REPORTER

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Analysis of Pesticides
by QuEChERS and SPE

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PERFORMANCE COMPARISON OF STORAGE CONTAINERS

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Cover Photo:
The analysis of pesticide residues continues to be a main focus of the food testing industry. For cleanup of difficult matrices prior to pesticide residue analysis, the new Supel™ Sphere Carbon/NH_2 and the Supel™ QuE Z-Sep sorbents for QuEChERS are described herein.

The QuEChERS Approach to Sample Preparation

Jennifer Claus

Solid Phase Extraction
Product Manager

Dear Colleague,

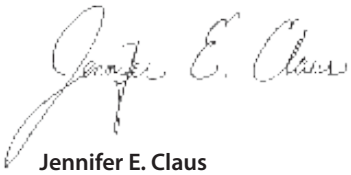
Nearly a decade ago, Steven J. Lehotay and Michelangelo Anastassiades changed the face of solid-phase extraction for food analysis with the introduction of the QuEChERS approach. The "Quick, Easy, Cheap, Effective, Rugged, Safe" QuEChERS methodology involves the use of bulk salts and SPE sorbents for the extraction and cleanup of homogenized food/agricultural samples with simple mixing and centrifugation steps. This technique has provided analytical laboratories with fast, cost-effective sample cleanup prior to pesticide residue analysis by LC-MS or GC-MS.

The QuEChERS approach to sample cleanup is now widely accepted and has been incorporated into a variety of contemporary laboratory methods. Extensive validation of two specific QuEChERS techniques has led to AOAC and EN official methods for pesticide residue analysis (AOAC Official Method 2007.01 and CEN Standard Method EN 15662). These methods have been adopted by numerous food testing labs throughout the world for various food matrices. In addition, although the QuEChERS method is used mainly for pesticide residue analysis, it has recently been expanded into areas including veterinary drug, PAH, PCB, PBDE and flame-retardant analysis.

Though the QuEChERS approach has yielded increased speed and sensitivity, lipid and pigment interferences in difficult matrices have remained obstacles in food analysis. The Supel™ QuE Z-Sep sorbents have recently illustrated the effective removal of more fat and color from sample extracts than traditional phases for QuEChERS (i.e. PSA and C18). Fatty matrix interferences are removed via the interaction between the polar group of the lipid and the proprietary bonded ion-exchange group of the Z-Sep sorbent as well as the interaction between the hydrophobic chains of the lipid and the hydrophobic group of the sorbent. By eliminating problematic matrix interferences, Z-Sep products improve limits of detection and provide more robust LC-MS and GC-MS methods. This proprietary technology can replace C18 and PSA phases in current methods without additional method development.

The article entitled "Reduce Matrix Background & Improve Overall Analyte Recovery for the Analysis of Pesticides in Beef Kidney" illustrates the use of Z-Sep+ for the removal of lipid interferences from a fatty matrix. To learn more about the Supel QuE Z-Sep sorbents for QuEChERS, refer to the aforementioned article in this issue, scan the QR code below, or visit sigma-aldrich.com/quechers

Sincerely,



Jennifer E. Claus
Product Manager, Solid Phase Extraction
jennifer.claus@sial.com

Benefits of Larger Fused-Core® Particles to Pharmaceutically Relevant Compounds

Gaurang Parmar
gaurang.parmar@sial.com

Non-steroidal anti-inflammatory drugs (NSAIDs) are pharmaceutically relevant compounds that inhibit the inflammation process without the use of steroid drugs. The most commonly used NSAIDs are aspirin, ibuprofen and naproxen.

NSAIDs often cause dangerous side effects. As reported to the US Food and Drug Administration, the majority of the undesired side effects are related to damage to the gastrointestinal (GI) tract and kidneys.[1] As a result, there is continuous interest in improving the analysis and characterization of NSAIDs.

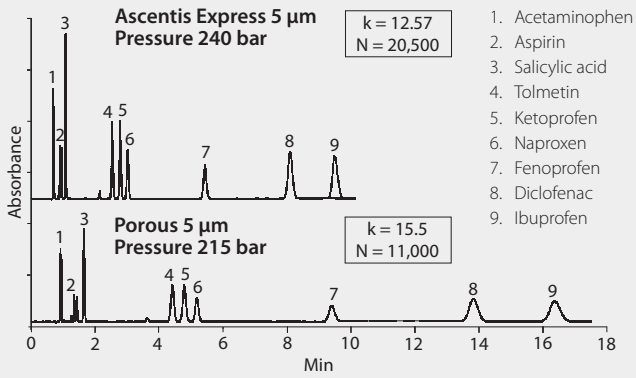
In this study, a simple UV-based HPLC method was developed using the new Ascentis® Express 5 µm Fused-Core HPLC column. A comparison was then made to a traditional 5 µm fully-porous HPLC column.

Experimental Conditions

An aliquot (2 µL) of NSAIDs mixture was injected on an Ascentis Express 5 µm and a regular porous 5 µm C18, 15 cm x 4.6 mm I.D. column. **Figure 1** shows the two chromatograms and details the experimental conditions.

Figure 1. Comparison of NSAIDs Analysis on Ascentis Express 5 µm and Regular Porous 5 µm Columns

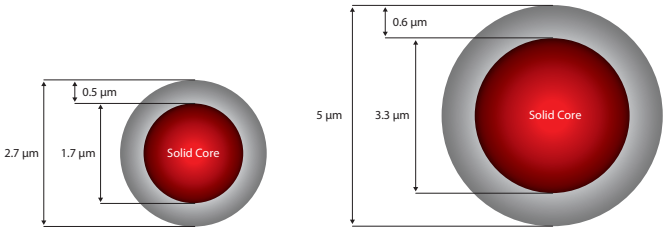
column: Ascentis Express C18, 5 µm (50537-U) and a regular porous C18, 15 cm x 4.6 mm I.D.
mobile phase: (A) 20 mM pH 2.5 potassium phosphate, (B) 50:50 ACN:MeOH; A:B = 48% A:52% B; instrument: Shimadzu Prominence UFLC XR
flow rate: 2.0 mL/min
injection volume: 2 µL
det: 254 nm
temp: 35 °C



Conclusion

The Fused-Core column shows the same selectivity as a regular 5-µm porous C18 HPLC column, but outperforms the fully-porous column in terms of efficiency, with almost twice the theoretical plates (N=20,500 vs. N=11,000). In addition to this, the 5-µm Fused-Core column exhibits greater resolution in about half the run time when compared to the fully porous column, all at about the same backpressure. The Fused-Core particle design provides an easy way to realize higher efficiencies and shorter run times. See **Figure 2** for the Fused-Core particle comparison of 2.7 µm vs. 5 µm.

Figure 2. Comparison of 2.7 µm vs. 5 µm Fused-Core Particles



Reference

1. Spac, A. F.; Dorneanu, V. HPLC Analysis of NSAIDs, *Encyclopedia of Chrome*, Edition 3, (**2009**) Chapter 129, 495

Featured Product

Description	Cat. No.
Ascentis Express C18, 15 cm x 4.6 mm I.D., 5 µm particles	50537-U

Related Products

I.D.	Length	C18	F5	C8	Phenyl-Hexyl	ES-Cyano
Ascentis Express 5 µm Columns						
2.1 mm	2 cm	50507-U	50603-U	50362-U	50442-U	50557-U
2.1 mm	3 cm	50508-U	50604-U	50363-U	50443-U	50558-U
2.1 mm	5 cm	50509-U	50605-U	50364-U	50446-U	50559-U
2.1 mm	10 cm	50517-U	50612-U	50368-U	50454-U	50563-U
2.1 mm	15 cm	50518-U	50613-U	50372-U	50455-U	50564-U
3.0 mm	3 cm	50522-U	50615-U	50376-U	50459-U	50567-U
3.0 mm	5 cm	50523-U	50616-U	50377-U	50464-U	50568-U
3.0 mm	10 cm	50526-U	50622-U	50381-U	50469-U	50570-U
3.0 mm	15 cm	50527-U	50623-U	50382-U	50470-U	50574-U
4.6 mm	3 cm	50529-U	50625-U	50386-U	50474-U	50577-U
4.6 mm	5 cm	50530-U	50626-U	50389-U	50477-U	50581-U
4.6 mm	10 cm	50536-U	50628-U	50391-U	50482-U	50585-U
4.6 mm	15 cm	50537-U	50631-U	50392-U	50483-U	50588-U

Description	Cat. No.
Universal guard holder w/EXP Titanium Hybrid Ferrule	53500-U

Narrow Bore Gel Filtration Columns for Protein and Aggregate Analysis

Roy Eksteen
roy.eksteen@sial.com

Introduction

Traditionally, in Gel Filtration Chromatography (GFC), the column diameter has been larger than the diameter of HPLC columns used in interactive modes of liquid chromatography. The main reason for this was that HPLC instruments were optimized for 4.6 mm I.D. columns that were 25 cm long. To prevent extra-column band broadening (ECBB) detracting from the efficiency of the column, it was (and still is) accepted practice to retain the solute of interest at least up to a retention factor of two, which means a peak volume that is three times the volume in which an unretained (low molecular weight) solute elutes from a GFC column. The most popular internal diameter of GFC columns is 7.8 mm. Since peak volume is proportional to the ratio of the square of the column diameters (7.8 and 4.6 mm), the volume in a 7.8 mm I.D. column is almost three times larger than that of a 4.6 mm I.D. column. In other words, extra-column band broadening was not a factor when 7.8 mm I.D. GFC columns were used with conventional HPLC instrumentation.

In recent years, HPLC and UHPLC instruments have been optimized to take advantage of higher column efficiencies provided by the Fused-Core® 2.7-µm particle technology available in Ascentis® Express columns and also by columns packed with fully porous 1.7-µm particles. When using such optimized instrumentation, the researcher can now take advantage of the primary benefit of smaller I.D. (gel filtration) columns, namely the ability to detect proteins at higher sensitivity when limited in sample mass. In this summary review of a poster from Tosoh Bioscience LLC,[1] we will illustrate this principle featuring the popular TSKgel® SuperSW3000 gel filtration columns.

Experimental

Columns

- TSKgel SuperSW3000, 4 µm, 1 mm I.D. x 30 cm (Cat. No. 821485)
- TSKgel SuperSW3000, 4 µm, 2 mm I.D. x 30 cm (Cat. No. 821845)
- TSKgel SuperSW3000, 4 µm, 4.6 mm I.D. x 30 cm (Cat. No. 818675)

Sample

Proteins and enzymes were purchased from Sigma-Aldrich®. The antibody was a gift from the Tosoh Research Center (Kanagawa, Japan).

Other

- UV cell: 2 µL (for 2 mm and 4.6 mm I.D.)
- UV cell: 35 nL (for 1 mm I.D.)
- Sample injector: Rheodyne 7520
- Tubing (injector hto column): 0.05 mm I.D. x 20 cm fused silica

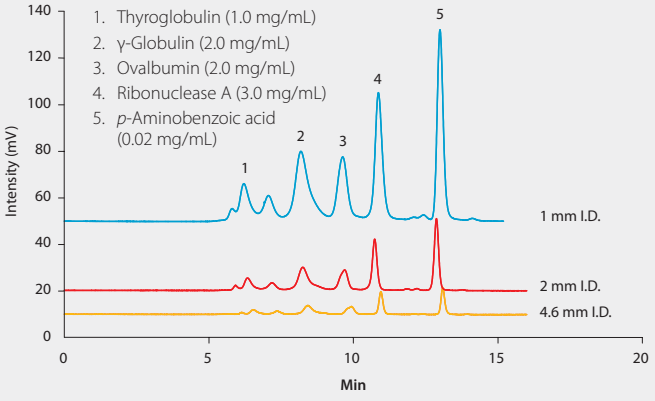
Results and Discussion

Gel filtration chromatography (size-exclusion chromatography in an aqueous mobile phase) is a powerful tool for analyzing biological polymers such as proteins, peptides, nucleic acids and their fragments. In biopharma companies, TSKgel SW series GFC columns are now routinely and widely used in research and in the final quality control of biotherapeutic drugs. In QC, the analyst is rarely sample-mass-limited. However, researchers performing proteomic studies routinely rely on the ability to detect very small amounts of proteins.

Like the conventional 4.6 mm I.D. column, the 1 mm and 2 mm I.D. TSKgel SuperSW3000 columns are filled with spherical 4-µm particles chemically bonded with diol-containing functional groups. The effect of sample mass on detection sensitivity is shown in **Figure 1** for a series of protein standards. The same protein mass and volume (1 µL) was injected on a 1 mm I.D., 2 mm I.D. and 4.6 mm I.D. TSKgel SuperSW3000 column. Approximately a five-fold increase in peak height of a standard protein mixture was observed when using a 2 mm I.D. x 30 cm TSKgel SuperSW3000 column compared to a 4.6 mm I.D. x 30 cm column. Clearly, the best sensitivity is obtained on the smallest bore (1 mm I.D.) column. Note that the same 2 µL UV detector cell volume was used for the 2 and 4.6 mm I.D. columns and a much smaller 35 nL cell volume for the 1 mm I.D. column.

