

Reporter

Volume 28.5



Separation of Closely Related Compounds



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

Liquid Chromatography
Sample Preparation
Gas Chromatography
Standards
Accessories
Chiral Chromatography

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What Supelco Brings to the Table



Wayne Way

Market Segment Manager
HPLC

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®** – One of the most well-known chromatography products worldwide which is benchmarked in many reference journals.
- **Kromasil® Eternity** – A silica platform column capable of wide pH operation that provides method development flexibility and increased loading of bases for purification.
- **Hamilton® PRP Columns** – Well-known polymeric columns that are written into many methods.
- **Other Important HPLC Column Brands** – Nucleosil®, Spherisorb®, and Lichrosorb®, just to name a few, are historically important products written into many HPLC methods.

Large Molecule HPLC Columns

- **TSKGEL® Columns** – World class leader in GFC, IEX column technology.
- **Discovery® HPLC Columns** – Supelco brand HPLC columns designed for protein and peptide separations.

Chiral HPLC Columns

- **Astec Chiral Columns** – CHIROBIOTIC® and CYCLOBOND™ columns for reversed-phase and LC-MS chiral applications developed in collaboration with Prof Dan Armstrong.
- **Kromasil AmyCoat™ and CelluCoat™ Columns** – For traditional normal phase chiral separations including scale-up and purification.

HPLC Accessories

- **Upchurch Fittings, Rheodyne® Valves, Optimize Guards and Pre-column Filters** – High-quality HPLC consumables. Available through our partnership with IDEX® and Optimize® Technologies.
- **Supel™-Fit connections** – Specialty interconnects that provide the most advanced performance for making all of your HPLC connections.

I hope to leave you with this message: You've trusted Supelco for over 40 years. Continue to look to us to be your supplier of innovative and reliable chromatography products to solve your analytical challenges and 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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Alternative Retention and Selectivity Using Fluorinated Stationary Phases

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 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.

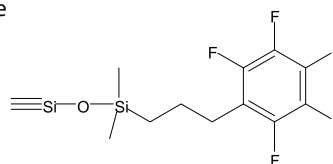
Fluorinated phases have been shown to exhibit greater ion-exchange character than their alkyl counterparts. Fluorinated phases often provide excellent chromatographic results when analytes to be separated differ in their ionization constants or where some ion-exchange is necessary for the retention of 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 utilize 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

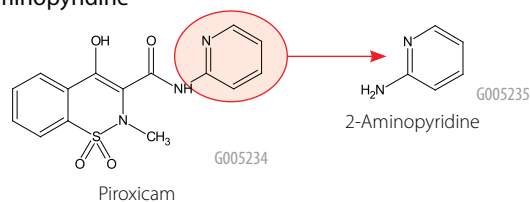
The structure of a popular form of fluorinated phases, pentafluorophenylpropyl (PFP or F5), is shown in **Figure 1**. The F5 bonded phase exhibits strong dipole potential (polar interaction) from the carbon-fluorine bonds, pi-pi interaction potential and the ability to interact via charge-transfer interactions 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

G005235

A common short-coming 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 ($\log P = 0.53 \pm 0.27$) 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

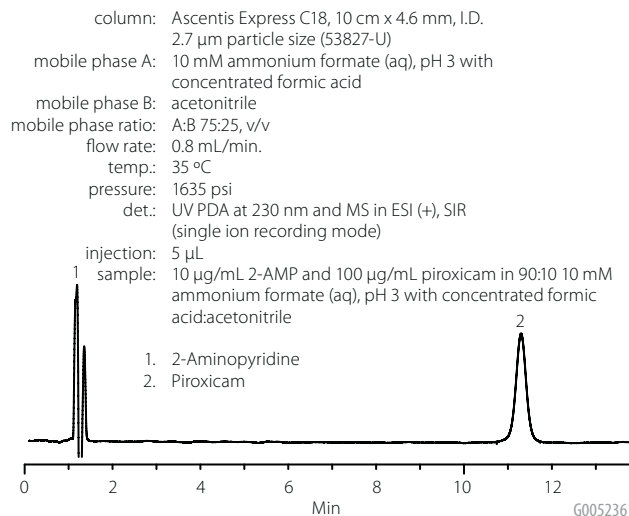
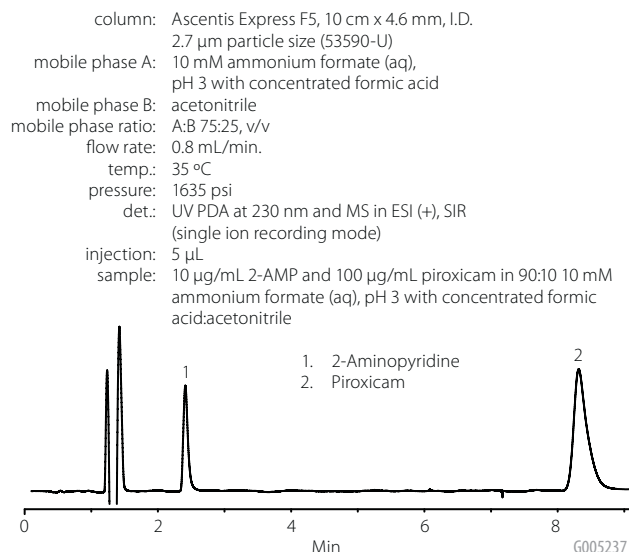


Figure 4. Separation of Piroxicam and 2-Aminopyridine on an F5 Column



(continued from page 3)

