	705489

Figure 1. Separation of 25-Hydroxyvitamin D_3 and 3-epi-25-Hydroxyvitamin D_3



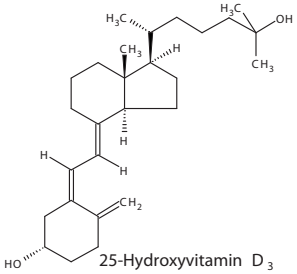
Conditions

column(s):	Ascentis Express F5, 10 cm x 3.0 mm I.D., 2.7 µm particles
mobile phase A:	methanol
mobile phase B:	water
mixing proportions:	A:B 75:25
flow rate:	0.6 mL/min.
pressure:	300 bar
temp.:	40 °C
det.:	ESI+ 100-1000m/z
injection:	1 µL
sample:	200 ng/mL in 75:25 methanol:water

Peak IDs

No.	Name	tR
1	25-hydroxyvitamin D3	4.4
2	3-epi-25-hydroxyvitamin	4.7

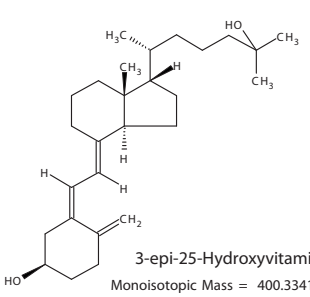
Structures



25-Hydroxyvitamin D_3

Monoisotopic Mass = 400.334131 Da

Molecular Formula = $C_{27}H_{44}O_2$



3-epi-25-Hydroxyvitamin D_3

Monoisotopic Mass = 400.334131 Da

Molecular Formula = $C_{27}H_{44}O_2$

Astec CLC-L and CLC-D

Copper Ligand Exchange HPLC Columns for Chiral Separations of Small Acids and Amino Acids

Astec CLC columns use the ligand-exchange concept described by Davankov to effect enantiomeric separation (1). The method uses a small, chiral bidentate ligand attached to the silica surface and a copper sulphate-containing mobile phase. The copper ions coordinate with the chiral selector stationary phase and certain functional groups on the analytes to form transient diastereomeric complexes in solution. The technique also has the advantage of giving small acids with no UV chromophore a strong 254 nm signal.

Astec CLC columns are ideal for analysis of α-hydroxy acids, such as lactic, malic, tartaric and mandelic acids, amino acids, other amines and bi-functional racemates, such as amino alcohols. Two versions of the column provide elution order reversal (see **Figure 1**). On the Astec CLC-D column, the L enantiomer generally elutes before D, with the exception of tartaric acid. The reverse is true on the Astec CLC-L column where D elutes before L. Proline and aspartic acid are particularly suited for low-level detection on the CLC column since the copper complex is detected at 254 nm UV. Both can be resolved on the Astec CLC-D or CLC-L in 5 mM $CuSO_4$ with reversal of elution order from the CLC-D to CLC-L. In theory, any analyte that can complete the coordination complex with the copper ion can be resolved.

Features:

- Separates α-hydroxy carboxylic acids, amino acids, and other α-bifunctional compounds
- High selectivity with simple mobile phases
- Copper complex gives strong UV 254 nm signal
- Simple reversal of elution order, CLC-L vs. CLC-D

Properties of Astec CLC-L and CLC-D:

- Bonded phase: Chiral bidentate ligand (L and D forms)
- Operating pH range: 3–6 (adjust pH of the 5 mM $CuSO_4$ mobile phase with acetic acid)
- Use with MeOH, EtOH or IPA as organic modifiers
- Particle type: High-purity spherical silica
- Particle diameter: 5 µm
- Pore size: 100 Å

Please visit ***sigma-aldrich.com/chiral*** to view our comprehensive product offering for chiral chromatography and chiral chemistry.

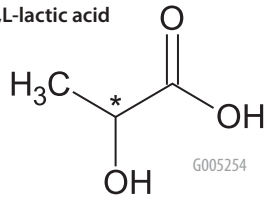
Reference

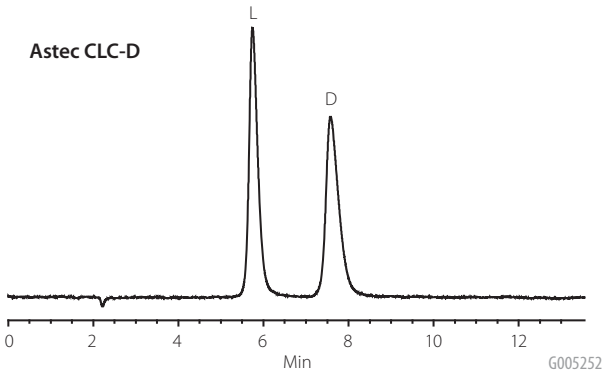
1. Davankov, V. A.; Rogozhin, S. V. Ligand chromatography as a novel method for the investigation of mixed complexes: Stereoselective effects in α-amino acid copper(II) complexes. J. Chrom. A. 1971, 60, 284–312.

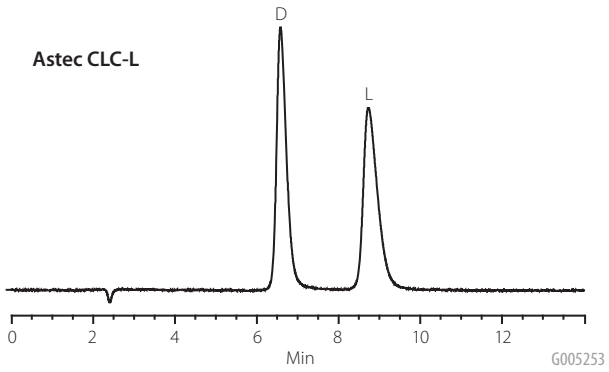
Figure 1. Reversal of Elution Order of Lactic Acid Enantiomers on Astec CLC-L and CLC-D

columns: Astec CLC-D (53023AST) and Astec CLC-L (53123AST), both 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 5 mM $CuSO_4$
flow rate: 1.0 mL/min
temp.: ambient
det.: UV at 254 nm
injection: 5 µL
sample: D,L-lactic acid (3 mg/mL)

Structure of D,L-lactic acid







Featured Products

Description	Cat. No.
Astec CLC-D, 15 cm x 4.6 mm I.D., 5 µm particles	53023AST
Astec CLC-L, 15 cm x 4.6 mm I.D., 5 µm particles	53123AST

Reversed-phase HPLC Buffers

High-quality buffers (solutions, solids or concentrates)

Shyam Verma
shyam.verma@sial.com

Consideration of the effects of pH on analyte retention, type of buffer to use, and its concentration, solubility in the organic modifier and its affect on detection are important in reversed-phase chromatography method development of ionic analytes. An improper choice of buffer, in terms of buffering species, ionic strength and pH, can result in poor or irreproducible retention and tailing in reverse-phase separation of polar and ionisable compounds.

Problems, such as partial ionisation of the analyte together with strong interaction between analytes and residual silanols on the stationary phases can be overcome by proper mobile phase buffering (maintaining the pH within a narrow range) and choosing the right ionic species and concentration (ionic strength) in the mobile phase (1-2). In sensitive LC-MS separations that depend heavily on the correct choice of acid base buffering species and other additives (3), a buffer must be chosen based on its ability to maintain, and not suppress analyte ionisation in the MS interface.

Buffer Selection

The typical pH range for reversed-phase on a silica-based packing is pH 2 to 8. Choice of buffer is typically governed by the desired pH. It is important that the buffer has a pK_a close to the desired pH since buffers control pH best at their pK_a. A rule of thumb is to choose a buffer with a pK_a value +/- 2 units from the analytes pK_a to keep control of the analytes ionisation state (see **Table 1**).

Table 1. HPLC Buffers, pK_a Values and Useful pH Range

Buffer	pK_a (25 °C)	Useful pH Range
TFA	0.5	<1.5
Sulphonate I	1.8	<1–2.8
Phosphate I	2.1	1.1–3.1
Chloroacetate	2.9	1.9–3.9
Formate	3.8	2.8–4.8
Acetate	4.8	3.8–5.8
Sulphonate II	6.9	5.9–7.9
Phosphate II	7.2	6.2–8.2
Ammonia	9.2	8.2–10.2
Phosphate III	12.3	11.3–13.3

Buffer Concentration: Generally, a buffer concentration of 10–50 mM is adequate for small molecules.

Buffer Solubility: A general rule is no more than 50% organic should be used with a buffer. This will depend on the specific buffer as well as its concentration.

Buffer's Effect on Detection: The choice of buffer is also dependent on the means of detection. For traditional UV detection, the buffer needs to be effectively transparent in this region, especially, critical for gradient separations. Buffers listed in **Table 1** have low enough absorption below 220 nm.