Figure 1. Effect of Sample Mass on Detection Sensitivity

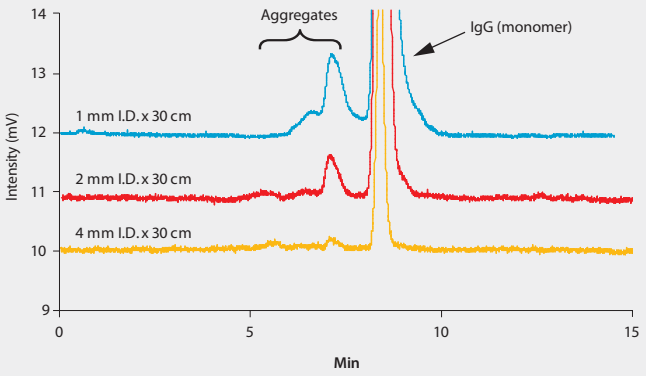
columns:	TSKgel SuperSW3000, 30 cm x 4.6 mm I.D. TSKgel SuperSW3000, 30 cm x 2 mm I.D. TSKgel SuperSW3000, 30 cm x 1 mm I.D.
eluent:	0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3 (pH 6.7)
flow rate:	0.350 mL/min (4.6 mm I.D.) 0.650 mL/min (2 mm I.D.) 0.016 mL/min (1 mm I.D.)
detection:	UV at 280 nm
detector cell volume:	2 µL (4.6 and 2 mm I.D.) 35 nL (1 mm I.D.)
temperature:	25 °C
injection volume:	1 µL



The same improvement in sensitivity as seen for protein standards was also evident when analyzing an IgG sample containing a low concentration of aggregates, as is shown in **Figure 2**. The fact that the calibration curves were linear (not shown) confirmed that nonspecific adsorption on the stationary phase was minimal. The detection limit of IgG was 18 ng using the 1 mm I.D. TSKgel SuperSW3000 column while still being able to detect small amounts of IgG aggregates. As with 1 mm I.D. columns, we found that reducing the injection volume of an IgG solution from 10 µL to 1 µL greatly improved efficiency of the 2 mm I.D. column, although at constant injection volume, efficiency did not vary with IgG concentration in the range of 1–5 mg/mL. In a separate study (results not shown) it was determined that trace analysis of biological components was possible when the 1 mm I.D. TSKgel SuperSW3000 column was utilized with an off-line SELDI/TOF/MS.

Figure 2. Improve IgG Aggregate Detection Limit

columns: TSKgel SuperSW3000, 30 cm x 4.6 mm I.D.
TSKgel SuperSW3000, 30 cm x 2 mm I.D.
TSKgel SuperSW3000, 30 cm x 1 mm I.D.
eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3 (pH 6.7)
flow rate: 0.350 mL/min (4.6 mm I.D.)
0.650 mL/min (2 mm I.D.)
0.016 mL/min (1 mm I.D.)
detection: UV at 280 nm
detector cell volume: 2 µL (4.6 and 2 mm I.D.)
35 nL (1 mm I.D.)
temperature: 25 °C
injection volume: 1 µL
sample: IgG (mouse, Mab, 1 mg/mL)



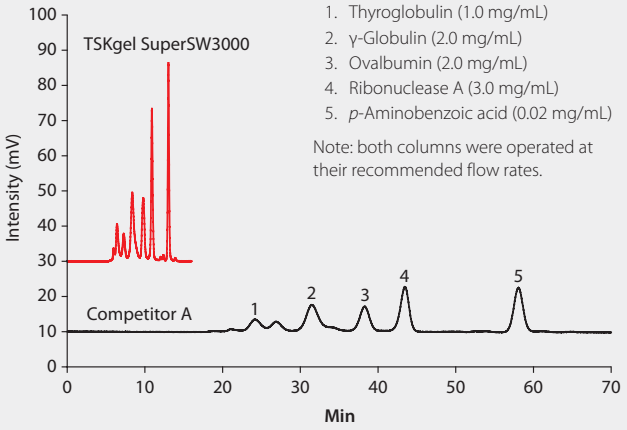
The performance of a competitor column filled with a composite matrix of dextran and agarose is compared in **Figure 3** with a 2 mm I.D. TSKgel SuperSW3000 column, each operated at their recommended flow rate. The result demonstrates that the silica backbone in SW-type TSKgel columns is best suited to deliver fast and efficient results in a high throughput situation.

Figure 3. Competitive Advantage

column: TSKgel SuperSW3000, 30 cm x 2 mm I.D.
flow rate: 65 µL/min
linear velocity: 124 cm/h
N: 30,000

column: Competitor A, 30 cm x 3.2 mm I.D.
flow rate: 40 µL/min
linear velocity: 30 cm/h
N: 11,000

eluent: 0.1 mol/L phosphate buffer + 0.1 mol/L Na_2SO_4 + 0.05% NaN_3 (pH 6.7)
detection: UV at 280 nm
temperature: 25 °C
injection volume: 0.2 µL



Conclusions

Narrow-bore TSKgel SuperSW3000 columns (1 mm and 2 mm I.D.) showed similar resolution for biological samples to what can be obtained on conventional 4.6 mm I.D. TSKgel SuperSW3000 columns. As expected, the highest sensitivity was achieved on the 1 mm I.D. narrow bore column. Linear calibration curves confirmed that nonspecific adsorption on the stationary phase was minimal. The detection limit of IgG was 18 ng using a 1 mm I.D. column while still being able to detect small amounts of IgG aggregates. The results indicate narrow-bore TSKgel SuperSW3000 columns are an excellent choice for the rapid separation of proteins and enzymes at micro scale and are a great fit for the trace analysis of biological components by LC-MS.

Reference

1. Based on work performed by Tomasek, C.; Moriyama, H.; Yamasaki, S.; Satoh, S. and Tomizawa, H. (Tosoh Corporation). For a complete report on this study, visit **sigma-aldrich.com/tsk**

Featured Products

Description	Cat. No.
TSKgel SuperSW3000 Columns, 4 µm	
1 mm I.D. x 30 cm	821485
2 mm I.D. x 30 cm	821845
4.6 mm I.D. x 30 cm	818675
TSKgel SuperSW Guard Column, 4 µm	
4.6 mm I.D. x 3.5 cm	818762

Teaming Seppro® and iTRAQ® for Biomarker Discovery

George Yeh
george.yeh@sial.com

In proteomics, protein depletion technologies, such as Seppro from Sigma-Aldrich®, have proven useful in increasing sample dynamic range via removal of high-abundance proteins that are not necessarily of immediate biomedical or clinical relevance. This is of particular importance in the context of biomarker discovery, where putative protein biomarkers are often present at low levels and thus are easily masked by highly abundant proteins.[1] After depletion of high-abundance proteins, tagging chemistries like iTRAQ, mTRAQ® and ICAT® from AB Sciex are available to label, isolate, and analyze the remaining protein pool to capture potentially useful biomarkers,[2] which can undergo further investigation with multiple reaction monitoring (MRM) studies.[3] In their systematic study of plasma sample analysis using iTRAQ, Ohlund et al. discussed the use of various protein depletion technologies, including Seppro, in conjunction with iTRAQ.[4]

Several very recent publications have looked at the tandem use of Seppro and iTRAQ in biomedical work. Müller et al. have studied the human skin suction blister fluid (SBF) proteome as a possible biomarker source for skin-related disorders. The authors tested two immunodepletion technologies, including Seppro IgY14, and combined immunodepletion with downstream iTRAQ treatment. This proof-of-principle investigation was the first publication on a gel-free, quantitative MS study of the SBF proteome.[5] Rehman, Evans and colleagues looked at human serum prostate cancer samples to probe for potential novel biomarkers, also with Seppro IgY14 and iTRAQ used in tandem. Their study revealed several potential candidate proteins, such as eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) and afamin.[6] Zhang et al. have investigated plasma samples from colorectal cancer (CRC) patients, using a workflow where they teamed Seppro technology with iTRAQ. They identified 13 potential CRC biomarkers, such as ORM2 and serpin peptidase inhibitor, clade D.[7]

Sigma-Aldrich R&D has recently collaborated with researchers at Fox Chase Cancer Center, Temple University, and National Jewish Medical & Research Center in a more novel combination of the Seppro and iTRAQ technologies in a single workflow. This newly published study extends the range of the Seppro-iTRAQ coupling by using both the IgY14 and the SuperMix Seppro technologies together, in order to deplete both the 14 most abundant proteins and moderately abundant proteins from plasma.[8] In principle, this additional level of protein depletion can enhance further potential detection of the lowest abundance proteins from plasma, although concerns do exist about ancillary depletion of proteins that can be potential biomarkers.[4] The publication, by Patel et al., looked at such concerns and noted more quantitatively the need to exercise caution in designing immunodepletion experiments, to factor in the possible association of low-abundance, potential biomarker proteins with immunodepleted, higher abundance proteins. This is consistent with earlier studies by Qian et al. on comparable Seppro tandem systems of IgY12 or IgY14 plus SuperMix (without subsequent iTRAQ treatment), where the authors advised that both the flow-through and bound protein fractions in immunodepletion should be evaluated in quantitative proteomics research.[9,10]

Sigma-Aldrich has partnered with AB Sciex to offer the iTRAQ product line as part of the Sciex iChemistry portfolio of reagents for quantitative proteomics. The iChemistry portfolio also includes the ICAT and mTRAQ tagging reagents. With product lines such as Seppro and iChemistry now available, Sigma-Aldrich provides you the necessary tools for the complete proteomics workflow.

References

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2. Li, Z.; Adams, R.M.; et al. Systematic Comparison of Label-Free, Metabolic Labeling, and Isobaric Chemical Labeling for Quantitative Proteomics on LTQ Orbitrap Velos. J. Proteome Res., 11(3), 1582–1590 (**2012**).
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6. Rehman, I.; Evans, C.A.; et al. iTRAQ Identification of Candidate Serum Biomarkers Associated with Metastatic Progression of Human Prostate Cancer. PLoS ONE, 7(2), e30885, doi:10.1371/journal.pone.0030885 (**2012**).
7. Zhang, X.; et al. The Potential Role of ORM2 in the Development of Colorectal Cancer. PLoS ONE, 7(2), e31868, doi:10.1371/journal.pone.0031868 (**2012**).
8. Patel, B.; et al. Assessment of Two Immunodepletion Methods: Off-Target Effects and Variations in Immunodepletion Efficiency May Confound Plasma Proteomics. J. Proteome Res., 11(12), 5947–5958 (**2012**).
9. Qian, W.-J.; et al. Enhanced Detection of Low Abundance Human Plasma Proteins Using a Tandem IgY12-SuperMix Immunoaffinity Separation Strategy. Mol. Cell. Proteomics, 7(10), 1963–1973 (**2008**).
10. Shi, T.; et al. IgY14 and SuperMix immunoaffinity separations coupled with liquid chromatography-mass spectrometry for human plasma proteomics biomarker discovery. Methods, 56, 246–253 (**2012**).

Featured Products

Description	Cat. No.
Seppro® Dilution buffer	S4199
Seppro IgY14	SEP010
Seppro IgY14 LC2	SEP020
Seppro IgY14 LC5	SEP030
Seppro IgY14 LC10	SEP040
Seppro Neutralization Buffer	S4449
Seppro Stripping Buffer	S4324
Seppro Supermix LC2	SEP050
Seppro Supermix LC5	SEP060
Spin Column for Seppro	S4574

Reduce Matrix Background and Improve Overall Analyte Recovery for the Analysis of Pesticides in Beef Kidney

Katherine K. Stenerson and Jennifer Claus
jennifer.claus@sial.com

The exposure of farm animals to agricultural pesticides continues to be a major concern among food producers. For example, organochlorine pesticides can still be detected at low levels in some agricultural products.[1] Lipophilic pesticides, such as organochlorine and some pyrethroids, can bioaccumulate in fatty tissues, thus finding their way into animal products consumed by the public.

The current methods for extracting pesticides from meat products require the use of organic solvents, consequently resulting in the co-extraction of undesired fatty matrix interferences. Cleanup of samples is then necessary by techniques such as freezing, liquid-liquid partitioning, solid phase extraction (SPE), gel permeation chromatography (GPC) or matrix solid phase dispersion (MSPD) prior to chromatographic analysis.[1,2]

QuEChERS Cleanup Sorbents

In the past several years, QuEChERS has become a very popular method for the extraction and cleanup of fruit, vegetable and some fatty matrices prior to pesticide residue analysis.[3–5] The extraction step uses acetonitrile and a salting-out effect (with magnesium sulfate). Cleanup is accomplished using dispersive SPE (dSPE), with sorbents such as:

- **PSA (primary-secondary amine)** – for removal of polar pigments, sugars and organic acids
- **Carbon** – for removal of chlorophyll and carotenoids
- **C18** – for removal of lipids and non-polar components

Typically, for reducing fatty matrix, a combination of PSA/C18 is used.[6] A new cleanup sorbent, Z-Sep+, was recently developed for removal of lipids and pigments. Supel™ QuE Z-Sep+ is a single material consisting of zirconia and C18 bonded to the same silica particles. The zirconia acts as a Lewis acid, attracting compounds with electron-donating groups, such as the -OH in mono and diglycerides, while the C18 binds fats through hydrophobic interaction.