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

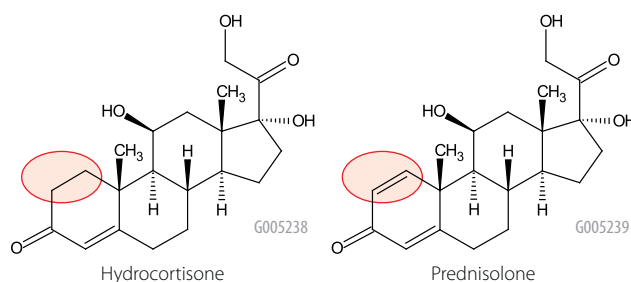
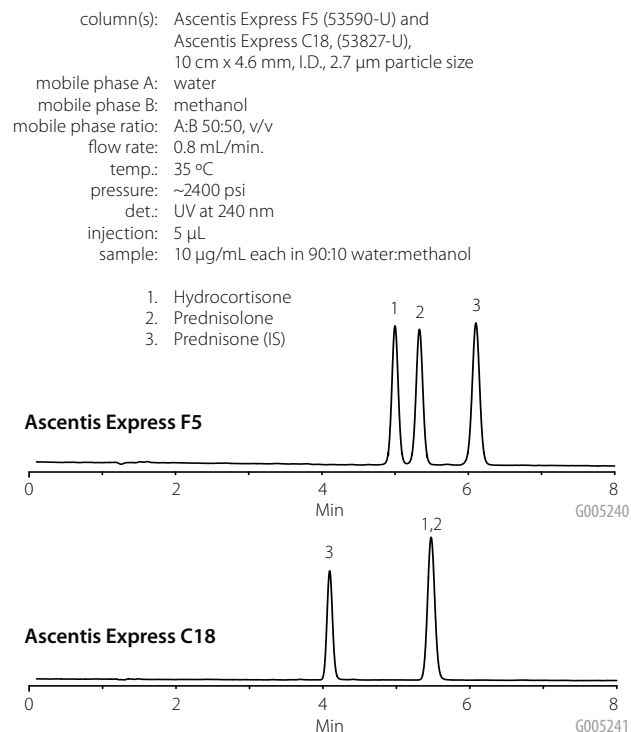


Figure 6. Comparison of C18 and F5 for the Separation of Closely Related Steroids



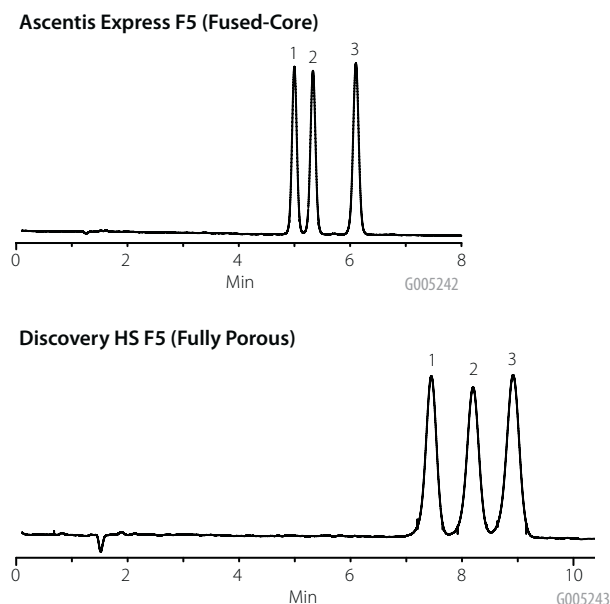


Alternate Selectivity with High Efficiency

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

TRADEMARKS: AmyCoat, CelluCoat, Kromasil – Eka Nobel AB; ASTM – American Society for Testing and Materials; Ascentis, CarboPack, CarboSieve, Carbotrap, Carboxen, CHIROBIOTIC, CYCLOBOND, Discovery, HybridSPE, Petrocol, SPB, Supelco, SUPELCOGEL, SUPELCOSIL, TDS³ – Sigma-Aldrich Biotechnology LP; Dynatherm – CDS Analytical; Fused-Core – Advanced Materials Technology, Inc.; GERSTEL – Gerstel GmbH; Gastight, Hamilton, Microliter – Hamilton Company; LiChrosorb – EM Science, Associate of Merck KGaA; Markes – MARKES International; MicroShot, MicroSeal – Merlin Instrument Company; Nucleosil – Machery Nagel GmbH & Co.; Optimize Technologies – Optimize Technologies, Inc.; PerkinElmer, TurboMatrix – PerkinElmer Corp.; Rheodyne – Rheodyne, Inc.; Shimadzu – Shimadzu Corp.; Spherisorb – Waters Associates, Inc.; Swagelok – Swagelok Co.; Tekmar – Tekmar Co.; TSKGEL – Tosoh Corporation; Upchurch – Upchurch Scientific



Efficient Conversion of HPLC Instruments between Normal-Phase and Reversed-Phase Solvents

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While reversed-phase (RP) HPLC is still by far the most common mode, normal-phase (NP) HPLC is increasing in popularity with the introduction of new highly polar columns with excellent retention, selectivity and stability. It is easy to inter-convert between RP and HILIC (a type of NP) because both modes employ polar aqueous mobile phases; however, many compounds are not polar enough to be retained under aqueous HILIC conditions. When aqueous solvents must be replaced with non-aqueous conditions to study non-polar samples, immiscibility situations can arise during changeover.

Customers often contact our Technical Service Department with a practical question that might go something like, "I need to change my HPLC instrument from RP mode to NP mode. Do you have any suggestions or guidelines for such a changeover?" This article describes our best practices for converting between reversed-phase and normal phase solvents, which are often immiscible.

The first best practice is to dedicate instruments to a specific mode. Significant seal wear-and-tear can be caused by expansion, contraction and extra friction of changing solvents. If possible, columns should also be dedicated to one mode for trouble-free operation. If dedicating the instrument or column is not possible, one should use the following procedure.

