The Next Evolution of Isocyanate Sampling
ASSET™ EZ4-NCO Dry Sampler

ILLICIT BATH SALTS IN URINE

PESTICIDE DETERMINATION IN ORANGES Supel QuE QuEChERs
Dear Colleague:

Since the 1930’s, our lives have changed for the better with the commercialization of polyurethane materials. Polyurethanes are used in paints, coatings, adhesives, fibers and spray foam applications, to name a few. They have also replaced metal and plastic parts; increased the life of mechanical equipment and reduced downtimes. Their many valuable physical properties such as abrasion resistance, weather resistance, tear resistance; oil and solvent resistance, electrical properties and flexibility have given us a better quality of life.

Due to the wide range of uses of polyurethane materials, employees who manufacture, convert, or apply these materials are at risk for exposure to isocyanate compounds. Isocyanates are a family of highly reactive, low molecular weight chemicals used in the production of polyurethane materials and are known to cause respiratory disorders such as asthma, irritation of the mucous membranes, skin inflammation and even death. Current sampling methods are inadequate to truly estimate workplace exposure of these compounds, due to incomplete derivatization of particles, field desorption, low capacity, poor shelf-life and stability, and inability to truly assess exposure during an eight-hour work shift.

Our new and innovative ASSET™ EZ4-NCO Dry Sampler for Isocyanates solves many issues of existing sampling devices. In 2011, we partnered with the inventors of the sampler, Drs. Gunnar Skarping and Marianne Dalene from the Institutet För Kemisk Analys Norden AB (IFKAN) in Hasselholm, Sweden. The ASSET EZ4-NCO sampler complies with ISO 17734-1: Determination of organonitrogen compounds in air using LC-MS Part 1: Isocyanates using dibutylamine derivatives. It is fully validated, easy-to-use and provides the ultimate sensitivity not capable with other sampling devices. We offer a full complement of calibration standards, deuterated internal standards, chemicals and analytical columns to support the ASSET Sampler.

We invite you to learn more about our innovative ASSET EZ4-NCO Dry Sampler by scanning the QR Code below or visiting sigma-aldrich.com/asset.

Kind regards,

Kristen Schultz
Air Monitoring Product Manager
Supelco
kristen.schultz@sial.com

Cover Photo:
New ASSET EZ4-NCO: A significant advance in industrial hygiene monitoring for work place exposure to isocyanates.

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 sigma-aldrich.com/analytical
Analysis of Isocyanates Using the ASSET™ EZ4-NCO Dry Sampler

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kristen.schultz@sial.com

Introduction

Isocyanates are used as a raw material to produce a number of products such as automotive paints, rigid and spray foam insulation and furniture, to name a few. Personal exposure can occur while the products made with isocyanates are being applied, or when the materials are removed by grinding or thermal degradation. Workers who are exposed to these compounds are at risk for respiratory disorders and asthma. The highly reactive nature of the isocyanate compounds and the low occupational exposure limits put high demands on both sampling and analytical techniques for monitoring of isocyanates in air.

The most common devices for sampling isocyanates are impingers and impregnated filters. Impingers are the least desired for personal sampling due to the risk of exposure to solvent vapors during sampling. There are also other issues such as glass breakage and difficulty shipping the needed reagents before and after sampling. Existing impregnated filter devices are safer for the worker to wear but have their own known issues, such as:

- Incomplete derivatization of particle bound analytes
- Insufficient capacity and/or breakthrough resulting in underestimation of isocyanate concentration
- Limited range of isocyanate compounds
- Field reagent addition and/or desorption necessary/recommended
- Unstable reagents and limited shelf-life

ASSET EZ4-NCO sampler (Figure 1) uses dibutyl amine (DBA) derivatization of isocyanates according to ISO17734-1*, the DBA-derivatives are very stable and the derivatization takes place during air sampling and does not require the use of additional liquids and reagents. The special sampler design ensures that both the vapor phase and particulate isocyanates are captured and derivatized during sampling. ASSET samplers can be kept at room temperature as long as 4 weeks prior to extraction and analysis. The analysis of 10 isocyanates and di-isocyanate compounds can be performed simultaneously using LC-MS or LC-MS-MS detection methods.

Figure 1. ASSET EZ4-NCO Dry Sampler

In this study, we investigated the detection limits and the sensitivity of analysis after the ASSET samplers were spiked with a mixture of DBA-isocyanate derivatives representing an air concentration of 5 μg/m³ at an assumed sample volume of 24 L (recommended flow range of the sampler is 0.1-0.25 L/min).

Spiking and Extraction of Isocyanates From The Sampler

The DBA-isocyanate standard solution was used for spiking the ASSET sampler at 0.12 μg for each compound investigated; the extraction procedure was then applied. The procedure is briefly described in Figure 2. Internal Standards (Deuterated DBA-isocyanates) are added prior to the extraction to get better precision for quantitation and to compensate for any change in the DBA-isocyanate concentration during the extraction process.

Figure 2. Summarized Procedure for Extraction of DBA-Isocyanate Derivatives from the ASSET Sampler

Remove the filter medium from ASSET sampler and place into a test tube
Add 1 mM sulfuric acid, methanol, toluene and deuterated I.S.
Shake, sonicate and centrifuge
Collect the top toluene layer, add more toluene and repeat the extraction
Collect all toluene layers and evaporate to dryness
Dissolve the sample in 1 mL acetonitrile for LC-MS(-MS) analysis

LC-MS Analysis

Calibration standards are prepared by spiking a matrix solution with both DBA-isocyanates and deuterated internal standards. The calibration standards are then taken through the extraction procedure. The concentrations used for calibration in this study were 5-280 ng/mL with respect to underivatized isocyanates. The I.S. concentration used in the final sample was 20 ng/mL. Table 1 lists the quantitation limits that were established based on the concentration of the extracted samples by using LC-MS and LC-MS/MS.