Experimental

In this study, the use of a new sorbent, Supel QuE Z-Sep+ is evaluated and compared to PSA/C18 for removal of fatty components from the beef kidney matrix prior to pesticide residue analysis by GC-MS. Lipophilic insecticides and fungicides common in beef tissues (organochlorines, pyrethroids, diphenyl fungicides and pesticide synergists) are analyzed in this study.[6, 7]

Extraction and cleanup procedures used in this study are based on AOAC method 2007.01 and summarized in **Table 1**.[3] Replicates of both unspiked and spiked kidney extract samples were processed. Spiked samples were prepared using a 10 µg/mL mixture of lipophilic pesticides in acetonitrile, spiking at 50 ng/g. Matrix-matched standards were prepared at 10 ng/mL, 20 ng/mL, 40 ng/mL and 60 ng/mL for each cleanup sorbent. Extracts underwent GC-MS analysis, and quantitation was performed using a calibration curve prepared with matrix-matched standards.

Table 1. Extraction and Cleanup Procedures

1. Place 10 g of a homogenized beef kidney sample into a 50 mL centrifuge tube (Cat. No. 55248-U). Add 50 µL of a 10 µg/mL spike solution if a spiked replicate.
2. Add 25 mL of acetonitrile (Cat. No. 34481) and shake for one minute.
3. Add the contents of an Acetate Extraction Tube (Cat. No. 55234-U) and shake for one minute.
4. Centrifuge for five minutes.
5. Transfer three mL of the supernatant into the appropriate cleanup tube, Z-Sep+ (Cat. No. 55296-U) or PSA/C18 (Cat. No. 55229-U).
6. Shake for one minute, then centrifuge for three minutes.
7. Inject the extract directly into the GC-MS for analysis.

Matrix Removal

Extracts were run in full scan mode to compare the backgrounds remaining before and after cleanup with Z-Sep+ and PSA/C18 (**Figure 1**). Heavy matrix interference observed in the uncleaned sample, consisting primarily of fatty acids, was significantly reduced by both cleanup sorbents. The large peak eluting around 30 minutes, identified as cholesterol, was notably reduced by cleanup. In a direct comparison of the two cleanups, the Z-Sep+ sorbent was found to remove more background than PSA/C18.

Chromatography

Consistent with the background comparison study, more matrix remained behind after cleanup with PSA/C18 than with Z-Sep+ in the total ion chromatograms (TICs) of the spiked samples run in SIM mode (**Figure 2**). The matrix enhancement effect was more pronounced in the samples cleaned with PSA/C18, especially for Endosulfan I and II, 4,4′-DDE, piperonyl butoxide, bifenthrin and permethrin.

Pesticide Recovery

As depicted in **Table 2**, recoveries for both cleanups fell in the generally acceptable range of 70–120%.[3] Reproducibility is comparable between both cleanup methods, as shown by the % RSD. Z-Sep+ exhibited higher average recovery values than PSA/C18 for a majority of the pesticides, with the exception of diphenylamine and 4,4′-DDE.

Conclusion

The new sorbent, Supel Que Z-Sep was found to reduce background effectively without significantly reducing recovery of lipophilic pesticides such as organochlorines and some pyrethroids. GC-MS full-scan data indicated that Z-Sep+ provided better cleanup in the form of reduced background than PSA/C18, and less interference in the GC-MS/SIM analysis of the target pesticides themselves.

Figure 1. GC-MS full scan chromatograms of beef kidney extract (a) with no cleanup (b) PSA/C18 cleanup (c) Z-Sep+ cleanup. All are on the same Y-scale.

column: SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm (28576-U)
oven: 70 °C (0.5 min), 25 °C/min to 125 °C, 10 °C/min to 200 °C, 5 °C/min to 300 °C (1 min)
inj. temp: programmed, 60 °C (0.28 min), 600 °C/min to 325 °C (5 min)
detector: MS
carrier gas: helium, 1 mL/min constant
injection: 10 µL, PTV solvent vent, 100 mL/min vent flow at 0.28 min, 5 psi vent pressure
liner: 4 mm I.D. FocusLiner™ with taper

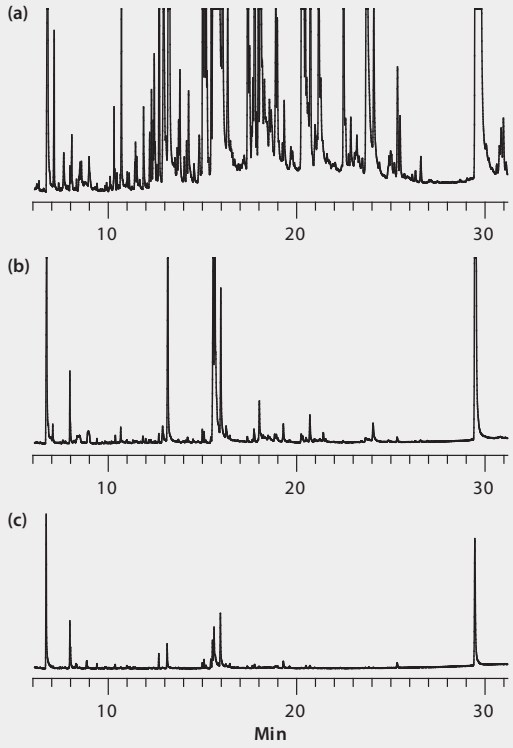

Figure 2. TICs of GC-MS/SIM Analysis of Pesticides at 50 ng/g in Beef Kidney Extract, Cleaned Using (a) Z-Sep+ and (b) PSA/C18.

Conditions same as Figure 1.

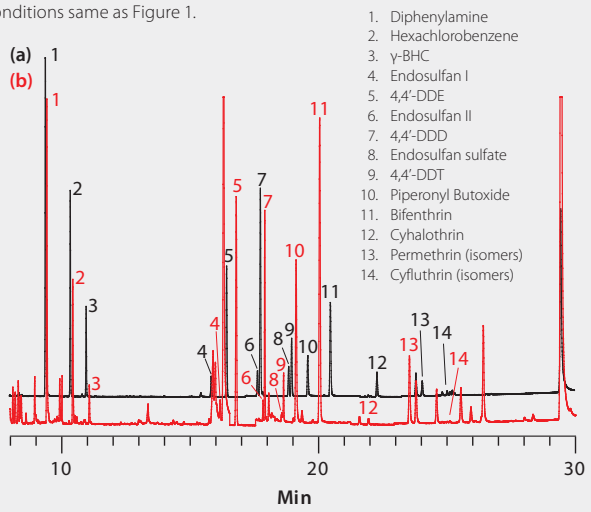

Table 2. Average Pesticide Recoveries from Beef Kidney Spiked at 50 ng/g, Average of n = 3 with (% RSD)

	Z-Sep +	PSA/C18
Diphenylamine	70 (1)	81 (1)
Hexachlorobenzene	74 (1)	73 (2)
γ-BHC (lindane)	81 (1)	77 (1)
Endosulfan I	106 (5)	79 (4)
4,4'-DDE	71 (2)	77 (2)
Endosulfan II	108 (6)	73 (5)
4,4'-DDD	91 (3)	78 (1)
Endosulfan sulfate	98 (4)	67 (4)
4,4'-DDT	83 (4)	74 (2)
Piperonyl butoxide	91 (5)	86 (2)
Bifenthrin	101 (7)	82 (2)
Cyhalothrin	101 (6)	87 (13)
Permethrin	99 (7)	86 (2)
Cyfluthrin	110 (8)	93 (2)

References

1. LeDoux, M. *J. Chromatogr. A*, *1218*, 1021–1036 (**2011**).
2. Lehotay, S.; Mastovska, K. *J. of AOAC International*, *88*, 630–638 (**2005**).
3. AOAC Official Method 2007.01, Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.
4. Lehotay, S. Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Approach for the Determination of Pesticide Residues. *Proceedings AOAC Annual Meeting*, St. Louis, MO USA, (**2004**).
5. Koesukwiwat, U.; Lehotay, S.; Mastovska, K.; Dorweiler, K.; and Leepipatpiboon, N. *J. of Agricultural and Food Chemistry*, *58*, 5950–5958 (**2010**).
6. *Pesticide Data Program Annual Summary, Calendar Year 2009*, United States Department of Agriculture, Agricultural Marketing Service, Science and Technology Programs, May (**2011**).
7. *The Pesticide Manual* 13th Edition, C.D.S. Tomlin, ed. (The British Crop Protection Council, Surrey, UK, **2003**).

Featured Products

Description	Cat. No.
Supel™ QuE QuEChERS Products	
Empty Centrifuge Tube, 50 mL, 50 ea	**55248-U**
Acetate Extraction Tube, 12 mL, 50 ea	**55234-U**
Z-Sep+ Cleanup Tube, 12 mL, 50 ea	**55296-U**
PSA/C18 Cleanup Tube, 12 mL, 50 ea	**55229-U**
SLB®-5ms Capillary GC Column	
20 m x 0.18 mm I.D., 0.36 µm	**28576-U**
Analytical Solvent	
Acetonitrile, for pesticide residue analysis	**34481**

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Rapid Cleanup of Spinach Extracts Using Gravity Elution SPE Prior to Pesticide Analysis

Katherine K. Stenerson and Jennifer Claus
jennifer.claus@sial.com

Prior to analysis for pesticide residues, extracts of highly pigmented foods, such as spinach, require a cleanup step for removal of matrix materials. Failure to do so can lead to ion suppression in LC-MS, inlet contamination in GC-MS and column contamination for both techniques. A dual-layer SPE tube containing graphitized carbon and aminopropyl on silica (NH_2) is often used.

- Carbon removes pigments and sterols
- NH_2 removes fatty acids, polar pigments, organic acids and sugars

Gravity Elution

Sample processing can be done either by applying a vacuum to pull the sample/solvents through the SPE tube, or by allowing gravity to pull the sample/solvents through. Gravity elution has the advantage of allowing more contact time between the extract and the sorbents. However, vacuum elution is more widely practiced due to limitations in sorbent particle technology.

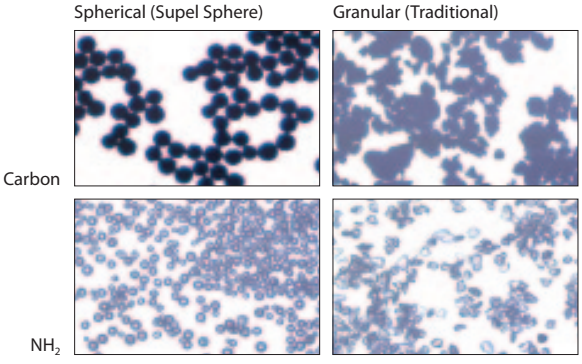
Spherical Particles

Current dual-layer SPE tubes contain granular materials. These materials have variations in particle size and shape, which can lead to slower flow, and more inconsistency in flow characteristics between tubes. Fines, which also contribute to flow and consistency problems, can be formed during manufacture and/or shipping due to the friable nature of granular materials.

A new dual-layer SPE tube, Supel™ Sphere Carbon/NH_2, was developed that uses spherical materials for both the carbon and NH_2 layers, providing fast, more consistent flows compared to tubes containing granular material. This allows gravity elution (instead of vacuum elution) to be applied, resulting in more contact time between the extract and the sorbents.

In this work, Supel Sphere Carbon/NH_2 tubes were compared to traditional tubes containing granular materials for flow characteristics, removal of matrix interferences and pesticide recovery for spinach extracts. The physical difference between spherical and granular materials is illustrated in **Figure 1**.

Figure 1. Spherical vs. Granular Materials



Experimental

Extraction and cleanup procedures derived from two published methodologies[1,2] are summarized in **Table 1**. Replicates of unspiked and spiked (5 ng/g) spinach samples were prepared, and allowed to sit for one hour prior to extraction. After extraction, extracts were split for cleanup using a tube containing spherical materials or a tube containing granular materials. Following cleanup, analysis was performed by GC-MS/SIM using large volume injection (LVI).

Quantitation was done using matrix-matched standards. The list of compounds evaluated included organophosphorus, organochlorine, acidic and hydrophobic pesticides.

