

# Reporter

Volume 28.3



## SPME-LC Fiber Probes



*SPME-LC Fiber Probes for in vivo plant and animal sampling can be used to identify pollutants directly from flowers.*

Liquid Chromatography

Sample Handling

Gas Chromatography

Standards

Accessories

Chiral Chromatography

# Reporter

Volume 28.3

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## The 20th Anniversary of SPME



Daniel Vitkuske  
Market Segment Manager

Dear Colleague:

20 years ago, SPME emerged as an innovative, solvent-free sample prep technique for measuring volatile and semi-volatile compounds in a fast and economical manner. SPME was invented by Dr. Janusz Pawliszyn at the University of Waterloo and first commercialized by Supelco. Since then it has become a mainstream sample prep technique ideal for difficult sample matrices. Today there are over 3500 application references to SPME and it is used in a broad range of applications in pharmaceutical, environmental, food, flavor and fragrance, forensics, clinical and academic research laboratories. Customer acceptance of SPME has expanded rapidly with support through partnerships with instrumentation and automation suppliers such as GERSTEL®, CTC Analytics, LEAP Technologies, and Varian®.

Since the early days, SPME has predominantly been a sample prep technique used with GC analysis. This is primarily due to the fact that the available SPME fiber coating materials were not compatible with common LC solvents. As we look to the future of SPME we are expanding into new areas of bioanalysis and *in vivo* sampling using LC for the final sample analysis. With new biocompatible fiber coatings such as C18 bonded silica in new low cost fiber probes, it's now possible to move into novel areas such as *in vivo* animal and plant sampling where single use fibers are desired or even required. This also opens up the possibility of using SPME for pharmacokinetic studies in small animals to minimize sample requirements and improve the data quality by using fewer animals.

We look forward to the next 20 years of SPME and the many new technologies jointly developed with Dr. Pawliszyn and the University of Waterloo; as well as new applications developed by researchers who are excited about the important advantages of SPME such as ease of use, sensitivity, and robustness in both *in vivo*, field and laboratory sampling conditions.

Visit [sigma-aldrich.com/spme](http://sigma-aldrich.com/spme) to learn more about SPME.

Kind regards,

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# Development of C18 SPME Fiber Probes for LC Applications\*

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## Product Description

Traditional solid phase microextraction (SPME) fibers were developed with common GC type phases which were primarily designed for extraction of volatile or semi-volatile compounds using thermal desorption as the means of introducing sample into the chromatographic column. In this article, the development of a new SPME fiber coating is being introduced specifically for extraction of polar and non-polar analytes using solvent desorption for LC applications. The newly developed fiber probes contain C18 silica particles embedded in a proprietary, non-swelling, biocompatible polymer. The benefit of this design enables minimized binding of macromolecules such as proteins and phospholipids, but allows extraction of most smaller analytes of interest.

The fiber core is made from a flexible metal alloy with shape memory properties and a diameter larger than typical SPME fibers. The larger diameter enables the fiber to be used both as the plunger and fiber core, thus simplifying the device construction and reducing the cost of the probe. The fiber is also sealed into a hypodermic needle with an attached hub to allow movement of the fiber for exposure and retraction into the needle. **Figure 1** shows the configuration of the fiber design. This design enables the fiber assembly to be pierced into a catheter shunt, vial septa or the fiber can be exposed for direct immersion into a liquid sample.

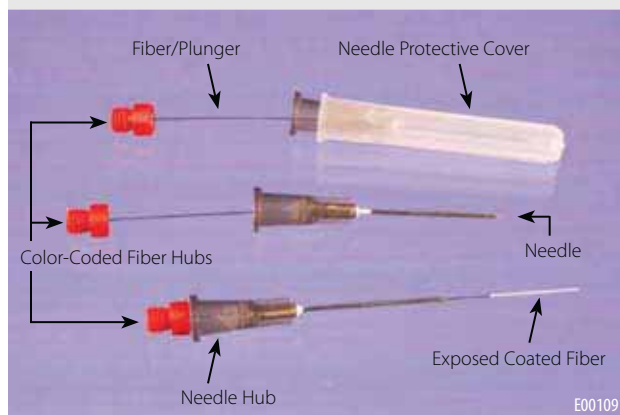
## Extraction of Drugs From Plasma

To demonstrate the properties of the LC fiber assembly, extraction studies were conducted using propranolol and the 4-hydroxy metabolite (4-HP) in both buffered water and plasma. Extraction fibers were then desorbed with solvent and analyzed by LC-MS-MS. The extraction and chromatographic conditions are detailed in **Table 1**.

Because SPME is primarily concentration dependent and not volume dependent, only small sample volumes are necessary to perform the extractions. In this study, both 100  $\mu$ L and 500  $\mu$ L sample volumes were evaluated with only minimal difference in analyte response. This ability to perform extractions on such a small sample size greatly reduces the need for larger sample volumes to be collected for analysis.

Because the new SPME fiber coating is designed to be biocompatible, the fiber can be inserted directly into plasma or other biological fluids without the need for performing protein precipitation or other tedious sample prep steps. In this study, only the pH of the plasma was adjusted to increase the extraction efficiency. **Figure 2** (pg. 4) depicts the response for propranolol and the 4-hydroxy metabolite obtained for the extraction from buffer and plasma.

**Figure 1. C18-SPME Fiber Probes**



**Table 1. Conditions for Extraction and Analysis of Propranol and Hydroxypropranol (4-HP)**

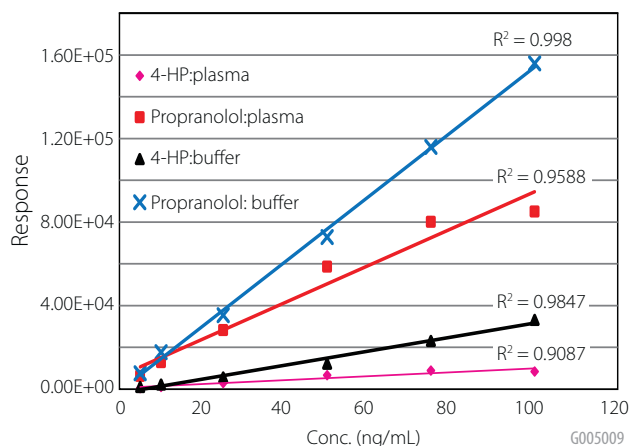
|                     |  |
|---------------------|--|
| Sample:             | 500 $\mu$ L and 100 $\mu$ L, spiked phosphate buffer, and rat plasma adjusted to pH 4.0 with 25% $H_3PO_4$ |
| Fiber Conditioning: | 15 min. in methanol, followed by 15 min. in water  |
| Fiber Coating:      | C18 (57281-U)  |
| Extraction:         | 10 min., static  |
| Desorption:         | 30 min. in 100 $\mu$ L 13 mM $NH_4OAc$ in 90:10 ACN:H <sub>2</sub> O                                       |
| Column:             | Discovery HS F5, 5 cm x 2.1 mm, 3 $\mu$ m  |
| Mobile Phase:       | 2 mM ammonium formate in 90:10 acetonitrile:water  |
| Flow:               | 200 $\mu$ L/min.   |
| Temperature:        | 35 $^{\circ}C$   |
| Injection Volume:   | 5.0 $\mu$ L  |
| Source Conditions:  | Turbo ion spray ESI +, MRM   |
| Q1 Mass (amu):      | Propranolol:260.21,<br>4-hydroxypropranolol:276.21   |
| Q3 Mass (amu):      | Propranolol:183.00,<br>4-hydroxypropranolol:173.10   |
| Dwell Time:         | 150 msec   |

The results show that the analytes can be extracted out of a small volume of either buffer or plasma with good linearity at low concentration levels. The lower response for the 4-hydroxy metabolite is primarily associated with a lower ionization efficiency than the parent drug under the LC-MS conditions. Also, the recovery of both analytes is lower from plasma when compared to buffer. This is primarily due to drug-protein binding in the plasma. It was shown that it could take multiple hours for

(continued on page 4)

\* This product and/or its uses may be covered by one or more of U.S. Patent Nos. 5,691,206, 7,232,689, 7,259,019, 7,384,794 and corresponding foreign patents.

**Figure 2. Linearity of Extractions of Propranolol and 4-HP from 100  $\mu$ L Samples**



(continued from page 3)

the binding equilibrium between the drugs and protein to be met. If the extraction occurs immediately after spiking the drugs into the plasma, the difference in recovery between buffer and plasma is much smaller. Better linearity is obtained if full protein binding equilibrium is obtained.

### Advantage of Biocompatibility

Phospholipids (PL) are large molecules that can interfere with the analysis of drugs by suppressing ionization in LC-MS electrospray ionization. The most common sample prep technique in bioanalysis is protein precipitation using an organic solvent to crash the proteins from the plasma sample. After filtration or centrifugation, the resulting supernatant is analyzed directly. Using this technique, PL are co-extracted with the analytes causing ion-suppression of the analytes resulting in irregular quantitation. To demonstrate the biocompatible properties of the C18 fibers, LC-MS-MS comparison of samples prepared using protein precipitation versus samples extracted using the C18 fibers were conducted. Comparisons were based upon total analyte response and phospholipid content. **Figure 3** depicts the MS-MS chromatograms for phospholipids along with propranolol and 4-hydroxymetabolite.

The results show the SPME fiber coating minimized the extraction of phospholipids as compared to the protein precipitation technique. The response of propranolol and the 4-hydroxy metabolite from the 10-minute SPME extraction was comparable to the response from direct injection following protein precipitation. This demonstrates that the large molecules are not retained by the fiber and do not inhibit the extraction of the analytes of interest.

### In-Vivo SPME Using Fiber Probes

Biocompatibility of the SPME assembly not only refers to the fiber coating resisting macromolecules, but it also refers to the materials used in the coating and used to make the fiber probe. All of the materials in the fiber probe are inert and approved for medical use. Because of the biocompatibility of the probes, it is possible to use them for *in-vivo* animal studies.

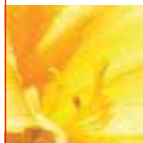
NoAb Biodiscoveries in Mississauga, ON Canada in conjunction with the University of Waterloo in Waterloo, ON have developed a method utilizing SPME in pharmacokinetic (PK) studies. In typical PK studies, a drug is administered to multiple mice or other animals. At various time intervals, blood is removed from the animal and the amount of the parent drug in the blood is measured at specific time intervals. Usually there are 6-7 time intervals in a study, ranging from time 0 minutes to 18 hours. The problem encountered is that usually 1 mL of plasma must be obtained for the SPE extraction or precipitated with acetonitrile followed by direct injection. To obtain 1 mL of plasma requires that approximately 1.5 mL of blood be removed from the mouse. In many cases, this amount of blood loss is detrimental to a mouse and may result in death. So in a typical PK study, 18-21 mice are sacrificed since there are triplicate reps at each time point. This can be extremely costly, especially if transgenic mice are used in the study. Also, the metabolism varies between mice that affects the breakdown of the drug and results in variability in the data.

PK studies utilizing SPME consist of an arterial catheter connected to a specially designed shunt device inserted into the carotid artery or jugular vein of mice or rats. This design enables the blood to pass through a port in the shunt. The SPME fiber probe is inserted into the port, the fiber exposed and the blood is pumped over the fiber at a specific rate for a given time period between 1-2 min. After extraction, the fibers are then rinsed in water and stored in a freezer for analysis at a later time.

In a preliminary study conducted by the University of Waterloo and NoAb, a comparison was made between terminal blood draw followed by extraction using SPE, to *in vivo* extraction using SPME with the shunt device. A 2 mg/Kg dose of carbamazepine (CBZ) was given to the mice and the level of the drug in the blood stream was monitored for 18 hr. In this study triplicate reps were made at each time point. This results in 3 mice being used for the SPME study and 18 mice being sacrificed in the terminal blood draw study, significantly reducing the number of animals harmed. **Figure 4** compares the results of the average response for CBZ at each time point between *in vivo* SPME and the terminal blood draw/SPE method.

The results show that there is good correlation for the response of CBZ in the whole blood between *in vivo* SPME testing and blood drawn followed by SPE testing. In addition to the benefits of *in vivo* SPME, some others were observed. One benefit is that SPME only measures the free form of the drug, the active form. Also, it was observed that when using *in vivo* SPME the metabolite of CBZ was detected. The metabolite was not observed when using SPE.