Table 1

<table>
<thead>
<tr>
<th>Isocyanate</th>
<th>Quantitation Limit (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ISO 17734-1: Determination of organonitrogen compounds in air using LC-MS Part 1: Isocyanates using dibutylamine derivatives
Isocyanate-derivative would equate to 5 μg/m³ air concentration if a 24 liter air sample was taken.

Table 1. Limits of Quantitation for LC-MS and LC-MS/MS Methods

<table>
<thead>
<tr>
<th>Compound</th>
<th>MS (ng/mL)</th>
<th>MS/MS (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA</td>
<td>5.00</td>
<td>1.00</td>
</tr>
<tr>
<td>MIC</td>
<td>25.00</td>
<td>3.00</td>
</tr>
<tr>
<td>EIC</td>
<td>15.00</td>
<td>4.00</td>
</tr>
<tr>
<td>PIC</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Phi</td>
<td>3.00</td>
<td>0.40</td>
</tr>
<tr>
<td>HDI</td>
<td>2.00</td>
<td>0.50</td>
</tr>
<tr>
<td>2,6-TDI</td>
<td>3.00</td>
<td>0.30</td>
</tr>
<tr>
<td>2,4-TDI</td>
<td>3.00</td>
<td>0.30</td>
</tr>
<tr>
<td>IPDI-1</td>
<td>4.00</td>
<td>0.40</td>
</tr>
<tr>
<td>IPDI-2</td>
<td>4.00</td>
<td>0.40</td>
</tr>
<tr>
<td>MDI</td>
<td>3.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Figure 3. Chromatogram of DBA Standard

- Column: Ascentis Express C18, 15 cm x 4.6 mm, 2.7 μm particles (53829-U)
- Mobile phase: 95:5 acetonitrile:water w/ 0.05% formic acid
- Gradient: 40% to 70% B in 3 min, 70% to 90% B in 2 min, hold at 90% for 6 min, 70% to 90% in 3 min, hold at 90% for 3 min
- Flow rate: 1 mL/min
- Column temp.: 35 °C
- Injection: 2 μL
- Detector: AB3200 QTrap MS, ESI(+), SIM

Table 2. Average Recovery (%RSD) from 0.12 μg Spiked Sampler (n=3)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MS (ng/mL)</th>
<th>MS/MS (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA**</td>
<td>98% (5)</td>
<td>117% (1)</td>
</tr>
<tr>
<td>MIC</td>
<td>101% (2)</td>
<td>97% (3)</td>
</tr>
<tr>
<td>EIC</td>
<td>95% (5)</td>
<td>95% (3)</td>
</tr>
<tr>
<td>PIC</td>
<td>78% (15)</td>
<td>102% (8)</td>
</tr>
<tr>
<td>PHI</td>
<td>95% (4)</td>
<td>100% (4)</td>
</tr>
<tr>
<td>HDI</td>
<td>125%</td>
<td>116% (2)</td>
</tr>
<tr>
<td>2,6-TDI</td>
<td>108% (13)</td>
<td>101% (4)</td>
</tr>
<tr>
<td>2,4-TDI</td>
<td>107% (6)</td>
<td>102% (4)</td>
</tr>
<tr>
<td>IPDI-1</td>
<td>102% (4)</td>
<td>103% (5)</td>
</tr>
<tr>
<td>IPDI-2</td>
<td>102% (4)</td>
<td>100% (2)</td>
</tr>
<tr>
<td>MDI</td>
<td>89% (13)</td>
<td>111% (9)</td>
</tr>
</tbody>
</table>

Overall, the recoveries for spiked filter sample with the DBA derivatives were consistent ranging 77.9-125% with RSD’s <16% for MS and averages of 95.0-117% with RSD’s < 9% for MS/MS analytical methods. Spiking the sampler with 0.12 μg of each isocyanate-derivative would equate to 5 μg/m³ air concentration if a 24 liter air sample was taken.

Conclusions

We investigated the performance of the LC-MS and LC-MS-MS methods in the analysis of isocyanates using the new ASSET™ EZ4-NCO Dry Sampler. We found that the analytical method can successfully reach the quantitation limit for most isocyanates of 5 ng/mL in the final sample when LC-MS-MS analysis was used and the quantitation limit of 10 ng/mL when LC-MS analysis was used. These numbers can translate, respectively, to 0.21 μg/m³ and 0.42 μg/m³ in air if a 24 liter air sample was taken. Both LC-MS and LC-MS-MS analysis gave reasonably low LOQs for the method. All 11 compounds were well-resolved chromatographically using a 15 cm Ascentis Express C18 column. The acceptable recoveries for the isocyanates demonstrated the overall efficient performance of the extraction and analytical method.