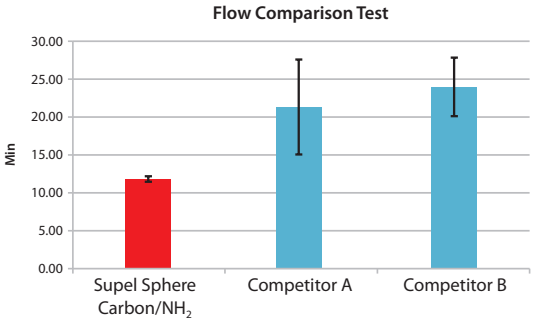
Table 1. Extraction and Cleanup Procedures

1. Place 10 g of homogenized spinach (spike added if a spiked replicate) and 10 mL of acetonitrile (Cat. No. 34481) into a 50 mL centrifuge tube (Cat. No. 55248-U), then shake for one minute.
2. Add content of an Acetate Extraction Tube (Cat. No. 55234-U), then shake for one minute.
3. Centrifuge at 3,200 rpm for five minutes.
4. Transfer the supernatant to a 12 mL tube containing 1 g of anhydrous magnesium sulfate (Cat. No. 63135).
5. Shake for one minute, then centrifuge at 3,200 rpm for five minutes.
6. Transfer 5 mL of the supernatant to a glass test tube and evaporate to 1 mL (at 40 °C).
7. Add 250 µL of toluene (Cat. No. 34494).
8. Condition a Supel Sphere Carbon/NH_2 Tube (Cat. No. 54283-U) or a traditional tube with 10 mL of acetonitrile:toluene (75:25).
9. Add sample extract from step 7, start eluent collection immediately.
10. Gravity elute tube with 20 mL of acetonitrile:toluene (75:25) and collect all eluent.

Flow Characteristics

The average time required for 25 mL of extraction solvent (75:25 acetonitrile:toluene) to be gravity eluted through each tube type is displayed in **Figure 2**. The range (slowest and fastest) of the five replicates is depicted by the vertical lines. Average flow times for the granular materials were similar, while flow through the Supel Sphere Carbon/NH_2 tubes was 50% faster. In addition, the Supel Sphere tube exhibited the best flow reproducibility between cartridges.

Figure 2. Average Timed Gravity Elution of 25 mL Solvent (n = 5)

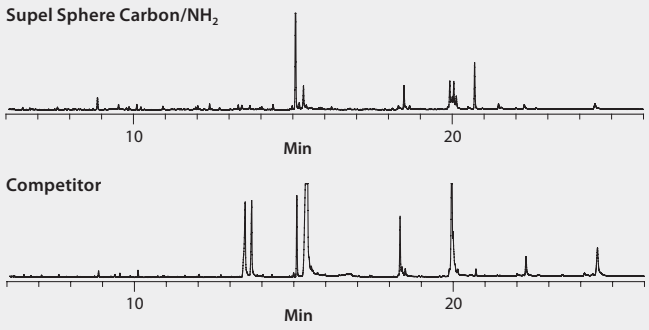


Removal of Matrix Interference

Full scan GC-MS runs of two extracts after cleanup are shown in **Figure 3**. The chromatogram of the extract when a Supel Sphere Carbon/NH_2 tube was used is notably cleaner. A cleaner background will translate to greater sensitivity and less instrument contamination, leading to longer periods of acceptable GC-MS performance before system maintenance is required.

Figure 3. Extracts of Spiked Spinach (full scan mode, same Y-axis)

column: SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm (28576-U)
oven: 70 °C (2 min), 15 °C/min to 325 °C (5 min)
inj. temp.: Programmed, 60 °C (0.28 min), 600 °C/min to 325 °C (5 min)
detector: MS, SIM mode
carrier gas: helium, 1 mL/min constant
injection: 10 µL LVI, PTV solvent vent, rapid injection speed; split vent flow: 100 mL/min (5 psi) until 0.28 min, 60 mL/min at 2.78 min
liner: 4 mm I.D., split/splitless type, single taper FocusLiner™ design (wool packed)



Pesticide Recovery

Table 2 compares average recoveries and % RSD values for three spiked samples after cleanup using Supel Sphere Carbon/NH_2 tubes and traditional tubes containing granular materials. As shown, recovery values when Supel Sphere Carbon/NH_2 tubes were used were similar or better for most pesticides. Reproducibility was also better for most pesticides.

Conclusions

Gravity elution may be preferred to vacuum elution for SPE as it allows a greater extract-sorbent contact time. When applied to the cleanup of spinach extracts and compared to traditional tubes containing granular materials, Supel Sphere Carbon/NH_2 SPE tubes exhibited superior and more consistent flow, removed as much or more background, and resulted in improved recovery and % RSD values for most pesticides.

References

1. *Multiresidue Method for Agricultural Chemicals by GC/MS (Agricultural Products)*, Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food: Syoku-An No. 0124001; Department of Food Safety, Japanese Ministry of Health, Labour and Welfare: January 24, (**2005**).
2. *Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate*. AOAC Official Method (**2007**.01).

Table 2. Average % Recovery (% RSD), n = 3

Analyte	Supel Sphere Tube (Spherical Material)	Traditional Tube (Granular Material)
Trifluralin	81 (2)	78 (3)
α-BHC	83 (1)	75 (10)
Thiometon	81 (5)	75 (3)
Hexachlorobenzene	43 (6)	55 (5)
Simazine	82 (2)	81 (4)
Quintozene	74 (8)	71 (6)
γ-BHC	84 (5)	80 (7)
Tolclofos-methyl	83 (4)	80 (3)
Heptachlor	72 (3)	69 (9)
Malathion	83 (11)	90 (5)
Metolachlor	83 (4)	82 (2)
Chloropyrifos	84 (3)	70 (4)
Endosulfan I	84 (8)	66 (11)
Oxadiazon	79 (3)	83 (3)
Ethion	81 (1)	79 (5)
Triazophos	73 (1)	83 (2)
4,4'-DDT	67 (3)	67 (4)
Phosmet	81 (3)	82 (4)
Methoxychlor	71 (4)	80 (3)
Coumaphos	79 (2)	79 (3)
Cyfluthrin isomer	66 (5)	71 (14)
Cypermethrin isomer	88 (5)	72 (8)
Deltamethrin	76 (7)	63 (36)

Featured Products

Description	Cat. No.
Supel Sphere SPE Product	
Carbon/NH_2 Tube, 6 mL, 30 ea.	54283-U
Supel QuE QuEChERS Products	
Acetate Extraction Tube, 12 mL, 50 ea.	55234-U
Empty Centrifuge Tube, 50 mL, 50 ea.	55248-U
SLB®-5ms Capillary GC Column	
20 m x 0.18 mm I.D., 0.36 µm	28576-U
Analytical Reagents and Solvents	
Anhydrous Magnesium Sulfate ($MgSO_4$), ≥97%	63135
Acetonitrile, for pesticide residue analysis	34481
Toluene, for pesticide residue analysis	34494

Related Products

Description	Cat. No.
Supel QuE QuEChERS Products	
Citrate Extraction Tube, 12 mL, 50 ea.	55227-U
Citrate/Sodium Bicarbonate Extraction Tube, 12 mL, 50 ea.	55237-U
SLB-5ms Capillary GC Columns	
30 m x 0.25 mm I.D., 0.25 µm	28471-U
30 m x 0.25 mm I.D., 0.50 µm	28473-U
20 m x 0.18 mm I.D., 0.18 µm	28564-U

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Ionic Liquid GC Column Option for the Analysis of Omega-3 and Omega-6 Fatty Acids

Craig Aurand
craig.aurand@sial.com
Katherine K. Stenerson, Michael R. Halpenny, Leonard M. Sidisky and Michael D. Buchanan
mike.buchanan@sial.com

Essential fatty acids are nutrients that must be obtained from the diet because humans lack the anabolic processes for their synthesis. Two closely related groups of essential fatty acids are the omega-3 and omega-6 fatty acids. These unsaturated fatty acids contain the initial double bond located directly after the third (omega-3) or the sixth (omega-6) carbon atom as measured from the methyl end of the compound.

Omega-3 and omega 6-fatty acids are typically analyzed using gas chromatography (GC) after their conversion to fatty acid methyl esters (FAMEs). Columns described in promulgated methods[1, 2] contain a stationary phase based on polyethylene glycol (PEG), and often have 'wax' in the name.

In this work, a new ionic liquid GC column, SLB®-IL60, is evaluated against an Omegawax® column for its suitability for this application. The SLB-IL60 column has selectivity similar to 'wax' columns, but is different enough to provide a unique elution pattern. It is not based on a PEG phase. Instead, it has various functional groups that allow an increased number of interaction mechanisms compared to a PEG phase. Specifications for both columns can be found in **Table 1**.

Table 1. Column Specifications

Omegawax
- Phase: Bonded; Poly(ethylene glycol)
- Temp. Limits: 50 °C to 280 °C (isothermal or programmed)

SLB-IL60
- Phase: Non-bonded; 1,12-Di(tripropylphosphonium)dodecane bis(trifluoromethylsulfonyl)imide
- Temp. Limits: 35 °C to 300 °C (isothermal or programmed)

Experimental

The GC conditions which were used are from the AOAC 991.39 and AOCS Ce 1i-07 methods. This was possible as both methods share the same set of run conditions. Two standard mixes were initially analyzed to gauge elution patterns. An individual FAME was also analyzed to confirm identification. Overall, the following standards were used:

1. Supelco® 37-Component FAME Mix
2. PUFA No. 3 (from menhaden oil), diluted in 1 mL of hexane
3. C22:5n3 methyl ester

Figure 1. C4-C24 FAME Standard

column 1: Omegawax, 30 m x 0.25 mm I.D., 0.25 µm (24136)
column 2: SLB-IL60, 30 m x 0.25 mm I.D., 0.20 µm (29505-U)
oven: 170 °C, 1 °C/min to 225 °C
inj. temp.: 250 °C
carrier gas: helium, 1.2 mL/min
det.: FID, 260 °C
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split/splitless type, single-taper wool-packed FocusLiner™ design
sample: Supelco 37-Component FAME Mix (47885-U) + C22:5n3, in methylene chloride

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

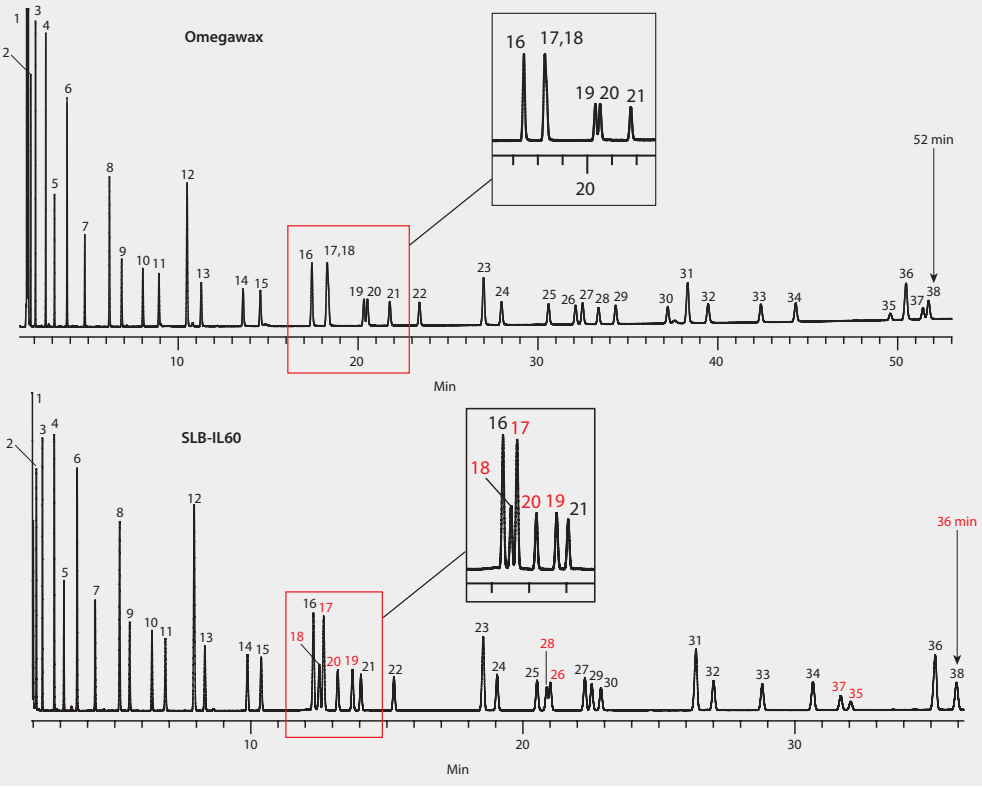
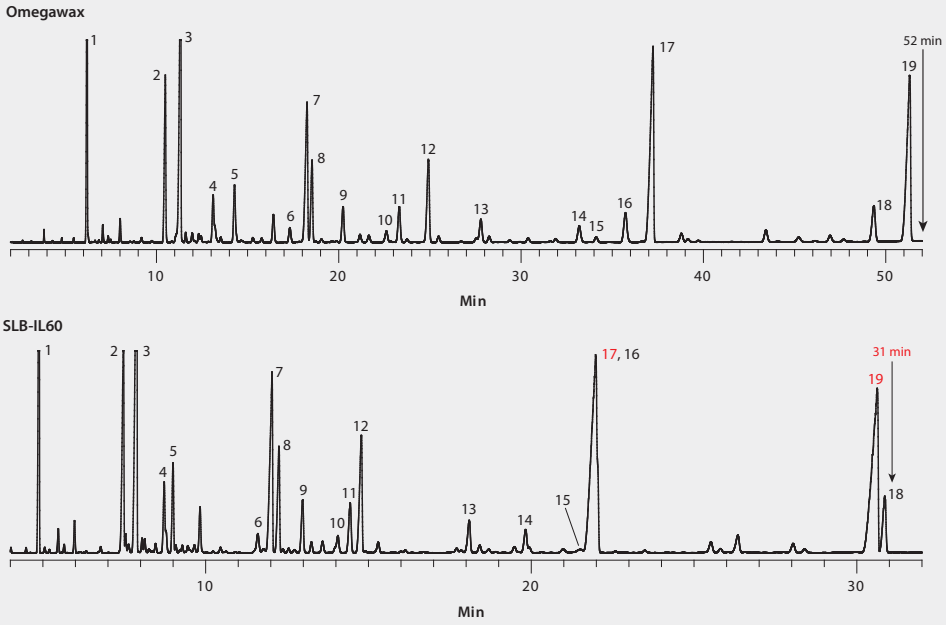