Phosphoric acid and its sodium or potassium salts are the most common buffer systems for reversed-phase HPLC. Phosphonate buffers can be replaced with sulphonate buffers when analysing organophosphate compounds. With the growing popularity of LC-MS, volatile buffer systems such as TFA used alone are not sufficiently volatile for MS. Instead, acetate, formate, and ammonia are frequently used due to their greater compatibility with MS detection. In regard to the issue of suppression of ionisation, formate and acetate are ideal choices for positive-ion mode detection. TFA, however, can negatively impact detector response even in positive-ion mode (4,5), while it strongly suppresses ionisation in the negative ion mode. To overcome the negative impact of TFA in negative-ion mode, a buffer consisting of formic acid (0.1%) and TFA (0.01%) can be used. Acetic acid is also a good choice for negative-ion mode. LC-MS applications can further determine buffer selection and buffer concentration.

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3. Analytix, Five-part series on Mobile Phase Additives for LC-MS, Issue 3, 2006 (***sigma-aldrich.com/analytix***).
4. Temesi, D., Law, B., 1999, The Effect of LC Eluent Composition on MS Response Using Electrospray Ionization, LC-GC, 17:626.
5. Apffel, A. et. al. 1995. Enhanced Sensitivity for Peptide Mapping with Electrospray Liquid Chromatography-Mass Spectrometry in the Presence of Signal Suppression Due to Trifluoroacetic Acid-Containing Mobile Phases, J. Chrom. A. 712:177.

Featured Products

Description	Qty.	Cat. No.
HPLC-grade Buffers and Additives from Sigma-Aldrich/Fluka®		
Ammonium acetate	50 g, 250 g	17836
Ammonium formate	50 g, 250 g	17843
Ammonium hydroxide solution in water	100 mL, 1 L	17837
Ammonium phosphate monobasic	250 g	17842
Ammonium trifluoroacetate	10 g, 50 g	17839
Potassium phosphate dibasic anhydrous	250 g	17835
Sodium formate	50 g, 250 g	17841
Sodium phosphate dibasic dehydrate	250 g	71633
Sodium phosphate monobasic anhydrous	50 g, 250 g	17844
Sodium trifluoroacetate	10 g	17840
Trifluoroacetic acid:Triethylamine 2M:1M	500 mL	09746
Trifluoroacetic acid:Triethylamine 2M:2M	100 mL	09747

For a complete list of HPLC buffers and additives, please refer to our online product catalogue: ***sigma-aldrich.com***

Analytical Tools to Determine the Ethanol Content of Denatured Fuel Ethanol by ASTM® D5501

Vicki Yearick, Steven P. Cecil, Katherine K. Stenerson, and Michael D. Buchanan
mike.buchanan@sial.com

Introduction

The desire for cleaner-burning fuels coupled with the desire to reduce use of non-renewable fossil fuels has required an increase in the production of bulk ethanol for fuel purposes. One use of ethanol in fuel applications is as an oxygenated additive in gasoline, resulting in a cleaner-burning fuel. Blends typically range from E10 (10% ethanol and 90% gasoline) to E25 (25% ethanol and 75% gasoline). (1,2) Another use of ethanol in fuel applications is in "flex-fuel" vehicles that can operate with higher ethanol percentages. In the USA, an E85 blend (85% ethanol and 15% gasoline) is used. (3) In Brazil, where ethanol is made from sugar-cane, over half of the cars can operate on E100. (2) Whether used as an additive or as a flex-fuel, the demand for bulk ethanol for fuel purposes is certain to increase in the coming decades.

ASTM Methodologies

Mandated fuel ethanol specifications are available for bulk producers and blenders, as outlined in ASTM D4806 – Standard Specification for Denatured Fuel Ethanol for Blending with Gasolines for Use as Automotive Spark-Ignition Engine Fuel. (4) Bulk ethanol producers are typically required by law to render the fuel ethanol unfit for human consumption by adding a denaturant, typically natural gasoline. ASTM D4806 requires the fuel ethanol to contain a minimum of 92.1% ethanol by volume with the denaturant volume ranging from 1.96% to 4.76%.

Producers and blenders must monitor and report the content of ethanol and the denaturant to show they are in compliance with local country laws. Monitoring is accomplished by following the analytical method ASTM D5501 – Standard Test Method for Determination of Ethanol Content of Denatured Fuel Ethanol by Gas Chromatography. (5)

Capillary GC Column

ASTM D5501, a gas chromatography (GC) method, specifies the use of a temperature programme and a flame ionisation detector to analyse the sample on a long polydimethylsiloxane capillary column, such as the Petrocol™ DH 150. This non-polar column is characterised by high efficiency and excellent reproducibility.

ASTM D5501 requires that peak identification be established, followed by the quantitation of ethanol. To perform this, a standard mixture containing known amounts of each alcohol in proportion to what is expected in the final blend is injected into the GC column, using n-heptane as a solvent. Retention times of the fuel ethanol sample are then compared to the analytical standard to verify identity.

Chemical Standard Kit

Quantitation per ASTM D5501 requires preparation of six multi-component calibration solutions, each containing ethanol, methanol and n-heptane in varying concentrations, to establish a linearity curve for the GC system. Because D4806 specifies a minimum ethanol content of 92.1% for denatured fuel ethanol, the ethanol content found in the six solutions range from 92 to 97%. N-heptane is included in the solutions in place of the denaturant.

Preparation of these calibration standards is time consuming and requires maintaining an inventory of high-purity raw materials. Calibration standards preparation is made easier by using the ASTM D5501 Denatured Fuel Ethanol Standards Kit from Sigma-Aldrich. This kit contains pre-made Supelco brand multi-component, quantitative solutions covering the required range for accurate calibration, per ASTM D5501. A certificate of analysis is provided for each calibration solution.

The resulting chromatogram from a Supelco® brand mid-level standard is shown in **Figure 1**. The chromatogram from the analysis of an E85 sample is shown in **Figure 2**. Per ASTM D5501, mass relative response factors for the fuel ethanol sample are calculated to the nearest 0.01 mass percent, and then compared to the values obtained for each of the six calibration solutions to determine the ethanol content in the denatured fuel ethanol sample for reporting purposes.

Did you know…?

The Renewable & Alternative Energy portal on the Sigma-Aldrich website contains a wealth of information for scientists looking for products and information to aid in their research.

sigma-aldrich.com/renewable

Figure 1. Calibration Standard

column: Petrocol™ DH 150, 150 m x 0.25 mm I.D., 1.0 µm (24155)
oven: 60 °C (15 min), 30 °C/min to 250 °C (23 min)
inj.: 300 °C
det.: FID, 250 °C
carrier gas: helium, 24 cm/sec @ 60 °C
injection: 0.5 µL, 150:1 split
liner: 4 mm I.D., single taper
sample: Denatured Fuel Ethanol Solution 4, from ASTM® D5501 Denatured Fuel Ethanol Standards Kit (40361-U)

1. Methanol, 95.00%
2. Ethanol, 4.70%
3. n-Heptane, 0.30%

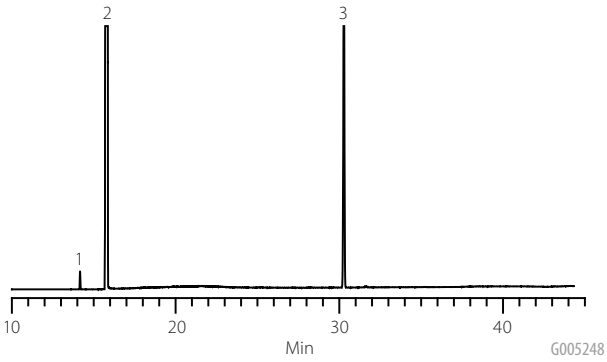
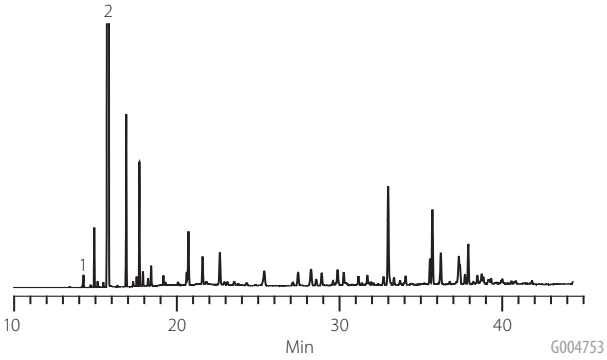


Figure 2. E85 Sample

sample: E85 denatured fuel ethanol

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



Conclusion

As the demand for bulk ethanol for fuel purposes increases, so will the need for testing. Supelco® capillary GC columns and chemical standards are the perfect complement to one another for this application.