Featured Products

- **Analytical Standards**
  - (in acetonitrile:methanol, 99:1, varied conc)
  - Ascentis Express C18, 15 cm x 4.6 mm, 2.7 μm particles 1 53829-U
  - DBA Isocyanate Mix 6 x 1 mL 40141-U
    - Isocyanic acid-di-n-butylamine (ICA-DBA), 1 μg/mL
    - Ethyl isocyanate-di-n-butylamine (EIC-DBA), 1 μg/mL
    - Hexamethylene diisocyanate-di-n-butylamine (HDI-2(DBA)), 1 μg/mL
    - Isophorone isocyanate-2(di-n-butyl amine) isomer 1 (IPDI-1), 0.28 μg/mL
    - 4,4’-Methylene diphenyl diisocyanate-2(di-n-butylamine) (4,4’-MDI-2(DBA)), 1 μg/mL
    - Methyl isocyanate-di-n-butylamine (MIC-DBA), 1 μg/mL
    - Phenyl isocyanate-di-n-butylamine (PhI-DBA), 1 μg/mL
    - Propyl isocyanate-di-n-butylamine (PIC-DBA), 1 μg/mL
    - 2,4-Toluene diisocyanate-2(di-n-butylamine) (2,4-TDI-2(DBA)), 1 μg/mL
    - 2,6-Toluene diisocyanate-2(di-n-butylamine) (2,6-TDI-2(DBA)), 1 μg/mL
- **d9-DBA Isocyanate Internal Standard Mix** 6 x 1 mL 40142-U
  - Isocyanic acid-di-n-butylamine-d9, ICA-DBA-d9, 1 μg/mL
  - Ethyl isocyanate-di-n-butylamine-d9, EIC-DBA-d9, 1 μg/mL
  - Hexamethylene diisocyanate-di-n-butylamine-d9, HDI-2(DBA-d9), 1 μg/mL
  - Isophorone isocyanate-2(di-n-butyl amine) isomer 1 (IPDI-1(DBA-d9)), 1 μg/mL
  - 4,4’-Methylene diphenyl diisocyanate-2(di-n-butylamine) (4,4’-MDI-2(DBA-d9)), 1 μg/mL
  - Methyl isocyanate-di-n-butylamine-d9, MIC-DBA-d9, 1 μg/mL
  - Phenyl isocyanate-di-n-butylamine-d9, PhI-DBA-d9, 1 μg/mL
  - Propyl isocyanate-di-n-butylamine-d9, PIC-DBA-d9, 1 μg/mL
  - 2,4-Toluene diisocyanate-2(di-n-butylamine) (2,4-TDI-2(DBA-d9)), 1 μg/mL
  - 2,6-Toluene diisocyanate-2(di-n-butylamine) (2,6-TDI-2(DBA-d9)), 1 μg/mL
- **DBA Isocyanates Mix Kit** 2 x 1 mL 40143-U
  - Included 1 mL of 40141-U and 1 mL of 40142-U listed above
Formaldehyde and Acetaldehyde Determination in Air Using Fully Automated On-Line Desorption and Analysis of DNPH Cartridges

Introduction
Airborne aldehydes and ketones are collected by passing air through a cartridge containing 2,4-dinitrophenylhydrazine (DNPH). Carbonyl compounds react with the DNPH to form hydrazones, which are immobilized on the cartridge. These compounds can be easily eluted from the cartridge with acetonitrile and analyzed by HPLC with UV detection. Traditionally, this analysis including the workup contains a series of manual steps, which can become time-consuming and could incur experimental error.

Method
In the discussed study, a required amount of air to be tested is passed through a Supelco® LpDNPH S10 cartridge. The manual desorption process is detailed as follows: Each cartridge is placed into a vacuum manifold and acetonitrile is used to elute the derivatized aldehyde or ketone from the cartridge. The extract is collected into a 5 mL volumetric flask and the flow of acetonitrile is stopped when 5 mL has been collected. This is performed by eye, checking that the top of the meniscus has reached the 5 mL mark. The volume of extract collected is critical as the concentration of the derivatized product will be dependent on the volume collected. The extract is then shaken to ensure that the solution is adequately mixed and subsequently filtered using a 0.2 micron PTFE filter, and a portion of this solution is then pipetted into a vial for HPLC analysis by UV detection.

To automate this method, a good seal is required between the DNPH cartridge and the injection needle from the syringe, which is attached to a Multi Purpose Sampler head (MPS). This will allow delivery of the solvent through the cartridge. Figure 1 shows a photograph of a DNPH cartridge with a sealing unit (transport adapter) and 0.2 micron filter. From previous experimental work, it has been found that the addition of 5.6 mL of acetonitrile to the cartridge will produce a 5 mL extract. A 5 mL extract is obtained as residual acetonitrile is left on the cartridge.

To automate this method, a good seal is required between the DNPH cartridge and the injection needle from the syringe, which is attached to a Multi Purpose Sampler head (MPS). This will allow delivery of the solvent through the cartridge. Figure 1 shows a photograph of a DNPH cartridge with a sealing unit (transport adapter) and 0.2 micron filter. From previous experimental work, it has been found that the addition of 5.6 mL of acetonitrile to the cartridge will produce a 5 mL extract. A 5 mL extract is obtained as residual acetonitrile is left on the cartridge. Figure 2 shows the instrumentation used to automate the DNPH process. In the development of this automated method, acetonitrile was collected from 20 different automated extractions and the precision for the volume of acetonitrile extracted was calculated to be 1.5% relative standard deviation on a 5 mL extract.

Airborne formaldehyde and acetaldehyde were collected onto various DNPH cartridges. The method to carry this out is proprietary. A transport adapter and filter were attached to the cartridge and placed onto the Anatune™ 300 unit. The cartridges were subsequently analyzed. Levels of the derivatized formaldehyde and acetaldehyde were quantified on the automated system using an external standard (T011/IP-6A Aldehyde/Ketone-DNPH Mix from Supelco).

After the DNPH cartridge has been aligned with an empty 10 mL vial, 5.6 mL of acetonitrile is added to the cartridge using a 5 mL syringe on an MPS head. Additional air is pushed through the cartridge. This is to maximize the amount of extract produced. The extract is then mixed and a 1 mL aliquot of the extract (using a 1 mL syringe) and is added to a sealed 2 mL HPLC vial which is sealed within the cooled tray. The extract is subsequently injected onto the HPLC. The LC method uses an Ascentis® Express C18, 5 cm x 4.6 mm I.D. column with a gradient starting at 30:70 acetonitrile:water (v/v) up to 95:05 acetonitrile:water (v/v) with UV detection at 365 nm. Full details of the method are given in Table 1. With the Prep-Ahead function, in the MAESTRO software, the automated sample preparation can be incorporated within the HPLC run time which is 12.5 minutes long.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Eluent A</th>
<th>% Eluent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>3.6</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>3.7</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>6.3</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>6.4</td>
<td>95</td>
<td>05</td>
</tr>
<tr>
<td>9.4</td>
<td>95</td>
<td>05</td>
</tr>
<tr>
<td>9.5</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>12.5</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

Up to 64 extractions including the HPLC analysis can be automated. The cooled tray, set to 4 °C, allows re-injection of the samples and standards if required. Figure 2 shows the instrumentation used together with a photograph of the how it is configured. Figure 3 shows a close up of the automated DNPH unit (Anatune 300).
Results

Figure 4 shows an example chromatogram of 0.075 ppm formaldehyde and acetaldehyde standard. Formaldehyde elutes at 3.7 minutes and acetaldehyde at 5.3 minutes. Six replicate injections of the 5 ppm standard were performed giving a percent relative standard deviation of 1.2% for formaldehyde and 1.3% for acetaldehyde. Levels of formaldehyde and acetaldehyde in the sample were consistent with the manual method. Figure 5 shows an example chromatogram of a sample containing formaldehyde and acetaldehyde. Table 2 shows the results obtained from five different sampled DNPH cartridges for formaldehyde and acetaldehyde using the automated process. Levels calculated from injecting an external 0.075 ppm standard of formaldehyde and acetaldehyde.