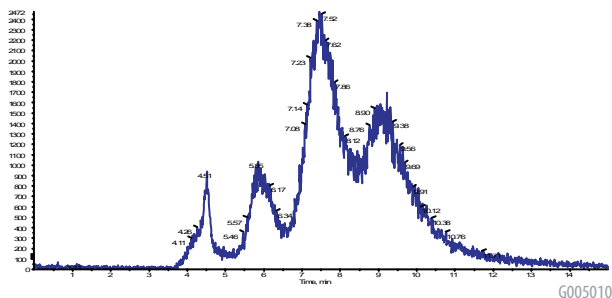
Validation studies are ongoing at NoAb Biodiscoveries to determine if *in vivo* SPME can be used as an alternative to terminal blood draw. Once these studies are completed *in vivo* SPME could be used for drug discovery studies.



**Figure 3. LC-MS Analysis of Drugs in Plasma: Comparison of SPME Extraction to Direct Injection on the Matrix Background and Detection of the Drugs**

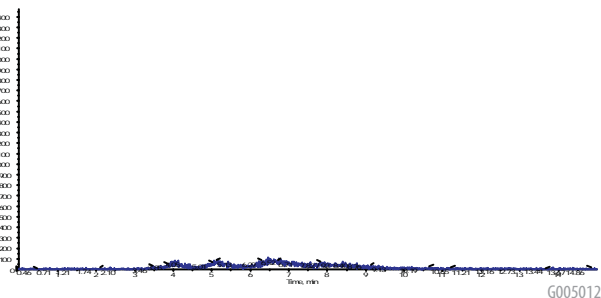
**Direct Injection After Protein Precipitation**

MRM 184/104 for Phospholipids

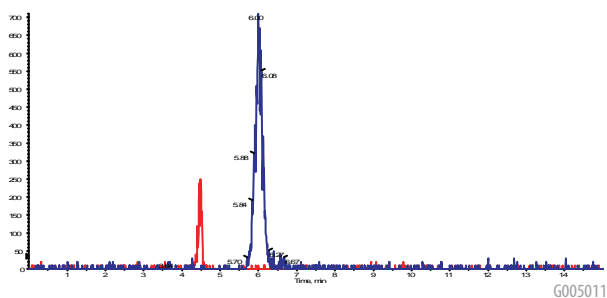


**SPME Extraction**

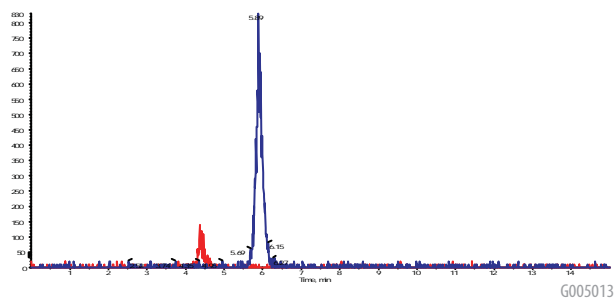
MRM 184/104 for Phospholipids



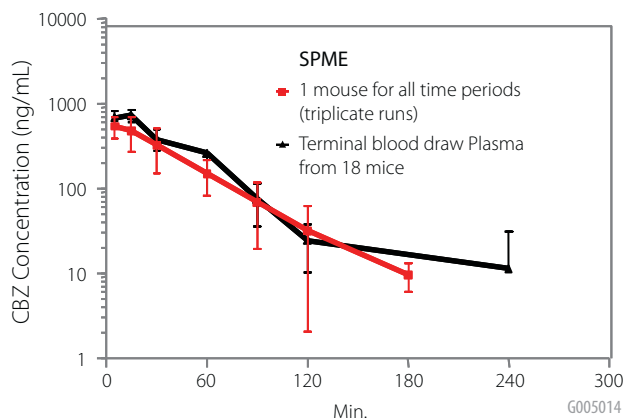
MRM 260/183.0 for Propranolol 276.21/173.1 for 4-HP



MRM 260/183.0 for Propranolol 276.21/173.1 for 4-HP



**Figure 4. Comparison of SPME *in vivo* Pharmacokinetics (PK) Study of Carbamazepine (CBZ) from Mice Whole Blood to Extracts of Plasma Removed from Mice**



Graph Courtesy of Ines de Lannoy-NoAb BioDiscoveries.

**Summary**

In this report the utility and application of newly developed C18 Silica Fiber Probes has been demonstrated. The goal was to offer an SPME fiber device specifically designed for solvent desorption instead of thermal desorption. This enables new possibilities for microextraction, where traditional SPME is not capable. These options could include monitoring of pesticides in plants, the uptake of contaminants in animal tissues and organs and other applications that simply require extraction out of a vial followed by solvent desorption and LC analysis.

**Reference**

1. Vuckovic, Dajana; Shirey, Robert; Chen, Yong; Sidisky, Len; Aurand, Craig; Stenerson, Katherine; Pawliszyn, Janusz. In vitro evaluation of new biocompatible coatings for solid-phase microextraction: Implications for drug analysis and in vivo sampling applications. *Analytica Chimica Acta* (2009), 638(2), 175-185.

**Acknowledgements**

1. Ines DeLannoy and Brad Gien - NoAb BioDiscoveries, Inc, Mississauga, ON L5N 8G4 Canada
2. Dajana Vuckovic and Janusz Pawliszyn -Department of Chemistry, University of Waterloo, Waterloo, ON N2L3G1 Canada

**+ Featured Product**

| Cat. No. | Description                       |
|----------|-----------------------------------|
| 57281-U  | SPME-LC C18 Fiber Probes, pk of 5 |

# Enrichment of Phospholipids in Biological Samples Using HybridSPE-PPT

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## Introduction

Phospholipids (PL) are one of the major building blocks of cell membranes and are important for signal transduction and many other biologic activities. Phospholipids are abundant in biological fluids with concentrations in human plasma as high as 1 mg/mL. The profiling of phospholipids in tissue and blood is significant in both functional and pathological studies.

HybridSPE-PPT has previously been used on biological samples, such as serum and plasma, to remove phospholipid interferences prior to LC-MS analysis. We found, however, that phospholipids retained on the sorbent could be easily recovered with a strong basic solution, such as ammonium hydroxide. As such, HybridSPE-PPT can also be used to enrich phospholipids for analysis and profiling. The interaction between HybridSPE-PPT and phospholipids is based on Lewis acid-base chemistry and has been thoroughly discussed in a previous Reporter, volume 26.3. This article will focus on the use of HybridSPE-PPT for phospholipid enrichment.

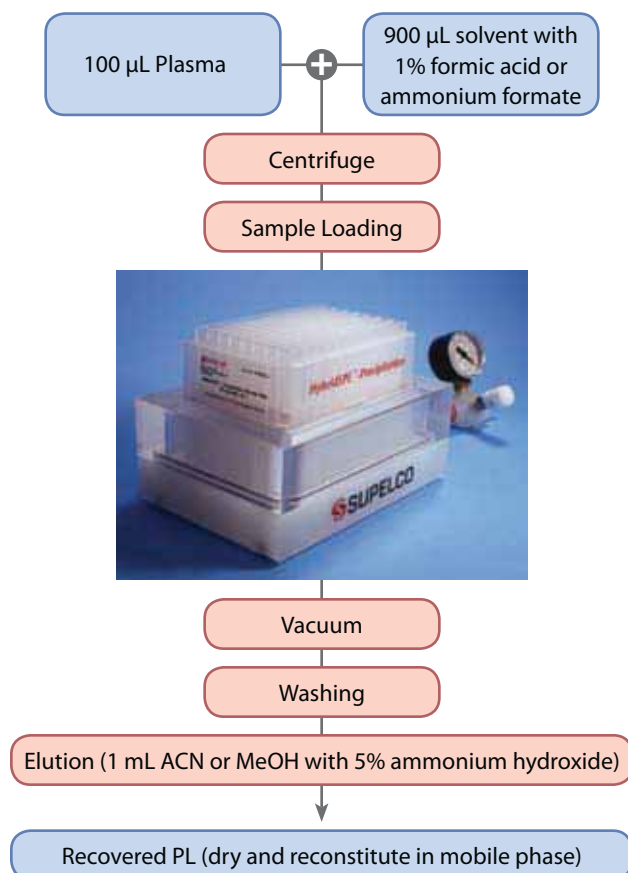
## Experimental

A 100  $\mu$ L aliquot of rabbit plasma was mixed with 900  $\mu$ L of acetonitrile with 1% formic acid in a 1.5 mL micro-centrifuge tube and vortexed for 30 seconds, followed by centrifugation at 5000  $\times$  g for 3 minutes. The resulting supernatant was transferred to a HybridSPE-PPT 96-well plate, and applied to vacuum at 10 in. Hg for 4 minutes. The flow-through was collected for LC-MS analysis. The sorbent was then washed with 1 mL of acetonitrile with 1% formic acid, and 1 mL of acetonitrile. The phospholipids retained on the HybridSPE-PPT sorbent were eluted with two consecutive 1 mL aliquots of acetonitrile with 5% ammonium hydroxide. The effluent was dried down with nitrogen and reconstituted in 50% acetonitrile with 0.1% formic acid.

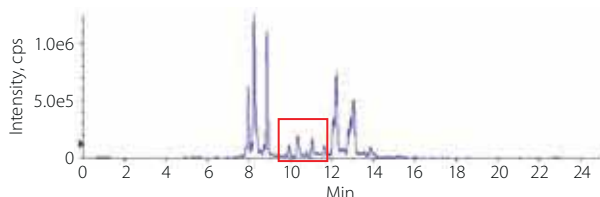
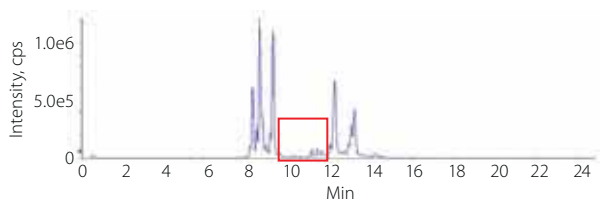
*HybridSPE-PPT can also be used to enrich phospholipids for analysis and profiling.*

The sample was also prepared with a similar protocol using methanol as the main reagent. A 100  $\mu$ L aliquot of plasma was mixed with 900  $\mu$ L of methanol with 1% formic acid. The sorbent was washed with 1 mL of methanol with 1% ammonium formate, followed by 1 mL of methanol. The phospholipids were eluted with two 1 mL aliquots of methanol with 5% ammonium hydroxide (See Figure 1 for flow chart).

**Figure 1. Experimental Flow Chart of Recovery of Phospholipids from Rabbit Plasma**



LC-MS was carried out on an Applied Biosystems QTrap 3200 mass spectrometer coupled with an Agilent 1100 HPLC. A 3  $\mu$ L sample was injected on an Ascentis® Express RP-Amide column (5 cm  $\times$  2.1 mm  $\times$  2.7  $\mu$ m, 53911-U). The HPLC was operated in linear gradient with mobile phases A (water with 0.1% formic acid) and B (methanol:acetonitrile, 1:1/0.1% formic acid) from 50%-100% B in 10 min. The phospholipids were monitored at MRM184 and 104 after in-source fragmentation. The mass spectrometric parameters were set as follows: CUR (35), IS (5000), TEM (350), GS1 (30), GS2 (30), ihe (ON), CAD (10), and CXP (4).

**Figure 2. Profile of Phospholipids in Rabbit Plasma Before Enrichment****Figure 3. Profile of Phospholipids in Rabbit Plasma After Enrichment**

## Results and Discussion

The Lewis acid-base interaction between HybridSPE-PPT and phospholipids can only be disrupted with strong basic solution, such as ammonium hydroxide. Since there are high amounts of proteins in biological samples, the proteins are crashed out with organic solvents, e.g. acetonitrile with 1% formic acid or methanol with ammonium formate before enrichment. This step can be done off-line if using a cartridge, or directly in the 96-well plate. Eluted phospholipids are monitored by the mass spectrometer at product ion 184 and 104 generated by in-source fragmentation as described by Little et al (1). We observed good separation of phospholipids on a polar-embedded RP-Amide column. It should be noted that Little's method only monitors phosphatidylcholines and lyso-phosphatidylcholines. We believe the same enrichment method would be applicable to other phospholipids in biological fluids since all phospholipids share the phosphate group, which interacts with the HybridSPE-PPT sorbent.

Figures 2 and 3 present the LC-MS profiles of phospholipids in rabbit plasma before and after enrichment. There are two major groups of peaks on the chromatograms. The early eluted group with retention time from 8.0 minutes to 9.0 min is comprised of mostly single-chained phospholipids (lyso-phosphatidylcholines), meaning there is only one fatty acid ester in the molecule. The later group with retention times greater than 12 minutes is comprised of mostly double-chained phospholipids, meaning there are two fatty acid esters in the molecule.

**Table 1. Recovery of Phospholipids from HybridSPE-PPT 96-well Plate at Each Stage of the Described Protocol**

| Stage       | Acetonitrile | Methanol |
|-------------|--------------|----------|
| 1st Elution | 91.5%        | 93.2%    |
| 2nd Elution | 4.0%         | 2.9%     |
| Loading     | 3.3%         | 1.9%     |
| Washing     | 1.3%         | 1.9%     |

Eluted with 5% Ammonium Hydroxide in Specified Solvent.