References

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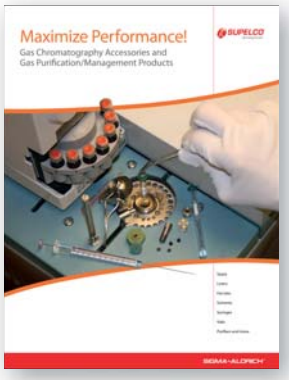
2. US DOE web site, Country Analysis Briefs: Brazil (***www.eia.doe.gov/cabs/Brazil/Full.html***).
3. US EPA website, SmartWay Grow & Go web page (***www.epa.gov***).
4. ASTM D4806; Standard Specification for Denatured Fuel Ethanol for Blending with Gasolines for Use as Automotive Spark-Ignition Engine Fuel, ASTM International (***www.astm.org***).
5. ASTM D5501; Standard Test Method for Determination of Ethanol Content of Denatured Fuel Ethanol by Gas Chromatography, ASTM International (***www.astm.org***).

Featured Products

Description	Cat. No.
Petrocol DH 150, 150 m x 0.25 mm I.D., 1.0 µm	24155
ASTM D5501 Denatured Fuel Ethanol Standard Kit Kit contains seven ampuls (prepared wt/wt). Solution 1 = Ethanol:Heptane:Methanol (92%:7.40%:0.60%) Solution 2 = Ethanol:Heptane:Methanol (93%:6.50%:0.50%) Solution 3 = Ethanol:Heptane:Methanol (94%:5.60%:0.40%) Solution 4 = Ethanol:Heptane:Methanol (95%:4.70%:0.30%) Solution 5 = Ethanol:Heptane:Methanol (96%:3.80%:0.20%) Solution 6 = Ethanol:Heptane:Methanol (97%:2.90%:0.10%) Solution 7 = Ethanol:Heptane:Methanol (98%:1.95%:0.05%)	40361-U

Related Products



For a list of commonly used GC items, refer to *Maximize Performance! Gas Chromatography Accessories and Gas Purification/Management Products* (T407103 JWE).

This brochure contains products such as GC septa, inlet liners, ferrules, solvents, autosampler syringes, autosampler vials, purifiers, and more, all designed to be used with the most common GC models, such as those manufactured by Agilent/HP, PerkinElmer, Shimadzu, Thermo, and Varian.

Related Information

Additional information about this, or other analytical methodologies used for bioethanol and biodiesel applications, can be found by visiting our biofuels web node at ***sigma-aldrich.com/biofuels***

HPLC Analysis of Fuel Ethanol Produced by Fermentation

One Source Solution of Column and Quantitative Calibration Standard

Steve Cecil
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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).

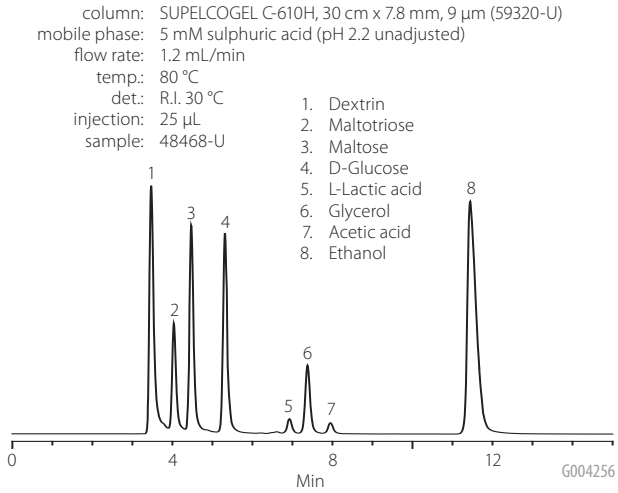
Many areas of the process are important to ensure a quality end product, such as the breakdown of the corn substrate to fermentable sugars and distillation. However, none are more critical than the ethanol-producing step of fermentation.

Optimised fermentation leads to increased ethanol yield and profitability of the biofuel facility. Residual sugars leave 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.

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



The HPLC analysis utilises a crosslinked polystyrene/divinylbenzene resin ion exchange column. Alternative 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.

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 as these 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.

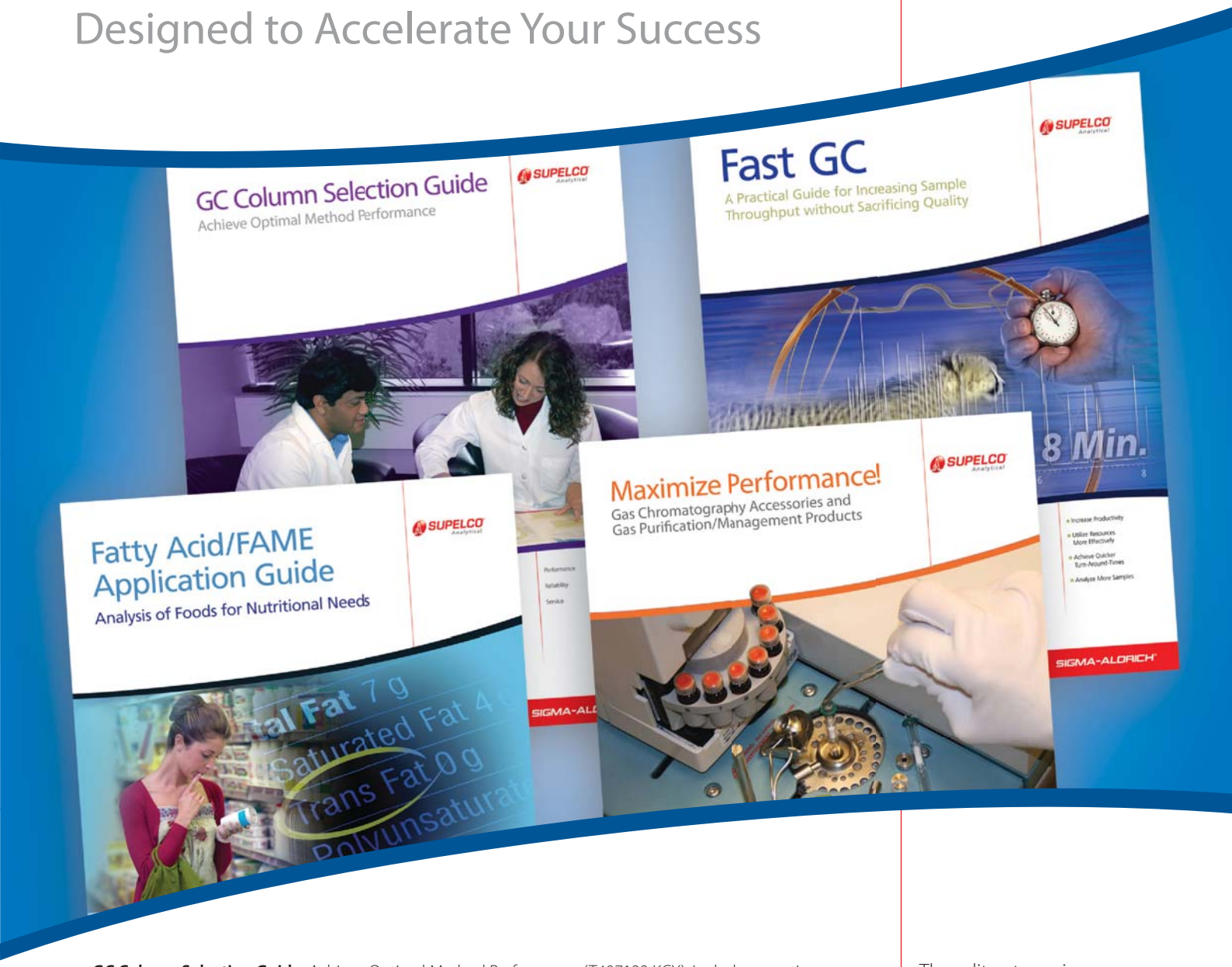
Utilising Supelco's Fuel Ethanol Residual Saccharides Mix in conjunction with the SUPELCOGEL C-610H column provide data for improved fermentation and higher ethanol yields.