Table 2. Formaldehyde and Acetaldehyde Results for Samples Using the Automated Process Described in the Methods Section

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Formaldehyde (ppm)</th>
<th>Acetaldehyde (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A-1</td>
<td>0.95</td>
<td>0.45</td>
</tr>
<tr>
<td>Sample A-2</td>
<td>0.91</td>
<td>0.49</td>
</tr>
<tr>
<td>Sample B-1</td>
<td>0.85</td>
<td>0.44</td>
</tr>
<tr>
<td>Sample B-2</td>
<td>0.85</td>
<td>0.44</td>
</tr>
<tr>
<td>Sample C-1</td>
<td>0.61</td>
<td>0.38</td>
</tr>
<tr>
<td>Sample C-2</td>
<td>0.61</td>
<td>0.37</td>
</tr>
<tr>
<td>Sample D-1</td>
<td>0.76</td>
<td>0.46</td>
</tr>
<tr>
<td>Sample D-2</td>
<td>0.75</td>
<td>0.45</td>
</tr>
<tr>
<td>Sample E-1</td>
<td>0.80</td>
<td>0.43</td>
</tr>
<tr>
<td>Sample E-2</td>
<td>0.79</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Conclusion

Automating the extraction of LpDNPH S10 cartridges and putting it in-line with the HPLC analysis will significantly reduce manual labor using this technique and this will improve reproducibility of the method by reducing potential experimental errors by the operator. The automation and unattended operation of the method leads to high throughput for determining airborne formaldehyde and acetaldehyde.

+ Featured Products

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty.</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascentis® Express C18, 5 cm x 4.6 mm I.D., 2.7 μm particles</td>
<td>1</td>
<td>53826-U</td>
</tr>
<tr>
<td>LpDNPH S10 Cartridge</td>
<td>10</td>
<td>21026-U</td>
</tr>
<tr>
<td>Acetonitrile, CHROMASOLV®, gradient grade</td>
<td>1</td>
<td>34851</td>
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<tr>
<td>Water, for HPLC</td>
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<td>95304</td>
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<tr>
<td>T011/IP-6A Aldehyde/Ketone-DNPH Mix</td>
<td>1</td>
<td>47285-U</td>
</tr>
</tbody>
</table>
Ascentis® Express 5 micron: A Fused-Core® Particle HPLC Column for Faster HPLC Separations with No Backpressure Concerns

Wayne K. Way and Carmen T. Santanasia
wayne.way@sial.com

Ascentis Express 5 micron columns provide a new choice for improving the performance of traditional HPLC systems. Based on Fused-Core particle technology, Ascentis Express provides the benefits of high speed and high efficiencies without the concerns of smaller particle columns. Due to the high efficiencies at low backpressures, Ascentis Express 5 micron can benefit conventional HPLC users with no drawbacks.

The new Fused-Core particle consists of a 3.3 μm solid core and a 0.6 μm porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.6 μm) compared to conventional fully porous particles. The short diffusion path reduces axial dispersion of solutes and minimizes peak broadening. In fact, Ascentis Express 5 micron columns are able to achieve greater speed and efficiency than any other 5 micron particle based column. This means that Ascentis Express 5 micron becomes the standard column for all of your 5 micron based methods.

Beyond the new standard column for all 5 micron based methods, Ascentis Express 5 micron is an excellent choice for bioanalytical LC/MS methods. The Ascentis Express 5 micron excels under high flow rates and the high throughput demands of these methods. Furthermore, the large particle format provides an extremely rugged HPLC column. Available in 17 dimensions from 2 cm x 2.1 mm I.D. to 25 cm x 4.6 mm I.D. and two introductory phases, C18 and F5, the conversion of current methods is seamless.

Experimental Conditions

Shown in Figure 1 are HPLC chromatograms of a mix of polar and non-polar analytes using both the new Ascentis Express 5 micron and a popular traditional 5 micron column. The chromatogram illustrates the high speed and high efficiency capabilities of the Ascentis Express at low back pressures. Table 1 provides a summary of the performance parameters of Figure 1.

Results and Discussion

Conventional HPLC users can obtain an immediate increase in efficiency with this “drop-in” replacement column. No need to change column dimensions, flow rates, mobile phase conditions, or sample prep. The Ascentis Express 5 micron column can improve the performance of all of your HPLC systems.

Conclusion

Ascentis Express 5 micron with Fused-Core particles is an excellent choice for standardizing all 5 micron methods. The combination of low backpressures and high efficiencies allows for greater column length and flow rate flexibility than offered by any 5 micron column currently available.
Impact of Mobile Phase Additives on LC-MS Sensitivity, Demonstrated using Spice Cannabinoids as Test Compounds

Xiaoning Lu, Craig Aurand, and David S. Bell
xiaoning.lu@sial.com

The article stresses the importance of using the highest quality solvents and reagents, along with high efficiency HPLC columns and effective sample prep methods, to provide the best MS data.

Introduction
Part 1 of our Topics in LC-MS published in Reporter 30.2 looked at leveraging column selectivity in developing robust LC-MS methods.1 Here, Part 2 examines the important role of mobile phase components, specifically the ionic modifiers. In this article, the LC-MS/MS optimization for analysis of spice cannabinoids is demonstrated using a range of mobile phase additives for increased analyte response and selectivity. A four-pronged approach leveraging HPLC column selectivity, solvent purity, effective sample prep, and reference standards, was used to develop a method to rapidly isolate and identify spice cannabinoids from plasma.