The recovery of phospholipids is over 95% using either acetonitrile or methanol as an elution solvent. We use 1% formic acid or ammonium formate to assist in the precipitation of proteins in the plasma sample. The wash step was used to clean any soluble proteins and endogenous substances in the sample. In either method, the total loss of phospholipids is less than 5% (Table 1).

## Conclusions

A simple method has been developed to enrich phospholipids from plasma samples. The method involves a HybridSPE-PPT 96-well plate that both retains phospholipids and removes precipitated proteins. The interaction between the HybridSPE-PPT sorbent and phospholipids is based on Lewis acid-base chemistry, and can be disrupted with a strong Lewis base, such as ammonium hydroxide. The recovery of phospholipids using this method is greater than 95%. The phospholipids can be profiled on a polar embedded reversed phase HPLC column and a triple Q mass spectrometer.

For more information on HybridSPE and available configurations, visit [sigma-aldrich.com/hybridspe-ppt](http://sigma-aldrich.com/hybridspe-ppt)

## Reference

1. J. Chromatogr. B 833, 219–230, 2006

## + Featured Products

| Cat. No. | Description   |
|----------|---|
| 575656-U | HybridSPE-PPT 96-well Plate, 50 mg/well                 |
| 53911-U  | Ascentis Express RP-Amide<br>5 cm x 2.1 mm I.D., 2.7 µm |

## Did you know...?

Archived issues of *Reporter*, including volume 26.3 referenced in this article, are available at [sigma-aldrich.com/thereporter](http://sigma-aldrich.com/thereporter)

TRADEMARKS: Ascentis, CHROMASOLV, Discovery, ENVI-Carb, HybridSPE, SLB, SPB, Supelclean, Supelco, Supel, Sylon, Thermogreen – Sigma-Aldrich Biotechnology LP; CD – QIS, Inc.; Fused-Core – Advanced Materials Technology, Inc.; GERSTEL – Gerstel; PEEK, PEEKsil – IDEX Health & Science, Inc.; Pico Pure Plus+ – AR Corporation; Varian – Varian, Inc.

# ENVI-Carb Plus for the Extraction of Propylene Glycol and Ethylene Glycol from Water

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## Introduction

Propylene glycol has a wide variety of applications, including uses as a solvent, emulsifier, and moisturizer in various chemical, food, and personal care products. Its toxicity to both humans and animals is relatively low. Ethylene glycol, which is widely used as automotive antifreeze, is moderately toxic and was included on the Drinking Water Contaminant Candidate List 3 Draft by EPA in 2008 (1). Due to the hydrophilic nature of ethylene and propylene glycol, traditional methods of extraction such as liquid/liquid and solid phase extraction (SPE) with most typical sorbents, cannot extract these compounds from aqueous samples. As a result, the standard method of analysis in water samples has been direct aqueous injection into a gas chromatograph (GC). This method, however, has numerous problems, including high detection levels, carryover, and chromatographic issues. For this reason, it would be advantageous to extract these glycols into an organic solvent for GC analysis. However, as stated previously, extracting them from an aqueous matrix is difficult. Since ethylene and propylene glycol are extremely polar, reversed phase and normal phase sorbents such as C18 and silica gel cannot retain them from water. Carbon sorbents, depending on their surface and structure, can retain analytes based on hydrophobicity and molecular size and shape. These unique characteristics give carbons the potential for use as sorbents to extract small glycols from water.

ENVI-Carb™ Plus is a microporous amorphous carbon molecular sieve. Its surface is less hydrophobic than other types of carbons, which gives it a higher affinity for water, and helps to draw analytes from aqueous solution into its pore structure. Elution of analytes is achieved by flooding the pores with a solvent in which the analyte is soluble. The efficiency of extraction is increased by the material's inert surface and minimal interstitial space (space between the individual particles). ENVI-Carb Plus was designed with a narrow particle size distribution that reduces interstitial space, thus allowing elution solvents to more thoroughly solvate the carbon's pore structure.

ENVI-Carb Plus was developed for the extraction of highly polar compounds from water, and has been found to work well for acephate, phenol, acrylamide, and 1,4-dioxane. In the case of 1,4-dioxane, it has been validated for use in US EPA Method 522, which details the extraction and analysis of this compound in drinking water (2). Considering the retention characteristics of ENVI-Carb Plus, it was considered to have potential for extracting glycols from water. In this study, we evaluated the use of this carbon for the extraction of ethylene and propylene glycol from water. The goal was to determine if the glycols could be retained from water and eluted with an organic solvent, thus allowing for easier GC analysis and subsequent sample concentration and solvent exchange if desired. Using the protocol described, ENVI-Carb Plus was found to retain both glycols from water, and

exhibited good recovery of propylene glycol from water and fair recovery of ethylene glycol.

## Experimental

Samples of deionized water were spiked at varying levels with propylene and ethylene glycols. Extraction was done with ENVI-Carb Plus reversible (these cartridges are fitted with female Luer inlets) cartridges using the protocol described in **Table 1**. Prior to the elution step, the cartridge was reversed. Just enough elution solvent was then drawn through the cartridge to wet the packing, and the vacuum was turned off and the cartridge was allowed to soak for 1 minute. The vacuum was then turned back on, and the remaining elution solvent was drawn through the cartridge and collected. GC analysis (**Table 2**) was performed directly on the extracts without further concentration or solvent exchange.

**Table 1. Extraction Procedure**

|               |   |
|---------------|---|
| Cartridge:    | ENVI-Carb Plus Reversible Tube, 400 mg, 1 mL, (54812-U)   |
| Conditioning: | Stepwise: 1 mL methylene chloride, 2 x 2 mL methanol, 3 mL deionized water (do not allow cartridge to go dry after first aliquot of methanol) |
| Sample:       | 5 mL water sample spiked with ethylene and propylene glycol   |
| Drying:       | 10 minutes  |
| Elution:      | 5 mL of 50:50 methanol:methylene chloride   |

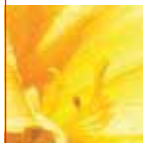
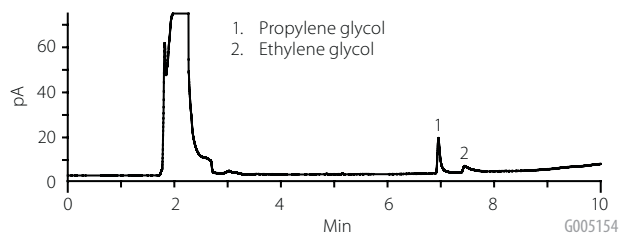
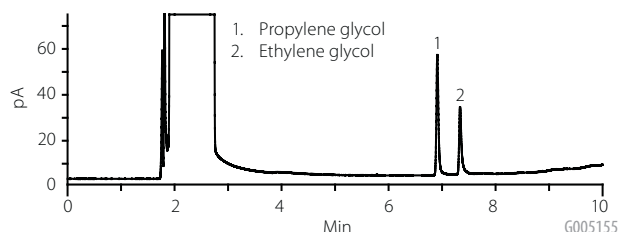
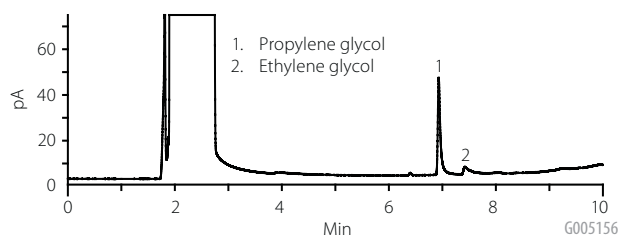
**Table 2. GC Analysis Conditions**

|              |  |
|--------------|--|
| Column:      | SPB™-1000, 30 m x 0.53 mm I.D., 1.5 µm (25445) |
| Oven:        | 100 °C (1 min.), 10 °C/min. to 200 °C          |
| Inj:         | 250 °C   |
| Det:         | FID, 220 °C                                    |
| Carrier Gas: | helium, 35 cm/sec constant                     |
| Injection:   | 1 µL, splitless                                |

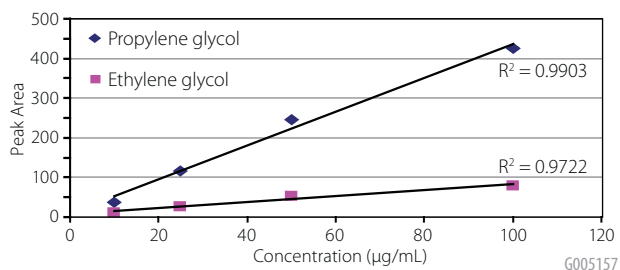
## Results and Discussion

As stated previously, aqueous injections are problematic in GC. **Figure 1** shows the result of an injection of a water sample containing 25 µg/mL of propylene and ethylene glycol. Water forms an extremely large vapor cloud in a heated GC inlet, and has a high boiling point compared to other solvents. As a result, sample focusing becomes difficult, which in turn affects peak shape and response. By comparison, if the glycols are injected in the organic solvent mixture used for elution of the ENVI-Carb Plus cartridges, as shown in **Figure 2**, peak shape and response are improved. For this reason, it was determined that GC analysis would be suitable directly after the elution step, and that no solvent exchange step was necessary.



**Figure 1. Direct Aqueous Injection of 25 µg/mL Spiked Water Sample****Figure 2. Injection of 25 µg/mL Glycol Standard in 50:50 Methanol:Methylene Chloride****Figure 3. Injection of 25 µg/mL Water Sample Extracted Using ENVI-Carb Plus****Table 3. Reproducibility and Recovery from Spiked Deionized Water, Using ENVI-Carb Plus**

| 25 µg/mL Spike | Propylene Glycol | Ethylene Glycol |
|----------------|------------------|-----------------|
| Avg. Recovery  | 97%              | 25%             |
| % RSD, n=3     | 4%               | 5%              |

**Figure 4. Concentration vs. Response of Spiked Water Samples Extracted Using ENVI-Carb Plus**

Propylene glycol retained well on ENVI-Carb Plus, while ethylene glycol, being smaller and more hydrophilic, did not retain as well. Replicate water samples spiked at 25 µg/mL were extracted and compared to the same concentration standard in elution solvent. As shown in **Table 3**, reproducibility was very good overall. Recovery of propylene glycol was significantly better than the more hydrophilic ethylene glycol. A chromatogram of an extracted spiked water sample is presented in **Figure 3**. The response and peak shape of propylene glycol was significantly improved over direct aqueous injection. The ethylene glycol, due to low recovery and response, did not show as dramatic an improvement.

The quantitative performance of the method was evaluated by determining the linearity of extracted water samples spiked at 10 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL. A plot of concentration vs. response is presented in **Figure 4**. Linearity was good for both glycols, and % RSD for the average response factors were 11% and 16% for propylene and ethylene glycol respectively.

## Conclusions

ENVI-Carb Plus reversible cartridges were able to extract both propylene and ethylene glycol from water, and the method demonstrated to be both quantitative and reproducible. Elution was achieved with a combination of organic solvents, offering further options for GC analysis such as sample concentration or solvent exchange. Direct injection of the elution solvent offered an improvement in response and peak shape over direct injection of an aqueous sample.

The extraction protocol shown here appears to be optimized for propylene glycol, as this compound exhibited a significantly better retention on ENVI-Carb Plus than ethylene glycol. Further investigation will be done to see if an alternative protocol can improve retention of ethylene glycol.

## References

- Environmental Protection Agency Drinking Water Contaminant Candidate List 3 – Draft Notice, Federal Register, Vol. 73, No. 35, Thursday, February 21, 2003.
- Munch, J, Grimmert, P, Determination of 1,4-Dioxane in Drinking Water by Solid Phase Extraction (SPE) and Gas Chromatography/Mass Spectrometry (GC/MS) With Selected Ion Monitoring (SIM). Part II US EPA Method 522-1 Sep. 2008.
- Betz, W.R., Keeler, M.J., Sarker, M., Aurand, C.R., Stenerson, K.K., Sidisky, L.M. Characterization of Polymer Carbon Sieves, Graphitized Polymer Carbons and Graphitized Carbon Blacks for Sample Preparation Applications; T408117, Sigma-Aldrich/Supelco.