Our regular practice is to replace the column with tubing or a union and flush extensively with isopropanol (IPA) before going over to water or hydrocarbon. Before beginning the

changeover process, remove the HPLC column, cap and store in the appropriate storage solvent unless the same column is to be used in the new mode. Columns such as Cyano and Fluorophenyl (F5) can work in either RP or NP mode and can remain installed if desired. After IPA flush, the column can be removed and capped to avoid excessive wear on the valuable component. In the flushing steps, be sure to include the entire fluid path (pump, autosampler, valves, detector, etc.). Also, include the sample loop and any other fluid paths that are encountered for the normal operation of making injections. This can vary considerably depending on whether the autosampler is an external loop design, or an internal loop design. As part of all the washes, make certain the injection needle gets washed as well. It is best to do several full loop injections of a solvent such as IPA that is miscible with both high aqueous and high organic mobile phases. The total volume of IPA needed will vary with instrument design, but the waste volume should be monitored (record the volume as a guide for future changeovers) and observed for uniform appearance. UV detectors may remain on (ca. 250 nm) during this step to indicate when the system has returned to a stable baseline.

The second wash step after the IPA should be with methanol (or ethanol). Follow the previous procedure that was used for the IPA wash before going to water. Methanol will help flush the IPA out faster than going directly from IPA to water. If excessive baseline noise or drift is observed with a UV detector, repeat the procedures and allow more time to flush out any poorly swept flow regions.

Table 1. Properties of Organic Solvents Commonly Used in HPLC

Solvent	Polarity	Miscible with Water?	UV Cutoff*	Refractive Index at 20 °C	Solvent Strength e° (silica)	Viscosity at 20 °C, C P	
Hexane	nonpolar	no	200	1.3750	0.00	0.33	
Isooctane	↓	no	200	1.3910	0.01	0.50	
Carbon tetrachloride		no	263	1.4595	0.14	0.97	
Chloroform		no	245	1.4460	0.31	0.57	
Methylene chloride		no	235	1.4240	0.32	0.44	
Tetrahydrofuran		yes	215	1.4070	0.35	0.55	
Diethyl ether		no	215	1.3530	0.29	0.23	
Acetone		yes	330	1.3590	0.43	0.32	
Ethyl acetate		poorly	260	1.3720	0.45	0.45	
Dioxane		yes	215	1.4220	0.49	1.54	
Acetonitrile		yes	190	1.3440	0.50	0.37	
2-Propanol		yes	210	1.3770	0.63	2.30	
Methanol		yes	205	1.3290	0.73	0.60	
Water		polar	yes	-	1.3328	>0.73	1.00

* typical values.



Some Dos and Don'ts for Solvent Changeover:

- Do remove all additives and start with 100% isopropanol in all reservoirs.
- Isopropanol is fully miscible with all common solvents and is the safest changeover solvent for either direction.
- Do use low flow - about half of normal to avoid excessive seal wear and damage due to over-pressuring.
- Don't use acetonitrile routinely as the changeover solvent- it is better than methanol, but is not fully miscible with pure hydrocarbons.
- Don't use methanol routinely as the organic- it is not fully miscible with many normal phase conditions.
- Either acetonitrile or methanol may be used to routinely change from reversed-phase and back (remember to remove additives).
- Do check miscibility (use small external vessel) with target mobile phase before starting, especially if IPA is not selected.
- Do use organic (such as IPA) in all lines of a gradient instrument to make certain that water or hydrocarbon is removed from all fluid areas.
- Do operate the injector valve and any other selector valves while doing the IPA flush procedure.
- Do monitor pressure and detector signals during changeover as these are excellent methods to confirm full system equilibration; evaporative detectors such as MS and ELSD cannot be used for this purpose.
- Incomplete mixing shows up as severe detector baseline noise or pressure fluctuations (globules of immiscible solvent can resemble bubbles or particles).
- Do flush detectors and all other components even if baseline is not monitored.
- Total time for changeover can vary but should take about an hour. Do not rush; this may actually slow down the process.
- Do record the volumes of solvent used during changeover for use as a future guide; if changeover is unsuccessful, use more solvent the next time.
- Don't expect fast changeover and baseline equilibration with refractive index detectors- they are extremely slow to equilibrate after changeover.
- Do check gradient blank runs for excessive baseline noise and drift that might indicate pockets of immiscible solvent.
- Good chromatography in the target mode is the most sensitive final test- start with simple binary mobile phases and standard test mixes and work toward real samples with mobile phase additives.

Finally, it is also good practice to contact your LC instrument manufacturer to be sure all details of the changeover are covered. The manufacturer may have additional details and tips for successful changeover to a different chromatographic mode. If columns and instruments are frequently used in different modes, adopt a labeling system to alert a new user about possible solvent compatibility issues.



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Astec CLC-L and CLC-D

Copper Ligand Exchange HPLC Columns for Chiral Separation of Acids and Amines

Astec CLC columns use the ligand-exchange concept described by Davankov to affect enantiomer 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, like lactic, malic, tartaric and mandelic acids, amino acids, other amines and bi-functional racemates, like 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 CuSO₄ with the usual reversal of elution order from the CLC-D to CLC-L. In theory, any analyte that can complete the coordination 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 CuSO₄ mobile phase with acetic acid)
- Particle type: High-purity spherical silica
- Particle diameter: 5 μ m
- Pore size: 100 Å