Background
Optimized mobile phases for LC-UV methods are not necessarily transferable to methods employing MS detection. Usually when developing LC-UV methods the main consideration is achieving adequate retention and resolution of the analytes while maintaining low UV background. However, for LC methods using electrospray ionization (ESI) detection, one not only has to consider retention and resolution, but also how the mobile phase components impact ionization. LC-ESI mobile phase components also must be volatile, so they are efficiently vaporized in the ESI source of the MS inlet, and free of impurities that can contribute to the background, reduce sensitivity by forming adducts, or otherwise impact the sensitivity and overall quality of the MS experiment. Typical mobile phase modifiers used in LC-MS include ammonium formate and ammonium acetate buffers and formic, acetic, and trifluoroacetic acids.

Spice Cannabinoids: Current Analytical Interest
Synthetic cannabinoids (Spice, Figure 1) are a relatively new type of designer drug used as a pseudo-legal means to get a cannabis-type high.2 In early 2011, the US Drug Enforcement Agency (DEA) placed several of the most popular synthetic cannabinoids such as JWH-018 and JWH-073 on their Schedule 1 list, making the possession or consumption of these compounds illegal. However, new synthetic cannabinoids are continually being introduced as suppliers tweak the molecular structures.

The ability to rapidly and reliably identify the continually changing population of these compounds in the blood or urine of suspected users is a significant analytical challenge facing forensic chemists.

Figure 1. Representative Spice Compounds

JWH-073 metabolite JWH-250

JWH-200 JWH-073 JWH-018

Study of the Mobile Phase Ionic Modifier on MS Signal
Method development employed screening the various Ascentis® Express phases. The Ascentis Express F5 gave the best resolution of the five spice cannabinoids under isocratic conditions. It was, therefore, used for the experiments to determine which mobile phase modifiers enabled the highest ESI-MS response for the five spice cannabinoids. Mobile phases comprising 5 mM ammonium acetate, 5 mM ammonium formate, 0.05% acetic acid, or 0.05% formic acid in 50:50 water:acetonitrile were evaluated for chromatographic resolution along with ESI-MS response.

Impact of Solvents and Additives on Resolution and Relative Response
Figure 2 shows the resulting chromatograms of the additive study. Resolution of the five compounds was obtained under each of the four conditions tested. Elution orders were comparable except for one compound, JWH-200, which had longer retention under the acidic conditions. (This is possibly due to the known ion exchange character of the F5 and the basic nature of the morpholine functional group of JWH-200. Further experiments are required to fully elucidate the retention mechanism.)
Regarding analyte response, the formate systems gave higher analyte response than the acetate systems, with the ammonium formate conditions giving the overall best results when retention of JWH-200 is taken into account (Table 1). Although coelution was not a problem in this example, for LC-MS separations it is generally advisable to select a method based on analyte response rather than chromatographic resolution when analytes are readily discriminated by specific MS/MS transitions, as is the case with these spice compounds.

### Table 1. Relative Response (Peak Height) in ESI(+) Mode of the Spice Compounds under Different Mobile Phase Conditions

<table>
<thead>
<tr>
<th></th>
<th>5 mM Ammonium Formate</th>
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<th>0.05% Formic Acid</th>
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<tr>
<td>1. JWH-073 metabolite</td>
<td>1.0</td>
<td>0.28</td>
<td>0.97</td>
<td>0.81</td>
</tr>
<tr>
<td>2. JWH-200</td>
<td>1.0</td>
<td>0.27</td>
<td>0.23</td>
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<tr>
<td>3. JWH-250</td>
<td>1.0</td>
<td>0.61</td>
<td>1.02</td>
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</tr>
<tr>
<td>4. JWH-073</td>
<td>1.0</td>
<td>0.35</td>
<td>1.08</td>
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</tr>
<tr>
<td>5. JWH-018</td>
<td>1.0</td>
<td>0.38</td>
<td>1.21</td>
<td>0.99</td>
</tr>
</tbody>
</table>

### Adduct Formation and Solvent Purity

Adduct formation is also an important consideration when optimizing LC-MS methods for sensitivity. Adduct formation of the target analyte, primarily with sodium and potassium in ESI(+), can sacrifice the formation of target protonated analyte ions, thus decreasing the overall sensitivity of the method and complicating the spectrum. Previous studies have discussed the adduct formation phenomenon in detail, stressing the importance of using solvents and eluent additives that are free of unwanted ions, a quality hallmark of the LC-MS CHROMASOLV line for mass spectrometry from Fluka. This study employed solvents from the newest member of the CHROMASOLV line, LC-MS Ultra CHROMASOLV, which is intended for UHPLC and UHPLC-MS analysis because the solvents are additionally free of particulates.

### Analysis of Spice Compounds in Plasma Samples

The optimized MS method on standards was then used on a spiked plasma sample. Endogenous proteins and phospholipids were first removed using HybridSPE®-Phospholipid. (Details of the sample preparation method and mechanism of the HybridSPE-Phospholipid can be found in reference 4.) Figure 3 shows the resulting LC-MS/MS chromatogram of the five spice compounds extracted from plasma and resolved on the Ascentis® Express F5 column.