Featured Products

Description		Cat. No.
Fuel Ethanol Residual Saccharides Mix w/v% varied conc., deionised 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

GC Literature from Supelco®

Designed to Accelerate Your Success



- **GC Column Selection Guide:** Achieve Optimal Method Performance (T407133 KCX). Includes a section on how to choose a column, 10 tables of phase recommendations by industry/application, a cross-reference chart, and details of each phase.
- **Fast GC:** A Practical Guide for Increasing Sample Throughput without Sacrificing Quality (T407096 JTW). Describes how to implement it, a theoretical discussion of why it works, 26 chromatograms spanning several industries/applications, and a brief listing of GC accessories.
- **Fatty Acid/FAME Application Guide:** Analysis of Foods for Nutritional Needs (T408126 KUK). Includes sections on free fatty acids, derivatisation to FAMEs, SPE fractionation, FAMEs by boiling point elution, FAMEs by degree of unsaturation, omega 3 and omega 6 FAMEs, and cis/trans FAME isomers.
- **Maximise Performance!** Gas Chromatography Accessories and Gas Purification/Management Products (T407103 JWE). A "must-have" for all GC labs! Lists all the common replacement items, such as septa, liners, ferrules, solvents, syringes, vials, purifiers, and much more.

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Headspace Solvents for Analysis of OVI

High Purity and Superior Performance

Katherine K. Stenerson and Shyam Verma
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The purity of dissolution solvents used in headspace analysis is essential for avoiding extraneous peaks in the subsequent chromatographic analysis, and preventing interference with the analytes of interest. Many protocols followed by laboratories doing Organic Volatile Impurity (OVI) analysis require the analysis of an acceptable blank, and some published methodologies (1–3) require the analysis of a blank to verify the absence of interfering peaks.

In earlier publications (4) we have reported the suitability of gas chromatography headspace grade (GC-HS) solvents offered by Sigma-Aldrich for use in the analysis of the OVIs listed in United States Pharmacopeia (USP) Method <467>, European Pharmacopoeia (EP) Method 2.4.24, and the International Conference on Harmonization (ICH) guidelines. By comparing headspace grade solvents with the conventional organic synthesis grade solvents, it was demonstrated that the GC-HS grade solvents produced cleaner blanks and showed no major interference peaks in the elution range of the target analytes.

Chromatograms of headspace blanks (4) using GC-HS and organic synthesis grades of DMSO are presented in **Figures 1** and **2**. Overall, the GC-HS grade blank had fewer peaks in the OVI elution range than the organic synthesis grade blank. Comparing the blanks with the chromatogram of a working OVI standard prepared in GC-HS grade DMSO, it was reported (4) that both blanks contained some dimethylformamide (DMF). The organic synthesis blank contained a peak eluting close to the retention time (t_R) of ethanol. This peak could potentially interfere with the proper detection and analysis of ethanol as an OVI. A peak corresponding to the t_R of 1,3-dimethyl-2-imidazolidinone (DMI) was detected in the GC-HS blank. This same peak was also detected in the OVI working standard prepared in GC-HS grade DMSO (4).

Figure 1. Headspace Blank, DMSO – Organic Synthesis Grade

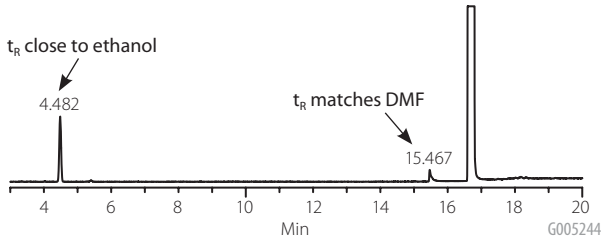
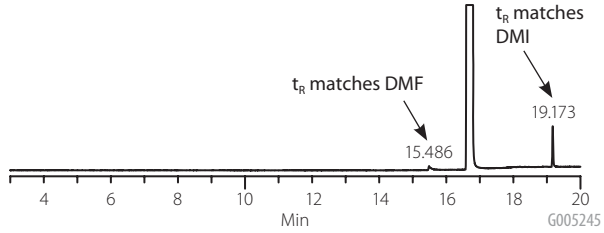


Figure 2. Headspace Blank, DMSO – Fluka® GC-HS Grade



Additional purity tests were done on both grades of DMSO using GC-MS. These tests were carried out to make tentative identification of impurities eluting in the primary range of OVIs. The analysis of impurities in these solvents was performed using solid phase microextraction (SPME) to do a headspace extraction. The samples of DMSO were diluted 1:1 with deionised water, and a 2 mL aliquot was extracted by headspace SPME and analysed by GC-MS using the conditions listed in **Table 1**.

Table 1. Headspace SPME, GC-MS Parameters

sample matrix:	1 mL DMSO + 1 mL deionised water in 4 mL vial
SPME fibre:	100 µm PDMS
extraction:	Headspace, 50 °C, 5 min with stirring
desorption process:	250 °C for 3 min
column:	SPB™-624, 30 m x 0.25 mm I.D., 1.4 µm (24255)
oven:	35 °C (3 min), 8 °C/min to 220 °C (10 min)
MSD interface:	220 °C
scan range:	m/z 40 – 450
carrier gas:	helium, 1 mL/min
liner:	0.75 mm I.D., SPME

Figure 3 presents Total ion chromatograms (TICs) from the analysis of GC-HS and organic synthesis grade DMSO. The scale of both TICs is the same, and the elution range prior to DMSO is shown. The baseline disturbances present in both TICs are a result of background artefacts resulting from the headspace SPME. The organic synthesis grade was found to contain many peaks not detected in the GC-HS grade DMSO. These peaks were tentatively identified by spectral library match and peaks 1 and 2 (pentane and hexane) are class 3 and 2 solvents respectively.

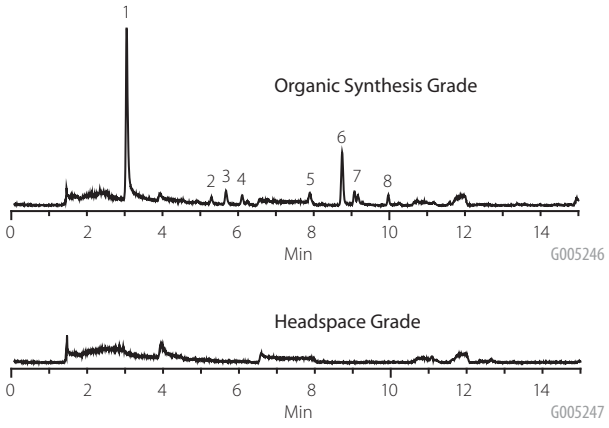
These results suggest that the GC-HS grade DMSO is more suitable for the analysis of OVIs. The GC-HS grade produced a cleaner headspace blank and did not show any major interference peaks in the elution range of the target analytes. In comparison, the organic synthesis grade showed a large peak in this range.

Additional purity testing of the solvents by headspace SPME/GC-MS was able to detect and tentatively identify compounds in the organic synthesis grade DMSO that were not present in the GC-HS grade. Two of these compounds were solvents listed in the ICH guidelines, USP Method <467>, and EP Method 2.4.24.

The GC-HS DMSO evaluated in this study was Fluka Brand and is specified for headspace use. DMSO, as well as other Fluka Brand solvents (see Featured Products next page) designated for headspace use are manufactured under strictly controlled processes, including micro-filtration, and packed under an inert atmosphere. This ensures their suitability for meeting the demands of headspace analysis.

Figure 3. SPME GC-MS Impurity Analysis of DMSO Grades

1. Pentane
2. Hexane
3. Isopropyl ether
4. Dimethylpentane
5. Isooctane
6. Diisobutylene
7. Trimethylcyclopentane
8. Dimethylcyclopentanone



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2. ICH Guidelines for Industry, Q3C Impurities: Residual Solvents, US Dept. of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), ICH December, 1997.
3. European Pharmacopoeia (EP) 5.0, Vol.1, (2004), 2.4.24 Identification and Control of Residual Solvents.
4. A. Quiroga, M. Dong, K. Stenerson, S. Verma. The Utility of Headspace Grade Solvents in the Analysis of Organic Volatile Impurities. Presented at the Eastern Analytical Symposium, Somerset, N.J., Nov. 2009; Supelco® publication T409180.