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

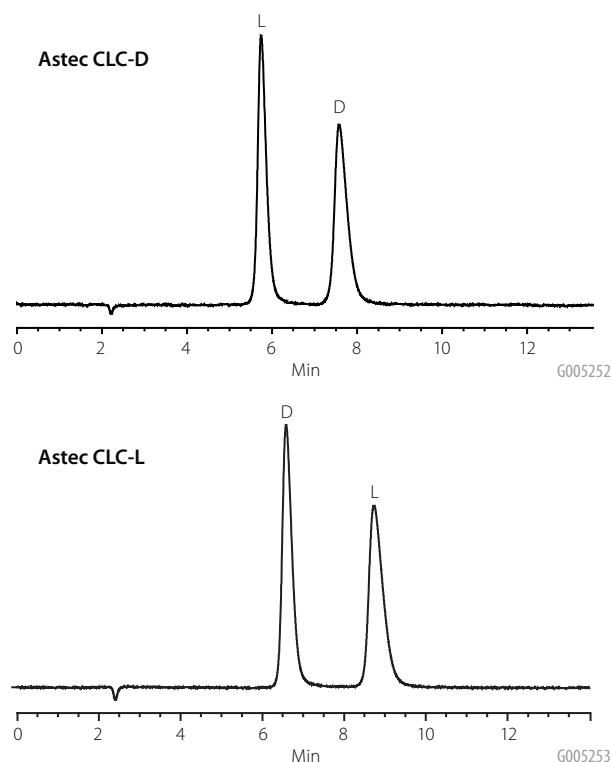
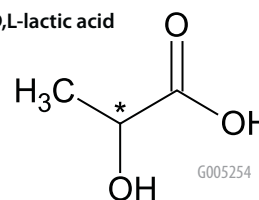
Reference

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 μ m particles
mobile phase: 5 mM CuSO₄
flow rate: 1.0 mL/min.
temp.: ambient
det.: UV at 254 nm
injection: 5 μ L
sample: D,L-lactic acid (3 mg/mL)

Structure of D,L-lactic acid



+ Featured Products

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

Reversed-phase HPLC Buffers

High-quality buffers (solutions, solids or concentrates)

Shyam Verma
shyam.verma@sial.com

Consideration of the affects 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 (RPC) 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 ionizable compounds.

Problems, such as, partial ionization of the analyte and strong interaction between analytes and residual silanols or other active sites 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 its 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 ionization in the MS interface.

Buffer Selection

The typical pH range for reversed-phase on 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 of the desired mobile phase pH (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
Sulfonate	1.8	<1-2.8
Phosphate	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
Sulfonate	6.9	5.9-7.9
Phosphate	7.2	6.2-8.2
Ammonia	9.2	8.2-10.2
Phosphate	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 upon 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 sulfonate buffers when analyzing organophosphate compounds. With the growth in popularity of LC-MS, volatile buffer systems, such as TFA, acetate, formate, and ammonia, are frequently used due to compatibility with mass spectral (MS) detection. In regard to the issue of suppression of ionization, 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 ionization with negative ion mode. Acetic acid is good for negative-ion mode. LC-MS applications further limit buffer selection and buffer concentration.

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+ 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 catalog: 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
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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 their 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 sure 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 program and a flame ionization detector to analyze the sample on a long polydimethylsiloxane capillary column, such as the Petrocol™ DH 150. This non-polar column is characterized by high efficiency and great reproducibility.

ASTM D5501 requires that peak identification be established, followed by the quantitation of ethanol. To perform this, a 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 Sigma-Aldrich's ASTM D5501 Denatured Fuel Ethanol Standards Kit. 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 web site 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 μ m (24155)
 oven: 60 $^{\circ}$ C (15 min.), 30 $^{\circ}$ C/min. to 250 $^{\circ}$ C (23 min.)
 inj.: 300 $^{\circ}$ C
 det.: FID, 250 $^{\circ}$ C
 carrier gas: helium, 24 cm/sec @ 60 $^{\circ}$ C
 injection: 0.5 μ L, 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%

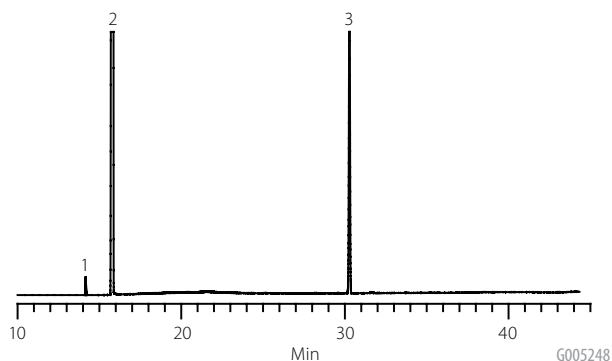
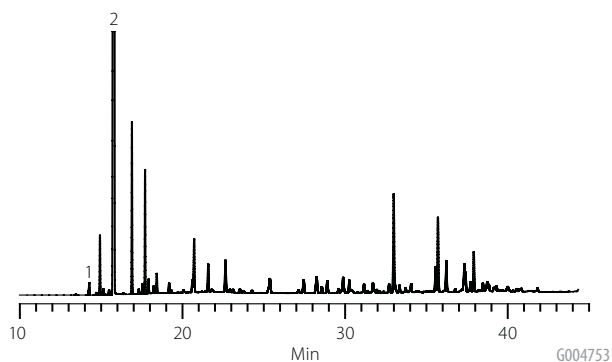


Figure 2. E85 Sample

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

1. US EPA web site, Fuels and Fuel Additives web page (www.epa.gov).
2. US DOE web site, Country Analysis Briefs: Brazil (www.eia.doe.gov/cabs/Brazil/Full.html).
3. US EPA web site, 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 μ m	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



Fuel Ethanol: Fermentation Analysis by HPLC

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).