Figure 2. PUFA No. 3 Standard

column 1: Omegawax, 30 m x 0.25 mm I.D., 0.25 µm (24136)
column 2: SLB-IL60, 30 m x 0.25 mm I.D., 0.20 µm (29505-U)
oven: 170 °C, 1 °C/min to 225 °C
inj. temp.: 250 °C
carrier gas: helium, 1.2 mL/min
det.: FID, 260 °C
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split/splitless type, single-taper wool-packed FocusLiner™ design
sample: PUFA No. 3 Mix (47085-U), diluted in 1 mL of hexane

1. C14:0
2. C16:0
3. C16:1n7
4. C16:2n4
5. C16:3n4
6. C18:0
7. C18:1n9
8. C18:1n7
9. C18:2n6
10. C18:3n4
11. C18:3n3
12. C18:4n3
13. C20:1n9
14. C20:3n3
15. C20:4n6
16. C20:4n3
17. C20:5n3
18. C22:5n3
19. C22:6n3



C4-C24 FAME Mix

A custom 38-component mix was prepared by the addition of C22:5n3 FAME to a stock 37-component FAME mix. The resulting mix was then analyzed on both columns under identical conditions. Resulting chromatograms are shown in **Figure 1**. Observations are that the SLB-IL60 column provides:

- Faster elution overall (36 minutes compared to 52 minutes)
- Resolution of C18:1n9c (peak 17) and C18:1n9t (peak 18)
- Elution of C18:1n9t (peak 18) before C18:1n9c (peak 17)
- Elution of C18:2n6t (peak 20) before C18:2n6c (peak 19)
- Elution of C20:3n3 (peak 28) before C20:3n6 (peak 26)
- Elution of C22:6n3 (peak 37) before C22:5n3 (peak 35) – confirmed by analysis of an individual C22:5n3 standard

PUFA No. 3 Mix

A qualitative polyunsaturated fatty acid (PUFA) methyl ester standard (made from menhaden oil) was also analyzed on both columns under identical conditions. **Figure 2** shows both chromatograms. Use of the SLB-IL60 column resulted in:

- Faster elution overall (31 minutes compared to 52 minutes)
- Coelution of C20:4n3 and C20:5n3 (peaks 16 and 17)
- Elution of C22:6n3 (peak 19) before C22:5n3 (peak 18) – confirmed by analysis of an individual C22:5n3 standard

Conclusion

Compared to existing 'wax' columns, the SLB-IL60 column offers unique selectivity, some *cis/trans* resolution, and the faster elution of analytes. The elution of *trans* isomers before *cis* isomers of the same carbon chain length plus degree and position of unsaturation demonstrates the analyte-stationary phase mechanism difference of the SLB-IL60. These differences make the SLB-IL60 a good column choice for the analysis of omega-3 and omega-6 fatty acid methyl esters.

References

1. AOAC Official Method 991.39, "Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters" *AOAC International* (**2003**).
2. AOCS Official Method Ce 1i-07, "Determination of Saturated, cis-Monounsaturated, and cis-Polyunsaturated Fatty Acids in Marine and Other Oils Containing Long Chain Polyunsaturated Fatty Acids (PUFAs) by Capillary GLC" *AOCS Official Methods and Recommended Practices*.

Featured Products

Description	Cat. No.
GC Columns	
Omegawax®, 30 m x 0.25 mm I.D., 0.25 µm	24136
SLB®-IL60, 30 m x 0.25 mm I.D., 0.20 µm	29505-U
Analytical Standards	
Supelco® 37-Component FAME Mix, 1 mL 10 mg/mL (total wt.) in methylene chloride Visit sigma-aldrich.com/fame for composition details.	47885-U
PUFA No. 3 (from menhaden oil), 100 mg Visit sigma-aldrich.com/fame for composition details.	47085-U
Methyl all-cis-7,10,13,16,19-docosapentaenoate (C22:5n3), >98.0%, 50 mg	17269
Analytical Solvents	
n-Hexane, for pesticide residue analysis	34484

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Investigating Matrix Interference in Analysis of Antiarrhythmic Cardiac Drugs in Plasma

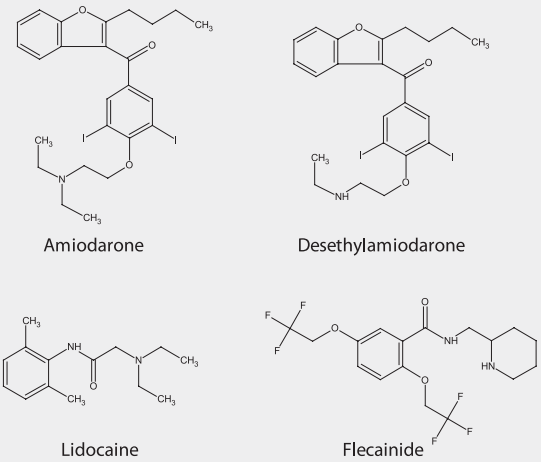
Craig Aurand
craig.aurand@sial.com

For efficient therapeutic drug monitoring, it is important for clinicians to have access to fast and robust analytical methods for accurate assessment of drug efficacy. Industrial trends toward highly specific LC-MS applications over traditional immunoassay have resulted in the need for high-speed chromatographic assays along with simplified sample preparation methods. Often the limitation of a bioanalytical technique is based upon the effectiveness of the sample preparation technique. Plasma and serum samples are often susceptible to assay irregularities due to matrix-induced interferences. In this study, the impact of matrix interference is investigated with respect to precision and accuracy of antiarrhythmic cardiac drugs from plasma samples.

Therapeutic Drug Monitoring of Antiarrhythmic Drugs

The focus of the study reported here was to develop a bio-analytical assay for antiarrhythmic cardiac drugs and associated metabolites from plasma samples. The goal was to develop a robust analytical technique that facilitates high throughput application with simplified sample processing. **Figure 1** shows the structures of the antiarrhythmic drugs evaluated in this study. The utility of these antiarrhythmic drugs vary: lidocaine is used for ventricular arrhythmias, flecainide is administered for tachyarrhythmia, while amiodarone is utilized in both ventricular and atrial cardiac dysrhythmias.[1] Having an analytical method capable of analyzing a range of antiarrhythmic agents permits simplified analysis while increasing the services the testing laboratory can offer. This pertains not only to LC-MS analysis, but also to sample collection and sample preparation.

Figure 1. Structures of Antiarrhythmic Compounds



Analytical Approach

The first part of the study focused on the chromatographic conditions for resolution of lidocaine, flecainide, amiodarone and the associated metabolite, desethylamiodarone. The basic nature of these antiarrhythmic agents makes them ideal candidates for HILIC (hydrophilic interaction) chromatographic separation.

The advantage of HILIC over traditional reversed-phase chromatography is two-fold for both sample introduction and analyte detection.[2] First, the high acetonitrile concentration of HILIC mobile phases allows direct analysis of precipitated plasma samples without the need for additional sample solvent exchange. Second, the high acetonitrile content provides increased analyte response in positive ESI MS detection. Of the various HILIC-mode columns tested, method development for this assay determined the Ascentis® Express HILIC, 2.7 µm particles, provided the best chromatographic resolution of the antiarrhythmic drugs while maintaining high peak efficiency for enhanced detection levels.

Once the chromatographic and LC-MS conditions had been optimized, the second part of this study was to evaluate the effectiveness of sample preparation and the impact of sample matrix on the assay. When dealing with plasma samples, endogenous matrix such as phospholipids often can cause irreproducibility in quantitation due to ionization-suppression effects. Coelution of endogenous matrix with target analytes can result in an arbitrary decrease in the response of target analytes, thus decreasing the overall accuracy of the method.[3] The determination of matrix interference is a critical aspect in the development of any bio-analytical method. In this case, a standard protein precipitation method was compared with hybrid zirconia coated silica particles in 96-well SPE format for processing the plasma samples prior to LC-MS analysis.

Experimental

Standard Solutions

Standard solutions were prepared from a stock standard in (3:1) 1% formic acid acetonitrile:water at levels of 10, 50, 100, 200 and 300 ng/mL.

Preparation and Extraction of Plasma Samples

Rat plasma stabilized with K_2EDTA was acquired from Lampire Biological Laboratories (Pipersville, Pennsylvania, USA). Plasma was spiked directly from stock standard to a level of 400 ng/mL of each analyte. Concentration of final sample work up in both techniques is equivalent to 100 ng/mL.

Standard protein precipitation method: Apply 100 µL of spiked plasma to centrifuge vial, followed by 300 µL of 1% formic acid in acetonitrile. Agitate via vortex for 2 minutes, place vial in centrifuge and spin at 15,000 rpm for 2.5 minutes. Collect supernatant and analyze directly.

HybridSPE®-Phospholipid 96-well method: Apply 100 µL of spiked plasma to the well, followed by 300 µL of 1% formic acid in acetonitrile. Agitate via vortex for four minutes, place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

Figure 2. LC-MS Analysis of Antiarrhythmic Drugs and Metabolite on Ascentis Express HILIC

LC-MS system:	Agilent 1290, 6210 TOF
column:	Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm (53939-U)
mobile phase:	(A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile, 5:95 (A:B), adjusted to pH 7.0 with formic acid
flow rate:	0.4 mL/min
pressure:	90 bar
column temp.:	35 °C
detection:	ESI+, 100-1000 m/z
sample:	each compound, 300 ng/mL in 75:25 (1% formic acid acetonitrile:water)
injection:	0.5 µL

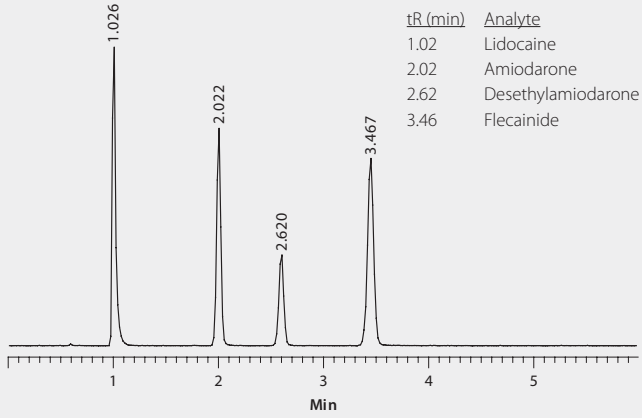
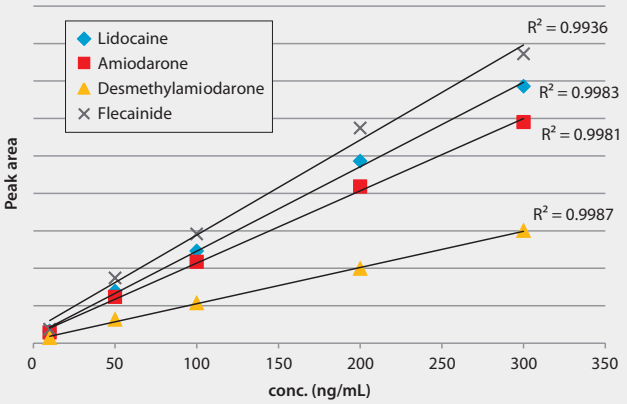


Figure 3. Antiarrhythmic Drugs Calibration Curve

Conditions same as Figure 2.



Results

Figure 2 shows the chromatographic resolution of the antiarrhythmic drugs on the Ascentis Express HILIC. Analysis was conducted on a time-of-flight (TOF) LC-MS and demonstrated a linear analyte response across the concentration range as shown in **Figure 3**.

Using the standard protein precipitation technique, matrix interference was observed with the overlap of endogenous phospholipids and the target analytes. As shown in **Figure 4**, the elution region of amiodarone, desethylamiodarone and flecainide overlaps with the elution of the endogenous phospholipids. This raises the issue as an area of concern for possible detection irregularities. As observed in the matrix trace, phospholipids are carried over from previous sample injections causing real concern of method reproducibility.