Featured Products

Description	Qty.	Cat. No.
N,N-Dimethylacetamide	1 L	44901
Dimethyl sulphoxide	1 L	51779
N,N-Dimethylformamide	1 L	51781
1,3-Dimethyl-2-imidazolidinone	1 L	67484
Water	1 L	53463

All products are puriss. p.a. for GC-HS

Related Products

Description	Qty.	Cat. No.
Column		
SPB-624, 30 m x 0.25 mm I.D., 1.4 µm	1	24255
SPME Fibre Assembly Polydimethylsiloxane (PDMS)		
100 µm, manual holder, 24 ga., fused silica/SS	3	57300-U
100 µm, manual holder, 23 ga., fused silica/SS	3	57342-U
100 µm, autosampler, 23 ga., fused silica/SS	3	57341-U
100 µm, autosampler, 23 ga., metal alloy/metal alloy	3	57928-U

Description	Concentration		Qty.	Cat. No.
Standards				
USP 467 Class 1 Residual Solvents Mix	DMSO, varied conc.		1 mL	40131-U
Benzene, 10,000 µg/mL	*1,2-Dichloroethane, 25,000 µg/mL*	*1,1,1 Trichloroethene, 50,000 µg/mL*		
Carbon tetrachloride, 20,000 µg/mL	*1,1-Dichloroethene, 40,000 µg/mL*			
USP 467 Class 2 Residual Solvents Mix A	DMSO, varied conc.		1 mL	40132-U
Acetonitrile, 2050 µg/mL	*1,4-Dioxane, 1900 µg/mL*	*Tetrahydrofuran, 3600 µg/mL*		
Chlorobenzene, 1800 µg/mL	*Ethyl benzene, 18400 µg/mL*	*Toluene, 4450 µg/mL*		
Cyclohexane, 1940 µg/mL	*Methanol, 1500 µg/mL*	*m-Xylene, 980 µg/mL*		
cis-Dichloroethene, 4700 µg/mL	*Methylcyclohexane, 5900 µg/mL*	*o-Xylene, 6510 µg/mL*		
trans-Dichloroethene, 4700 µg/mL	*Methylene chloride, 3000 µg/mL*	*p-Xylene, 1520 µg/mL*		
USP 467 Class 2 Residual Solvents Mix B	DMSO, varied conc.		1 mL	40133-U
Chloroform, 300 µg/mL	*2-Hexanone, 250 µg/mL*	*Tetralin, 500 µg/mL*		
1,2-Dimethoxymethane, 500 µg/mL	*Nitromethane, 250 µg/mL*	*Trichloroethene, 400 µg/mL*		
Hexane, 1450 µg/mL	*Pyridine, 1000 µg/mL*			

Selective Phospholipid Extractions for Cleanup or Enrichment Using HybridSPE®-Phospholipid

Michael Monko
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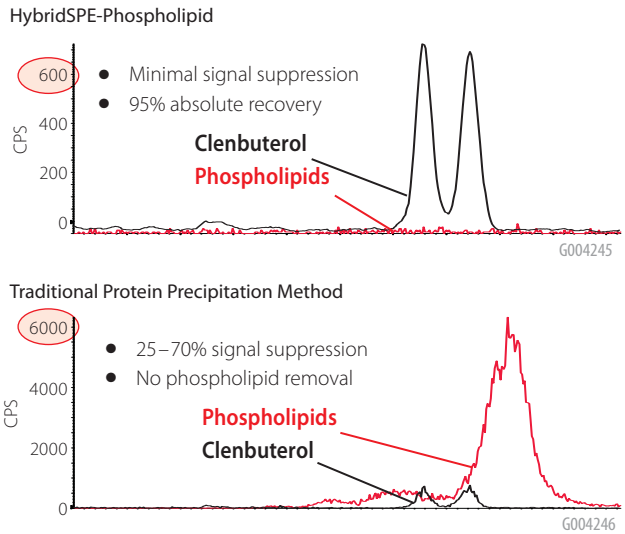
Introduction

Since phospholipids are one of the major components of cell membranes, they are typically present in nearly all biological samples encountered by an analytical chemist. This is important in several ways. First, phospholipids typically represent interferences for analysts trying to quantify pharmaceutical molecules by LC-MS in samples such as serum or plasma. Secondly, phospholipids can be important in many pathological and functional studies and therefore need to be carefully isolated and quantitatively analysed. This dual perspective on phospholipids as an interference in some samples, but as the analyte of interest in other samples, presents a challenge for sample preparation. As such, there is a need for a sample preparation technique that allows for the removal of phospholipids from samples where they are interferences, while allowing for isolation and recovery of phospholipids in applications where they are the analyte of interest. HybridSPE-Phospholipid has demonstrated the ability to efficiently and effectively accomplish both tasks by simple manipulation.

As discussed previously in The Reporter (1), the selectivity of the HybridSPE sorbent for phospholipids is based on Lewis-acid-base interactions between phospholipids and the zirconia-coated surface of the particle, moderated by a weaker Lewis-base such as formic acid.

Figure 1. Comparison of the Extraction of Clenbuterol Using HybridSPE-Phospholipid vs. Traditional PPT Method



column: Chirobiotic™ T 100 mm X 2.1 mm, 5 µm (12018AST)
mobile phase: 10 mM Ammonium Formate/Methanol
flow: 300 µl/min
temp.: 30 °C
detection: MS-MRM

Phospholipids as Contaminants

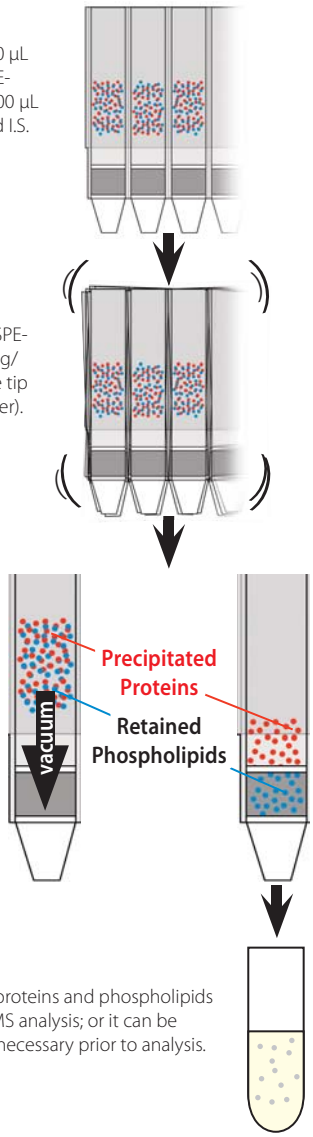
Traditional sample preparation methods such as protein precipitation (PPT) are only effective at removing proteins, leaving phospholipid contamination that cause ion suppression in LC-MS (**Figure 1**). HybridSPE-Phospholipid is highly efficient at removing phospholipid contaminants from biological matrices. The standard protocol for this application requires only a few steps and minimal method development to implement into a regular sample preparation routine (**Figure 2**). This primary technique is highly effective for the vast

Figure 2. Standard Protocol for Phospholipid Removal Using HybridSPE-Phospholipid

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

2) Mix by vortexing/shaking HybridSPE-Phospholipid 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 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.

majority of analytes in bioanalytical samples, but it should be noted that occasionally a secondary protocol needs to be employed. In cases where analytes of interest are strongly acidic or chelating compounds, the use of 0.5% citric acid in acetonitrile as crash solvent has been shown to significantly improve analyte recovery. In cases where analytes of interest are strongly basic, the use of 1% ammonium formate in methanol has been shown to improve analyte recovery. (2)

Phospholipids as Analytes

HybridSPE®-Phospholipid also allows for a simple and effective method to isolate and enrich phospholipids from biological samples such as plasma. (3) This method utilises the same Lewis-acid-base interactions that selectively retain phospholipids, but also to efficiently desorb them from the HybridSPE sorbent. A strong Lewis base such as 5% ammonium hydroxide in methanol is employed to disrupt the interactions of the phospholipids and the HybridSPE sorbent. The standard protocol (**Figure 3**) requires minimal method development and results in phospholipid recoveries near 95%. (3) This highly selective retention and desorption process allows for clean and efficient recovery of phospholipids for analysis.

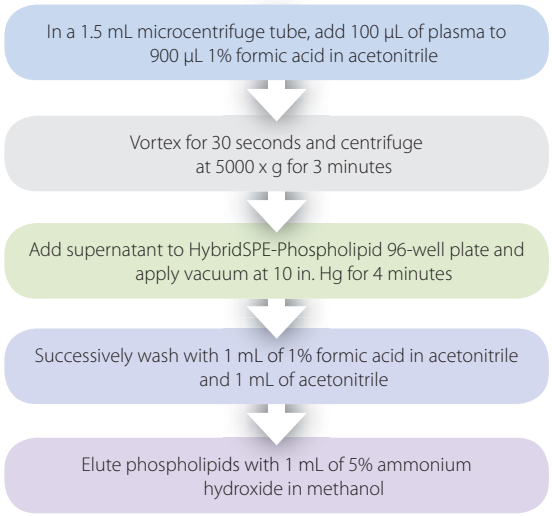
Conclusion

Phospholipids are significant as interferences in LC-MS, but also can be analytes of interest. Traditional sample preparation techniques (like standard SPE, liquid-liquid-extraction, standard PPT) typically lack the selectivity to effectively extract phospholipids. HybridSPE-Phospholipid, based on Lewis-acid-base interactions, provides the selectivity to extract and remove phospholipids as contaminants, and also to isolate and recover phospholipids when they are the analyte of interest.