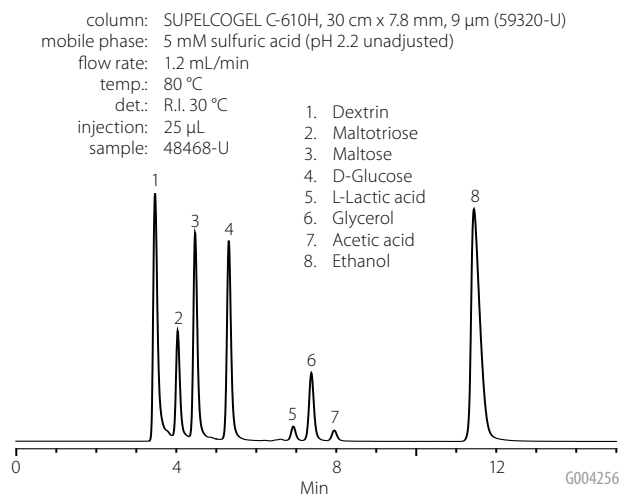
Many areas of the process are important to ensure a quality end product, such as the breakdown of the corn substrate to fermentable sugars and distillation. However, none are more critical than the ethanol-producing step of fermentation.

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.

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



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.

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

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, derivatization 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.
- **Maximize 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.

These literature pieces can be downloaded at no-charge from sigma-aldrich.com/gc

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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 the 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 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. 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).

Additional purity tests were done on both grades of DMSO using gas chromatography-mass spectrometry (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 deionized water, and a 2 mL aliquot was extracted by headspace SPME and analyzed by GC-MS using the conditions listed in **Table 1**.

Table 1. Headspace SPME, GC-MS Parameters

sample matrix:	1 mL DMSO + 1 mL deionized water in 4 mL vial
SPME fiber:	100 μ m PDMS
extraction:	Headspace, 50 $^{\circ}$ C, 5 min. with stirring
desorption process:	250 $^{\circ}$ C for 3 min.
column:	SPB TM -624, 30 m x 0.25 mm I.D., 1.4 μ m (24255)
oven:	35 $^{\circ}$ C (3 min.), 8 $^{\circ}$ C/min. to 220 $^{\circ}$ C (10 min.)
MSD interface:	220 $^{\circ}$ 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 artifacts 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 packaged under an inert atmosphere. This ensures their suitability for meeting the demands of headspace analysis.

Figure 1. Headspace Blank, DMSO – Organic Synthesis Grade

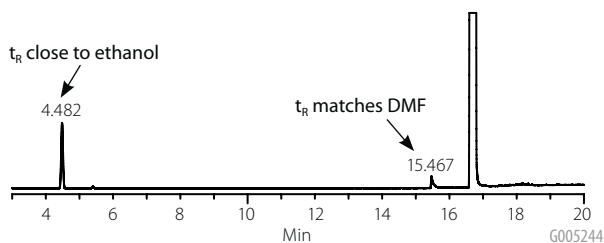


Figure 2. Headspace Blank, DMSO – Fluka GC-HS Grade

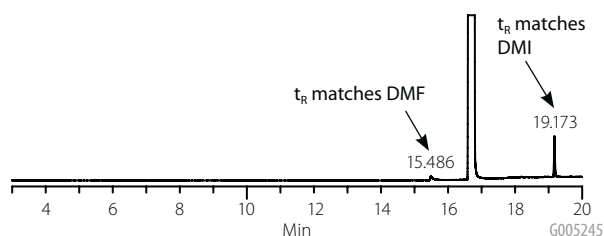
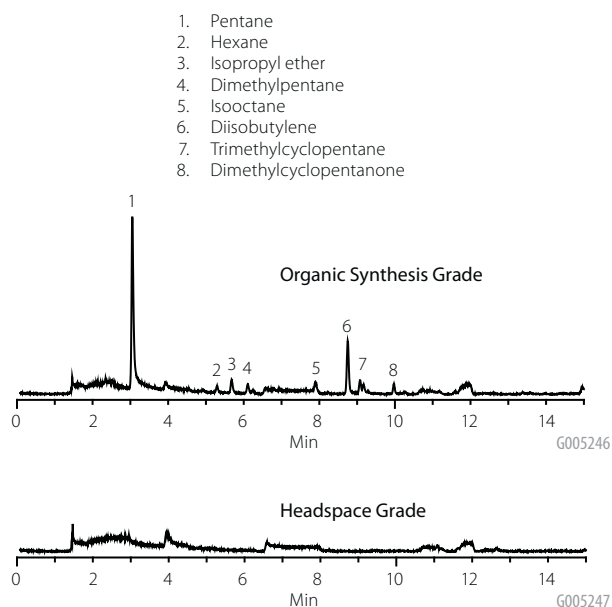




Figure 3. SPME GC-MS Impurity Analysis of DMSO Grades



References

1. United States Pharmacopeia (USP), 31st Edition (2008), <467> Residual Solvents.
2. ICH Guidelines for Industry, Q3C Impurities: Residual Solvents, US Dept. of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), ICH December, 1997.
3. 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 sulfoxide	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-MS

+ Related Products

Description	Qty.	Cat. No.
Column		
SPB-624, 30 m x 0.25 mm I.D., 1.4 µm	1	24255
SPME Fiber Assembly Polydimethyl (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		

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Selective Phospholipid Extractions for Cleanup or Enrichment Using HybridSPE-Phospholipid

