High Resolution LC-MS Profiling of Serum Lipids using Ascentis Express
Dear Colleague:

In the editorial in the last volume of The Reporter newsletter I told you of our vision of Supelco
serving the analytical world through our innovative products and technologies. In this space, I
want to introduce you to our strategic marketing team, each one of whom plays a special role
in carrying out this vision.

Roy Eksteen, Market Segment Manager, Bioanalytical - roy.eksteen@sial.com

Bioanalytical may have different meaning depending on whom you are
addressing. It can mean the analysis of compounds in biological matrices;
forensic, clinical, or metabolomic analyses, for example. It can also mean the
analysis of biomolecules; like the characterization of proteins, glycans, or
DNA. One of Roy’s roles is to apply his extensive separations experience to
define our message to each of these important, chromatography-intensive
application areas.

Shyam Verma, Market Segment Manager, Reagents - shyam.verma@sial.com;
and Vicki Yearick, Market Segment Manager, Standards - vicki.yearick@sial.com

I’m discussing Shyam and Vicki together for good reason: Reagents and standards are used nearly universally across
all market and separations segments. Shyam and Vicki work
closely with our Fluka colleagues and manage our high-
quality solvents & reagents, and standards, respectively,
to make our innovative separation products work that
much better!

Dan Vitkuske, Market Segment Manager, Sample Prep - daniel.vitkuske@sial.com

Our sample prep product line is large and extremely diverse, attributes that are
necessary because of the myriad types and complexity of samples, analytes,
matrices, and detection methods routinely encountered by our customers.
Dan manages the sample prep marketing group and product lines.
The diversity in sample prep applications is why Dan also has responsibility for
our market segmentation strategy and positioning all of our products, sample
collection, sample prep, separation, and other relevant products, into the
analytical workflow for these markets.

Wayne Way, Market Segment Manager, Chromatography - wayne.way@sial.com

Besides managing the HPLC and GC marketing group and product lines,
Wayne is responsible for strategic positioning our innovative products within
the Technology Adoption Life Cycle. To that end, Wayne works with innovators
and early adopters, those people who take new technologies for a scientific
test drive, and who are always looking for new and better ways of breaking the
analytical sound barrier, so to speak. If that describes you, please drop Wayne
an email!

I enjoy working with my strategic marketing team members who, along with our R&D group, are
committed to serving the analytical world with innovative products and application solutions.
Please let us know if we can help you solve any of your analytical challenges!

Paul Ross, Ph.D.
Director of Marketing & R&D
Analytical Separations
paul.ross@sial.com
Serum Lipidomics Profiling using an Ascentis Express Fused-Core C18 Column

**Contributed Article**

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

Susan S. Bird and Bruce S. Kristal
Department of Neurosurgery, Brigham and Women’s Hospital and, Department of Surgery, Harvard Medical School, 221 Longwood Avenue, LMRC-322, Boston, Massachusetts 02115
bkristal@partners.org

**Introduction**

Lipid diversity in a given biological system is quite great as there are both charged and neutral lipids in addition to species of varying degrees of chemical polarity. The LIPID Metabolites And Pathways Strategy (LIPID MAPS) consortium (www.lipidmaps.org) has systematically defined and classified lipids into eight categories, with multiple subclasses of each covering both eukaryotic and prokaryotic sources (Figure 1) (1). This standardization has assisted in the progress in developing analytical methods for untargeted lipidomic profiling studies where all lipids are of potential importance and therefore require sensitive and robust detection.

The current study uses a Fused-Core® C18 RP-LC column to achieve lipid separations in biological samples. The Fused-Core technology offers more robust particle size distribution, in comparison to traditional porous particles, that allow for sharper peaks by minimizing the eddy diffusion through the column. Additionally, this type of particle has a reduced diffusion path that provides sharper peaks at higher flow rates allowing for increased sample throughput (4).

Lipidomic analysis of serum samples from a rat diet study (n=192) were analyzed using RP-LC-MS in order to achieve comprehensive lipid coverage, relative quantitation of lipid species between diets and characterization of unknown lipids. The methods ability to reproducibly separate and detect 86 unique triglycerides in addition to lipids from the other 6 categories found in plasma will be highlighted.

**Methods**

**Animal Study**

Sets of male Fisher 344 x Brown Norway F1 (FBNF1) rats (n=8 per set), aged 7-9 weeks, were fed ad libitum one of 24 isocaloric diets that differed in fat and carbohydrate composition (total n=192).

**Lipid Extraction and LC-MS Analysis**

Lipid extracts were extracted from 30 µL of rat serum using a modified Bligh and Dyer liquid-liquid extraction and separated on an Ascentis® Express C18, 15 cm x 2.1 mm I.D., 2.7 µm column (Sigma-Aldrich, St. Louis, MO) connected to a Thermo Fisher Scientific PAL autosampler, Accela quaternary HPLC pump and an Exactive benchtop orbitrap mass spectrometer (Thermo Fisher, San Jose, CA) equipped with a heated electrospray ionization (HESI) probe. The HPLC was run at 260 µL/min and the column was held at 550 °C. Mobile phase A consisted of 60:40 acetonitrile:water in 10 mM ammonium formate and 0.1% formic acid and mobile phase B consisted of 90:10 isopropanol:acetonitrile also with 10 mM ammonium formate and 0.1% formic acid. Details of the LC-MS method and SIEVE analysis have been described previously (6, 7).

**Results**

**Lipid Class Separation**

Because many lipid species are isobaric, an understanding of the chromatographic retention time pattern can facilitate the identification and characterization of unknowns. The Ascentis Express reversed phase C18 column separated lipids by both head group polarity and acyl side chain composition in an efficient manner yielding sharp and robust peaks. **Figure 2** shows representative total ion current (TIC) chromatograms of a serum sample pooled from all 192 animals in our study. Sections of the chromatograms are labeled with the lipid categories detected, indicating the regions where each will elute using the LC-MS method. Highlighted in italics are some specific lipid classes (continued on page 4)
which are found from those categories that are observed in the serum pool samples. The diverse nature of the serum lipidome suggested that comprehensive LC-MS profiling methods utilize both positive and negative ionization modes.

**Figure 2.** Panels A and B show the total ion chromatogram (TIC) separation of the same serum pool sample using LC-MS performed in negative and positive ionization modes, respectively.