References

1. Aurand, C., et al. Introducing HybridSPE-Precipitation Technology for Pharmaceutical Bioanalytical Sample Preparation. The Reporter Volume 26.3. page 3 (2008).
2. Aurand, C., et al. Increased Bioanalytical Throughput Using Fused-Core® HPLC with Selective Phospholipid Depletion. Poster from HPLC (2009).
3. Lu, X.; Ye, M. Enrichment of Phospholipids in Biological Samples Using HybridSPE-PPT. The Reporter Volume 28.3 page 6. (2010).

Figure 3. Standard Protocol for Phospholipid Enrichment Using HybridSPE-Phospholipid



Featured Products

Description	Qty.	Cat. No.
HybridSPE-Phospholipid 96-well Plate, 50 mg/well	1	575656-U
HybridSPE-Phospholipid 96-well Plate, 15 mg/well	1	52794-U
HybridSPE-Phospholipid Cartridges, 30 mg/1 mL	100	55261-U
HybridSPE-Phospholipid Cartridges, 500 mg/6 mL	30	55267-U
NEW* HybridSPE-Phospholipid ULTRA Cartridges, 30 mg/1 mL	100	55269-U

* Suitable for in tube protein precipitation as is possible with the 96-well plates.

Related Information

To maximise the speed and sensitivity of your bioanalytical assays, you need proven consumables. For more information about sample prep, HPLC columns, and LC-MS buffers and solvents to help you achieve maximum LC-MS performance, please visit ***sigma-aldrich.com/bioanalytical***

Preventing Contamination of Thermal Desorption Tubes During Storage Using TDS³™ Storage Containers

Jamie Brown and Kristen Schultz
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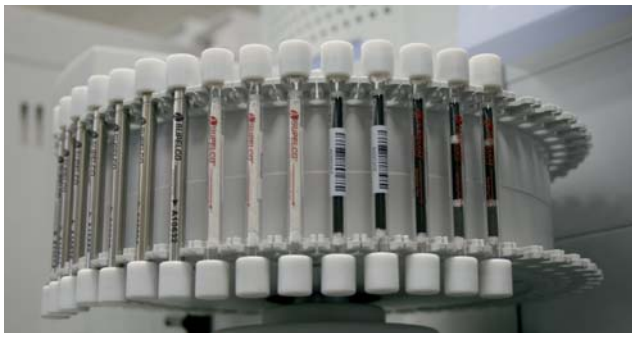
Carbotrap 300 Thermal Desorption Tubes in a TDS³ Container and Sealed with Brass Swagelok Endcaps

Introduction

The goal of this study was to evaluate the usefulness of the Supelco® "Thermal Desorption Tube Sampling and Storage System" (TDS³) in preventing thermal desorption tubes from becoming contaminated if stored in a highly contaminated atmosphere. It's crucial that the thermal desorption tube is kept clean prior to sampling, and that new contaminants are not adsorbed by the adsorbents after sampling and during transport back to the laboratory. The TDS³ storage container was tested along with the popular brass Swagelok® endcap with a PTFE one-piece ferrule. The TDS³ container offers users an alternative to brass endcaps. They are 84% lighter (reduced shipping cost) and the clear body allows the user to conveniently read the serial number or the barcode* on the tube without the need to remove it from the container.

This study challenges the TDS³ storage container by subjecting pre-conditioned tubes to a highly contaminated atmosphere for seven days. The tubes were then analysed to determine if any contaminants migrated through the storage container and were adsorbed by the adsorbents in the tube. This study was conducted using Supelco's new glass-fritted 89 mm long thermal desorption tubes packed in the Carbotrap™ 300 configuration (29532-U).



A Variety of Supelco Thermal Desorption Tubes in Autosampler

Experimental

Nine Carbotrap 300 tubes were conditioned for 4 hours at 350 °C. Four tubes were sealed in the TDS³ storage containers, and four tubes were sealed with brass endcaps. Three of each type were placed in the high contamination atmosphere of a laboratory solvent flame cabinet, and one of each type was placed in a clean metal paint can with a charcoal filter in the bottom to serve as storage blanks.

The last tube remained unsealed and was placed in the flame cabinet on the sixth day of the study to illustrate the high contamination atmosphere of the flame cabinet.** The flame cabinet contained a variety of volatile organic solvents that posed an environment more challenging than typically encountered when tubes are stored before and after sampling.

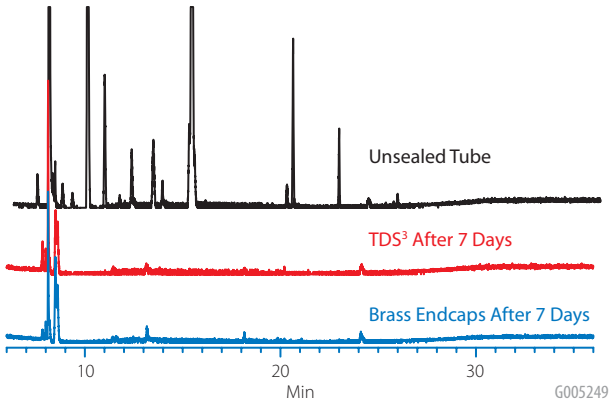
To assess the concentration, a toluene calibration curve was performed that spanned 1, 10, 25, and 100 nanograms per tube. The background contamination was defined as any extraneous peaks not present in the control blank.

Results

After seven days, the tubes were removed from the flame cabinet, along with the storage blanks from the paint can. The tubes were analysed using a SPB-HAP capillary GC column. This column has a 4.0 µm film, causing very volatile analytes to focus on the front of the column. It was selected for this study to eliminate the need to employ cryogenic focusing techniques. Results of the GC analysis showed no extraneous peaks above the 1 ng toluene calibration level on any of the six tubes sealed with the TDS³, or with the brass endcaps.

The top chromatogram in **Figure 1** shows what analytes were passively collected on the tube without a storage container. There were several peaks exceeding > 100 nanograms (acetonitrile, ethyl ether, methylene chloride, hexane, heptane, and toluene).

Figure 1. Comparison of Unsealed Tube to TDS³ and Brass Endcaps



The middle and bottom chromatograms of **Figure 1** show the results of a tube sealed in the TDS³™ and a tube sealed with brass endcaps after being stored in the flame cabinet for seven days.

Figure 2 compares the results of the tubes sealed with the TDS³ storage container. There was no significant difference between the storage blank tube stored in the paint can (top) and the tube stored inside the flame cabinet for seven days (bottom). Note: the peaks at 8 and 8.5 minutes are system peaks of carbon dioxide.

Figure 2. Comparison of TDS³ Blank to Exposure of Flame Cabinet

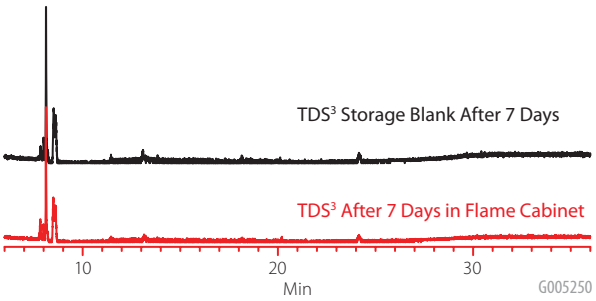
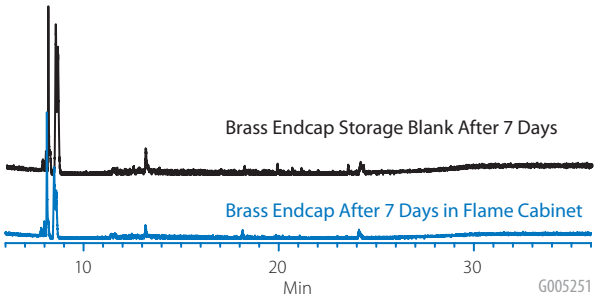


Figure 3 compares the results of the tubes sealed with the brass endcaps. There was no significant difference between the storage blank tube stored in the paint can (top) and the tube stored inside the flame cabinet for 7 days (bottom). Note: the peaks at 8 and 8.5 minutes are system peaks of carbon dioxide.