## + Featured Products

| Cat. No.       | Description   |
|----------------|---|
| <b>54812-U</b> | Supelclean™ ENVI-Carb Plus Reversible Tube<br>400 mg, 1 mL, pk. of 30 |
| <b>25445</b>   | SPB-1000<br>30 m x 0.53 mm I.D., 1.5 µm                               |

## + Related Products

| Cat. No.     | Description      | Pk. Size    |
|--------------|------------------|-------------|
| <b>12279</b> | Propylene glycol | 1 mL, 5 mL  |
| <b>85978</b> | Ethylene glycol  | 5 mL, 10 mL |

# Melamine and Related Compounds in Dog Food Using GC-MS

Katherine K. Stenerson and Michael D. Buchanan

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## Introduction

Melamine contamination in food became an issue in recent years after the discovery of it and related compounds in pet food and baby formula. It was discovered that melamine was intentionally added to inflate nitrogen content, often the sole measure of the amount of protein in these products. The tainted food led to numerous illnesses, several fatalities, and massive product recalls. Currently, imported raw materials, namely wheat gluten and rice protein used to make these foods, as well as the actual consumer-ready foods, may undergo testing to ensure the absence of these compounds. We have detailed preparation and analytical procedures for these adulterants using HPLC-MS-MS instrumentation in previous publications (1,2). In this article, we focus on the analysis of melamine and related compounds with the use of more economical gas chromatography-mass spectrometry (GC-MS) instrumentation.

## GC-MS Method

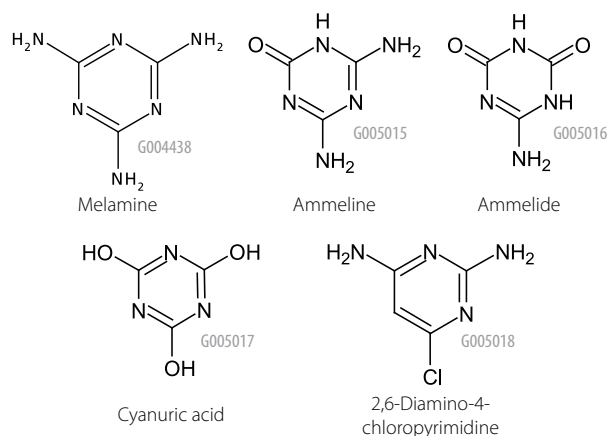
The United States Food and Drug Administration (US FDA) adopted a screening method in October 2008 for the GC-MS analysis of melamine and related compounds in a variety of matrices (3). Per the method, 0.5 g of the sample is mixed thoroughly with 20 mL of an extraction solvent mixture (10:40:50 diethylamine:water:acetonitrile). Following sonication (30 minutes) and centrifugation (10 minutes), an aliquot is filtered and evaporated to dryness. Sylon BFT and pyridine are then added along with an internal standard. The extract is then incubated (70 °C for 45 minutes) so that trimethylsilyl (TMS) derivatives of each analyte are formed. The resulting derivatized extract is then analyzed by GC-MS. The method allows the operation of the MS in the scan mode ( $m/z$  from 50-450 amu) or the selected ion monitoring (SIM) mode. **Table 1** shows the structures of the four analytes (melamine, ammeline, ammelide, and cyanuric acid) plus 2,6-diamino-4-chloropyrimidine, the internal standard (I.S.) specified by the method.

## Experimental

For this work, we choose a common dry dog food obtained from a local grocery store. The following samples were prepared:

1. Three calibration standards, each containing all four analytes, were made at levels of 10 ng/mL, 50 ng/mL, and 100 ng/mL (I.S. added at 1000 ng/mL in each), derivatized, and then used to perform a three-point calibration of the instrument.
2. A laboratory blank was extracted (I.S. added at 1000 ng/mL), derivatized, and then analyzed to show cleanliness.

**Table 1. Melamine and Related Compounds**



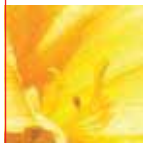
3. A 0.5 g dog food sample was extracted (I.S. added at 1000 ng/mL), derivatized, and then analyzed to determine analyte levels.
4. A second 0.5 g dog food sample (spiked with each analyte at 10 µg/g) was extracted (I.S. added at 1000 ng/mL), derivatized, and then analyzed to determine method sensitivity and accuracy.

All standards and extracts were analyzed with the MS operating in the scan mode, and again later with the MS operating in the SIM mode.

## Results

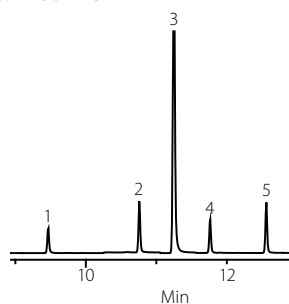
The following results were observed:

- **MS Mode:** Sensitivity was noticeably better when the MS was operated in the SIM mode. All chromatograms shown are from SIM mode analyses.
- **Instrument Calibration:** The 100 ng/mL standard is shown in **Figure 1**. Note the symmetrical peak shape for each analyte and the I.S., achieved because the activity of amide functional groups was minimized when TMS derivatives were formed, and also due to the inert nature of the capillary GC column.
- **Laboratory Blank (Figure 2):** Trace levels of each of the four target analytes were detected in the laboratory blank only when the MS was operated in the SIM mode.
- **Dog Food Sample (Figure 3):** The detection of analytes was at a level consistent with that observed in the laboratory blank. We concluded that this dog food was not contaminated with any of the target analytes.
- **Spiked Dog Food Sample (Figure 4):** The percent recoveries of each of the four target analytes are summarized in **Table 2**. Good recovery was obtained for each analyte.

**Figure 1. 100 ng/mL Calibration Standard (SIM Mode)**

column: SLB™-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)  
 oven: 115 °C (3 min.), 10 °C/min. to 325 °C (6 min.)  
 inj.: 250 °C  
 MSD interface: 325 °C  
 scan range: SIM  
 carrier gas: helium, 1 mL/min., constant  
 injection: 1 µL, splitless  
 liner: 4 mm I.D. single taper  
 sample: 100 ng/mL standard containing each analyte at 100 ng/mL,  
 then I.S. added at 1000 ng/mL, in pyridine:Sylon™ BFT to  
 form TMS derivatives

1. Cyanuric acid
2. Ammelide
3. 2,6-Diamino-4-chloropyrimidine (I.S.)
4. Ammeline
5. Melamine

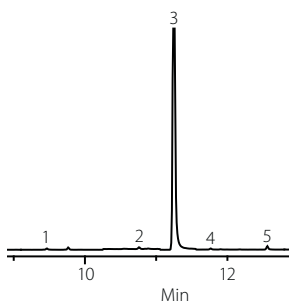


G005019

**Figure 2. Laboratory Blank (SIM Mode)**

sample: extract of a laboratory blank, then I.S. added at 1000 ng/mL,  
 in pyridine:Sylon BFT to form TMS derivatives

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

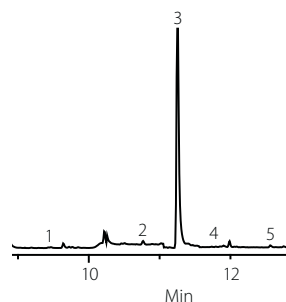


G005020

**Figure 3. Dog Food Sample (SIM Mode)**

sample: extract of dog food, then I.S. added at 1000 ng/mL,  
 in pyridine:Sylon BFT to form TMS derivatives

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

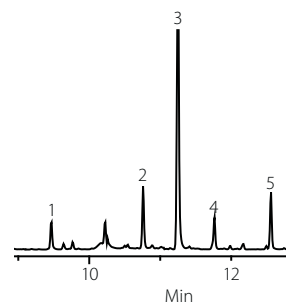


G005021

**Figure 4. Spiked Dog Food Sample (SIM Mode)**

sample: dog food spiked with each analyte at 10 µg/g, then  
 extracted, then I.S. added at 1000 ng/mL, in pyridine:  
 Sylon BFT to form TMS derivatives

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



G005022

**Table 2. Recovery from Dog Food Spiked at 10 µg/g**

| Analyte       | % Recovery |
|---------------|------------|
| Melamine      | 73         |
| Ammeline      | 77         |
| Ammelide      | 105        |
| Cyanuric acid | 97         |

## Conclusion

Our observation is that the method is very easy to perform and provides good sensitivity. In particular, the use of the specified extraction solvent mixture was found to be very effective in solubilizing and extracting all target analytes. Additionally, the formation of TMS derivatives allows these analytes to be analyzed by GC, with symmetrical peak shapes, high signal-to-noise ratios, and low detection levels.

(continued on page 12)

(continued from page 11)

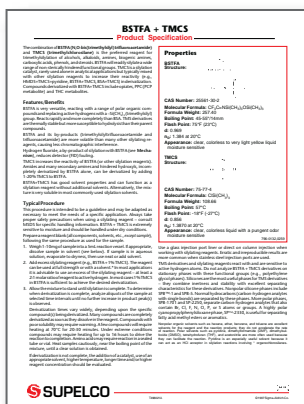
## References

1. O. Shimelis and C. Santasania, "Analysis of Melamine in Milk" Analytix, 2009, Issue 1, lit. code T409021 LCX, pages 6-7.
2. O. Shimelis, C. Santasania, and A. Trinh, "The Extraction and Analysis of Melamine in Milk-Based Products using Discovery DSC-SCX SPE and Ascentis Express HILIC LC-MS/MS" Sigma-Aldrich Technical Report T408188 LEN, 2008.
3. J. J. Litzau, G. E. Mercer, and K. J. Mulligan, "GC-MS Screen for the Presence of Melamine, Ammeline, Ammelide, and Cyanuric Acid" US FDA Laboratory Information Bulletin, LIB No. 4423, Volume 24, October 2008.

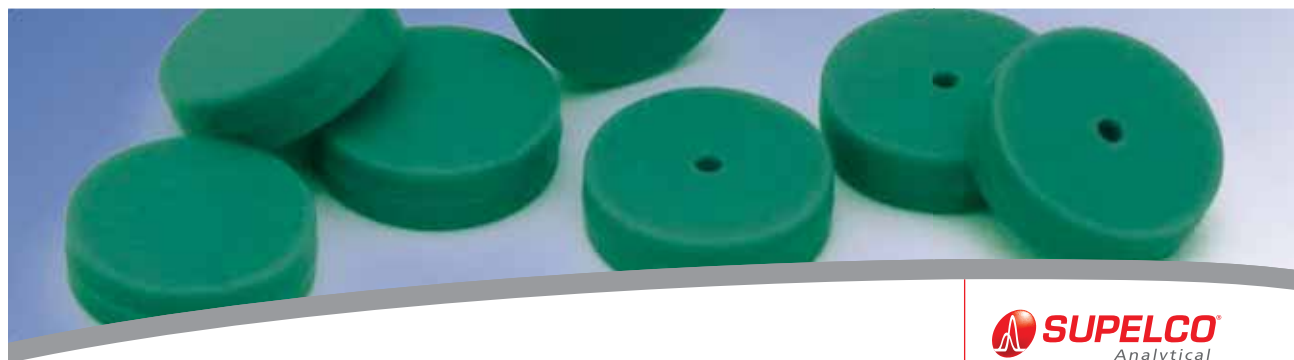
 Related Information

The BSTFA+TMCS (Sylon BFT) Product Information Sheet (T496021, AWK), describing the use of this derivatization reagent, can be obtained at [sigma-aldrich.com/derivatization](http://sigma-aldrich.com/derivatization)

Detailed information concerning the many features and benefits of the SLB-5ms line of capillary GC columns can be found at [sigma-aldrich.com/slb](http://sigma-aldrich.com/slb)


 Featured Products

| Cat. No.                                    | Description  |
|---|--|
| <b>Solvents</b>                             |  |
| <b>471216</b>                               | Diethylamine [109-89-7], >99.5%                        |
| <b>360570</b>                               | Pyridine [110-86-1], ACS reagent, >99.0%               |
| <b>34481</b>                                | Acetonitrile [75-05-8], PESTANAL, for residue analysis |
| <b>0.45 µm Nylon Filters</b>                |  |
| <b>Z290815-100EA</b>                        | 25 mm diameter, pk of 100                              |
| <b>Z290793-100EA</b>                        | 47 mm diameter, pk of 100                              |
| <b>Z290785-25EA</b>                         | 90 mm diameter, pk of 25                               |
| <b>BSTFA+TMCS, 99:1 (Sylon BFT) Reagent</b> |  |
| <b>33148</b>                                | 20 x 1 mL ampuls                                       |
| <b>33155-U</b>                              | 25 mL bottle   |
| <b>Calibration Standards</b>                |  |
| <b>52549-250MG</b>                          | Melamine [108-78-1], >99.0%, 250 mg                    |
| <b>45613-250MG</b>                          | Ammeline [645-92-1], PESTANAL, 250 mg                  |
| <b>16614-250MG</b>                          | Cyanuric acid [108-80-5], >98.0%, 250 mg               |
| <b>C33204-5G</b>                            | 2,6-Diamino-4-chloropyrimidine [156-83-2], 98%, 5 g    |
| <b>Capillary GC Column</b>                  |  |
| <b>28471-U</b>                              | SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm                  |



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Hillel Brandes  
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Discovery BIO PolyMA polymer-based ion-exchange particles have discriminating hydrophilic surface chemistry making them ideally suited for separating proteins, peptides, and other biomolecules. Two ion-exchangers, Discovery BIO PolyMA-SCX for cation-exchange, and Discovery BIO PolyMA-WAX for anion-exchange, complement the Discovery BIO and Ascentis Express Peptide silica-based materials.