**Triacylglyceride Separation**

In the positive ion TIC (Figure 2) the majority of signal is due to the highly abundant serum triglycerides (TGs). There is a growing understanding that there are TG species-specific implications for health (8) therefore our priority was to identify all serum TGs found in our animal dietary macronutrient study. Identification was achieved by first analyzing a standard mixture of 6 TGs —TG (8:0)₃, TG (10:0)₃, TG (12:0)₃, TG (14:0)₃, TG (15:0)₃, and TG (16:0)₃—and determining the elution profile and retention time (RT) reproducibility of these species. From Figure 3, you can see that separation is based on acyl chain length. From repeat injections of these standards over 5 days we determined the RT coefficients of variation to be < 0.3%. This experiment allows us to recognize regions in the chromatogram where we can expect the TGs to be found, noting that the biologically relevant TGs will contain longer acyl side chains and be located between 22-25 minutes.

**Unknown Triacylglyceride Identification**

When analyzing our unknown rat serum data, exact mass measurement and retention time pairs from SIEVE that fell into the predetermined regions of the chromatogram were searched through online databases for possible TG hits. All ion fragmentation MS was then used to fully characterize these hits. Extracted ion chromatograms (XIC) of the fragment and parent masses were aligned to determine the FA side chains on each TG molecule.

**Figure 4** shows an example of this type of identification. A lipid with m/z 852.8015 found at RT 24.06 minutes, is found to match the mass of TG (50:0) as an [M+NH₄]⁺ ion when searching the human metabolome database and yields two possible TG isomers. In panels B and C, the diacylglycerol fragments chromatographically align with the parent ion to confirm the structure as TG (16:0/16:0/18:0). We present the specifics on the MS studies involved in the characterization of TGs, in a recent paper in Analytical Chemistry (7).

**Conclusion**

In a profiling LC-MS method, lipid separation is very important since it is natural for co-elution to occur when a large number of species elutes over a limited time period. In addition to identifying lipids from 6 major categories found in rat serum, this profiling method was able to identify 86 unique TGs with 62 having their individual side-chains characterized. This is a significant improvement over past methods where not only were far fewer TGs identified; they were merely characterized by their total number of carbons and double bonds. This enhancement is a reflection of the added chromatographic resolution observed with the Ascentis Express Fused-Core column and the high resolution accurate mass detection of our MS system.
Figure 4. The XIC of parent ion m/z 852.8015 is shown in panel A, with panels B and C showing XICs of the diacylglycerol fragments of the molecule. Chromatographic alignment of the parent ion peak at 24.05 minutes can be seen with fragments m/z 579 and m/z 551.

References


HPLC Method for the Separation on Nine Key Components of Milk Thistle

Dave Bell and Hugh Cramer
dave.bell@sial.com

Introduction

Silymarin, derived from the milk thistle plant, has been used as a natural remedy for the treatment of a number of liver diseases as well as in the protection of the liver from potential toxins. More recently, silymarin has been connected to antitumor promoting activity (1). It is therefore of interest to have available analytical methods for the analysis of silymarin components in various matrices.

The silymarin complex consists of several closely related, biologically active components. The objective of this study was to investigate several modern stationary phase chemistries for optimal selectivity toward the development of an efficient method for the analysis of silymarin. The method would optimally provide baseline resolution for nine of the known components of silymarin in less than 15 minutes.

Initial screening experiments were used to select a single combination of stationary phase and organic modifier that demonstrates the greatest potential for the separation. The information from subsequent optimization experiments was processed with simulation software to predict an optimized set of conditions. Finally the predicted conditions were confirmed using the analysis of an herbal supplement obtained from a local drug store.

Experimental

The screening protocol utilized five Ascentis Express stationary phases including C18, C8, RP-Amide, Phenyl-Hexyl and pentafluorophenylpropyl (F5). Gradient elution with two distinct organic modifiers was performed. Injections of a standard containing nine of the known constituents of silymarin (Figure 1) were made under each condition.

The HPLC screening method employed gradient elution from 5% - 95% of either methanol or acetonitrile. The aqueous component of the mobile phase was 0.1 % formic acid (pH 2.6).

In order to optimize the separation, separate chromatographic analyses at two different gradient slopes and two temperatures were performed. These four runs were then processed using ACD LC-Simulator software (Toronto, ON, Canada) and a predictive model was used to develop conditions that would provide baseline resolution of all peaks in less than 15 minutes. The conditions were then confirmed using both standards and a commercially available milk thistle herbal supplement.

Results and Discussion

Using a simple peak counting approach, the combination the C18 stationary phase along with methanol as the organic modifier resulted in the most visible peaks with nine. A representative chromatogram is shown in Figure 2. Other combinations of stationary phase and organic modifier produced between six and eight peak responses.

Four separate chromatographic runs using the C18 stationary phase and methanol modifier were conducted where the gradient slopes were varied (5% and 10% ramp) at two different temperatures (30 °C and 60 °C). The data was then analyzed using ACD LC-Simulator to predict the most suitable optimized conditions. Comparison to the experimental data shown in Figure 3 confirms good agreement between the predicted (not shown) and actual chromatography. Because the suggested conditions ran to just 45% organic, an additional step to 100% organic was included to elute potential hydrophobic components from a natural sample.
Figure 2. Initial Separation of Silymarin Components using C18 Stationary Phase and Methanol as the Organic Modifier

Figure 3. Experimental Confirmation of Predicted Optimized Conditions

- **Column**: Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particle size
- **Mobile Phase A**: Water with 0.1% formic acid
- **Mobile Phase B**: Methanol
- **Gradient**:
  - %A: 65 → 35
  - %B: 0 → 65
  - Min: 0 → 15

- **Flow Rate**: 0.6 mL/min
- **Temperature**: 35 °C
- **Detection**: UV, 254 nm
- **Injection**: 20 µL
- **Sample**: 9 milk thistle related 20 µg/µL in 86:14, water:methanol

Finally an herbal supplement labeled as containing milk thistle along with dandelion, fennel and licorice was extracted using water:ethanol 50:50, v/v and analyzed. As shown in Figure 4, all 9 of the targeted components could be observed in the herbal supplement material.

Conclusions

Stationary phase screening at the onset of method development, especially when dealing with a complex set of analytes, provides a facile means of analytical method development. As shown in the study, a few short experiments, coupled with powerful prediction software provided chromatographic conditions suitable for the analysis biologically active milk thistle components. The developed conditions should prove useful in natural supplement, raw material and/or biological monitoring of silymarin complex components.