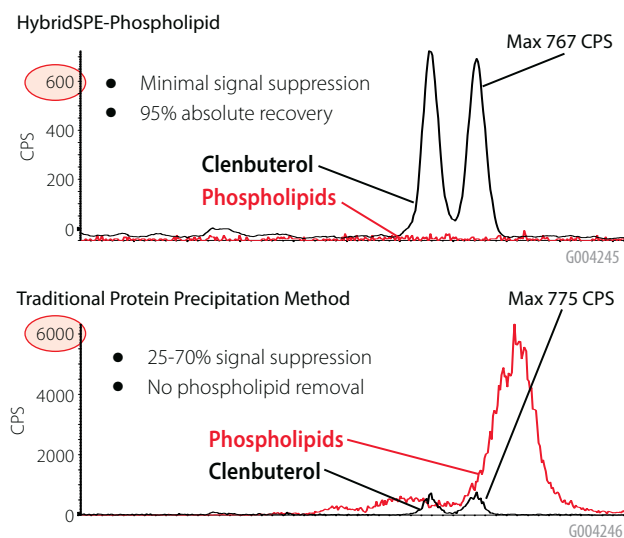
Michael Monko
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Introduction

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 like serum or plasma. Secondly, phospholipids can be important in many pathological and functional studies and therefore need to be carefully isolated and quantitatively analyzed. 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.

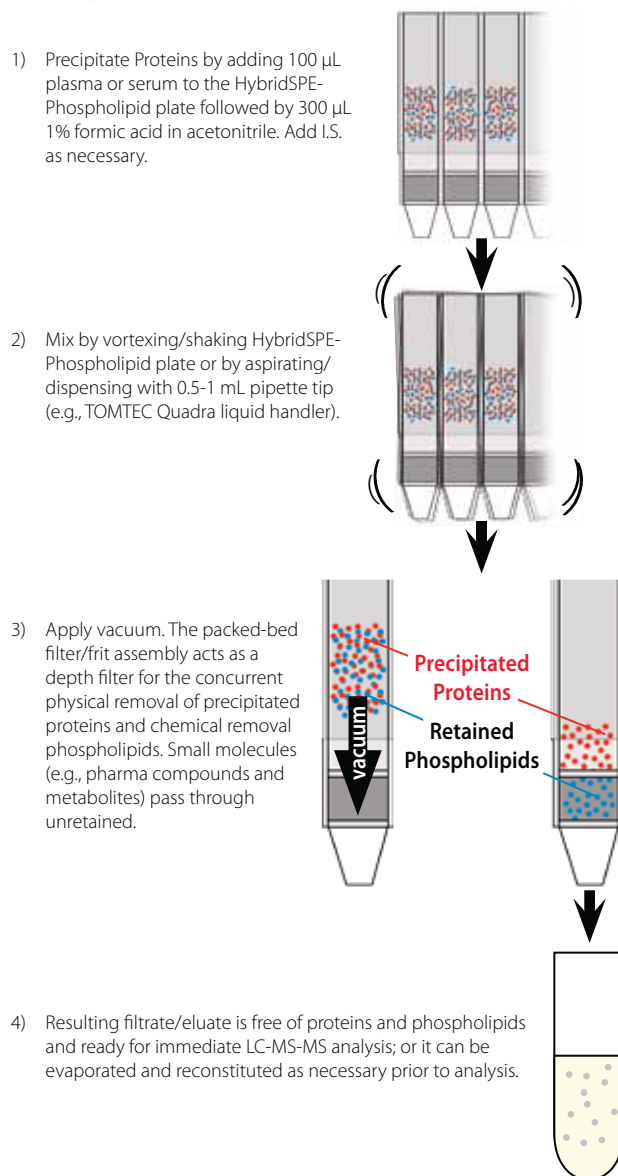
Figure 1. Comparison of the Extraction of Clenbuterol Using HybridSPE-Phospholipid vs. Traditional PPT Method



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

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



technique is highly effective for the vast 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 utilizes 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.

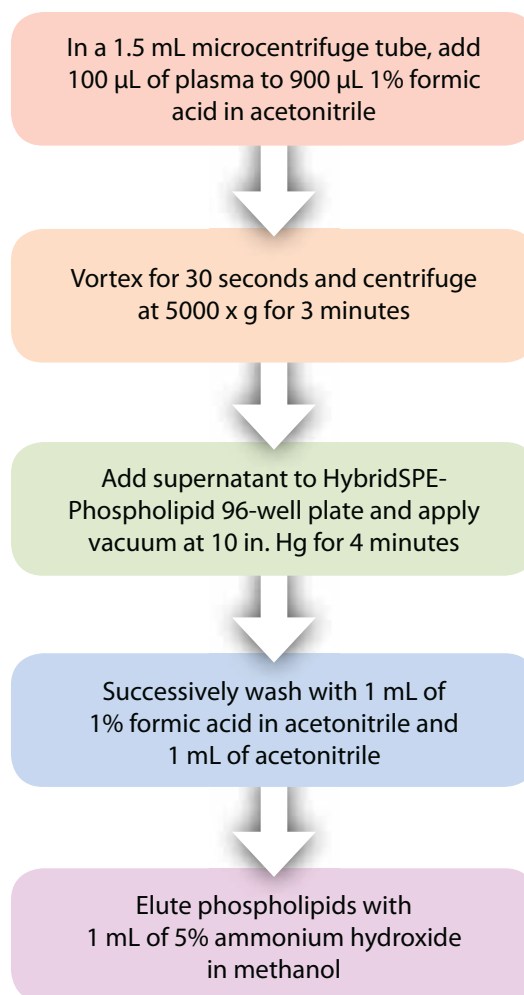
Conclusion

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. p3 (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 p6. (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

+ Related Information

To maximize 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 analyzed 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.