The unique hydrophilic surface chemistry of Discovery PolyMA particles offers subtle ionic selectivity characteristics that are not available from the typical polystyrene-divinylbenzene (PS-DVB) and standard polymethacrylate based ion-exchange resins. In contrast to silica-based packings, Discovery BIO PolyMA is resistant to chemical degradation at acidic and basic pH extremes. Significant Discovery BIO PolyMA features and benefits include:

- Excellent separations of peptides and proteins, even protein isoforms
- Optimal pore diameter allows full access to small peptides and very large proteins and protein aggregates
- High resolution at low sample load
- Hydrophilic surface eliminates protein adsorption for quantitative recovery
- High efficiency
- Wide pH range
- Excellent balance of protein capacity and recovery
- Long column lifetime
- High column reproducibility

For most applications, Discovery BIO PolyMA columns provide higher efficiency (**Figure 1**) and better recovery (**Table 1**) than competitive columns.

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**Table 1. Comparison of Fibrinogen Recovery**

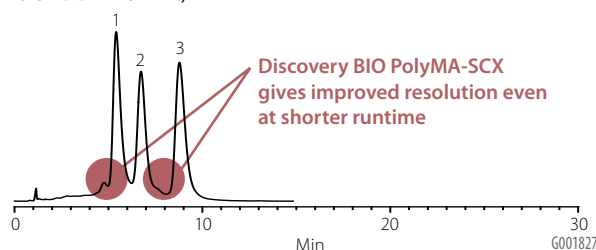
| Column  | 1 <sup>st</sup> Inj. | 2 <sup>nd</sup> Inj. | 5 <sup>th</sup> Inj. |
|---|----------------------|----------------------|----------------------|
| Discovery BIO PolyMA-WAX (5 cm x 4.6 mm, 5 µm particles)            | 88.9%                | 89.9%                | 92.9%                |
| Competitive polymethacrylic DEAE (7.5 cm x 7.5 mm, 10 µm particles) | 59.1%                | 75.7%                | 82.1%                |

Mobile Phase: Gradient of 0 – 0.5M NaCl in 20 mM Tris-HCl (pH 8) over 30 min., Flow: 1 mL/min., Ambient Temperature, Sample: 40 µg fibrinogen (F-4753) in 20 µL starting mobile phase

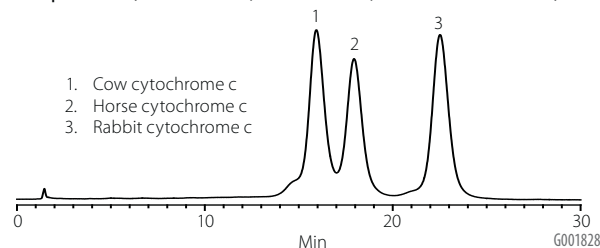
**Figure 1. Comparison of Efficiency: Cytochrome c Variants**

columns: Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm I.D., 5 µm particles (59601-U) & competitive polymeric-SCX columns of similar dimensions  
mobile phase A: 50 mM MOPS/KOH, pH 7.0  
mobile phase B: 50 mM MOPS/KOH, 0.5 M KCl, pH 7.0  
linear velocity: 3.01 cm/min. (flow rates appear in Figure)  
temp.: 35 °C  
det.: UV, 280 nm  
sample: 10 µg each variant  
gradient profile: 0.6% B per minute. See Figure for details.

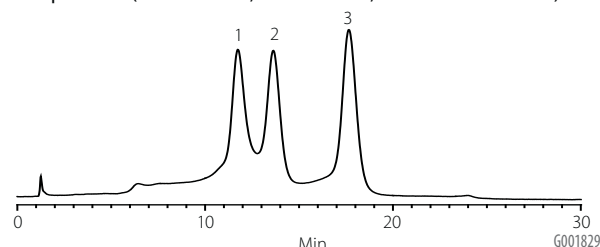
**Discovery BIO PolyMA-SCX (5 cm x 4.6 mm, 0.5 mL/min, 28-37 %B in 15 min.)**



**Competitor 1 (5 cm x 5 mm, 0.59 mL/min, 28-46 %B in 30 min.)**



**Competitor 2 (5 cm x 5 mm, 0.59 mL/min, 28-43 %B in 25 min.)**



## + Featured Products

| Cat. No.               | Description  |
|------------------------|--|
| <b>Cation Exchange</b> |  |
| <b>59601-U</b>         | Discovery BIO PolyMA-SCX<br>5 cm x 4.6 mm I.D., 5 µm |
| <b>Anion Exchange</b>  |  |
| <b>59602-U</b>         | Discovery BIO PolyMA-WAX<br>5 cm x 4.6 mm I.D., 5 µm |

# Metabolomic Profiling of *Neurospora crassa* Fungi Using HILIC and Reversed-Phase LC-MS

Craig R. Aurand<sup>1</sup>, David S. Bell<sup>1</sup>, Teresa Lamb<sup>2</sup> and Deborah Bell-Pedersen<sup>2</sup>

<sup>1</sup>Sigma Aldrich/Supelco, <sup>2</sup>Texas A&M University

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Complex metabolome profiling by LC-MS can be facilitated using advanced instrumentation and software. The choice of the HPLC column is also important. This article shows the benefit of choosing highly-efficient Ascentis Express phases with orthogonal selectivities to provide the most information from the LC-MS experiments.

## Introduction

The general aim of metabolomic profiling is to document the set of metabolites from a defined sample for determination of physiological changes. The specific sample can be characterized by a variety of descriptors or parameters, such as cell type, organelle, age, tissue, treatment, etc. In this study, *Neurospora crassa* cultures grown over a specified time period in the dark were compared and contrasted for a set of identified components. Much is known regarding the genome of *Neurospora crassa*, specifically in the determination of circadian rhythms. However, little is known regarding how the metabolome changes over the course of the day under control of the circadian clock. Endogenous circadian biological clocks program 24 h rhythms in biochemical, physiological and behavioral processes of living entities, including animals, plants, and fungi. These cyclic processes typically occur with an approximate 24-hour period, but this period can be impacted by light-to-dark and temperature cycles. When the organism is maintained in constant environmental conditions, such as constant dark, they will free run with an endogenous period. For *Neurospora*, the free running period is 22.5 hrs. The goal of this study was to profile the change in the metabolome of *Neurospora crassa* as a function of the circadian clock. The small molecule metabolites from time series experiments are evaluated to determine possible influences or artifacts of the circadian rhythms in the *neurospora* cultures.

## Experimental

Time-series experiments were conducted at Texas A&M University. Each time series was performed in triplicate from the same starting culture, labeled series A, B, or C. The experiments consisted of an initial culture that was subdivided into 27 cores; all individual cores were then inoculated at the same time. Culture sets were shifted from a light environment to a dark environment at various times (three per time period) as described in **Table 1** to obtain cultures representing different times of the day. For example, 12 hours of darkness represents dawn and 28 hours of darkness represents midnight. Total incubation period for the experiment was 48 hours. After the incubation period, tissue samples were processed, packaged in dry ice, and shipped to the laboratory. Tissue samples were kept frozen until they were extracted. The tissue samples were extracted by placing 20 mg of tissue into to a 2 mL centrifuge tube. Next, 100  $\mu$ L of 50:50 methanol:water was added making the

**Table 1. *Neurospora crassa* Incubation Period**

| Sample No. | Hours in Dark |
|------------|---------------|
| 1          | 12            |
| 2          | 16            |
| 3          | 20            |
| 4          | 24            |
| 5          | 28            |
| 6          | 32            |
| 7          | 36            |
| 8          | 40            |
| 9          | 44            |

final concentration for all samples 200 mg extracted tissue per mL. Samples were then vortexed to thoroughly mix sample and placed in refrigerator for 1 hour. The samples were then vortexed and centrifuged for 3 minutes at 15,000 rpm. The resulting supernatant was collected and analyzed directly.

Profiling of the *Neurospora* samples was conducted using high-performance liquid chromatography (HPLC) in both reversed-phase (RP) and hydrophilic interaction (HILIC) modes utilizing accurate mass time-of-flight (TOF) mass spectrometry. The concept behind utilizing both RP and HILIC HPLC is to facilitate a more accurate determination of an actual sample component versus a chromatographic artifact, without relying specifically on accurate mass resolution. By leveraging the selectivity differences between two (or more) different or orthogonal chromatographic modes, sample components that co-elute, do not retain, or do not elute on one mode may be resolved using the other mode. In this study, RP and HILIC separations were carried out using Fused-Core™ Ascentis Express RP-Amide and Ascentis Express HILIC columns, respectively. The polar embedded group of the amide was chosen over traditional C18 phases to increase the retention of the polar components. The Ascentis Express HILIC was chosen for alternative selectivity for polar analytes. Because of the large amount of unknown components in the samples, using orthogonal chromatographic separation in combination with accurate mass enabled better dissemination of components of interest from sample matrix and chromatographic anomalies.

Samples were analyzed by LC-MS RP (**Figure 1**) and HILIC (**Figure 2**) modes. The data were deconvoluted and pushed into the Mass Profiler™ software programs. Mass Profiler enables sets of experiments to be compared to each other. This can be performed using individual data files or batch files. By performing batch processing, samples can be compared for common components within all samples from the batch. Batch processing can also identify components that are common to only one set of samples, or attributed to a subset within the batch. There are several permutations of the comparisons that can be made. In this particular example, all samples from series A, B, and C were compared to each other for components common to all samples. The study also incorporated a blank chromatographic run to cancel out anomalies from the chromatographic system.

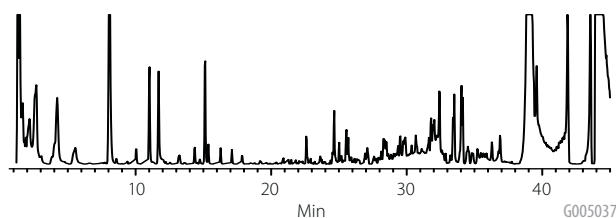
**Figure 1. RP-HPLC Separation of *Neurospora crassa* Extract on Ascentis Express RP-Amide**

column: Ascentis Express RP-Amide, 15 cm x 2.1 mm I.D., 2.7  $\mu$ m particles (53914-U)  
 instrument: Agilent 1200SL Rapid Resolution interfaced with Agilent 6210 TOF  
 mobile phase A: 10 mM ammonium formate, pH 4.2 with formic acid  
 mobile phase B: 10 mM ammonium formate 95:5 acetonitrile:water  $S_w$  pH 4.2 with formic acid  
 temp.: 35  $^{\circ}$ C  
 flow rate: 0.2 mL/min.  
 det.: LC-MS TOF, ESI (+)  
 injection: 2  $\mu$ L  
 gradient:

| time (min.) | %A | %B  | Flow |
|-------------|----|-----|------|
| 0           | 98 | 2   | 0.2  |
| 2           | 98 | 2   | 0.2  |
| 40          | 0  | 100 | 0.2  |
| 50          | 0  | 100 | 0.2  |
| 50.01       | 98 | 2   | 0.4  |
| 60          | 98 | 2   | 0.2  |

**LC-MS TOF conditions**

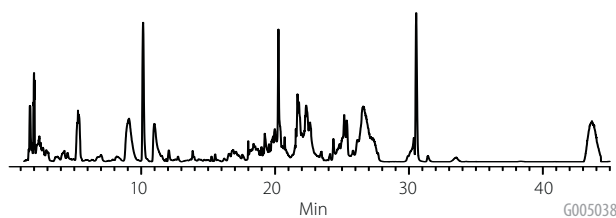
ionization: ESI (+)  
 sampling rate: 1.0 spectra/second, 9528 transients/spectra  
 reference ions: 322.0481, 622.0289  
 data acquisition: Agilent Mass Hunter™  
 data deconvolution: Agilent Mass Profiler™



**Figure 2. HILIC Separation of *Neurospora crassa* Extract on Ascentis Express HILIC**

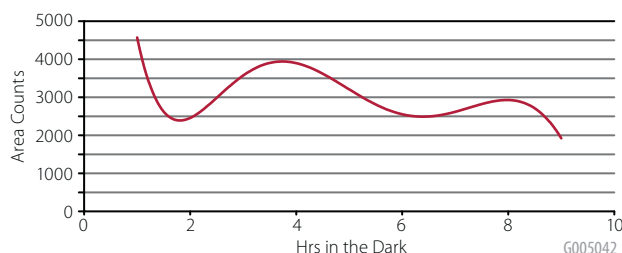
Conditions same as Figure 1, except:

column: Ascentis Express HILIC, 15 cm x 2.1 mm I.D., 2.7  $\mu$ m particles (53946-U)  
 mobile phase A: 2 mM ammonium formate in 95:5 acetonitrile:water  
 mobile phase B: 2 mM ammonium formate in 50:50 acetonitrile:water



**Figure 3. Time Series A for 326.1945 Component**

Signal intensity of component m/z 326.1945 plotted as a function of incubation period for series A



The comparison was based upon the accurate mass of the components along with chromatographic retention time of either the RP or HILIC separations.