Reference


+ Featured Products

<table>
<thead>
<tr>
<th>ID</th>
<th>Length (mm)</th>
<th>Length (cm)</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascentis Express C18 Columns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>2</td>
<td>53799-U</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>3</td>
<td>53802-U</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>5</td>
<td>53822-U</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>7.5</td>
<td>53804-U</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>10</td>
<td>53823-U</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>15</td>
<td>53825-U</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>3</td>
<td>53805-U</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>5</td>
<td>53811-U</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>7.5</td>
<td>53812-U</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>10</td>
<td>53814-U</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>15</td>
<td>53816-U</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>3</td>
<td>53818-U</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>5</td>
<td>53826-U</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>7.5</td>
<td>53819-U</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>10</td>
<td>53827-U</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>15</td>
<td>53829-U</td>
<td></td>
</tr>
</tbody>
</table>
Solvent impurities are the most common cause of extraneous peaks and unstable baseline. Background noise is typically caused by impurities that enter the system from the sample, by leaching from system components (bottles, tubes, valves, vials, etc.), and from solvents and additives. Solvent-derived impurities do not condition out over time and can interfere in the analysis in multiple ways, such as: a) collect on head of the column and elute as a distinct peak or as baseline rise, b) cause general elevation in baseline, lowering sensitivity of analysis, c) foul or damage sensitive instrument components and d) cause cluster ion formation that prevents reliable identification and quantification. For minimizing or eliminating these issues, sensitive tests like UHPLC, LC-MS and GC Headspace requires the use of highly pure solvents and additives.

Our Fluka brand has added another product line to the family of high-purity CHROMASOLV® solvents, blend and additives for sensitive UHPLC applications. This new product line is tested for UHPLC performance, including gradient applications, under both UV and MS detection. These solvents and reagents, designed for use in these sensitive analytical methods, as well as the sensitive GC-headspace analysis, are manufactured with utmost precision and are tested under strict quality control requirements.

**LC-MS Ultra CHROMASOLV Grade Solvents**

These solvents are tested for performance in UHPLC gradient separations, as well as UV, and positive and negative ion mode MS detection (Figure 1). Compared to standard LC-MS grade solvents, this new grade has significantly lower baseline and fewer minor peaks. In general, these solvents offer the following advantages:

- High purity for extremely low detection limits under any detection mode together with UHPLC
- Tested for UHPLC-MS
- Suitability tested by UHPLC-MS TOF
- High lot-to-lot reproducibility
- Reliable data, high performance and eliminate system downtime

**LC-MS CHROMASOLV Solvents**

The LC-MS CHROMASOLV solvents undergo 34 distinct and relevant tests to ensure solvent requirements of sensitive LC-MS analyses. Some of the most important features are:

- Application-tested for LC-MS using the reserpine tests (Figure 2)
- Very low level of inorganic and metal ions for high sensitivity spectra
- Particle/non-volatile compound-free (maintain system integrity)
- Low gradient baseline with your own optimized protocols
- Significantly reduced level of phthalate contaminants

**Pre-Blended LC-MS Solvents**

Our pre-blended solutions of the most commonly-used LC-MS mobile phases are prepared with precision and unsurpassed attention to quality. Using the precisely blended solvents eliminates time-consuming mobile phase preparation, and can eliminate lost sample information and instrument downtime caused by impure mobile phase. A special formulation assures that no precipitation or decomposition of the additive occurs under normal laboratory conditions. These high-quality pre-blended solvents offer:

- Time savings
- Accurate composition
- Minimized baseline and artifacts
LC-MS Mobile Phase Additives

Fluka brand offers a wide range of high-purity additives for LC-MS applications that are already tested for LC-MS application. These high-purity additives offer many advantages for both small and large molecule analysis.

- Acids, bases, volatile salts and sodium sources
- High purity and LC-MS application tested
- Improved ionization and resolution
- Sodium ion additives for uniform/stable molecular ions

GC-Headspace Solvents

An important application of GC-Headspace (GC-HS) is for the determination of residual volatile organic impurities (OVIs) in active drug substances or excipients in drug formulations. The allowable limits for these OVIs are listed by the United States Pharmacopeia (USP), European Pharmacopoeia (Ph.Eur.) and in the International Conference on Harmonization (ICH) guidelines.

In the GC-HS method, the composition and purity of the sample solvent have significant effects on the recovery and quality of the chromatogram. Sigma-Aldrich/Fluka developed high-purity solvents specifically for GC-HS applications (Figure 3). These solvents, microfiltered at 0.2 μm and packed under inert gas, offer the following benefits:

- High purity and longer shelf life
- Cleaner blanks and improved analyte recoveries
- No major interference peaks in elution range
- Specifications matching USP, Ph.Eur. & ICH guidelines

Fluka Brand headspace solvents are manufactured under strictly controlled processes, including micro-filtration and packaging under an inert atmosphere. This ensures their suitability for meeting the demands of headspace analysis.

**Featured Products**

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-MS Ultra CHROMASOLV Solvents and Additives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>≥ 99.9%*</td>
<td>1 L, 2 L</td>
<td>14261</td>
</tr>
<tr>
<td>Methanol</td>
<td>≥ 99.9%</td>
<td>1 L, 2 L</td>
<td>14262</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>1 L, 2 L</td>
<td>14263</td>
</tr>
<tr>
<td>Trifluoroacetic acid</td>
<td>≥ 99.0% **</td>
<td>1 mL, 2 mL</td>
<td>14264</td>
</tr>
<tr>
<td>Formic acid</td>
<td>≥ 98%**</td>
<td>1 mL, 2 mL</td>
<td>14265</td>
</tr>
<tr>
<td>Ammonium formate</td>
<td>**</td>
<td>25 g</td>
<td>14266</td>
</tr>
<tr>
<td>Ammonium acetate</td>
<td>**</td>
<td>25 g</td>
<td>14267</td>
</tr>
</tbody>
</table>

* tested for UHPLC-MS
** suitable for UHPLC-MS

**Description**

<table>
<thead>
<tr>
<th>LC-MS CHROMASOLV Solvents</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>39253</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>34967</td>
</tr>
<tr>
<td>Methanol</td>
<td>34966</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>34965</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>34972</td>
</tr>
</tbody>
</table>

**LC-MS CHROMASOLV Solvent Blends**

<table>
<thead>
<tr>
<th>LC-MS CHROMASOLV Mobile Phase Additives</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile with 0.1% TFA</td>
<td>34976</td>
</tr>
<tr>
<td>Methanol with 0.1% TFA</td>
<td>34974</td>
</tr>
<tr>
<td>Acetonitrile with 0.1% formic acid</td>
<td>34668</td>
</tr>
<tr>
<td>Acetonitrile with 0.1% ammonium acetate</td>
<td>34669</td>
</tr>
<tr>
<td>Acetonitrile with 0.1% formic acid and 0.01% TFA</td>
<td>34676</td>
</tr>
<tr>
<td>Water with 0.1% TFA</td>
<td>34978</td>
</tr>
</tbody>
</table>