When analyte levels were calculated against the calibration curve, the concentration of desethylamiodarone and flecainide were substantially lower than the spiked levels. Significant ion suppression was observed due to the endogenous phospholipids in the plasma samples. The coefficient of variation (% C.V.) value was extremely high for both desethylamiodarone and flecainide, further confirming the concern about matrix interference.

To confirm that the decreased detected levels of desethylamiodarone and flecainide were due to phospholipid ion suppression, plasma samples were processed using the HybridSPE-Phospholipid 96-well plate. This technology utilizes hybrid zirconia-coated silica particles to facilitate the selective extraction of phospholipids from biological samples via Lewis acid/base interaction between the phosphate moiety of the phospholipids and the zirconia surface. This interaction is highly selective toward the isolation of phospholipids while remaining non-selective towards target analytes. Sample processing using the HybridSPE-Phospholipid consists of the addition of formic acid acetonitrile to the plasma samples, followed by mixing and filtration by applying vacuum to the 96-well plate. This technique allows direct depletion of sample proteins and phospholipids, resulting in a simplified and effective sample preparation. Samples processed using the HybridSPE-Phospholipid technique were depleted of phospholipid matrix with no additional sample processing.

Figure 5 shows the analyte and phospholipid trace of plasma samples processed using the HybridSPE-Phospholipid technique. Notice the increased peak response of both desethylamiodarone and flecainide, as compared to samples processed using the standard protein precipitation technique. The calculated levels were consistent with the spiked levels. There was no phospholipid matrix observed in the HybridSPE-Phospholipid processed samples, confirming that the reduced response of desethylamiodarone and flecainide in the standard protein precipitation technique was a result of the endogenous phospholipids. The consistency in the calculated levels of all three antiarrhythmic agents and metabolite with the spiked level, coupled with low CV values verifies the precision and accuracy of the HybridSPE-Phospholipid sample preparation.

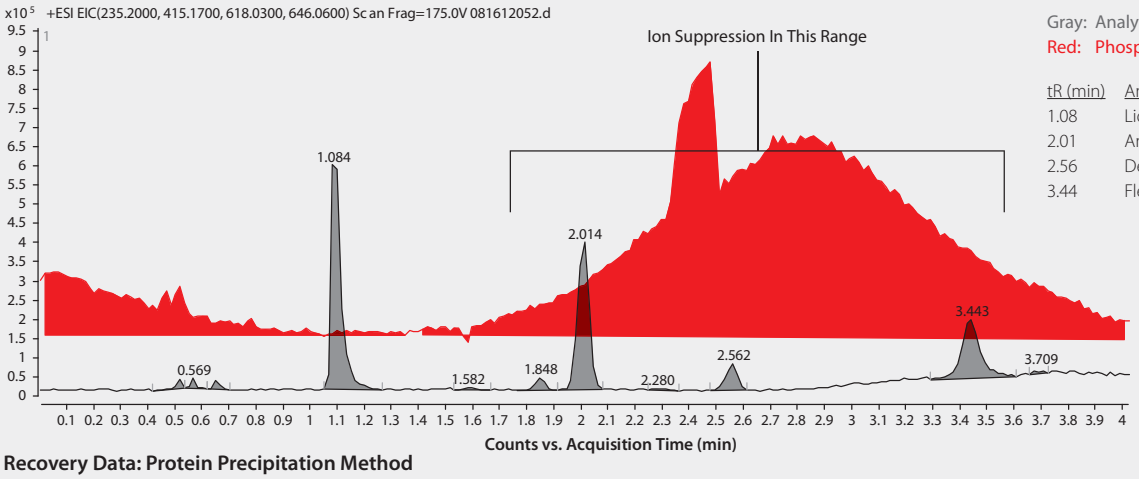
Conclusions

Endogenous phospholipids can dramatically impact the precision and accuracy of a bio-analytical method. During development, the impact of sample matrix on analyte detection should be a priority in method validation. Failure to do so can result in inaccuracies in reported levels, thus impacting the assessment of patient health. Eliminating matrix effects in LC-MS is imperative for producing reliable and accurate bio-analytical methods. The targeted phospholipid selectivity of the HybridSPE-Phospholipid technique enables simplified sample processing with no phospholipid matrix interference, while exhibiting excellent recovery from plasma and serum samples. This application demonstrates how selectivity in both sample preparation and chromatographic separation allows a simplified and efficient bioanalytical method, resulting in a highly precise and accurate assay. The fast HILIC chromatography on Ascentis Express paired with the selective sample preparation of HybridSPE-Phospholipid, LC-MS Ultra CHROMASOLV® solvents and Cerilliant® Certified Reference Materials resulted in a robust and precise bio-analytical method.

Figure 4. LC-MS Analysis of Antiarrhythmic Drugs in Spiked Plasma Following Sample Prep Using Protein Precipitation

LC-MS system: Agilent 1290, 6210 TOF
column: Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm (53939-U)
mobile phase: (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile, 5:95 (A:B), adjusted to pH 7.0 with formic acid
flow rate: 0.4 mL/min
pressure: 90 bar

column temp.: 35 °C
detection: ESI+, 100-1000 m/z
sample/matrix: rat plasma, unfiltered K_2EDTA, spiked with each compound at 100 ng/mL (3:1, plasma: 1% formic acid in acetonitrile)
injection: 0.5 µL



Gray: Analytes
Red: Phospholipids

tR (min)	Analyte
1.08	Lidocaine
2.01	Amiodarone
2.56	Desethylamiodarone
3.44	Flecainide

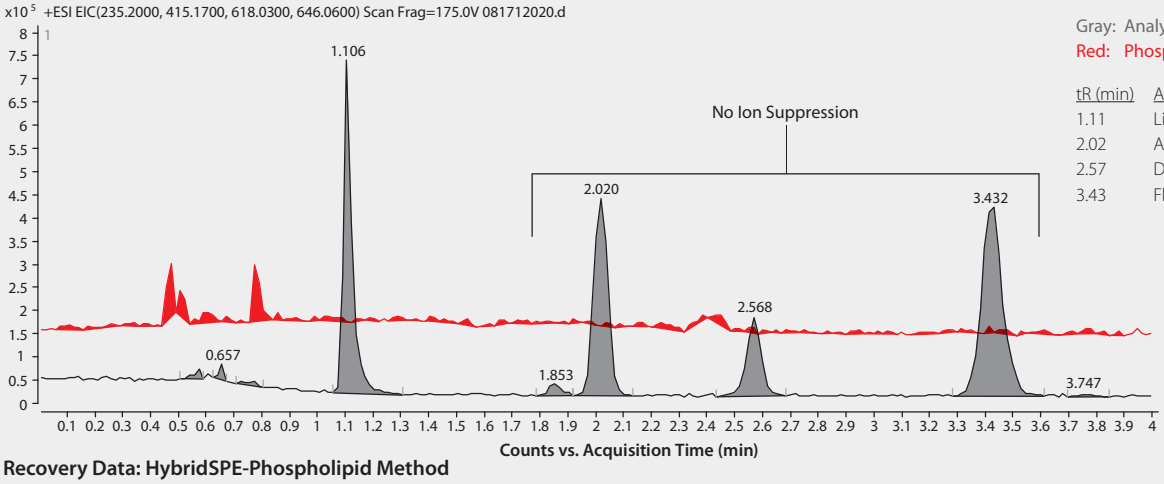
Recovery Data: Protein Precipitation Method

Protein Precipitation Plasma Recovery (n=16)	Lidocaine Calc. Con. (ng/mL)	Amiodarone Calc. Con. (ng/mL)	Desethylamiodarone Calc. Con. (ng/mL)	Flecainide Calc. Con. (ng/mL)
Average	118.5	105.5	52.9	44.7
Std. Dev.	9.6	1.5	21.8	20.5
% C.V.	8.1	1.5	41.2	45.9

Figure 5. LC-MS Analysis of Antiarrhythmic Drugs in Spiked Plasma Following Sample Prep Using HybridSPE-Phospholipid

LC-MS system: Agilent 1290, 6210 TOF
column: Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm (53939-U)
mobile phase: (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile, 5:95 (A:B), adjusted to pH 7.0 with formic acid
flow rate: 0.4 mL/min
pressure: 90 bar

column temp.: 35 °C
detection: ESI+, 100-1000 m/z
sample/matrix: rat plasma, unfiltered K_2EDTA, spiked with each compound at 100 ng/mL (3:1, plasma: 1% formic acid in acetonitrile)
injection: 0.5 µL



Gray: Analytes
Red: Phospholipids

tR (min)	Analyte
1.11	Lidocaine
2.02	Amiodarone
2.57	Desethylamiodarone
3.43	Flecainide

Recovery Data: HybridSPE-Phospholipid Method

HybridSPE Plasma Recovery (n=16)	Lidocaine Calc. Con. (ng/mL)	Amiodarone Calc. Con. (ng/mL)	Desethylamiodarone Calc. Con. (ng/mL)	Flecainide Calc. Con. (ng/mL)
Average	108.4	109.8	104.8	119.0
Std. Dev.	5.4	4.4	4.7	7.2
% C.V.	4.9	4.0	4.5	6.1

References

1. American Heart Association, Medications for Arrhythmia. http://www.heart.org (accessed Nov. 26, **2012**).
2. Ascentis Express HILIC Guide. Faster Analysis of Polar Compounds. (**2012**). Sigma-Aldrich Literature Code OMI.
3. Aurand, C. Understanding, Visualizing, and Reducing the Impact of Phospholipid-Induced Ion Suppression in LC-MS. *Supelco Reporter*, (**2012**), 30.2, 10–12.

Featured Products

Description	Cat. No.
Ascentis® Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles	53939-U
HybridSPE®-Phospholipid 96-well Plate, bed wt. 50 mg, volume 2 mL	575656-U
Acetonitrile, LC-MS Ultra CHROMASOLV®, tested for UHPLC-MS, 1 L	14261
Water, LC-MS Ultra CHROMASOLV, tested for UHPLC-MS, 1 L	14263
Formic acid, LC-MS Ultra eluent additive, 1 mL	14265
Ammonium formate, LC-MS Ultra eluent additive, 25 g	14266

Description	Cat. No.
Cerilliant® Certified Reference Materials*	
Amiodarone HCl, 1.0 mg/mL (as free base) in methanol, 1 mL ampule	A-060
Amiodarone-D_4 HCl, 100 µg/mL (as free base) in methanol, 1 mL ampule	A-082
N-Desethylamiodarone HCl, 1.0 mg/mL (as free base) in methanol, 1 mL ampule	D-055
N-Desethylamiodarone-D_4 HCl, 100 µg/mL (as free base) in methanol, 1 mL ampule	D-056
(±)-Flecainide, 1.0 mg/mL in methanol, 1 mL ampule	F-017
Lidocaine, 1.0 mg/mL in methanol, 1 mL ampule	L-018

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* Standards from Cerilliant, a Sigma-Aldrich Company, can be ordered from **sigma-aldrich.com/cerilliant**. For technical enquiries, email **cerilliantEU@sial.com**

Improvement in LC-MS/MS Analysis of Vitamin D Metabolites in Serum by Leveraging Column Selectivity and Effective Sample Preparation

Craig R. Aurand
craig.aurand@sial.com

Clinical interest in vitamin D stems from its implications in an ever-widening range of human health conditions.[1] This heightened interest has spawned the need for analytical strategies to assess an individual's vitamin-D status. Because LC-MS/MS has overcome many of the limitations of traditional immuno-assay (e.g. cross-reactivity, matrix interferences), it is becoming more and more the analytical method of choice for certain clinical assays, including vitamin D. To maximize the effectiveness of LC-MS/MS experiments, it is important to consider factors that reduce measurement sensitivity and accuracy, restrict throughput and cause instrument downtime. This brief report presents a rapid and sensitive LC-MS/MS method for accurate determination of 25-hydroxyvitamin D_2, 25-hydroxyvitamin D_3, and 3-*epi*-25-hydroxyvitamin D_3 in serum.

Importance of Resolving Vitamin D Homologs

The metabolic pathways of vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) involve many compounds with varying degrees of biological activity and clinical interest. Chromatographic resolution of the various homologs of vitamin D_2 is necessary for accurate quantitation, especially considering that several key metabolites are isobaric and not distinguishable by MS alone. For example, separation of the active 25-hydroxyvitamin D_3 from the 3-*epi*-25-hydroxyvitamin D_3, whose biological role is currently unclear, may provide more accurate information for treatment and prevention.[2] The aim of this study was to identify an HPLC stationary phase that would resolve vitamin D homologs, especially isobars, with short analysis time and high efficiency.