## Results and Discussion

Approximately 310 components, or features, were observed in the RP separation (Figure 1), while approximately 670 components were observed in the HILIC separation (Figure 2). When data from both RP and HILIC methods were compared, twelve major components were found in all sample extracts. The goal of the study was to determine if there was a correlation between the intensity of the major components and the time of day. The experiment was designed to track intensity changes of the major common components throughout the time series. To simplify the experiment, this study did not target components that decreased completely, nor did it track the formation of new components. The signal intensity vs. incubation period was plotted for the twelve common components to determine if intensity levels exhibited circadian rhythm behavior. An example of this data for one component is shown in Figure 3. Here, the signal intensity of component m/z 326.1945 was plotted as a function of incubation period for series A. The trend in the time series is a cyclic change in the intensity of m/z 326.1945. At this stage of the study, the exact identity of m/z 326.1945 has yet to be determined nor has the influence from the circadian rhythm been identified. This work is still ongoing.

## Conclusions

Profiling of metabolic changes in biological samples can be a complex and tedious task, even with the most advanced instrumentation and software. Every advantage should be utilized to help simplify the deconvolution process, including sample preparation and enhanced chromatographic resolution. The approach of using orthogonal chromatographic separation modes greatly increases the opportunity for distinguishing true sample components from chromatographic anomalies. By doing so, this simplifies the data interpretation while increasing the confidence level of tracking components of interest. The use of the high-resolution Ascentis Express HPLC columns greatly aids in the resolution of components in even the most complex sample matrix. An added benefit of Ascentis Express columns is their durability, which makes them less susceptible to fouling and therefore highly suited for the long-term analysis of complex biological matrixes, such as those encountered in this study.

For additional information on sample profiling using orthogonal chromatographic modes, please see the Reporter 27.2 article on "Profiling of *Stevia rebaudiana* Extract by Accurate Mass Using HILIC and Reversed-Phase Chromatography."

### + Featured Products

| Cat. No.       | Description   |
|----------------|---|
| <b>53914-U</b> | Ascentis Express RP-Amide<br>15 cm x 2.1 mm I.D., 2.7 $\mu$ m particles |
| <b>53946-U</b> | Ascentis Express HILIC<br>15 cm x 2.1 mm I.D., 2.7 $\mu$ m particles    |

Other phases and dimensions of Ascentis Express are available. Please visit [sigma-aldrich.com/express](http://sigma-aldrich.com/express) for details.

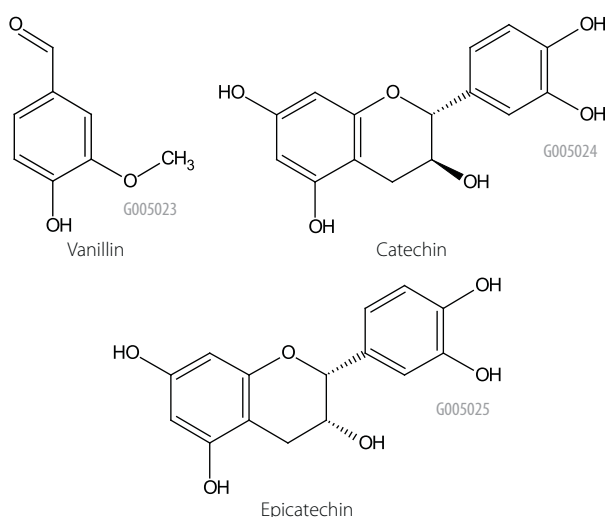
# Analysis of Polyphenolic Compounds and Vanillin in Cocoa Using Ascentis Express RP-Amide and MS Detection

Carmen T. Santasania  
carmen.santasania@sial.com

Cocoa, the principal component of chocolate, is reported to contain more than 800 different compounds (1). This complexity makes HPLC with mass spectrometric detection (LC-MS) an important analytical tool. In this short study, we looked at a few compounds (polyphenolics and vanillin) found in cocoa by LC-MS analysis. We chose Ascentis Express HPLC columns for their speed, efficiency, and ruggedness. The unique RP-Amide chemistry is ideal for these polar compounds.

**Figure 1** shows the compounds that were examined in this study: the polyphenolic, anti-oxidant compounds catechin and epicatechin, which have reported health benefits. The extraction method was that described by Risner (2). A milk chocolate and an extra dark chocolate bar were tested. A 1 gram sample of the chocolate was added to 25 mL of warm (60 °C) water and allowed to melt with moderate stirring. A 2 mL aliquot of this solution was filtered through a 0.5 µm PVDF filter and cooled to ambient temperature prior to LC-MS analysis. **Figure 2** shows the extracted ion chromatograms of a dark and milk chocolate sample by monitoring  $m/z$  289.07. The analysis was run in negative ion mode on a single quadrupole mass spectrometer. The two samples are shown on the same scale to show the different amounts of catechins in the two types of chocolates analyzed. The observed ratio confirms literature reports that the darker the chocolate, the higher the levels of polyphenolic compounds (3).

**Figure 1. Compounds Examined in the Study**

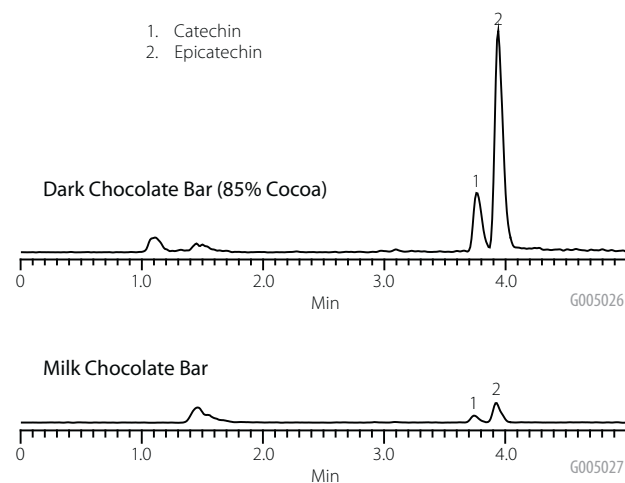


**Figure 3** shows a comparison of vanillin content. Vanillin is a flavor enhancer that is added to many foods, including chocolate. Our results show higher levels of vanillin in the milk chocolate sample; not surprising since vanillin is known to reduce the bitterness of chocolate.

In this brief report, we showed a simple extraction procedure followed by LC-MS analysis using Ascentis Express RP-Amide columns. Differences in catechin, epicatechin, and vanillin levels were observed in two types of chocolate. Additional applications using Ascentis Express and LC-MS to characterize chocolate samples are currently underway.

**Figure 2. Polyphenolic Compounds: LC-MS Extracted Ion Chromatogram of Dark and Milk Chocolate Samples**

|                 |  |    |    |
|-----------------|--|----|----|
| column:         | Ascentis Express RP-Amide, 10 cm x 4.6 mm I.D., 2.7 µm particles (53929-U) |    |    |
| instrument:     | Waters 2690 HPLC and Micromass MS  |    |    |
| mobile phase A: | 95:5, 0.1 % acetic acid in water:acetonitrile                              |    |    |
| mobile phase B: | 0.1 % acetic acid in acetonitrile  |    |    |
| temp.:          | 35 °C  |    |    |
| flow rate:      | 0.8 mL/min.  |    |    |
| injection:      | 5 µL   |    |    |
| det.:           | ESI (-) in SIR mode, $m/z$ 289.07  |    |    |
| pressure:       | 2200 psi   |    |    |
| sample:         | chocolate bar aqueous extract  |    |    |
| gradient:       | time (min.)  | A% | B% |
|                 | 0  | 95 | 5  |
|                 | 10   | 5  | 95 |
|                 | 12   | 95 | 5  |



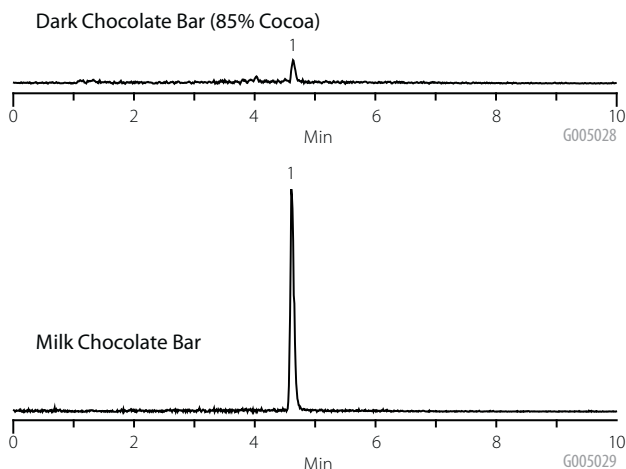


**Figure 3. Vanillin: LC-MS Extracted Ion Chromatogram of Dark and Milk Chocolate Samples**

Conditions same as Figure 2 except:

det.: ESI (-) in SIR mode, m/z 151.04

1. Vanillin

**References**

1. <http://pubs.acs.org/cen/whatstuff/stuff/7849sci5.html>, accessed 5/1/2010.
2. Risner, Charles H., Kiser, Melissa J., High performance Liquid Chromatography Procedure for the Determination of Flavor Enhancers in Consumer Chocolate Products and Artificial Flavors. *J. Sci. Food Agric.* 88:1423-1430 (2008).
3. <http://www.hersheys.com/nutrition/antioxidants.asp>, accessed 5/1/2010.

**+ Featured Products**

| Cat. No.       | Description  |
|----------------|--|
| <b>53929-U</b> | Ascentis Express RP-Amide<br>10 cm x 4.6 mm I.D., 2.7 µm particles |

**+ Related Products**

| Cat. No.     | Description | Pk. Size   |
|--------------|-------------|------------|
| <b>C0567</b> | Catechin    | 5 mg       |
| <b>E4018</b> | Epicatechin | 1 mg, 5 mg |

## New Fittings for Ultra High Performance Liquid Chromatography



E001129

The efficiency of separations performed with low-volume columns is highly dependent on the system having components designed to minimize bandwidth. With UHPLC systems it is good laboratory practice to install the proper fittings, ferrules, and other accessories to ensure the analytical results show no extra column effects created by improperly assembled accessories. In our commitment to provide the most up-to-date liquid chromatography accessories, we have introduced new fittings for use in UHPLC. These high performance fittings are user-friendly, compact, and for use with PEEK/PEEKSil™ and stainless steel tubing.

This newly expanded line of fittings for high sensitivity analytical applications help to maximize the efficiency of your analysis and protect your column investment. These new products complement the vast array of HPLC accessory products currently offered by Supelco.

**+ Featured Products**

| Cat. No.       | Description   |
|----------------|---|
| <b>51359-U</b> | Supel™-Connect Better Nut   |
| <b>51361-U</b> | Supel™-Connect Better Nut with Stainless Steel Ferrule                |
| <b>51365-U</b> | Supel™-Connect Better Nut with PEEK Ferrule                           |
| <b>51366-U</b> | Supel™-Connect Short High Performance Fitting for PEEK/PEEKSil Tubing |
| <b>51367-U</b> | Supel™-Connect Long High Performance Fitting for PEEK/PEEKSil Tubing  |
| <b>51368-U</b> | Supel™-Connect High Performance Fitting for Stainless Steel Tubing    |
| <b>51369-U</b> | Ferrule Crimping Tool for Supel™-Connect High Performance Fittings    |

**+ Related Products**

| Cat. No.     | Description  | Pk. Size                 |
|--------------|--------------|--------------------------|
| <b>49199</b> | Acetic acid  | 50 mL                    |
| <b>39253</b> | Water        | 1 L, 4x4 L, 20 L         |
| <b>34967</b> | Acetonitrile | 250 mL, 1 L, 2.5 L, 20 L |

# Supelco Introduces the Hamilton Company Line of Polymeric HPLC Columns

For more than 30 years, Hamilton Company has been the leading provider of polymer-based HPLC particles. Now, Supelco is pleased to add the renowned Hamilton products to our line-up of quality HPLC columns. This includes the highly-popular Hamilton PRP-1 – the gold standard in polymer reversed-phase columns.