**LC-MS CHROMASOLV Mobile Phase Additives**

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trifluoroacetic acid, puriss. p.a.</td>
<td>40967</td>
</tr>
<tr>
<td>Formic acid, puriss. p.a.</td>
<td>56302</td>
</tr>
<tr>
<td>Acetic acid, puriss. p.a.</td>
<td>49199</td>
</tr>
<tr>
<td>Ammonium formate, puriss. p.a.</td>
<td>55674</td>
</tr>
</tbody>
</table>

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

**Figure 3. Fluka’s High Purity Headspace Grade vs an Organic Synthetic Grade - Using GC-MS and SPME on DMSO**

The organic synthesis grade showed many peaks not detected in the GC-HS grade DMSO. The GC-HS grade produced a cleaner headspace blank and showed no major interference peaks in the elution range of the target analytes.
Increased Bioanalytical Throughput and Recovery Utilizing HybridSPE-PL Small Volume Plates

Craig Aurand
craig.aurand@sial.com

Often a major concern in developing bioanalytical methods is addressing the affect of biofluid matrix on the detection of desired analytes. The impact of matrix affects in bioanalysis has been well documented. In the majority of cases, co-extracted interferences directly affect the quantitation of analytes due to ionization effects induced by the extracted matrix. This extracted matrix can impact the chromatographic analysis, but more often results in a chromatographic build-up that leads to irregularities in both retention and quantitation. To address these issues, organic gradient elution is often utilized to ‘wash’ adsorbed contaminants from the column. In most cases gradient elution is not required for resolution of desired analytes, but instead required only to elute extracted matrix from the analytical column.

Performing a more thorough sample cleanup enables faster chromatographic analysis and thus increases the overall sample throughput. Using the HybridSPE-Phospholipid (PL) platform for selective phospholipid depletion eliminates the need for gradient elution of adsorbed matrix from the analytical column, resulting in the ability to perform isocratic chromatographic separation with a dramatic increase in throughput.

This study evaluates the performance of the newly developed HybridSPE-PL-Small Volume 96-well plate for preparation of small volumes of rat plasma. The HybridSPE-PL-Small Volume plate accommodates plasma volumes of 20-40 µL, ideally suited for bioanalytical testing of mouse plasma. It makes use of the zirconia-coated silica stationary phase as is also used in the standard HybridSPE-PL plate for phospholipid depletion. The HybridSPE-PL-Small Volume plate is a scaled down version with a 1 mL well volume and a 15 mg packed stationary bed. A 0.45 µm polishing filter is also used for fine particle removal. The narrow internal well diameter of the plate, along with small packed bed, results in minimal holdup volume (typically 20-40 µL). This enables sufficient volume recovery when handling small volume plasma samples.

In this study, rat plasma samples spiked with methadone and metabolites 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) were processed with the HybridSPE-PL-Small Volume 96-well plate and compared against standard protein precipitation methods. The analysis was conducted on an Agilent 1200SL Rapid Resolution system coupled to an Agilent 6210 TOF LC/MS. Chromatographic separation was performed on the Ascentis Express RP-Amide. The high sensitivity of methadone and metabolites enable for direct small volume injection of the processed sample without the need for evaporation or reconstitution.
Sample Handling

Each spiked level sample was prepared n=8 for both the HybridSPE-PL-Small Volume technique and the standard protein precipitation method. Samples processed using the HybridSPE-PL-Small Volume technique were collected directly into an Agilent low volume 96-well collection plate, average sample volume recovery from the plate was 40 µL. To ensure that sufficient sample was drawn into the injector, the autosampler was set for bottom well sensing. Samples were assayed for content of methadone and metabolites along with matrix monitoring for phospholipids. In this particular study, monitoring for 1-palmitoylglycerophosphatidylcholine, m/z 496.3375, was conducted as a representative phospholipids matrix ion.

Samples prepared using the HybridSPE-PL-Small Volume plate demonstrated high recovery across the concentration range. These samples were not affected by the matrix buildup due to the complete depletion of phospholipids. No signal suppression was observed using the HybridSPE-PL-Small Volume technique. As shown in Tables 1, 2, and 3 nearly equivalent calibration slopes between the standard solution and spiked plasma processed with the HybridSPE-PL-Small Volume technique was achieved. In Table 3, some drug protein binding was observed for the EDDP metabolite, resulting in the slight decrease in response. The recovery of the standard solution (red) was included in this chart to show the high recovery of EDDP from the HybridSPE-PL-Small Volume plate.

(continued on page 12)
Phospholipid matrix affect was evident with samples prepared using the standard protein precipitation technique. Often when performing ballistic gradient methods, the high organic content elutes a portion of the matrix from the column in a broad range. When performing isocratic methods, phospholipid buildup is continuous and results in an overall background increase, due to phospholipids gradually leaching from the column. As demonstrated in Figure 3, monitoring of m/z 496.3375 representing 1-palmitoylglycerophosphatidylcholine, an increase in background from none detected to over 3000 count was observed over the 40 sample injection range of the standard protein precipitation technique. The gradual increase in background phospholipids is the cause for the dramatic decreased signal response with increasing spike level samples. Samples were analyzed in order from lowest spike level to highest spike level. The highest level spiked samples were then subject to the highest amount of chromatographic buildup of phospholipid. Significant signal reduction was observed for the standard protein precipitation method due to the background phospholipids.

This study has demonstrated the detrimental effect of phospholipid buildup and resulting matrix ionization effect when performing standard protein precipitation techniques. By utilizing the HybridSPE-PL-Small Volume plate, excellent recovery of methadone and associated metabolites across the concentration range along with depletion of proteins and phospholipids from the plasma samples was achieved. The combination of facile protein precipitation/phospholipid depletion and fast analysis using modern chromatographic particles shows great promise in increasing the throughput for bioanalytical methods. The ability to perform selective matrix removal enables the use of optimized chromatographic elution conditions without the need for gradient elution of sample matrix, resulting in shorter run times and more rugged bioanalytical methods.