### Summary

Special consideration of mobile phase components must be made when optimizing methods for LC-MS applications. Using the socially problematic spice cannabinoids as the test case, the study reported here demonstrates the impact of various mobile phase modifiers on the separation, with the formate modifiers outperforming acetate in terms of MS signals (or sensitivity) and chromatographic resolution. This study is prime example of the benefit of using high-purity CHROMASOLV solvents with the HybridSPE-Phospholipid plates for effective sample cleanup, Ascentis Express UHPLC Columns for rapid, efficient separations, and Cerilliant standards for reliable characterization and quantification of analytes in biological matrices.
Fig 3. LC-MS/MS Analysis of Spice Compounds from Plasma on Ascentis® Express F5 after SPE using HybridSPE-Phospholipid

Conditions same as Figure 2, Panel B except as follows:
- sample/matrix: rabbit plasma, unfiltered K$_2$-EDTA, spiked with spice cannabinoids (5 ng/mL each)
- SPE: HybridSPE-Phospholipid, 96-well plate (575656-U)
- sample addition: to each well add 100 μL plasma, followed by a 300 μL of 1% formic acid in acetonitrile, agitate on orbital shaker for two minutes
- elution: attach collection plate and apply vacuum at 10” Hg for four minutes

1. JWH-073 metabolite
2. JWH-200
3. JWH-250
4. JWH-073
5. JWH-018

References

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</table>

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Isolation and LC-MS Characterization of Illicit Bath Salts in Urine

Craig Aurand
craig.aurand@sial.com

Introduction
With the rapid development of unregulated designer and synthetic compounds, the field of illicit drug testing has recently been met with a changing environment. Of most concern has been the development of a class of phenethylamine and cathinone compounds being marketed as “bath salts,” “transposed consumption,” “jewelry cleaner,” or “plant food.” Though sold as “not for human consumption,” these compounds are reported to generate stimulating effects similar to that of methamphetamine, heroin, and 3,4-methylenedioxymethamphetamine (MDMA also known as ecstasy).1,2 For a period of time, these compounds could be acquired legally through the internet and head shops due to a lack of direct legal control. In the US, both state and local governments have instituted bans on the sale of these bath salt compounds.2 Forensic testing facilities often experience difficulty in testing these compounds due to the fact that they are not detected under normal ELISA testing methods; additional more specific LC-MS methods are necessary. The challenge for LC-MS detection of these particular bath salts resides in three sets of isobaric compounds, which require chromatographic resolution for positive confirmation and quantitation. For example, in Figure 1, both butylone and ethylone have the same monoisotopic mass, making these compounds indistinguishable, even when using time of flight mass spectrometry (TOF-MS).

Figure 1. Structure of the Bath Salt Analytes

Figure 2. Analysis of Bath Salts on Ascentis® Express HILIC (Si)

column: Ascentis Express HILIC (Si), 10 cm x 2.1 mm, 2.7 μm (53939-U)
mobile phase: (A) 5 mM ammonium formate acetonitrile, (B) 5 mM ammonium formate water (98:2, A:B)
flow rate: 0.6 mL/min
pressure: 127 bar
column temp: 35 ºC
detector: MS, ESI(+), 100-1000 m/z
injection: 1 μL
sample: 200 ng/mL in acetonitrile

In this article, the analysis of bath salts from urine samples is demonstrated using polymeric solid phase extraction (SPE) sample preparation, followed by hydrophilic interaction liquid chromatography (HILIC) analysis with TOF-MS detection. HILIC conditions on the Ascentis Express HILIC (Si) phase are used for fast, high-resolution separation of nine synthetic bath salts. The polar basic nature of the bath salts makes these compounds prime subjects for HILIC separation. These compounds are difficult to retain on traditional reversed-phase C18 and even polar embedded stationary phases. Under reversed-phase conditions, high aqueous conditions are necessary to achieve retention, often leading to decreased ionization and detection in ESI-MS sources. High organic mobile phases are preferred for ESI-MS detection due to faster analyte desolvation and more efficient analyte ionization with their use. HILIC conditions not only enable increased retention of such polar basic compounds, but the high organic conditions also translate to increased response in ESI-MS. Figure 2 depicts the separation of all nine bath salts within six minutes on the Ascentis Express HILIC (Si) column.

Supel™-Select SCX SPE was used for the processing and sample cleanup of the urine samples. The Supel-Select SCX is a polymeric cation exchange absorbent, containing a strong cation exchange sulfonic acid functionality. The selective retention of the bath salts is based upon the ion exchange mechanism between the anion functionality of the Supel-Select SCX and the basic functionality of the bath salts.
The strong ionic interaction with the analytes enables high organic wash solvents to be used for displacement of endogenous matrix, while maintaining retention of the analytes. Elution of the bath salts is achieved with the addition of a basic organic solvent. Only with these basic conditions are the bath salts eluted from the Supel™-Select SCX material. This approach results in a highly clean sample.

Experimental
Urine samples were collected from a volunteer donor and verified not to contain illicit bath salts. Control water samples, along with urine samples, were spiked to a level of 100 ng/mL with each target analyte making up the bath salt mixture. To ensure full ionization of the analytes, spiked samples were treated with formic acid to a final concentration of 0.1% formic acid. For comparison purposes, two separate sample prep techniques were performed prior to LC-MS analysis: SPE using the Supel-Select SCX and a “dilute and shoot” technique. The Supel-Select SCX SPE method is detailed in Table 1. For the dilute and shoot sample, 500 μL of spiked urine was mixed with 500 μL of acetonitrile and analyzed directly by LC-MS.

Table 1. Sample Preparation Conditions for Supel-Select SCX Tube
Vacuum manifold was used to pull solvent through cartridge at a rate of 1 drop per 3 seconds.

- **condition:** 1 mL 1% formic acid acetonitrile
- **load:** 1 mL spiked water blank or urine
- **wash:** 1 mL water
- **wash:** 1 mL 1% formic acid acetonitrile
- **elute:** 2 mL 10% ammonium hydroxide in acetonitrile

Cartridge eluent was thoroughly mixed via vortex agitation, and a 1 mL aliquot of eluent was evaporated and reconstituted into 0.5 mL of acetonitrile.