Figure 3. Comparison of Brass Endcaps Blank to Exposure of Flame Cabinet



Conditions

Thermal Desorption

desorber:	PerkinElmer® TurboMatrix™ 150
primary tube:	Carbotrap™ 300 Fritted Glass Tube (29532-U)
desorption temp.:	330 °C for 5 min
focusing tube:	p/w Carbopack™ Y & Carbopack B & Carboxen 1000 (Custom)
desorption temp.:	Low -20 °C, High -330 °C for 5 min
valve temp:	175 °C
transfer line:	175 °C
desorb flow:	25 mL/min
inlet split:	5 mL/min
outlet split:	5 mL/min

Chromatography

column:	SPB-HAP, 60 m x 0.32 mm I.D. x 4.0 µm film (25020-U)
oven:	35 °C (5 min) 5 °C/min to 100 °C (0 min) 15 °C/min to 220 °C (10 min)
MSD interface:	230 °C
scan range:	m/z 35–265
carrier gas:	helium, 2 mL/min

Conclusion

The TDS³ storage containers are as effective as the brass endcaps at protecting the adsorbents of the thermal desorption tubes from contaminated environments that occur in normal transportation and storage. Additionally, the clear TDS³ body protects the outer surface of the tube from contamination that can enter the analytical systems during desorption on some thermal desorption instruments. The TDS³ are significantly lighter than the brass endcaps, which reduces the costs associated with shipping tubes to and from the lab. The polymer construction also helps to prevent breakage of glass thermal desorption tubes during shipping and handling.

* Barcode with a visual serial number is currently only available the new line of ¼ "O.D. x 89 mm long glass fritted tubes from Supelco®.

** The unsealed tube was only exposed to the inside of the flame cabinet for one day for fear it may overload the chromatographic system if it was exposed for seven days.

Featured Products

Description	Qty.	Cat. No.
Capillary GC Column		
SPB-HAP, 60 m x 0.32 mm I.D., 4.0 µm		25020-U
TDS³ Storage Container by Instrument Manufacturer/Model		
Supelco, DANI, Markes, PerkinElmer, Shimadzu®	1	25097-U
CDS/Dynatherm™ Standard Tubes	1	25096-U
Chrompack TD Tubes	1	25098-U
GERSTEL® TDS/TDS2/TDSA Tubes	1	25095-U
Teledyne/Tekmar® AERO trap 6000 Tubes	1	25095-U
TDS³ Storage Container Accessories		
Sampling Caps w/washers for ¼ in. OD Tubes	10	25069
Replacement Septa for all TDS³ Containers	50	25073
Male Luer Plug	12	504351
Female Luer Cap	12	57098

Related Information

ATIS
Adsorbent Tube Injector System

The ATIS system allows a simple gas phase spiking of analytes onto adsorbent tubes. It evaporates liquid standards in a heated cuvette and transfers them with inert gas onto the tube. With the optional humidifier module, humid sampling environments can be simulated.

For more information call our Technical Service, visit us at the website below and refer to "Tube Spiking System – ATIS" or request our air monitoring brochure (**KQV**).

New *Trace*CERT® Organic Certified Reference Materials for Environmental and Food Analysis

Matthias Nold, Product Manager Analytical Standards, *matthias.nold@sial.com*
Alexander Rück, Senior Scientist R&D Europe, *alexander.rueck@sial.com*
Christine Hellriegel, Senior Scientist R&D Europe, *christine.hellriegel@sial.com*

The use of traceable certified reference materials is gaining increased importance, especially for accredited testing labs in the areas of food and beverage analysis and environmental analysis. We now present several new CRMs as part of the organic ***Trace*CERT** line. These products, including pesticides, PAHs (polycyclic aromatic hydrocarbons), fatty acids, and antibiotics are intended for use as chromatography standards.

Recently, the innovative new product line of organic ***Trace*CERT** standards (21 amino acids) was presented [1]. These highest quality certified reference materials (CRMs) are certified in a double accredited laboratory fulfilling both ISO/IEC 17025 and ISO Guide 34 using **high-performance quantitative NMR** for content determination with direct traceability to NIST and SI.

The organic ***Trace*CERT** reference materials have several benefits:

- Certified content by quantitative NMR (qNMR)
- Superior level of accuracy, calculated uncertainties and lot-specific values
- Traceability to NIST
- Comprehensive documentation delivered with the product (certification according to ISO Guide 31)

The major advantage of ^{1}H quantitative NMR as a relative primary method is that the integrals of the proton signals are completely independent of the chemical structure. Therefore, traceability can be established between two different compounds, in contrast to most other analytical techniques.

Thus, with a small set of NIST traceable internal standards, it is possible to certify basically any organic compound by measuring a gravimetrically produced mixture of the analyte and the internal standard. The precise mass to mass ratio then allows for the calculation of the analyte content with a very high precision. Typical uncertainties range from 0.5% down to 0.1%.

Certification of PAHs by qNMR spectroscopy

Polycyclic aromatic hydrocarbons (PAHs), occur in oil, coal, and tar deposits, and are produced as byproducts of fuel burning (whether fossil fuel or biomass). As a pollutant, they are of high concern because some of these combustion compounds have been identified to be carcinogenic, mutagenic, or teratogenic. The first PAH CRMs launched as ***Trace*CERT** products include Acenaphthene, Fluorene, Naphthalene, Phenanthrene and Pyrene.

After extensive preliminary tests, the material was quantified by weighing ten separate subsamples together with a suitable reference standard traceable to NIST, dissolved in deuterated solvent, and analysed by NMR spectroscopy. After the content value was determined, a comprehensive uncertainty evaluation was completed, leading to an ISO Guide 31 conform certificate associated with the products.

New organic *Trace*CERT products

Table 1 lists the most recent product additions, including not only polycyclic aromatic hydrocarbons, but also pesticides, fatty acids/FAME and antibiotics. We are continually working to expand our product portfolio, and new products are added regularly. Please visit our website (***sigma-aldrich.com/organiccrm***) where you can find an up-to-date product list and download example certificates and technical articles.

Table 1: NEW Organic Neat CRMs ***Trace*CERT**

Description	Cat. No.	Package Size	Product Group
Acenaphthene	05426	100 mg	PAH
Fluorene	56849	100 mg	PAH
Naphthalene	91489	100 mg	PAH
Phenanthrene	73338	100 mg	PAH
Pyrene	18868	100 mg	PAH
Malathion	91481	100 mg	Pesticides
Diazinon	68486	100 mg	Pesticides
Stearic Acid	76137	100 mg	Fatty Acids / FAME
Methylstearate	75533	100 mg	Fatty Acids / FAME
Sulfamethoxazole	76177	100 mg	Antibiotics

References:

1. ***Trace*CERT®** Organic Certified Reference Materials, Analytix, Vol 2 and 3, 2010.

Seminars on Food Analysis in Europe

These seminars provide an overview on current topics from food analysis such as determination of pesticides, fatty acids, and flavors. Presentations on the most recent developments in GC, HPLC and sample handling will complete the program.

The seminars will take place in different locations in Austria, Czech Republic, Germany, Hungary, Poland and Switzerland during May and June.

Please find more details on the seminars at: ***sigma-aldrich.com/events***

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Description	Qty.	Cat. No.
General use, 2 mL crimp-top glass vials with aluminum seal, large 6.0 mm opening		
Clear vial with PTFE/rubber septa	100	29124-U
Clear vial with PTFE/silicone septa	100	29125-U
Clear vial with PTFE/silicone/PTFE septa	100	29126-U
Amber vial with PTFE/rubber septa	100	29127-U
Amber vial with PTFE/silicone septa	100	29128-U
Amber vial with PTFE/silicone/PTFE septa	100	29129-U
General use, 2 mL screw thread glass vials (8-425, standard opening) with polypropylene caps		
Clear vial with PTFE/silicone septa	100	29104-U
Clear vial with PTFE/silicone/PTFE septa	100	29107-U
Amber vial with PTFE/silicone septa	100	29106-U
Amber vial with PTFE/silicone/PTFE septa	100	29108-U
General use, 2 mL screw thread glass vials (10-425, large opening) with polypropylene caps		
Clear vial with PTFE/rubber septa	100	29116-U
Clear vial with PTFE/silicone septa	100	29118-U
Clear vial with PTFE/silicone/PTFE septa	100	29120-U
Amber vial with PTFE/rubber septa	100	29117-U
Amber vial with PTFE/silicone septa	100	29119-U
Amber vial with PTFE/silicone/PTFE septa	100	29121-U

High-Purity DNPH Standards for Monitoring of Atmospheric Carbonyls

Ketones and aldehydes are released into the atmosphere on a daily basis by motor vehicle emissions, building materials, household products, cigarette smoke and the photo-oxidation of volatile organic compounds. Short-term exposure to these carbonyl compounds can result in burning sensations to the eyes, nose and throat, fatigue and nausea. Long-term exposure may result in cancer as carbonyls are considered probable carcinogens.