Sample Prep to Remove Interferences and Enhance MS Sensitivity

The hydrophobic character of vitamin D and its metabolites requires mobile phases with high concentrations of organic modifier, conditions that also elute endogenous interferences such as phospholipids. This phospholipid co-elution causes ion suppression and/or enhancement in the MS instrument that reduces sensitivity and accuracy.[3] Additionally, serum phospholipids and proteins foul HPLC and UHPLC columns and can cause instrument downtime. It is therefore important to remove them prior to analysis. In this study, commonly used protein precipitation and solid-phase extraction methods were compared in terms of their ability to remove these interferences and improve detection accuracy.

Materials and Methods

- **HPLC:** Ascentis® Express F5, 10 cm x 2.1 mm I.D., 2.7 µm
- **Sample Prep Device:** HybridSPE®-Phospholipid, 96-well plates, 50 mg/well
- **Protein Precipitation Method:** Apply 100 µL of plasma to centrifuge vial followed by 300 µL of 1% formic acid in acetonitrile. Agitate via vortex for two minutes. Centrifuge 2.5 minutes at 15,000 rpm. Collect supernatant and analyze directly.
- **HybridSPE®-Phospholipid 96-Well Method:** Apply 100 µL of spiked plasma to the well, followed by 300 µL of 1% formic acid in acetonitrile. Agitate via vortex for 4 minutes, place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

Results

A pentafluorophenyl HPLC phase (Ascentis Express F5) was chosen because of its ability to rapidly resolve the vitamin D homologs tested, especially the 25-hydroxyvitamin D_3 and the 3-*epi*-25-hydroxyvitamin D_3 (**Figure 1**) that coelute on C18 stationary phases. **Figure 2** shows the separation of 25-hydroxyvitamin D_2, 25-hydroxyvitamin D_3 and 3-*epi*-25-hydroxyvitamin D_3 on the Ascentis Express F5 column. Note that the co-elution of 25-hydroxyvitamin D_2 and 3-*epi*-25-hydroxyvitamin D_3 is not an issue, because they are resolved by the mass spectroscopy. **Figure 3** shows the phospholipid monitoring chromatograms of co-extracted matrix from standard protein precipitation, and using the HybridSPE-Phospholipid technique. Comparing the sample prep methods, the simple and straight-forward HybridSPE-Phospholipid method was found to be far superior to standard protein precipitation. HybridSPE-Phospholipid selectively depleted the phospholipid matrix and precipitated proteins, providing no interference from the serum matrix. In contrast, the protein precipitation technique contained a large amount of co-extracted phospholipid matrix, resulting in interference that eluted in the retention range of 25-hydroxyvitamin D_2, 25-hydroxyvitamin D_3, and 3-*epi*-25-hydroxyvitamin D_3. This co-elution reduces sensitivity and reproducibility, resulting in irregularities in quantitation, as confirmed by the recovery and reproducibility data reported in **Table 1**.

Table 1. Sample Prep Analyte Recovery Comparison

	25-OH Vitamin D_3	3-*epi*-25-OH Vitamin D_3	25-OH Vitamin D_2
HybridSPE-Phospholipid Method			
% Recovery	70.4	65.6	55.8
% Std. Dev.	8.2	5.8	12.1
Protein Precipitation Method			
% Recovery	53.0	55.7	33.9
% Std. Dev.	12.1	7.9	118.6

Conclusion

Chromatographic resolution of analytes still plays an important role in LC-MS applications; especially when dealing with isobaric compounds. The unique selectivity of the Ascentis Express F5 provided a fast and efficient method for the analysis of 25-hydroxyvitamin D and homologs from serum samples. The selective phospholipid depletion of the HybridSPE-Phospholipid method enabled an efficient sample cleanup, increasing method reproducibility and accuracy.

Figure 1. HPLC Separation of 25-Hydroxyvitamin D_2 and 25-Hydroxyvitamin D_3 on Ascentis Express F5

column: Ascentis® Express F5, 10 cm x 2.1 mm I.D., 2.7 µm (53569-U)
mobile phase: (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in methanol; (25:75, A:B)
flow rate: 0.4 mL/min
column temp.: 40 °C
detector: ESI(+), m/z 100-1000
injection: 1 µL, each compound 20 µg/mL in methanol

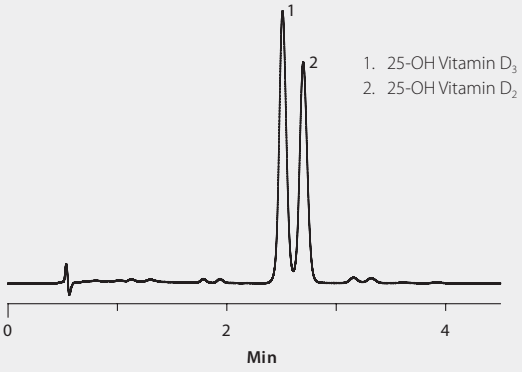


Figure 2. HPLC Separation of 25-Dihydroxyvitamin D_2, 25-Hydroxyvitamin D_3, and 3-*epi*-25-Hydroxyvitamin D_3 on Ascentis Express F5

Conditions same as in Figure 1.

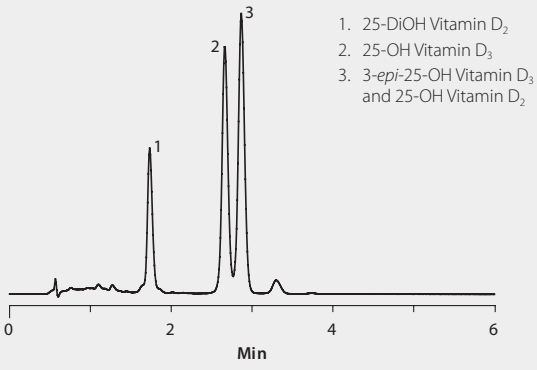
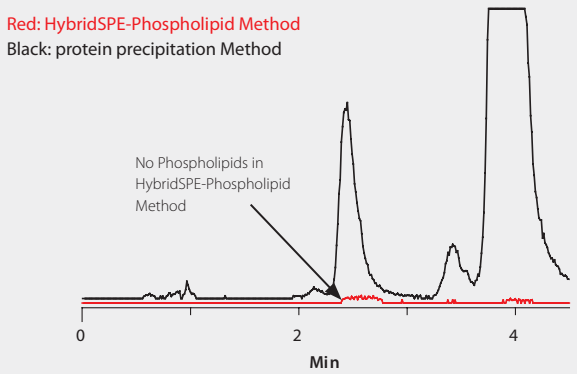


Figure 3. Serum Extracted Using HybridSPE-Phospholipid or Protein Precipitation

Conditions same as in Figure 1 except:
phospholipid monitoring (m/z): lysophosphatidylcholines, 496.3, 524.3 m/z; glycerophosphocholines, 758.5, 786.5, 806.5, 810.5 m/z



This approach demonstrates how selectivity, in both chromatographic and sample preparation, allows for efficient analysis that would otherwise be unattainable with traditional reversed-phase approaches. The combination of this novel sample prep technique along with the unique selectivity of the Ascentis Express F5 enables a fast and simplified bioanalytical method for associated vitamin D metabolites. For further reading and details of this method, please see reference 2.

References

1. Wang, S. Epidemiology of vitamin D in health and disease. *Nutr. Res. Rev.*, (**2009**). *22(2)*, 188–203.
2. Aurand, Craig R.; Bell, David S.; Wright, Michael. Highly selective isolation and separation of 25-hydroxyvitamin D and 3-epi-25-hydroxyvitamin D metabolites from serum. *Bioanalysis*, (**2012**), 4 *(22)*, 2681–2691.
3. Aurand, C. Understanding, Visualizing, and Reducing the Impact of Phospholipid-Induced Ion Suppression in LC-MS. *Supelco Reporter*, (**2012**), *30.2*, 10–12.

Featured Products

Description	Cat. No.
Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm	53569-U
Guard cartridge for Ascentis Express F5, 2.1 mm I.D., 2.7 µm, pk. of 3	53594-U
Universal Guard Holder	53500-U
HybridSPE®-Phospholipid 96-well Plate, bed wt. 50 mg, volume 2 mL	575656-U
Methanol, LC-MS Ultra CHROMASOLV®, tested for UHPLC-MS, 1 L	14262
Acetonitrile, LC-MS Ultra CHROMASOLV, tested for UHPLC-MS, 1 L	14261
Water, LC-MS Ultra CHROMASOLV, tested for UHPLC-MS, 1 L	14263
Ammonium formate, LC-MS Ultra eluent additive, 25 g	14266
3-*epi*-25-Hydroxyvitamin D_3, 1 mg (neat)	705993
Cerilliant® Certified Reference Materials*	
25-Hydroxyvitamin D_2, 50 µg/mL in ethanol, 1 mL/ampoule	H-073
25-Hydroxyvitamin D_3, 100 µg/mL in ethanol, 1 mL/ampoule	H-083
d_6-25-Hydroxyvitamin D_3 (26,26,26,27,27,27-d_6), 50 µg/mL in ethanol, 1 mL/ampoule	H-074
25-Hydroxyvitamin D_2, 5 µg/mL in ethanol, 1 mL/ampoule	H-087
25-Hydroxyvitamin D_3, 5 µg/mL in ethanol, 1 mL/ampoule	H-086
1α,25-Dihydroxyvitamin D_2, 5 µg/mL in ethanol, 1 mL/ampoule	H-090
1α,25-Dihydroxyvitamin D_3, 5 µg/mL in ethanol, 1 mL/ampoule	H-089
Vitamin D_2 (Ergocalciferol), 1.0 mg/mL in ethanol, 1 mL/ampoule	V-024
d_3-Vitamin D_2 (6,19,19-d_3), 100 µg/mL in ethanol, 1 mL/ampoule	V-026
Vitamin D_3 (Cholecalciferol), 1.0 mg/mL in ethanol, 1 mL/ampoule	V-025

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Radiello® the Passive Sampler as a Tool for the Measurement of Atmospheric Ammonia Concentrations and Dry Deposition

Contributed Article

The following was generated with the assistance of an outside source using Sigma-Aldrich® products. Content was provided from the public information published on the Ammonia Monitoring Network website. http://nadp.isws.illinois.edu

Kristen Schultz

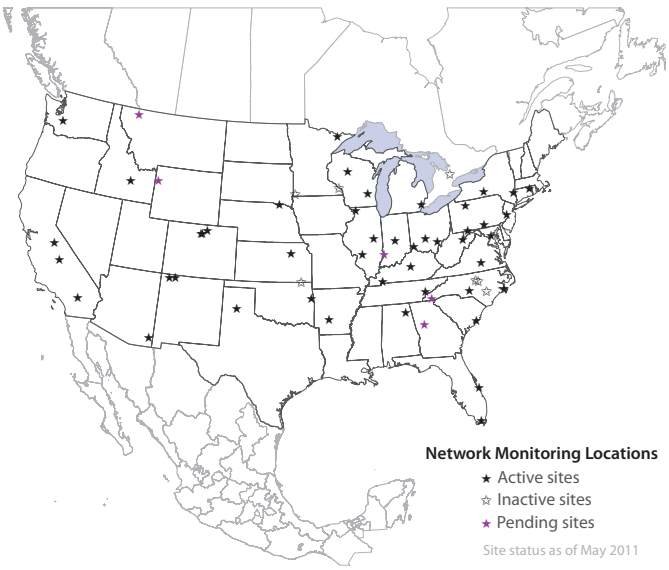
kristen.schultz@sial.com

Introduction

The National Atmospheric Deposition Program (NADP) launched the Ammonia Monitoring Network (AMoN) in 2010 but first piloted a passive sampling program beginning in 2007 with 16 test sites, experimenting with different types of passive sampling devices. Illinois Water Survey (IWS) operates as the Central Analytical Laboratory (CAL) for NADP's AMoN and deploys and analyzes the ammonia test samples and reports the analytical data back to the NADP.

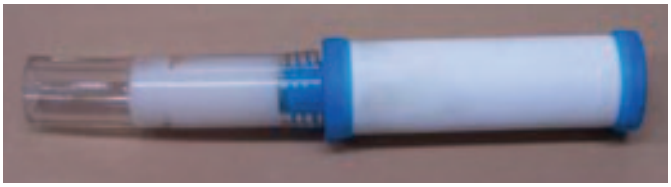
Ammonia is emitted by many sources, including very important agricultural sources of fertilizer application and meat production. NADP'S AMoN provides useful information to the agricultural community to assess the fate and transport of ammonia in the atmosphere. Its goal is to measure atmospheric ammonia concentrations and estimate dry deposition over North America. The network uses the low-cost passive diffusion sampler, radiello, deployed every two weeks to 54 locations (**Figure 1**), providing an integrated and quality-assured measurement of ammonia in air.

Figure 1. NADP Ammonia Monitoring Network (AMoN)



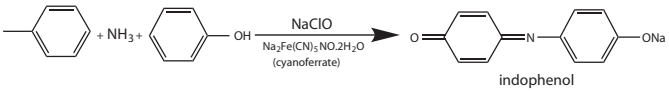
Radiello Ammonia Adsorbing Cartridge

The cartridge adsorbent (RAD168) is made of microporous polyethylene material and impregnated with phosphoric acid. Ammonia is adsorbed as ammonium ion. *Airborne ammonium salts dispersed as particulate matter do not cross the diffusive membrane of radiello.*



Ammonium ion is quantified by visible spectrometry as indophenol: at the basic buffered pH, ammonium ion reacts with phenol and sodium hypochlorite, with pentacyanonitrosylferrate catalysis (in the following *cyanoferrate*), to form indophenol. The reaction product is intensely colored in blue, and its absorbance measured at 635 nm. (**Figure 2**).