**Featured Products**

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HybridSPE-PL-Small Volume 96-well plate, 15 mg/well</td>
<td>S2794-U</td>
</tr>
<tr>
<td>96-Square/Deep Well Collection Plates, 1 mL, PP</td>
<td>S75652-U</td>
</tr>
<tr>
<td>IKA® MS 3 Digital Orbital Shaker</td>
<td>Z645036-1EA</td>
</tr>
</tbody>
</table>

**Related Product**

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm</td>
<td>S3913-U</td>
</tr>
<tr>
<td>HybridSPE-Phospholipid 96-well Plate, 50 mg/well</td>
<td>S75656-U</td>
</tr>
<tr>
<td>HybridSPE-Phospholipid Cartridge, 30 mg/1 mL</td>
<td>S5261-U</td>
</tr>
<tr>
<td>96-well Protein Precipitation Filter Plate</td>
<td>S5263-U</td>
</tr>
<tr>
<td>Supelco PlatePrep Vacuum Manifold</td>
<td>S7192-U</td>
</tr>
<tr>
<td>96-Square/Deep Well Collection Plates, 0.35 mL, PP</td>
<td>S75651-U</td>
</tr>
<tr>
<td>96-Square/Deep Well Collection Plates, 2 mL, PP</td>
<td>S75653-U</td>
</tr>
<tr>
<td>96-Square Well Pierceable Cap Mats</td>
<td>S75655-U</td>
</tr>
<tr>
<td>Methadone, 50 mg and 1 g</td>
<td>M0267</td>
</tr>
<tr>
<td>EDDP, 10 mg</td>
<td>E5264</td>
</tr>
</tbody>
</table>

**Related Information**

For more information on the HybridSPE-PL products, visit sigma-aldrich.com/hybridspe-pl

**Did you know ...**

Supelco’s Ascentis Express is a perfect complement to HybridSPE-PL sample prep cartridges and 96-well plates for bioanalytical LC/MS-MS assays. If you have an interest in these products, please complete the survey at sigma-aldrich.com/bioanalysis-request.
NEW! SLB-IL111 Column Literature
Two Technical Reports are available:

- SLB-IL111 for Petroleum Applications (T411138 ODY) describes the benefits of this column for measuring benzene and other aromatics in gasoline, and for determining the fatty acid methyl ester (FAME) profile in blended biodiesel.
- SLB-IL111 for Fatty Acid Methyl Ester (FAME) Applications (T411139 ODZ) contains 13 chromatograms, and describes the benefits of this column for detailed separations of cis/trans FAME isomers, and for edible oil analysis.

Request your copies by email (techservice@sial.com), or download at sigma-aldrich.com/il-gc

Special Limited-time Offer
40% OFF SLB-IL111 columns!
Valid until June 30, 2012.
To learn how, visit sigma-aldrich.com/gc-offers

New Syringe Selection Guide
Overwhelmed with syringe anxiety? No worries! The Syringe Selection Guide can help you decide upon the optimal syringe for your analytical needs.

- Includes a newly designed syringe selection decision tree
- Highlights a vast range of both manual and autosampler syringes
- Features leading brands: Hamilton®, SGE and Valco® VICI

To request a free copy of the Syringe Selection Guide, please visit sigma-aldrich.com/syringes
EN 14110 (Determination of Methanol Impurity in B100 Biodiesel) Using SPME on the Equity-1

Katherine K. Stenerson and Michael D. Buchanan
mike.buchanan@sial.com

Introduction
Biodiesel is a renewable, alternative diesel fuel produced from vegetable oils, animal fats, or recycled restaurant grease. This non-toxic, biodegradable liquid fuel consists of mono-alkyl esters of long chain fatty acids (also known as fatty acid methyl esters, or FAMEs) and may be used alone or blended with petroleum-based diesel fuels. The most common process for producing biodiesel involves two steps:

1. Through the transesterification reaction, triglycerides (i.e. oils or fats) are chemically reacted with an alcohol, usually methanol, in the presence of a catalyst, like sodium or potassium hydroxide, yielding fatty acid methyl esters (FAMEs) and glycerin by-product.
2. The FAMEs and glycerin by-product are then separated and purified. Biodiesel is the name given to the FAME fraction retained for use as fuel. The glycerin fraction is sold for use in soaps and other products.

The resulting biodiesel contains no sulfur or fossil fuel aromatics. Biodiesel is almost 10% oxygen, making it an oxygenated fuel, which aids combustion in fuel-rich circumstances. Biodiesel can be used pure (B100 biodiesel = 100% biomass-based diesel) or blended (for example, B20 biodiesel = 20% biomass-based diesel and 80% petroleum-based diesel).

Before being used or blended, B100 biodiesel must be tested for trace levels of contaminants that may cause problems in diesel engines. One of these contaminants is methanol. Because methanol is commonly used as the alcohol for the transesterification reaction during manufacturing, it may remain at residual levels in the final B100 biodiesel product. Too much methanol in a fuel will cause engine stress. Therefore, its level must be below set specifications for a fuel to be acceptable.

DIN EN 14110
European Standard Method DIN EN 14110 describes the headspace gas chromatography (GC) analysis of biodiesel for methanol, and is used to verify the residual methanol level is below set guidelines (1). A poly(dimethylsiloxane) phase is one of the column chemistries recommended in EN 14110 for the analysis methanol in biodiesel. This phase will elute analytes primarily according to boiling point, so under the proper analysis conditions, methanol and the internal standard 2-propanol should be resolved. Our Equity®-1 columns are made with poly(dimethylsiloxane) phase, and are available in a variety of dimensions to suit the needs of the application. For this analysis, a column with the dimensions described in the analytical conditions of the EN method was used.

Solid Phase Microextraction (SPME)
EN 14110 specifies the use of headspace analysis with a 45 minute sample equilibration time at 80 °C prior to GC analysis. In this study, solid phase microextraction (SPME) was evaluated as a possible alternative to headspace.

Linearity Evaluation
The three calibration standards described in EN 14110 were made and analyzed. Each standard consisted of a B100 biodiesel sample spiked with methanol plus 2-propanol (internal standard). Each standard was extracted using SPME and analyzed by GC on an Equity-1. An 85 µm polyacrylate fused silica fiber assembly was selected, as this phase possesses good affinity for polar analytes. A five minute extraction time at 60 °C was found to yield sufficient response for the lowest level calibration standard. The resulting chromatogram for the mid-level calibration standard is shown in Figure 1.

Figure 1. Spiked B100 Biodiesel Sample

| sample/matrix: | 2 g of B100 biodiesel containing 0.01% methanol and 0.0785% 2-propanol in a 4 mL vial |
| SPME fiber: | 85 µm polyacrylate (57304) |
| extraction: | headspace, 60 °C for 5 min. |
| desorption process: | 250 °C for 0.75 min. |
| column: | Equity-1, 30 m x 0.32 mm I.D., 1.0 µm (28057-U) |
| oven: | 50 °C |
| inj: | 250 °C |
| det: | FID, 200 °C |
| carrier gas: | helium, 30 cm/sec |
| liner: | 0.75 mm I.D., SPME type, straight design (unpacked) |

[1] sigma-aldrich.com/biofuels

Calibration factors were calculated per EN14110, and then the average, standard deviation, and %RSD of the calibration factors were determined. Per the method, the %RSD value must be <15 for the calibration to be acceptable. A 5 %RSD was obtained with the SPME calibration, showing that SPME is a viable extraction technique for this method. Complete calibration data is shown in Table 1.
Table 1. 3-Point Calibration Results

<table>
<thead>
<tr>
<th>Methanol (wt/wt %)</th>
<th>Methanol Area Counts</th>
<th>2-Propanol Area Counts*</th>
<th>Calibration Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>515</td>
<td>1947</td>
<td>0.4819</td>
</tr>
<tr>
<td>0.1</td>
<td>4718</td>
<td>1787</td>
<td>0.4826</td>
</tr>
<tr>
<td>0.5</td>
<td>28995</td>
<td>2410</td>
<td>0.5294</td>
</tr>
</tbody>
</table>

Average (Calibration Factor) = 0.4979
Standard Deviation (Calibration Factor) = 0.0273
%Relative Standard Deviation (Calibration Factor) = 5%
*Note: 2-propanol at 0.0785% in all standards

Conclusion

Whether headspace or SPME is used for the extraction of methanol from biodiesel, the Equity-1 column can be used in the subsequent GC analysis, as it provides sufficient resolution of the methanol and 2-propanol internal standard.