Hamilton polymer-based HPLC columns are available for reversed-phase, anion exchange, cation exchange, and ion exclusion separations, and are well-suited for many application areas, including:

- High and low pH operation (pH 1-13)
- Inorganic anions
- Metal cations
- Strong silanophiles and chelators
- Carboxylic acids
- Alcohols
- EDTA

All Hamilton HPLC packings are available as bulk resin or packed into analytical, semi-preparative, and preparative columns. Both bulk and packed column resins are tested for chromatographic capacity and efficiency.



## Extend the Range of Your QuEChERS Applications with Supelco's Custom Services

Supelco offers a custom QuEChERS service with a wide range of salts, SPE sorbents, and package sizes, to fit nearly any dispersive SPE application. The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe), is already popular in multi-residue pesticide testing of food and agricultural products. Supelco's custom service fully extends the range of applications possible using this technique.

For more information, please visit our website:  
[sigma-aldrich.com/custom-quechers](http://sigma-aldrich.com/custom-quechers)

### + Featured Products

| Cat. No.   | Particle Size | Dimensions and Hardware Composition |
|--|---------------|-------------------------------------|
| <b>Hamilton PRP-1 (Polymeric Reversed Phase)</b>   |               |                                     |
| Ideal for high pH (pH 8-13) operation or with analytes that give poor chromatography on silica-based columns. USP Code L21 |               |                                     |
| <b>82013-U</b>   | 5 µm          | 15 cm x 4.1 mm I.D., SS hardware    |
| <b>82021-U</b>   | 5 µm          | 25 cm x 4.1 mm I.D., SS hardware    |
| <b>82030-U</b>   | 5 µm          | 15 cm x 4.6 mm I.D., PEEK hardware  |
| <b>82025-U</b>   | 5 µm          | 25 cm x 4.6 mm I.D., PEEK hardware  |
| <b>82016-U</b>   | 7 µm          | 25 cm x 4.1 mm I.D., SS hardware    |
| <b>82024-U</b>   | 7 µm          | 25 cm x 4.6 mm I.D., PEEK hardware  |
| <b>82018-U</b>   | 10 µm         | 15 cm x 4.1 mm I.D., SS hardware    |
| <b>82012-U</b>   | 10 µm         | 25 cm x 4.1 mm I.D., SS hardware    |
| <b>Hamilton PRP-X100</b>   |               |                                     |
| Ion chromatography columns for anion separation. USP Code L47  |               |                                     |
| <b>82017-U</b>   | 5 µm          | 15 cm x 4.6 mm I.D., PEEK hardware  |
| <b>82023-U</b>   | 5 µm          | 25 cm x 4.6 mm I.D., PEEK hardware  |
| <b>82026-U</b>   | 10 µm         | 10 cm x 4.1 mm I.D., SS hardware    |
| <b>82014-U</b>   | 10 µm         | 15 cm x 4.1 mm I.D., SS hardware    |
| <b>82011-U</b>   | 10 µm         | 25 cm x 4.1 mm I.D., SS hardware    |
| <b>82028-U</b>   | 10 µm         | 15 cm x 4.6 mm I.D., PEEK hardware  |
| <b>82020-U</b>   | 10 µm         | 25 cm x 4.6 mm I.D., PEEK hardware  |
| <b>Hamilton PRP-X200</b>   |               |                                     |
| For inorganic and organic, mono or divalent cations. USP Code L17  |               |                                     |
| <b>82019-U</b>   | 10 µm         | 15 cm x 4.1 mm I.D., SS hardware    |
| <b>82027-U</b>   | 10 µm         | 25 cm x 4.1 mm I.D., SS hardware    |
| <b>Hamilton PRP-X300</b>   |               |                                     |
| For alcohols and organic acids. USP Code L17   |               |                                     |
| <b>82015-U</b>   | 7 µm          | 25 cm x 4.1 mm I.D., SS hardware    |
| <b>Hamilton HC-75 (Ca<sup>2+</sup> form)</b>   |               |                                     |
| For the separation of mono and disaccharides, organic acids, sugars, and sugar alcohols. USP Code L19                      |               |                                     |
| <b>82031-U</b>   | 9 µm          | 25 cm x 4.1 mm I.D., SS hardware    |
| <b>82022-U</b>   | 9 µm          | 30 cm x 7.8 mm I.D., SS hardware    |

**Note:** A complete listing, which includes guard columns, can be found on our web site at [sigma-aldrich.com/hplc](http://sigma-aldrich.com/hplc)

# Heavy Wall CD Vials Increase Sample Recovery

Heavy wall center drain (CD™) vials are an excellent choice of vials for analysts seeking to increase sample recovery when performing high temperature and/or high-pressure applications.

The CD vial has a unique conical interior bottom that allows the sample to completely drain to the center bottom of the vial. This patented design allows maximum extraction of the sample using a syringe or micropipette, with less than 4 µL of sample being left behind.

These vials are manufactured from 2.5 mm wall borosilicate glass tubing that provides a more durable vessel than standard wall vials for high pressure and/or high temperature applications that include lyophilization, derivatization and other small-scale reactions. This durability allows the heavy wall CD vial to be used as a replacement for V-vials and Micro Reaction vessels that are traditionally used in high temperature applications.

Heavy wall CD vials are also priced 30% less than the comparable V-vials and Micro Reaction vessels providing analysts with a quality alternative at a considerable savings.

Heavy wall CD vials have the following benefits

- Greater sample recovery
- Economically priced
- Autoclavable
- Temperature range of -70 °C to 160 °C
- Manufactured from Type 1 borosilicate glass
- Vial, cap and septa are pre-assembled

## Did you know...?

The Pico Pure Plus+™ cap is a polyethylene closure that was designed for LC-MS single injection applications requiring low extractables. It punctures with far less force than a PTFE/silicone septum, and eliminates the possibility of silicone contamination.

For more information on these products, email our Technical Service Department at [techservice@sial.com](mailto:techservice@sial.com) or visit [sigma-aldrich.com/vials](http://sigma-aldrich.com/vials)



E001128



E001112

The heavy wall CD vials are available in a pack of 12 with a black cap and PTFE/silicone septa.

| Cat. No.   | Volume | O.D. x Height (mm) | Thread Size |
|--|--------|--------------------|-------------|
| <b>CD Vials with Graduations, clear glass</b>    |        |                    |             |
| <b>29362-U</b>                                   | 1 mL   | 13 x 41            | 13/425      |
| <b>29363-U</b>                                   | 2 mL   | 16 x 58            | 15/415      |
| <b>29364-U</b>                                   | 2 mL   | 20 x 40            | 20/400      |
| <b>29365-U</b>                                   | 3 mL   | 20 x 46            | 20/400      |
| <b>29366-U</b>                                   | 5 mL   | 20 x 61            | 20/400      |
| <b>CD Vials without Graduations, clear glass</b> |        |                    |             |
| <b>29356-U</b>                                   | 1 mL   | 13 x 41            | 13/425      |
| <b>29357-U</b>                                   | 2 mL   | 16 x 58            | 15/415      |
| <b>29358-U</b>                                   | 2 mL   | 20 x 40            | 20/400      |
| <b>29359-U</b>                                   | 3 mL   | 20 x 46            | 20/400      |
| <b>29361-U</b>                                   | 5 mL   | 20 x 61            | 20/400      |
| <b>CD Vials without Graduations, amber glass</b> |        |                    |             |
| <b>29367-U</b>                                   | 1 mL   | 13 x 41            | 13/425      |
| <b>29368-U</b>                                   | 2 mL   | 20 x 40            | 20/400      |
| <b>29369-U</b>                                   | 3 mL   | 20 x 46            | 20/400      |
| <b>29370-U</b>                                   | 5 mL   | 20 x 61            | 20/400      |

# Phenolic Calibration Standards for Monitoring Wastewaters



Phenolic compounds are important raw materials for the manufacturing of phenolic resins, pharmaceuticals, laboratory reagents, dyes, germicidal paints, and general disinfectants. Due to their toxicity to both man and animals, manufacturers using these raw materials may be required by local or regional government regulatory agencies to monitor wastewater discharges for these materials. Analysis of these compounds at low levels in waste effluents can be complex, requiring extensive sample preparation, followed by gas chromatography (GC). High-quality analytical standards are essential for routine daily calibration of the GC instrument.

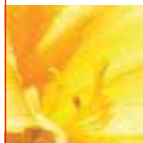
Sigma Aldrich offers high-quality analytical phenol and substituted phenol standards in the form of neat, single-component solutions, and multi-component solutions. These standards are suitable for use when monitoring phenolic compounds in accordance with the United States Environmental Protection Agency, the Canadian Ministry of Environment and the European Union environmental contaminant methodologies. The single and multi-component calibration standards are tested for purity, identity, and concentration. Each component is guaranteed to be within 0.5% of the stated concentration. Documentation is provided free-of-charge for all neat reference materials and calibration solutions.

The table below lists a sampling of the many phenolic calibration standards and neat reference materials available from Sigma-Aldrich. Additional calibration standards can be found by visiting us online at [sigma-aldrich.com/standards](http://sigma-aldrich.com/standards)

If you prefer, we can also formulate, test, and package phenol calibration standards per your specifications in a relatively short time, saving you valuable time and resources. To request a quote, please email our Technical Service group at [techservice@sial.com](mailto:techservice@sial.com), or visit our website: [sigma-aldrich.com/custom-standards](http://sigma-aldrich.com/custom-standards)

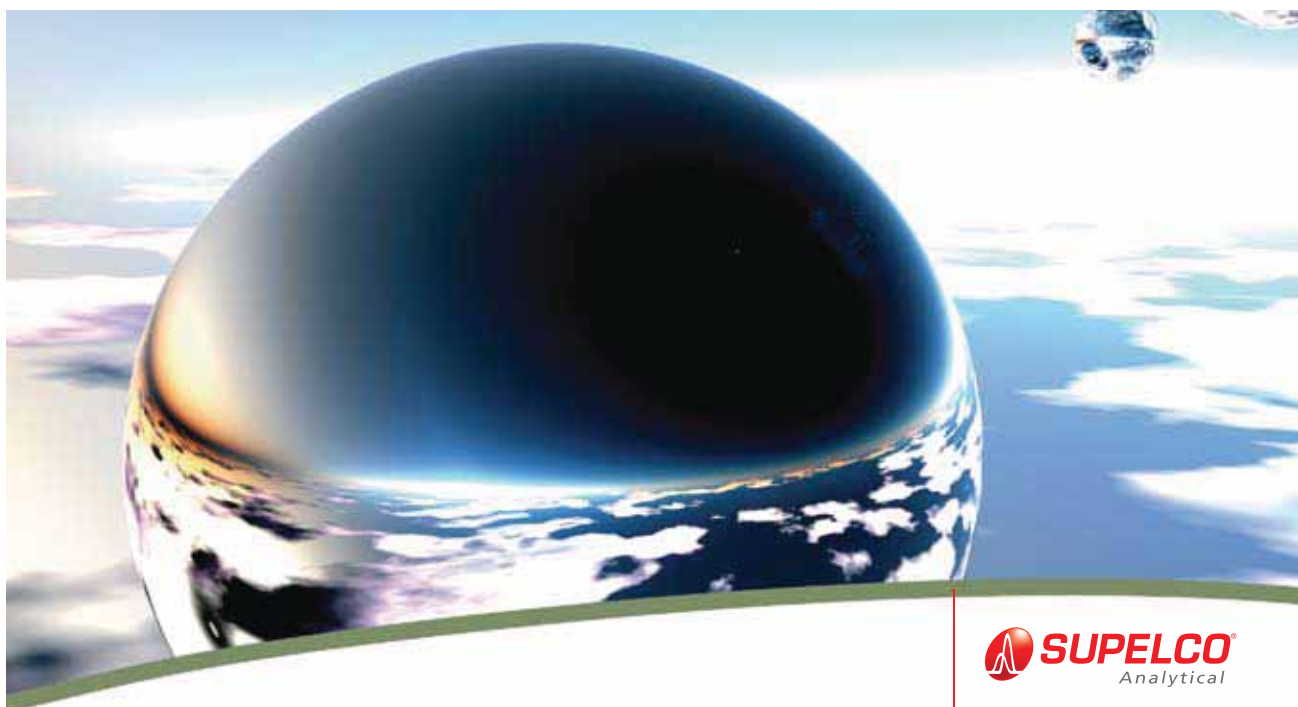
| Cat. No.                         | Description  |
|----------------------------------|--|
| <b>Multi-component Solutions</b> |  |
| <b>43240-U</b>                   | DL 152/2006 (ex. DM 471) phenol mixture - 100 µg/mL, in methanol, 1 mL<br><i>2-Chlorophenol, 4-Chlorophenol, 2,4-Dichlorophenol, 2-Methylphenol, 3-Methylphenol, 4-Methylphenol, Pentachlorophenol, Phenol, 2,4,6-Trichlorophenol</i>  |
| <b>48859</b>                     | EPA Phenols Mix - Varied conc., in methanol, 1 x 1 mL<br><i>4-Chloro-3-methylphenol (2500 µg/mL), 2-Chlorophenol (500 µg/mL), 2,4-Dichlorophenol (500 µg/mL), 2,4-Dimethylphenol (500 µg/mL), 2,4-Dinitrophenol (1500 µg/mL), 2-Methyl-4,6-dinitrophenol (2500 µg/mL), 2-Nitrophenol (500 µg/mL), 4-Nitrophenol (2500 µg/mL), Pentachlorophenol (2500 µg/mL), Phenol (500 µg/mL), 2,4,6-Trichlorophenol (1500 µg/mL)</i> |
| <b>48751</b>                     | EPA 604-M Phenols Kit - Individual 1 mL solutions prepared at 500 µg/mL each in methanol<br><i>4-Chloro-3-methylphenol, 2-Chlorophenol, 2,4-Dichlorophenol, 2,4-Dimethylphenol, 2,4-Dinitrophenol, 2-Methyl-4,6-dinitrophenol, 2-Nitrophenol, 4-Nitrophenol, Pentachlorophenol, Phenol, 2,4,6-Trichlorophenol, EPA Phenol Mix (48859)</i>  |
| <b>48130-U</b>                   | MISA Group 20 Phenols Mix A - 2000 µg/mL each in methanol, 1 x 1 mL<br><i>2,6-Dichlorophenol, 2,4-Dimethylphenol, 4,6-Dinitro-o-cresol, o-Cresol, p-Cresol, 2,3,4,6-Tetrachlorophenol, 2,3,5,6-Tetrachlorophenol, 2,3,4-Trichlorophenol, 2,3,5-Trichlorophenol, 2,4,5-Trichlorophenol</i>  |
| <b>48131</b>                     | MISA Group 20 Phenols Mix B - 2000 µg/mL each in methanol, 1 x 1 mL<br><i>p-Chloro-m-cresol, 2-Chlorophenol, m-Cresol, 2,4-Dichlorophenol, 2,3,4,5-Tetrachlorophenol, 2,4-Dinitrophenol, 4-Nitrophenol, Pentachlorophenol, Phenol, 2,4,6-Trichlorophenol</i>   |