Table 2. Analyte Recovery of Nine Bath Salts for the Supel-Select SCX Processed Samples and Spiked Urine Sample

<table>
<thead>
<tr>
<th>Sample (100 ng/mL standard)</th>
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<th>Spiked Urine Calculated Concentration (ng/mL)</th>
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<tr>
<td>MDVP</td>
<td>79.3</td>
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<td>Buphedrone</td>
<td>90.6</td>
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<td>3-Fluoromethcathinone</td>
<td>76.2</td>
<td>67.4</td>
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<td>Butylone</td>
<td>89.5</td>
<td>80.8</td>
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<tr>
<td>Ethylone</td>
<td>88.5</td>
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<td>4-Fluoromethcathinone</td>
<td>83.1</td>
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<tr>
<td>Methedrone</td>
<td>89.9</td>
<td>80.3</td>
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</table>

Results and Discussion
Table 2 demonstrates the recovery of all nine bath salts for the control samples and spiked urine sample. Using the external calibration method, recoveries greater than 65% were observed for all analytes except MDPV (43.7%). Figure 3 illustrates the detection of bath salts in the spiked urine sample after SPE sample cleanup. Notice there are no interfering peaks in the chromatogram, demonstrating the effectiveness of the SPE sample cleanup. As a reference, Figure 3 also depicts the monitored bath salt ions for the blank urine sample. Again, there are no interfering peaks that could cause irregularities in analyte detection.
Common practice for preparation of urine samples is just dilution with mobile phase prior to analysis. For comparison, an acetonitrile-diluted urine sample was analyzed directly to show the difference in analyte detection. Figure 3 illustrates the monitored bath salt ions for the “dilute and shoot” urine sample. The anticipated peak response for the dilution sample prep technique should be half that of the SPE method, but notice in the chromatogram there are no distinguishable peaks in this sample. Not only is there a significant amount of matrix interference, but also the high aqueous content of the mobile phase has diminished the HILIC separation. Solvent mismatch between the chromatographic system and the injected sample should always be considered, regardless of HILIC or reversed-phase chromatographic modes of separation. Sample preparation using the Supel™-Select SCX not only allows for effective removal of interfering sample matrix, it also allows for easy analyte exchange into an organic solvent.

Conclusion
The combination of the ion-exchange SPE with the HILIC separation provides a novel approach for the testing of problematic bath salt compounds. The isocratic Ascentis Express HILIC (Si) separation produces fast resolution of the isobaric compounds, thus, enabling the accurate quantitation of all nine bath salts. The Supel-Select SCX sample preparation method allows for efficient urine matrix removal while maintaining high analyte recovery. By utilizing ion-exchange mechanisms for sample cleanup, and taking advantage of the unique selectivity of chromatographic modes such as HILIC, analytical chemists can greatly improve the selectivity and sensitivity of their difficult bioanalytical applications.

References
1. Coppola, M.; Mondola, R. Synthetic cathinones: chemistry, pharmacology, and toxicology of a new class of designer drugs of abuse marketed as “bath salts” or “plant food” Toxicology Letters 2012, 211, 144-149.

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The Potential of SPE for Fractionation of Serum Metabolome into Phospholipids and Other Metabolites

Contributed Article

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Tracy Ascah
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Introduction

The high concentration of phospholipids (PLs) in blood serum is a frequent limitation in metabolomic analysis based on mass spectrometric detection, mainly owing to their ionization suppression effects. Thus, PLs mask the detection of less-abundant metabolites that may be potential biomarkers in clinical applications. This justifies a fractionation step for their removal when dealing with analysis of less-abundant metabolites. On the other hand, metabolism of PLs has been closely associated with different pathologies, such as obesity, cancer disorders, atherosclerosis, diabetic nephropathy, kidney failure, arterial hypertension, neurodegenerative and neurological disorders such as Alzheimer’s and Niemann-Pick’s diseases, metachromatic leukodystrophy, as well as multiple sclerosis, among others.1 For this reason, the determination of PLs is of enormous clinical interest.

PL Enrichment vs. Interference Removal

Two important points can be derived from the above discussion: First, when PLs are the target analytes, it is necessary to isolate them from other sample components, such as proteins, sugars, and other small molecules, that interfere with the chromatography of the PLs. Second, removal of PLs is necessary with MS detection when they are not the target analytes.

Among the techniques that are used in the clinical field for analyte enrichment or interference removal, solid-phase extraction (SPE) is arguably the most popular. This is due to the high efficiency, easy handling, affordable price, and wide variety of commercially available sorbents with very different selectivities and particle compositions from which the analyst can choose. Versatility is also an important characteristic. One such sorbent that meets these criteria is HybridSPE®-Phospholipid.

The purpose of this study was to investigate the potential of HybridSPE-Phospholipid for the selective isolation and characterization of two serum fractions: one that contains all of the PLs in the sample (an enrichment application), and one where the PLs are removed to enable determination of other, non-PL, metabolites (an interference removal application).

Experimental

An Agilent 1200 Series LC system on-line coupled to an Agilent 6540 UHD QqTOF hybrid accurate mass spectrometer equipped with an electrospray ionization (ESI) source was the platform for this analysis. MassHunter Workstation software (Agilent) was used to process raw MS data, including feature extraction, molecular formula generation, and database searching.

HybridSPE-Phospholipid Method

Both cartridge and plate formats of the HybridSPE-Phospholipid possess a top frit that acts as a filter for the physical retention of precipitated proteins while allowing metabolites to pass through. The top frit also impedes flow of the sample before vacuum application. The bed comprises zirconia-coated silica particles that selectively retain the PLs. The cartridge format was used in this study with the following protocol:

1. Load 300 μL of serum and 700 μL of 1% (v/v) formic acid in acetonitrile to the top of the HybridSPE-Phospholipid cartridge (Supelco Cat. No. 55269-U). The acetonitrile acidified with 1% formic acid added to the serum not only acts as a precipitation agent, but also as a modifier to inhibit the interaction of most acidic compounds with Zr sites on the particles.
2. Vortex cartridge 30 seconds, rest cartridge for 2 minutes.
3. Apply vacuum, collect fraction FA1 (non-retained, flow-through).
4. Add 1 mL of 1% (v/v) formic acid in acetonitrile to the cartridge, apply vacuum, collect fraction FA2.
5. Add 1 mL pure acetonitrile to the cartridge, apply vacuum, collect fraction FA3.
6. Add 1 mL of PL elution solvent (methanol, ethanol, or acetonitrile) containing 5% (v/v) ammonium hydroxide to the cartridge, apply vacuum, collect PL fraction.