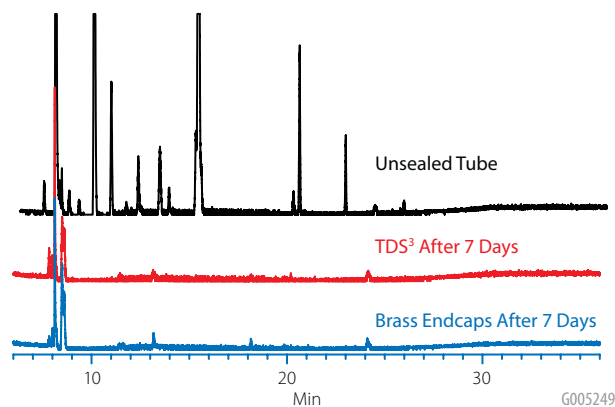
To assess the concentration, a toluene calibration curve was performed that spanned 1, 10, 25, and 100 nanograms per tube. The background contamination was defined as any extraneous peaks not present in the control blank.

Results

After seven days, the tubes were removed from the flame cabinet, along with the storage blanks from the paint can. The tubes were analyzed 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

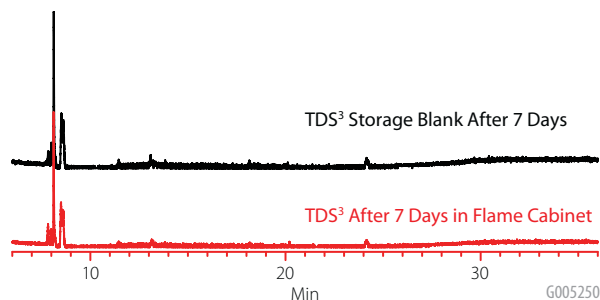
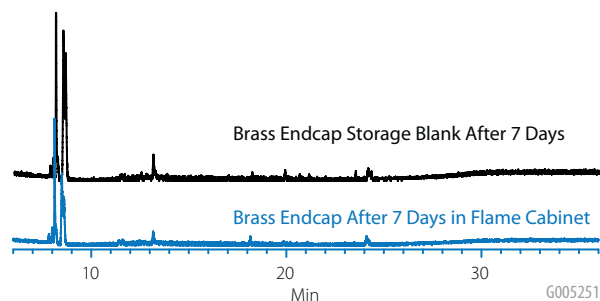


Figure 3 compares the results of the tubes sealed with the brass endcaps. There was no significant difference between the storage blank tube stored in the paint can (top) and the tube stored inside the flame cabinet for 7 days (bottom). Note: The peaks at 8 and 8.5 minutes are system peaks of carbon 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 Carboxen Y & Carboxen 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 on Supelco's new line of ¼" O.D. x 89 mm long glass fritted tubes.

** 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 TDS ³ Containers	50	25073
Male Luer Plug	12	504351
Female Luer Cap	12	57098
Tubing Adapter for Use With		
1/8 in. tubing to male luer	20	21016
3/16 in. tubing to male luer	20	23364
1/4 in. tubing to male luer	10	24856
Luer Coupler		
Male to male luer	20	25064-U



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Particle/Pore Size	Description	Pack Size*	Cat. No.
40-63 $\mu\text{m}/60 \text{ \AA}$	High purity silica gel	1KG	60737-1KG
		25KG	60737-25KG
40-63 $\mu\text{m}/60 \text{ \AA}$	High purity silica gel With 0.1% calcium	1KG	60752-1KG
		25KG	60752-25KG
35-70 $\mu\text{m}/60 \text{ \AA}$	High purity silica gel	1KG	60738-1KG
		25KG	60738-25KG
63-200 $\mu\text{m}/60 \text{ \AA}$	High purity silica gel	1KG	60741-1KG
		25KG	60741-25KG

* Other pack sizes are available.

For additional details please contact your Sigma-Aldrich sales representative or your local Sigma-Aldrich office.

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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

Precise Manual Injections Every Time with the Merlin MicroShot Injector



The new Merlin MicroShot™ Injector delivers fast and reproducible manual syringe injections for gas chromatography. Using a ball-end gas chromatography autosampler syringe, the unique trigger mechanism of the Merlin MicroShot makes each injection automatic when the needle is inserted into the injection port. The plunger displacement is fixed for precise volume delivery, confirming reproducibility each and every injection, and reducing dwell time in the injection port that will minimize sample discrimination. Each Merlin MicroShot Injector is calibrated to deliver a stroke of the plunger slide within +/- 0.025 mm of nominal.

Available in five convenient volume delivery sizes, the Merlin MicroShot injector is compatible with a ball-end 23 or 26 gauge fixed or removable needle syringe (syringe not included).

For a complete listing of autosampler syringes, please visit sigma-aldrich.com/syringes

+ Featured Products

Volume	Cat. No.
Merlin MicroShot Injector	
0.1 µL	29464-U
0.2 µL	29466-U
0.5 µL	29468-U
1.0 µL	29471-U
2.0 µL	29472-U

+ Related Products

Description	Volume	Needle	Cat. No.
Hamilton® 700 Series Microliter™ Fixed Needle, Cone Tip			
	10 µL	23ga	21313
	10 µL	23 – 26 ga	24573
Hamilton 700 Series Microliter Removable Needle, Cone Tip			
	10 µL	23 ga	21312
	10 µL	23 – 26 ga	24575
	10 µL	23 ga	21323-U
Hamilton 1700 Series Gastight® Fixed Needle, Cone Tip			
	10 µL	23 ga	26719
	10 µL	23 – 26 ga	24579