Figure 2. Reaction of Ammonia on Adsorbent Cartridge



Field Deployment of radiello Samplers

The sampler shelter (design available from Central Analytical Laboratory) consists of an inverted plastic shelter permanently fastened to an aluminum U-channel mounting bracket (**Figure 3**). The shelter is erected so that the lower edge is two metres (80 inches) above surrounding surfaces. Triplicate radiello ammonia samplers are mounted within the shelter (**Figure 4**). *Samplers are not mounted near plumbing stacks, chimneys, vents, exhausts or other possible sources of direct ammonia emissions.*

Figure 3. Shelter Schematic

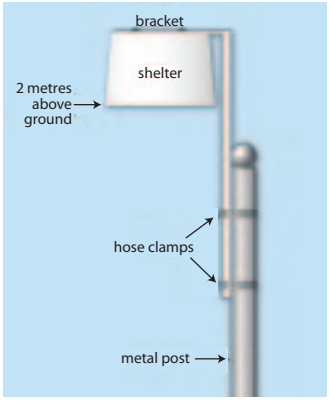


Figure 4. Installation of radiello Cartridges in Shelter

The vertical arm of the aluminum bracket can be fastened to a metal fence post. Alternatively, the shelter can be mounted on a suitable wooden post or pole by using screws (or small lag bolts) driven through the pre-drilled holes in the lower part of the bracket.

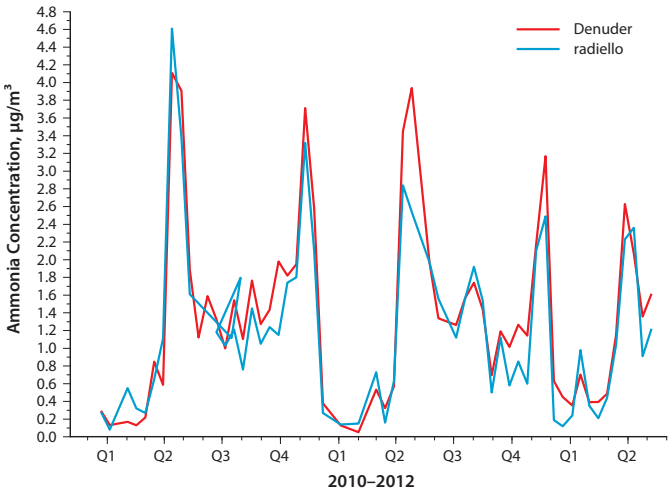
Laboratory Methods

The radiello ammonia samplers are returned to the Central Analytical Laboratory and analyzed. The cartridges are removed and the ammonia (as NH_4^+) is removed by sonication in deionized water, using Flow Injection Analysis (FIA).

Suitability of radiello for Ammonia Concentration Measurements

The NADP selected radiello samplers for the ease of use and deployment (no sampling pump or calibration requirement) and the quality of data. In order to ensure that the radiello samplers perform according to the AMoN criteria, it is compared to a denuder reference method at the Illinois field site. A denuder differs from radiello in that it is an active sampling method requiring a sampling pump. The denuder consists of a coated glass sampler. Below is a plot comparing the data from radiello and denuder for ammonia measurements (**Figure 5**).

Figure 5. Comparison of radiello and Denuder Measurements at Champaign County, Illinois



Conclusion

The radiello ammonia sampler impregnated with phosphoric acid represents a low-cost, reliable and simple tool for assessing the atmospheric deposition of ammonia at parts per billion (ppb) concentration levels.

Reference

1. National Atmospheric Deposition Program (NADP) Ammonia Gas Monitoring Network Factsheet (AMoN), Rev 4/29/11 http://nadp.isws.illinois.edu/amon/AMoNfactsheet.pdf

Featured Products

Description	Qty.	Cat. No.
Cartridge Adsorbent – Ammonia	20	RAD168
Diffusive Body – White	20	RAD120
radiello Accessories		
Triangle Support Plate	20	RAD121
radiello Clips	20	RAD195
Bar Code Labels	198	RAD190

Related Information

For more information on the NADP AMoN network or to participate in the program, contact:

Christopher M.B. Lehmann, PhD
Director, Central Analytical Laboratory

National Atmospheric Deposition Program
Illinois State Water Survey; Prairie Research Institute
University of Illinois at Urbana-Champaign
2204 Griffith Dr, Champaign, IL 61820-7495
Phone: +1-217-265-8512, Fax: +1-217-333-0249
Email: clehmann@illinois.edu
http://nadp.isws.illinois.edu

Performance Comparison of TDS³™ Storage Containers to Swagelok® Fittings and Glass Storage Containers

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

Introduction

The typical analytical process for air sampling using thermal desorption tubes almost always involves shipping and storing the sampling tube before and after sampling, prior to analysis. The most common approach to preventing contamination of the tube during shipment and storage has been to attach Swagelok end-cap fittings, using PTFE ferrules, to both ends of the tubes before and after sampling. Another common technique is to place the sampling tube in a glass vial-like container, constructed to seal at one end with a PTFE-faced screw cap.

The TDS³ (Thermal Desorption Storage and Sampling System) offers advantages over both the Swagelok end-cap fittings and glass storage containers because it is designed to eliminate internal dead volume, minimize the area of migration of the sample from the adsorbent during the storage period, and eliminate breakage risks when shipping and handling, in contrast to glass storage containers bearing this same risk. The TDS³ storage container holds the tube in its hard polycarbonate shell and seals with inert end-caps fitted with PTFE-faced silicone septa that are easily replaced. This eliminates the need for extensive cleaning or thermal conditioning of the device before it can be used for storing another tube.

Experimental

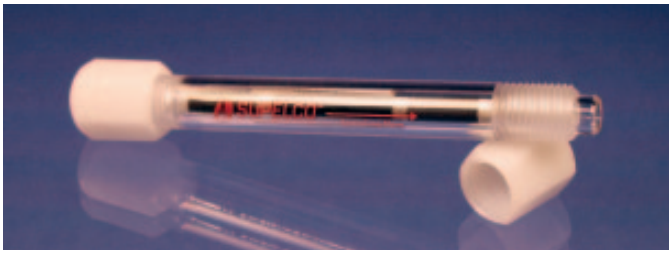
The performance of the TDS³ storage container was measured by its effectiveness for storing a collected sample relative to Swagelok end-cap fittings and glass storage containers.

A mix of twelve analytes were spiked onto twelve Carbotrap® 300 thermal desorption tubes (6 mm O.D. x 4 mm ID x 11.5 cm L), containing three carbonaceous adsorbents: Carbotrap C, Carbotrap B and Carbosieve® S-III. The sampling tubes were spiked with 40 ng of each analyte in 0.2 µL of methanol, using flash vaporization and 0.5 litres of inert nitrogen (50 mL/min for 10 min) to transfer the vaporized analytes onto the sampling tube.

The twelve tubes were assigned to three sets, each stored in a different storage device. Tubes in Set One were stored in TDS³ storage containers, Set Two were fitted with brass Swagelok nut and end-cap fittings, using PTFE ferrules, and Set Three were stored in a threaded glass vial-type container which seals with a PTFE screw cap at one end.

After spiking, the tubes were quickly sealed and placed in a paint can (all tubes in the same can) containing a small amount of activated charcoal and placed in a laboratory freezer for 14 days at -24 °C. After 14 days, the samples were removed from the paint can and thermally desorbed to a gas chromatograph.

Figure 1. TDS³ Container with Carbotrap 300 Glass-Fritted TD Tube



The clear body of the TDS³ container is shorter than the actual tube (as shown), so the septa seals on the end of the thermal desorption tube, creating an air-tight seal.

Results

Percent recoveries in **Table 1** were calculated by comparing peak areas for the desorbed analytes to those for calibration standards spiked onto the Carbotrap 300 tubes in the same manner on the day of analysis. Values shown are means for the three samples. Area counts for the first six listed compounds were normalized to an internal standard, bromodichloromethane; the last six compounds were normalized to 1,3-dichlorobenzene.

Table 1. Recovery of Analytes Stored for 14 Days Conclusion

	Set One	Set Two	Set Three
Analyte	TDS³ Storage Container	Swagelok Fittings with PTFE Ferrules	Glass Storage Container
Chloroform	97.7 ± 6.8	97.9 ± 2.3	99.2 ± 3.5
1,1,1-Trichloroethane	102.4 ± 1.7	102.6 ± 1.5	104.4 ± 1.4
Carbon tetrachloride	98.7 ± 2.8	95.8 ± 1.3	98.8 ± 2.6
1,2-Dichloroethane	98.6 ± 3.0	95.8 ± 1.7	98.6 ± 1.0
Trichloroethylene	100.7 ± 2.8	96.7 ± 0.7	105.0 ± 2.0
1,2-Dichloropropane	99.3 ± 2.5	97.5 ± 1.4	102.9 ± 2.7
1,3-Dichloropropane	101.3 ± 6.0	94.4 ± 9.2	87.8 ± 9.4
Tetrachloroethylene	99.5 ± 5.3	101.7 ± 4.4	93.8 ± 6.6
Ethylene dibromide	114.2 ± 6.2	97.1 ± 18.3	76.2 ± 8.7
Chlorobenzene	95.4 ± 4.4	95.9 ± 6.9	95.0 ± 5.7
Bromoform	107.7 ± 11.3	98.9 ± 5.5	91.7 ± 10.4
Bromobenzene	96.0 ± 4.8	95.4 ± 8.4	91.1 ± 6.8

The results in **Table 1** demonstrate that the TDS³ storage containers are equivalent to both Swagelok end-cap fittings and glass storage containers in terms of sample stability during storage.

Did you know . . .

In addition to its ability to maintain sample integrity, the TDS^3 storage container offers the user versatility during the sampling process. It can be converted to a sampling device by installing optional sampling caps that allow the user to easily connect the tube in the TDS^3 container to a sampling pump. An optional tube holder is also available.

Figure 2. Carbotrap 300 TD Tubes with Escort™ Elf Pump and Twin Port Sampler

Featured Products

Description	Qty.	Cat. No
TDS^3 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
Gerstel 60 mm Tubes	1	28307-U
Teledyne/Tekmar® AEROTtrap 6000 Tubes	1	25099-U
Envirochem, 810 Tubes	1	25100-U
TDS^3 Storage Container Accessories		
Sampling caps w/washers for 1/4 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
Tubing Coupler		
For use with male to male Luer	20	25064-U

Related Products

Description	Qty.	Cat. No.
Equipment		
Escort Elf Sampling Pump	1	28160-U
Gemini Twin Port Sampler	1	28118-U
12 Volt Battery Charger	1	28155-U
110 Volt Battery Charger	1	28158-U
240 Volt Battery Charger	1	28159-U
Chemical Standards		
Chloroform	5 mL	02487
Carbon tetrachloride	1 mL	02671
1,2-Dichloroethane	1 mL	02562
Trichloroethylene	5 mL	46267
1,2-Dichloropropane	1 mL	02577
1,3-Dichloropropane	250 mg	45439
Tetrachloroethylene	1 mL	02666
Ethylene dibromide	1 g	31040
Chlorobenzene	5 mL	08650
Bromoform	1 g	36972
Bromobenzene	500 mg	442495

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 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**
Bar Code Labels	25 mg	**442441**
2-Butanone-2,4-DNPH	100 mg	**442339**
Formaldehyde-2,4-DNPH	100 mg	**442597**

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**

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*		

Sigma-Aldrich Contact Details

Austria
Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium
Free Tel: 0800 14747
Free Fax: 0800 14745
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Czech Republic
Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark
Tel: (+45) 43 56 59 00
Fax: (+45) 43 56 59 05

Finland
Tel: (+358) 9 350 9250
Fax: (+358) 9 350 92555

France
Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 88
Fax: (+33) 474 95 68 08

Germany
Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1160

Hungary
Ingyenes telefonszám: 06 80 355 355
Ingyenes fax szám: 06 80 344 344
Tel: (+36) 1 235 9063
Fax: (+36) 1 269 6470

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

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

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

Norway
Tel: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10

Poland
Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal
Free Tel: 800 202 180
Free Fax: 800 202 178
Tel: (+351) 21 924 2555
Fax: (+351) 21 924 2610

Russia
Tel: (+7) 495 621 5828
Fax: (+7) 495 621 5923

Slovakia
Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

South Africa
Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain
Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden
Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland
Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2828
Fax: (+41) 81 755 2815

United Kingdom
Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 1747 833 000
Fax: (+44) 1747 833 313

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sigma-aldrich.com

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World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(+1) 314-771-5765
sigma-aldrich.com

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