SPME required considerably less equilibration time than the headspace method (5 min. vs. 45 min.), and exhibited excellent sensitivity at the low end of the required calibration range. SPME also proved to be quantitative, as evidenced by the linearity evaluation done using the three standard levels described in EN 14110.

References

1. DIN EN 14110, “Fat and Oil Derivatives - Fatty Acid Methyl Esters (FAME) - Determination of Methanol Content”

Featured Products

Description | Cat. No.
--- | ---
SPME Fiber Assemblies, 85 µm Polyacrylate Fused Silica, 3 ea | 57304
85 µm polyacrylate fused silica, 24 ga for manual holder, 3 ea | 57330-U
SPME Fiber Holder for manual use, 1 ea | 28057-U
Equity-1 Capillary GC Column 30 m x 0.32 mm I.D., 1.0 µm, 1 ea | 2637505
Molded Thermogreen™ LB-2 Septa 11 mm diameter, 50 ea | 2637505
Inlet Liner 0.75 mm I.D., direct (SPME) type, straight design, for Agilent®, 5 ea | 2637505

Related Products

Description | Cat. No.
--- | ---
SPME Fiber Assemblies, 85 µm Polyacrylate Fused Silica, 3 ea | 57294-U
23 ga for Autosampler Holder and Merlin Microseal™ Systems | 57305
SPME Fiber Holders, 3 ea | 57331
For CTC CombiPAL, GERSTEL® MPS 2, and Thermo® TriPlus™ Autosamplers | 57347-U
For Varian® Autosamplers | 57331
Equity-1 Capillary GC Columns, 1 ea | 22582
30 m x 0.25 mm I.D., 0.25 µm | 28046-U
60 m x 0.25 mm I.D., 0.25 µm | 28047-U
60 m x 0.32 mm I.D., 1.00 µm | 28058-U
Merlin Microseal Systems (required a nut and a SPME septum) For Agilent (nut only) | 22582
For Varian 1079 Injector (1 nut, 1 inlet adapter, 1 o-ring, and 1 non-SPME septum) | 22609-U
For Varian CP-1177 Injector (1 nut, 1 inlet adapter, 1 o-ring, and 1 non-SPME septum) | 24818-U
Molded Thermogreen LB-2 GC Septa, 50 ea | 22609-U
9.5 mm diameter | 28331-U
10 mm diameter | 28333-U
11.5 mm diameter | 29446-U
17 mm diameter | 29452-U
Inlet Liners, 0.75 mm I.D., Direct (SPME) Type, Straight Design, 5 ea | 2637505
For Finnigan (9001GCQ) | 2637505
For PerkinElmer® (AutoSystem™) | 2631205
For Shimadzu® (9A, 15A, and 16, with SPL-G9/15 Injector) | 2632905
For Shimadzu (14, 15A, and 16, with SPL-14 Injector) | 2633505
For Shimadzu (17A, with SPL-17 Injector) | 2633905
For Thermo (ThermoQuest 8000 and TRACE®) | 2876605-U
For Varian (1075 and 1077 Injector) | 2635805
For Varian (1078 and 1079 Injector) | 2637805
For Varian (1093-94 SPI Injector) | 2636405
For Varian (CP-1177 Injector) | 2637505

Related Information

Additional information about this, or other analytical methodologies used for biofuel (biodiesel or bioethanol) is available at our web node: sigma-aldrich.com/biofuels

NEW! Handbook of SPME by Janusz Pawliszyn

This new 400-page book contains comprehensive descriptions of the fundamental principles of solid phase microextraction (SPME), recent applications, SPME devices and procedures published to date. SPME protocols are presented in a step-by-step fashion, providing useful tips and potential pitfalls. The important steps in SPME method development and optimization including calibration are clearly discussed to assist new users of the technology. This handbook enables researchers at all stages of their careers to effectively apply this convenient and solvent-free sample preparation technique to solve their analytical challenges in an effective way.

This up-to-date handbook contains 13 chapters with topics including: Theory of SPME, SPME devices and fiber coatings, commercial devices and coatings, automated SPME systems, calibration of extraction step, SPME method development, ligand-receptor binding, in-vivo SPME, review of different application areas including: environmental, food and fragrance, forensic and drug analysis, as well as SPME protocols.
US EPA Method 8141 (Organophosphorous Pesticides) on the SLB-5ms and SPB-608

Katherine K. Stenerson and Michael D. Buchanan
mike.buchanan@sial.com

Introduction
Several methods and multiple literature references exist for the analysis of pesticides and herbicides, as these compounds are widely used against specific animal, insect, or plant life. The most commonly used pesticides and herbicides contain chlorine substitution. Therefore, the use of an electron capture detector (ECD), a specialized detector for detecting chlorinated analytes, is employed during analysis. Non-chlorinated pesticides and herbicides, those which contain phosphorous or nitrogen substitution, are also used to deter the spread of specific biologics. Due to the different chemistry of this group, different methodology must be employed for their analysis.

Methodology
US EPA Method 8141 describes the analysis of a variety of organophosphorus pesticides and triazine herbicides (sometimes collectively referred to as OP-Pest) using gas chromatography (GC) following their extraction from solid waste or ground water samples (1). This method requires the use of either a nitrogen phosphorous detector (NPD) or a flame photometric detector (FPD), two specialized detectors that should be used in conjunction with stable, low bleed columns for maximum sensitivity.

This method also requires confirmatory analysis; the analysis of all standards, sample extracts, and QA/QC extracts on two columns, each with a different selectivity. If a peak is found within an analyte’s retention time window on the primary column, it must be “confirmed” on the confirmatory column in order to be considered positive. This technique reduces the occurrence of false positives when confirmation by mass spectrometry (MS) detection and spectral library matching is not feasible.