Analysts monitoring for atmospheric carbonyls follow prescribed methods published by the US Environmental Protection Agency, the California Air Resource Board, and the American Society for Testing and Materials (ASTM®). These methods call for trapping carbonyls on an adsorbent coated with dinitrophenylhydrazine (DNPH). The captured carbonyls react with the 2,4-DNPH compound to form a more stable dinitrophenylhydrazone derivative that can be analysed by HPLC-UV using reference standards.

Sigma Aldrich offers more than fifty 2,4-DNPH carbonyl derivative reference standards for use in the quantitation of atmospheric carbonyl compounds. They are available in the form of neat substances, single component solutions and method specific mixtures. A Certificate of Analysis stating both the DNPH derivatised and non-derivatised concentration for each carbonyl is supplied with each purchase. Free data packets detailing the preparation and testing of both the raw materials and the final product are available upon request. To view a complete listing of all DNPH-derivative products, please visit ***sigma-aldrich.com/standards***

Featured Products

Description	Qty.	Cat. No.
Neats		
Acetaldehyde-2,4-DNPH	100 mg	442434
Acetone-2,4-DNPH	50 mg	442436
Acrolein-2,4-DNPH	25 mg	442441
2-Butanone-2,4-DNPH	100 mg	442339
Formaldehyde-2,4-DNPH	100 mg	442597

Description	Concentration		Qty.	Cat. No.
European Standards				
Carbonyl DNPH Mix 1	20 µg/mL in acetonitrile (except where noted)		1 mL	47672-U
Acetaldehyde-2,4-DNPH	*2-Butanone-2,4-DNPH*	*Methacrolein-2,4-DNPH*		
Acetone-2,4-DNPH	*Crotonaldehyde-2,4-DNPH*	*Propionaldehyde-2,4-DNPH*		
Acrolein-2,4-DNPH	*Formaldehyde-2,4-DNPH (40 µg/mL)*	*p-Tolualdehyde-2,4-DNPH*		
Benzaldehyde-2,4-DNPH	*Hexaldehyde-2,4-DNPH*	*Valeraldehyde-2,4-DNPH*		
Butyraldehyde-2,4-DNPH				
California Air Resource Board				
CARB Method 1004 DNPH Mix 1	3 µg/mL each component in acetonitrile		1 mL	47650-U
CARB Method 1004 DNPH Mix 2	30 µg/mL each component in acetonitrile		1 mL	47651-U
Acetaldehyde-2,4-DNPH	*2-Butanone-2,4-DNPH*	*Methacrolein-2,4-DNPH*		
Acetone-2,4-DNPH	*Crotonaldehyde-2,4-DNPH*	*Propionaldehyde-2,4-DNPH*		
Acrolein-2,4-DNPH	*Formaldehyde-2,4-DNPH*	*m-Tolualdehyde-2,4-DNPH*		
Benzaldehyde-2,4-DNPH	*Hexaldehyde-2,4-DNPH*	*Valeraldehyde-2,4-DNPH*		
Butyraldehyde-2,4-DNPH				
US Environmental Protection Agency				
TO-11/1P-6A Aldehyde & Ketone DNPH Mix	15 µg/mL each component in acetonitrile		1 mL	47285-U
Acetaldehyde-2,4-DNPH	*Crotonaldehyde-2,4-DNPH*	*Propionaldehyde-2,4-DNPH*		
Acetone-2,4-DNPH	*2,5-Dimethylbenzaldehyde-2,4-DNPH*	*o-Tolualdehyde-2,4-DNPH*		
Acrolein-2,4-DNPH	*Formaldehyde-2,4-DNPH*	*m-Tolualdehyde-2,4-DNPH*		
Benzaldehyde-2,4-DNPH	*Hexaldehyde-2,4-DNPH*	*p-Tolualdehyde-2,4-DNPH*		
Butyraldehyde-2,4-DNPH	*Isovaleraldehyde-2,4-DNPH*	*Valeralddehyde-2,4-DNPH*		

Did you know …?

Supelco® offers a wide range of DNPH air-sampling cartridges designated for sampling carbonyls (like formaldehyde) in ambient, indoor and industrial hygiene atmospheres. Our low back pressure LpDNPH cartridges are suitable for use in the following regulatory methods: NIOSH 2016, ASTM D5197, US EPA 100, IP-6A, and TO-11A. For more information, visit ***sigma-aldrich.com/lpdnph***

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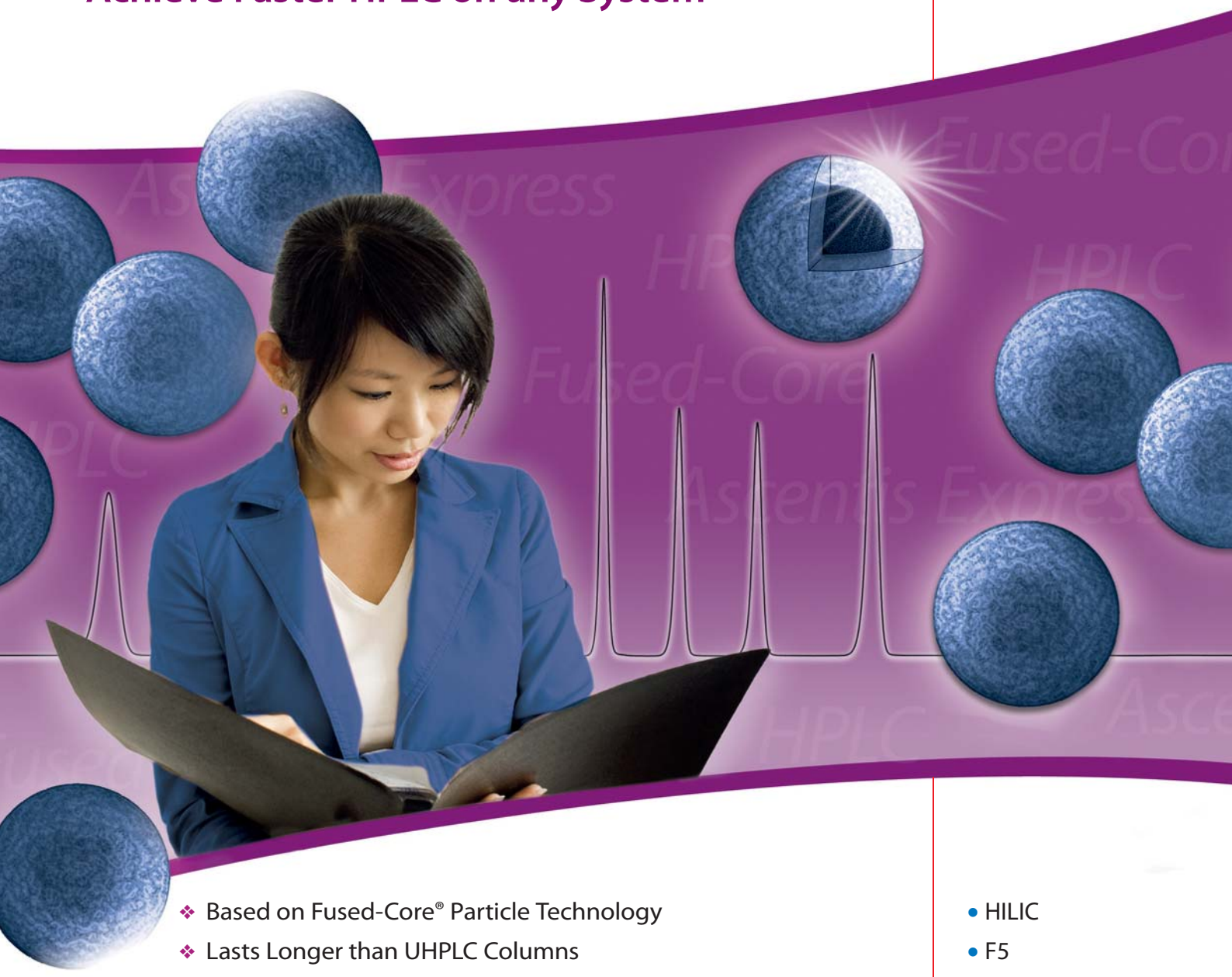

- Based on Fused-Core® Particle Technology
- Lasts Longer than UHPLC Columns
- 7 available selectivities

- HILIC
- F5
- PhenylHexyl
- RP-Amide
- C8
- C18
- C18 ES Peptide (160 Å pore size)

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For technical resources, instructional videos, applications, products, and pricing, contact our technical experts at **EurTechServ@sial.com** for advice on choosing the right column for your needs.

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