Accessories



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 analyzed 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, single component solutions and method specific mixtures. A Certificate of Analysis stating both the DNPH derivatized and non-derivatized 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
<i>Acetaldehyde-2,4-DNPH</i>	<i>2-Butanone-2,4-DNPH</i>	<i>Methacrolein-2,4-DNPH</i>	
<i>Acetone-2,4-DNPH</i>	<i>Crotonaldehyde-2,4-DNPH</i>	<i>Propionaldehyde-2,4-DNPH</i>	
<i>Acrolein-2,4-DNPH</i>	<i>Formaldehyde-2,4-DNPH (40 µg/mL)</i>	<i>p-Tolualdehyde-2,4-DNPH</i>	
<i>Benzaldehyde-2,4-DNPH</i>	<i>Hexaldehyde-2,4-DNPH</i>	<i>Valeraldehyde-2,4-DNPH</i>	
<i>Butyraldehyde-2,4-DNPH</i>			
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
<i>Acetaldehyde-2,4-DNPH</i>	<i>2-Butanone-2,4-DNPH</i>	<i>Methacrolein-2,4-DNPH</i>	
<i>Acetone-2,4-DNPH</i>	<i>Crotonaldehyde-2,4-DNPH</i>	<i>Propionaldehyde-2,4-DNPH</i>	
<i>Acrolein-2,4-DNPH</i>	<i>Formaldehyde-2,4-DNPH</i>	<i>m-Tolualdehyde-2,4-DNPH</i>	
<i>Benzaldehyde-2,4-DNPH</i>	<i>Hexaldehyde-2,4-DNPH</i>	<i>Valeraldehyde-2,4-DNPH</i>	
<i>Butyraldehyde-2,4-DNPH</i>			
US Environmental Protection Agency			
TO-11/1P-6A Aldehyde & Ketone DNPH Mix	15 µg/mL each component in acetonitrile	1 mL	47285-U
<i>Acetaldehyde-2,4-DNPH</i>	<i>Crotonaldehyde-2,4-DNPH</i>	<i>Propionaldehyde-2,4-DNPH</i>	
<i>Acetone-2,4-DNPH</i>	<i>2,5-Dimethylbenzaldehyde-2,4-DNPH</i>	<i>o-Tolualdehyde-2,4-DNPH</i>	
<i>Acrolein-2,4-DNPH</i>	<i>Formaldehyde-2,4-DNPH</i>	<i>m-Tolualdehyde-2,4-DNPH</i>	
<i>Benzaldehyde-2,4-DNPH</i>	<i>Hexaldehyde-2,4-DNPH</i>	<i>p-Tolualdehyde-2,4-DNPH</i>	
<i>Butyraldehyde-2,4-DNPH</i>	<i>Isovaleraldehyde-2,4-DNPH</i>	<i>Valeraldehyde-2,4-DNPH</i>	

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 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.



Drinking Water Odor Standards

Musty odors in drinking water are often caused by the presence of geosmin, methylisoborneol, isopropyl-3-methoxy pyrazine, and isobutyl-3-methoxy pyrazine. These analytes are produced by a variety of microbes, including blue-green algae (cyanobacteria), which is commonly found in lakes and reservoirs. These analytes are released into the water when the organism dies. Some people can smell the odor analytes in drinking water at concentrations of 10 parts per trillion (ppt). To eliminate this problem, many water utility companies and beverage manufacturers monitor for these analytes at concentrations as low as 1 – 3 ppt.

Sigma-Aldrich offers calibration standards to aid in the monitoring of water for odors. These quantitative standards are prepared from raw materials that have been carefully evaluated for identity and purity. A Certificate of Analysis is included with each product.

Description (100 µg/mL in methanol)	Qty.	Cat. No.
(±)-Geosmin solution	1 mL	47522-U
(±)-Geosmin solution	5 x 2 mL	4M7522-U
2-Methylisoborneol solution	1 mL	47523-U
2-Methylisoborneol solution	5 x 2 mL	4M7523-U
(±)-Geosmin and 2-Methylisoborneol	1 mL	47525-U
2,4,6-Trichloroanisole solution	1 mL	47526-U
2-Isopropyl-3-methoxy pyrazine solution	1 mL	47527-U
2-Isobutyl-3-methoxy pyrazine solution	1 mL	47528-U
Drinking Water Odor Standards Kit	6 x 1 mL	47529-U
Contains one each of the individual solutions listed above.		

Did you know...?

Solid phase microextraction (SPME) is commonly used for the analysis of trace odor components such as geosmin, MIB, IPMP and IBMP from drinking water at parts per trillion (ppt) concentration for analysis by gas chromatography/mass spectrometry (GC/MS). The simple-to-use, and cost-effective method, 6040D, developed by the American Water Works Association (AWWA) describes the use of SPME for odor determination in drinking water. For more details request Application Note 147 or click on the Downloadable SPME Literature link at sigma-aldrich.com/spme

+ Related Products

Glass Magnet Vial Holder

The Glass Magnet Vial holder helps to prevent sample and reference material vials and ampuls from being accidentally tipped over.

This product is made from a tacky material that temporarily anchors glass products to any dry surface. Simply press it to your work surface, then press the dry bottom of the glass vessel onto a smooth portion of the magnet. The glass vessel holds securely in place while you remove the cap or withdraw the sample.



P000392

Description	Cat. No.
Glass Magnet Vial Holder	57270

Silanization Service for Vials

High concentrations of silanol groups (Si-O-H) on untreated glass vial surfaces can catalyze decomposition of unstable compounds or adsorb polar compounds through hydrogen bonding. Quantitative analyses of these sensitive compounds become unreliable, recoveries are reduced and analyses can be complicated by decomposition byproducts.

Supelco uses an environmental friendly process to silanize glass vials. This organosilanization process derivatizes surface silanols at a high temperature, shielding the active groups from contact with the active hydrogens on the sample components.

Our experience includes silanizing vials ranging from 2 mL to 40 mL, and maintaining a stock of popular 2 mL and 4 mL silanized vials. If your application requires a different vial,

you may choose from the large assortment of Supelco brand vials, or send us your own vials for treatment. To obtain a quote or learn more about our silanization process, please contact our Technical Service department at techservice@sial.com



P000376

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