Chromatographic Results
Figure 1 shows the GC-NPD analysis of many of the target compounds listed in US EPA Method 8141 on a non-polar SLB™-5ms. No coelutions were noted under these run conditions, making this column a viable choice as the primary column for this method. The same mix analyzed on an intermediate polar SPB™-608 column is shown in Figure 2. The alternative selectivity of this column results in a different elution order, making this column a suitable choice as a confirmatory column for this method. Identical run conditions were used to generate both chromatograms, allowing a direct comparison of elution orders and retention times. This also allows both columns to be used in the same GC oven.

One Injection, Two Columns
Instead of separate injections on each column, it is possible to perform a single injection and split it to each column. This is only possible if the run conditions for each column are identical. Simply follow this procedure:
1. Connect a short guard column/retention gap from the injection port to a “Y” connector (the deactivation of the fused silica should match the polarity of the injection solvent)
2. Connect each column to the “Y” connector
3. Connect each column to its detector
4. Weekly, clip 4-6 inches off the front of the guard column/retention gap
5. When the guard column/retention gap becomes shorter than 9 inches in length:
   a. Replace the guard column/retention gap
   b. Replace the “Y” connector
   c. Clip 4-6 inches off the front of each analytical column

Applying a small drop of polyimide sealing resin on the outside of guard columns/retention gaps and analytical columns prior to inserting into “Y” GlasSeal™ connectors makes very durable permanent seals.

Conclusion
As shown here, an SLB-5ms/SPB-608 column set can be used to perform US EPA Method 8141, and similar methods, for the confirmatory analysis of organophosphorous pesticides. This is the same column combination recommended for the GC analysis of chlorinated pesticides (2).

References

Related Information
To view our full-line of GC columns for environmental applications, visit sigma-aldrich.com/gc-enviro
Figure 1. OP-Pest Standard on the SLB-5ms

column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
oven: 60 °C (1 min.), 5 °C/min. to 200 °C, 15 °C/min. to 300 °C (5 min.)
inj.: 20 µL, splitless (0.75 min.)
carrier gas: helium, 1.5 mL/min.
sample: 4 mm I.D., single taper

1. Dichlorvos
2. Hexamethyl phosphoramide (HMPA)
3. 1-Bromo-2-nitrobenzene (i.S.)
4. Mevinphos isomers
5. Trichlorfon
6. Tetraethyl pyrophosphate (TEPP)
7. Thionazin (Zinoprophos)
8. Demeton-O
9. Ethoprop
10. Tributyl phosphate (sur.)
11. Naled

Figure 2. OP-Pest Standard on the SPB-608

column: SPB-608, 30 m x 0.25 mm I.D., 0.25 µm (24103-U)

Did you know . . .
You can receive 40% off GC columns for environmental applications.
Visit sigma-aldrich.com/gc-offers to learn how.
Broader Selection of Quality Pesticide Standards Now Available from Sigma-Aldrich

Sigma-Aldrich now provides analysts with one of the broadest offerings of standards for pesticide analysis available. Recent additions to our portfolio include proficiency testing samples, NIST traceable certified reference materials and matrix standards for monitoring sample preparation methods. These products, along with our 2000 plus pesticide standards, allow Sigma-Aldrich to fulfill the needs of many laboratories, including those that are ISE/ISO 17025 accredited.

NEW! Certified Reference Materials (CRMs)

Our newly expanded pesticide standard line includes certified reference materials in the form of neats and matrix standards.

Fluka TraceCERT® pesticide CRMs are certified in a double accredited laboratory fulfilling ISO/IEC 17025 and ISO Guide 34 using high performance quantitative NMR (HP-qNMR®) for content determination with direct traceability to NIST and SI. This measurement technique for purity offers a superior level of accuracy, calculated uncertainties and lot-specific values when compared to other CRMs in the marketplace.

Sigma-Aldrich RTC pesticide CRMs are available as either "real world" soil or sediment natural matrices or natural matrices in which selected analytes have been fortified to give analytical profiles that meet the needs of the market. A robust statistical program is used in determining the certified values of these CRMs, utilizing data developed from multi-laboratory analysis supported by in-house data. Most CRMs are method specific and are supplied with certificates that show an uncertainty, expanded uncertainty confidence interval, and a prediction value, in addition to the mean value and standard deviation from the mean.

Neat and Single-Component Solutions

We stock more than 1800 isotope-labeled and non-isotope labeled neat and single component solutions for shipment every day, including Fluka brand PESTANAL® grade standards. PESTANAL grade products are high-purity standards of herbicides, pesticides and their metabolites. Most have a purity >99%. Each PESTANAL standard is formulated for single use to reduce waste and packaged in glass ampuls. A batch specific assay for each standard is provided on the product label, and details of this assay are found on the certificate of analysis accompanying each purchase.

Second Source Products

To complement our PESTANAL grade standards, we provide Chem Service brand pesticide and pesticide metabolite standards. Many other second source pesticide standards are available from within Sigma-Aldrich. It is our goal to provide these products to you in a timely manner, making it convenient to order all your chromatography supplies from Sigma-Aldrich. Standards are shipped with documentation and expiration dates.

Multi-Component Calibration Mixtures

Analysts performing multi-residue pesticide analyses can select from a variety of Supelco brand pesticide calibration mixtures for their application. These quantitative mixtures eliminate the time and money associated with sourcing individual raw materials, preparing the mix, and then disposing of unused hazardous materials.

Manufacturing and testing information is available for each Supelco brand calibration mixture in the form of a data packet. Each data packet documents the rigorous analytical methods used to verify raw material identity and purity, and provides verification of analyte purity and final concentration accuracy. Data packets are free of charge.

The composition of Sigma-Aldrich RTC Proficiency Testing Samples and Certified Reference matrix standards will vary from lot-to-lot. This is because these products are prepared from natural matrices; some are fortified.

NEW! Proficiency Test Samples

Through Sigma-Aldrich RTC, accredited ISO 17025 testing laboratories can now source certified soil, water and sludge proficiency test (PT) samples to evaluate their laboratory’s competency for performing pesticide analyses and calibration. Laboratories can choose to participate in our quarterly or semi-annual PT programs, or simply purchase PT samples.

The advantages of choosing Sigma-Aldrich RTC PTs include:

- Most PT samples are supplied in duplicate. The duplicate can be used to report additional methods or be retained as a QC standard, using the study values.
- Advanced tracking – methods, analysts, accreditation and equipment can be reported for the same analyte at no extra charge
- Evaluation reports can be sent to your Accreditation Authority (AA) for no additional charge.