| Cat. No.   | Description  |
|--|--|
| <b>Single-component Solutions, all offered as 1 mL</b> |  |
| <b>48689</b>   | 2-Chlorophenol solution, 500 µg/mL in methanol               |
| <b>40029</b>   | 2,4-Dichlorophenol solution, 5000 µg/mL in methanol          |
| <b>40302</b>   | 2,6-Dichlorophenol solution, 5000 µg/mL in methanol          |
| <b>48719-U</b>   | 2-Fluorophenol solution, 2000 µg/mL in methylene chloride    |
| <b>48697</b>   | 2-Methyl-4,6-dinitrophenol solution, 500 µg/mL in methanol   |
| <b>40058</b>   | 2-Methyl-4,6-dinitrophenol solution, 5000 µg/mL in methanol  |
| <b>40250-U</b>   | 2-Methylphenol solution, 5000 µg/mL in methanol              |
| <b>40251-U</b>   | 3-Methylphenol solution, 5000 µg/mL in methanol              |
| <b>40252-U</b>   | 4-Methylphenol solution, 5000 µg/mL in methanol              |
| <b>48694</b>   | 2-Nitrophenol solution, 500 µg/mL in methanol                |
| <b>40055</b>   | 2-Nitrophenol solution, 5000 µg/mL in methanol               |
| <b>48695</b>   | 4-Nitrophenol solution, 500 µg/mL in methanol                |
| <b>40056</b>   | 4-Nitrophenol solution, 5000 µg/mL in methanol               |
| <b>48692</b>   | Pentachlorophenol solution, 500 µg/mL in methanol            |
| <b>40062</b>   | Pentachlorophenol solution, 5000 µg/mL in methanol           |
| <b>48718</b>   | Pentafluorophenol solution, 2000 µg/mL in methylene chloride |
| <b>48688</b>   | Phenol solution, 500 µg/mL in methanol                       |
| <b>48154</b>   | 2,3,4-Trichlorophenol solution, 2000 µg/mL in methanol       |
| <b>40179</b>   | 2,4,5-Trichlorophenol, 5000 µg/mL in methanol                |
| <b>48691</b>   | 2,4,6-Trichlorophenol, 500 µg/mL in methanol                 |
| <b>40019</b>   | 2,4,6-Trichlorophenol, 5000 µg/mL in methanol                |



| Cat. No. | Description             | Qty.    |
|----------|-------------------------|---------|
| Neats    |                         |         |
| 442405   | 4-Bromophenol           | 1000 mg |
| 506761   | 4-tert-Butyl phenol     | 1000 mg |
| 48519    | 4-Chloro-3-methylphenol | 5000 mg |
| 48521    | 2-Chlorophenol          | 5000 mg |
| 36747    | 3-Chlorophenol          | 1000 mg |
| 442411   | 4-Chlorophenol          | 500 mg  |
| 442312   | 2,4-Dibromophenol       | 1000 mg |
| 442324   | 2,6-Dibromophenol       | 1000 mg |
| 442291-U | 2,3-Dichlorophenol      | 1000 mg |
| 48528    | 2,4-Dichlorophenol      | 5000 mg |
| 442375   | 3,4-Dichlorophenol      | 1000 mg |
| 442378   | 3,5-Dichlorophenol      | 1000 mg |
| 48531    | 2,4-Dimethylphenol      | 500 mg  |
| 442350   | 2-Fluorophenol          | 1000 mg |
| 442361   | 2-Methylphenol          | 1000 mg |

| Cat. No. | Description           | Qty.    |
|----------|-----------------------|---------|
| Neats    |                       |         |
| 442391   | 3-Methylphenol        | 1000 mg |
| 442418   | 4-Methylphenol        | 1000 mg |
| 48548    | 2-Nitrophenol         | 5000 mg |
| 48549    | 4-Nitrophenol         | 500 mg  |
| 442873   | Nonylphenol           | 100 mg  |
| 442850   | 4-Octylphenol         | 500 mg  |
| 442858   | 4-tert-Octylphenol    | 500 mg  |
| 48555-U  | Pentachlorophenol     | 5000 mg |
| 442745   | Pentafluorophenol     | 1000 mg |
| 48556    | Phenol                | 1000 mg |
| 506842   | 4-Phenylphenol        | 1000 mg |
| 442287   | 2,3,6-Trichlorophenol | 1000 mg |
| 48518    | 2,4,6-Trichlorophenol | 5000 mg |
| 442373   | 3,4,5-Trichlorophenol | 25 mg   |
| 442306   | 2,4,6-Trimethylphenol | 1000 mg |



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# Headspace Grade Solvents

## High Purity for OVIs Analysis

Shyam Verma  
shyam.verma@sial.com

Static headspace GC (HS-GC), a commonly used technique in the analysis of organic volatile impurities (OVIs), concentrates volatile analytes to allow their analysis free from sample matrix. Samples to be analyzed by HS-GC are dissolved in a suitable solvent that must allow for sufficient sensitivity of the analytes of interest in the headspace. Also, the vapor pressure of the dissolution solvent itself should be sufficiently low so it will not affect detection of OVI analytes by "flooding" the headspace.

USP <467> and European Pharmacopoeia (EP) methodologies list procedures for both water soluble and water-insoluble samples (1-2). For water-insoluble samples, USP <467> designates the use of the solvents dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). Other dissolution solvents that have been found to be useful for headspace analysis of water-insoluble samples include dimethylacetamide (DMAC) and 1,3-dimethyl-2-imidazolidinone (DMI), and the later is described for use in EP Method 2.4.24 (2). These solvents have significantly lower vapor pressure than many other high boiling-point organic compounds and they elute later than most OVI analytes in chromatographic analyses.



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Solvent purity is essential to avoid extraneous peaks in the chromatographic analysis, and prevent interference with the analytes of interest. A recent publication (3) on our joint work with Genentech, Inc. reported chromatogram results of comparing the headspace grade to the conventional organic synthesis grade solvents. The cleanliness of the headspace grade solvents as well as their compatibility for use in the analysis of the OVIs listed in United States Pharmacopoeia (USP) Method <467>, European Pharmacopoeia (EP) Method 2.4.24, and the International Conference on Harmonization (ICH) guidelines were demonstrated. This publication documented the following benefits of Fluka's headspace grade solvents.

- Headspace grade solvents produced cleaner blanks
- Headspace grade solvents did not produce any major interference peaks in the elution range of the target analytes
- Headspace grade DMSO, DMF, DMAC and DMI solvents evaluated were suitable for the analysis of OVIs by HS-GC

### References

1. United States Pharmacopoeia (USP), 31st Edition (2008), <467> Residual Solvents.
2. European Pharmacopoeia (EP) 5.0, Vol.1, (2004), 2.4.24 Identification and Control of Residual Solvents.
3. A. Quiroga, M. Dong, K. Stenerson, S. Verma, The Utility of Headspace Grade Solvents in the Analysis of Organic Volatile Impurities. Supelco Publication T409180, November 2009.

### + Featured Products

| Cat. No. | Description                    | Boiling Pt. | Pkg. Size |
|----------|--------------------------------|-------------|-----------|
| 44901    | N,N-Dimethylacetamide          | 166 °C      | 1 L       |
| 51779    | Dimethyl sulfoxide             | 189 °C      | 1 L       |
| 51781    | N,N-Dimethylformamide          | 153 °C      | 1 L       |
| 67484    | 1,3-Dimethyl-2-imidazolidinone | 225 °C      | 1 L       |
| 53463    | Water                          | 100 °C      | 1 L       |

All products are puriss. p.a.

# LC-MS Solvents, Blends and Additives

## High Purity and Pre-tested

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Demand on sensitivity, specificity and speed of analysis requires use of high-purity chemicals for sample preparation, mobile phase and post-column additives, especially for LC-MS technique. Impure mobile phase solvents are the most common source of extraneous peaks and unstable LC-MS baseline. Potential LC-MS contaminants include inorganic ions, decomposition products, closely related compounds present in the manufacturing process, microbes and compounds they excrete, particulate matter from improper filtration and precipitation, and compounds adsorbed from exposure to atmosphere.

Irrespective of the source, impurities in LC-MS mobile phase solvents can result in the following situations:

- Build-up on the head of the HPLC column and elution as distinct peaks or as baseline rise
- A general elevation in baseline, lowering the sensitivity of the analysis
- Foul or damage sensitive instrument components
- Cause cluster ion formation that prevents reliable identification and quantification

A study reported earlier (1) demonstrated that use of higher purity LC-MS CHROMASOLV® solvents do not foul the source, minimizes instrument downtime, and allows complex spectral analysis. On the other hand, lower quality solvents (e.g. standard HPLC grade brand) contaminate the detection source.

LC-MS CHROMASOLV solvents from Sigma-Aldrich undergo 34 distinct and relevant tests to ensure they meet the criteria required for sensitive LC-MS analysis. These high-purity, particulate-free solvents deliver stable, minimal LC-MS baselines. Convenient pre-mixed and pre-tested blends are easy-to-use and reduce variability due to mixing.

### Reference

1. C. T. Santasania and S. Verma, Avoid LC-MS Source Contamination by Using High Quality CHROMASOLV Solvents and Blends, Supelco Reporter, Vol. 25.2: pgs. 8-9.



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### + Featured Products

| Cat. No.  | Description                                     |
|---|---|
| <b>LC-MS CHROMASOLV Solvents</b>                |   |
| 39253   | Water   |
| 34967   | Acetonitrile                                    |
| 34966   | Methanol  |
| 34965   | 2-Propanol                                      |
| 34972   | Ethyl acetate                                   |
| <b>LC-MS CHROMASOLV Solvent Blends</b>          |   |
| 34978   | Water with 0.1% TFA                             |
| 34976   | Acetonitrile with 0.1% TFA                      |
| 34974   | Methanol with 0.1% TFA                          |
| 34668   | Acetonitrile with 0.1% formic acid              |
| 34670   | Acetonitrile with 0.1% ammonium acetate         |
| 34676   | Acetonitrile with 0.1% formic acid and 0.1% TFA |
| <b>LC-MS CHROMASOLV Mobile Phase Additives*</b> |   |
| 40967   | Trifluoroacetic acid                            |
| 56302   | Formic acid                                     |
| 49199   | Acetic acid                                     |
| 55674   | Ammonium formate                                |

\* puriss. p.a.

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