Custom Standard Formulation Services

We recognize that a stock product may not always be appropriate for your applications. That’s why we have dedicated staff ready to formulate, test, and package pesticide calibration standards according to your exact specifications. Our chemists will gladly discuss stability and solubility concerns with you, and make suggestions where needed to improve the quality of your purchase. If you are interested in a customized pesticide standard, please feel free to contact us by email: customstandards@sial.com. Or, use the quote request form at our website sigma-aldrich.com/standards
**Certified Reference Materials (CRMs)**

**CRM matrix standards**
Ordering information for matrix standards varies by country.
Please visit sigma-aldrich.com/rtccrm for details or contact your local Sigma-Aldrich office.

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAHs, PCBs &amp; Pesticides on Fresh Water Sediment</td>
<td>50 g</td>
<td>CNS391</td>
</tr>
<tr>
<td>Pesticides - Loamy Sand 1</td>
<td>50 g</td>
<td>CRM846</td>
</tr>
<tr>
<td>PAHs, PCBs &amp; Pesticides on Sewage Sludge</td>
<td>50 g</td>
<td>CNS312</td>
</tr>
<tr>
<td>PCBs &amp; PDBEs on Fresh Water Sandy Loam Sediment</td>
<td>50 g</td>
<td>CNS329</td>
</tr>
<tr>
<td>Pesticides - Loamy Sand 1</td>
<td>50 g</td>
<td>CNS529</td>
</tr>
</tbody>
</table>

**CRM Neats**

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine, CRM, TraceCERT</td>
<td>50 mg</td>
<td>90935</td>
</tr>
<tr>
<td>Biphenyl, CRM, TraceCERT</td>
<td>100 mg</td>
<td>96996</td>
</tr>
<tr>
<td>Chlorpyrifos, CRM, TraceCERT</td>
<td>100 mg</td>
<td>94114</td>
</tr>
<tr>
<td>Cypermethrin, CRM, TraceCERT</td>
<td>50 mg</td>
<td>51991</td>
</tr>
<tr>
<td>Diazinon, CRM, TraceCERT</td>
<td>50 mg</td>
<td>68486</td>
</tr>
<tr>
<td>Deltamethrin, CRM, TraceCERT</td>
<td>50 mg</td>
<td>05995</td>
</tr>
<tr>
<td>Fipronil, CRM, TraceCERT</td>
<td>50 mg</td>
<td>16785</td>
</tr>
<tr>
<td>Malathion, CRM, TraceCERT</td>
<td>50 mg</td>
<td>91481</td>
</tr>
<tr>
<td>ProChlorox, CRM, TraceCERT</td>
<td>50 mg</td>
<td>64947</td>
</tr>
</tbody>
</table>

**NEATs (non-certified)**

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine, PESTANAL</td>
<td>250 mg</td>
<td>45330</td>
</tr>
<tr>
<td>Azadirachtin (technical mixture)</td>
<td>10 mg</td>
<td>PS2075</td>
</tr>
<tr>
<td>µ-Cyhalothrin PESTANAL</td>
<td>100 mg</td>
<td>31058</td>
</tr>
<tr>
<td>Cypermethrin (mixture of isomers), PESTANAL</td>
<td>100 mg</td>
<td>36128</td>
</tr>
<tr>
<td>o,p'-DDE</td>
<td>50 mg</td>
<td>PS695</td>
</tr>
<tr>
<td>Glufosinate ammonium, PESTANAL</td>
<td>100 mg</td>
<td>45520</td>
</tr>
<tr>
<td>Glyphosate, PESTANAL</td>
<td>250 mg</td>
<td>45521</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>100 mg</td>
<td>P578</td>
</tr>
<tr>
<td>Imidacloprid, PESTANAL</td>
<td>100 mg</td>
<td>31058</td>
</tr>
<tr>
<td>Oxymethon-methyl</td>
<td>50 mg</td>
<td>PS641</td>
</tr>
<tr>
<td>Paraquat dichloride, PESTANAL</td>
<td>100 mg</td>
<td>36541</td>
</tr>
<tr>
<td>Paraoxon-ethyl PESTANAL</td>
<td>100 mg</td>
<td>36186</td>
</tr>
<tr>
<td>Thiachlordiprid</td>
<td>100 mg</td>
<td>37905</td>
</tr>
</tbody>
</table>

**Mixtures**

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT – Endrin Mixture</td>
<td>1 mL</td>
<td>48282</td>
</tr>
<tr>
<td>Triazines Mixture</td>
<td>1 mL</td>
<td>48392</td>
</tr>
<tr>
<td>Ametryl</td>
<td>500 µg/mL each component in methanol</td>
<td>1 mL</td>
</tr>
<tr>
<td>Propetryl</td>
<td>4,4′-DDD</td>
<td>Endrin</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>β-HCH</td>
<td>4,4′-DDD</td>
</tr>
<tr>
<td>Atrazine</td>
<td>4,4′-DDE</td>
<td>Endrin ketone</td>
</tr>
<tr>
<td>Prometon</td>
<td>Lindane</td>
<td>Dieldrin</td>
</tr>
<tr>
<td>Paraoxon-ethyl PESTANAL</td>
<td>δ-BHC</td>
<td>α-Endosulfan</td>
</tr>
<tr>
<td>Paraoxon-ethyl PESTANAL</td>
<td>a-Chlordane</td>
<td>β-Endosulfan</td>
</tr>
<tr>
<td>Paraoxon-ethyl PESTANAL</td>
<td>γ-Chlordane</td>
<td>Endosulfan sulfate</td>
</tr>
</tbody>
</table>

**Proficiency Testing (PT) Samples**

Ordering information for PT products varies by country.
Please visit sigma-aldrich.com/rtccrm for details or contact your local Sigma-Aldrich office.

**Water Pollution**

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphorus Pesticides – WP</td>
<td>1 x 2 mL</td>
<td>PE1161</td>
</tr>
<tr>
<td>Organophosphorus Pesticides (Low Level) - WP</td>
<td>1 x 2 mL</td>
<td>PE1377</td>
</tr>
<tr>
<td>Pesticides 1-WP</td>
<td>1 x 2 mL</td>
<td>PE1280</td>
</tr>
<tr>
<td>Pesticides 2-WP</td>
<td>1 x 2 mL</td>
<td>PE1201</td>
</tr>
</tbody>
</table>

**Water Supply**

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamate Pesticides – WS</td>
<td>1 x 2 mL</td>
<td>PE1507</td>
</tr>
<tr>
<td>Organonitrogen Pesticides – WS</td>
<td>1 x 2 mL</td>
<td>PE1400</td>
</tr>
</tbody>
</table>

**Soil**

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated Pesticides in Soil</td>
<td>50 g</td>
<td>SPE009</td>
</tr>
<tr>
<td>Organophosphorus Pesticides</td>
<td>50 g</td>
<td>SPE021</td>
</tr>
</tbody>
</table>