LC-QqTOF MS/MS Analysis of Serum Fractions

All chromatographic parameters are described in Figure 1. A 20 μL aliquot of the prepared sample was injected into the LC and separated on a reversed-phase (C18) column. Two different chromatographic gradient profiles were programmed for independent analysis of the individual (FA1, FA2, and FA3), combined (FA), and PL fractions. Accurate mass spectra were acquired in the m/z range 100-1100, typical for metabolomics experiments, at 3.5 spectra/sec. Analyses were carried out both in positive and negative ionization modes with the appropriate continuous internal calibration. Collision energy was varied from 15 to 30 eV to obtain different MS/MS profiles which should support PL identification.
Results and Discussion

The strategy selected for this fractionation approach was based on the selective retention capability of PLs on the zirconia-coated silica particles that comprise HybridSPE-Phospholipid, and absence of retention by those particles of the wide range of basic, neutral, and acidic compounds (e.g., metabolites) in serum. The packed-bed filter/frit assembly acts as an effective filter to facilitate the simultaneous removal of both PLs and precipitated proteins during the extraction process.

The flow-through portion, FA1, together with the two rinsing solutions, FA2 and FA3, showed total absence of PLs indicating the HybridSPE-Phospholipid sorbent was very effective at removing PL interferences. Additionally, both negative and positive ionization modes showed fractions FA2 and FA3 to be essentially devoid of molecular entities of any type. This demonstrated an important feature of the HybridSPE-Phospholipid sorbent: It does not deplete the sample of compounds other than phospholipids. A caveat, however, the acetonitrile crash solvent must be acidified with 1% (v/v) formic acid to inhibit the interaction of most acidic compounds with Zr sites on the particles.

Elution of PLs from the cartridge was assayed by methanol, ethanol, or acetonitrile [in all cases the eluent contained 5% (v/v) ammonium hydroxide as modifier]. Figure 2A shows the Venn diagram of the molecular entities detected in the eluates using the negative ionization mode, in which the better performance of methanol as eluent is clear.

The effective fractionation of PLs and non-PLs metabolites in serum is shown by the Venn diagram in Figure 2B. The two fractions had no molecular entities in common. This behavior is corroborated in Figure 1, which corresponds to the ion chromatograms extracted from LC-TOF/MS monitoring of lysophosphatidylcholine (LPC) 18:0 (m/z 568.3619) and arachidonic acid (m/z 320.2351) in the FA and methanol-eluted fractions in negative ionization mode. The target PL was not detected in the FA, thus supporting the highly effective retention of PLs by the HybridSPE-Phospholipid cartridge. On the other hand, other metabolites elute unretained with the flow-through sample portion and rinsing solutions, free from PLs.

Figure 1. Extracted Ion Chromatograms from LC–TOF/MS monitoring of LPC 18:0 (m/z 568.3619) and Arachidonic acid (m/z 320.2351) in FA and Methanol-Eluted Fractions (negative ionization mode)

- **Sample/Matrix**: Human serum
- **SPE Tube**: HybridSPE®-Phospholipid cartridge
- **Sample Addition**: 300 μL serum, 700 μL 1% (v/v) formic acid in acetonitrile
- **Elution**: FA1: Flow-through from sample addition; FA2: 1 mL 1% (v/v) formic acid in acetonitrile; FA3: 1 mL acetonitrile; PL: 1 mL methanol, ethanol, or acetonitrile, each with 5% (v/v) ammonium hydroxide
- **Column**: C18, 10 cm x 4.6 mm I.D., 3 μm particles
- **Mobile Phase**: (A) water; (B) acetonitrile, both containing 0.1% (v/v) formic acid
- **Gradient (FA fraction)**: 4% B for 2 min, to 100% B in 18 min, held at 100% B for 5 min
- **Gradient (PL fraction)**: 20% B for 2 min, to 100% B in 16 min, held at 100% B for 7 min
- **Flow Rate**: 0.8 mL/min
- **Column Temp.**: 25 ºC
- **Injection**: 20 μL
- **Detector**: MS
- **MS Conditions**: nozzle, capillary and focusing voltage set at ±2 kV, 3.5 kV and 175 V; fragmentor, skimmer and octapole voltages were fixed at 175, 65, and 750 V; nebulizer gas at 40 psi, drying gas flow rate and temperature were 10 L/min and 325 °C

Figure 2. Venn Diagrams with the Count of Molecular Entities Detected

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<tr>
<td>FA</td>
<td>18</td>
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<tr>
<td>Ethanol</td>
<td>24 entities</td>
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Figure 2A. After selective SPE of PLs and elution with 5% ammonium hydroxide in methanol, ethanol, or acetonitrile, showing methanol most effective PL eluant

Figure 2B. FA (combined FA1-3) and methanol-eluant PL fractions showing no molecular entities in common and underscores the selectivity of the method
Conclusion
From these results, it can be concluded that this SPE protocol using HybridSPE®-Phospholipid followed by LC-MS/MS is useful for global metabolome analysis by fractionation, here into non-PL and PL metabolites. Therefore, the multipurpose use of HybridSPE-Phospholipid cartridges commonly used for interference removal has been demonstrated.

Complete details on this research and the PLs detected and confirmed by MS/MS, can be found in the reference.

Reference

Acknowledgments
The Spanish Ministerio de Ciencia e Innovación (MICINN), FEDER European Program and Junta de Andalucía are thanked for financial support through projects CTQ2009-07430 and FQM2010-6420. F.P.C. is also grateful to the MICINN for a Ramón y Cajal contract (RYC-2009-03921).

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96-well plates were not used in this study.
Clinical Application of SPME: Analysis of VOCs in Exhaled Breath as Cancer Biomarkers

Introduction
Characteristic odors in breath have long been used as a tool for medical diagnosis. Familiar examples are diabetes with the odor of overripe apples, renal diseases with the fishy smell of amines and ammonia, and dental or liver diseases with the cabbage-like odor of organic sulfides. Linus Pauling applied formal science to the analysis by using gas chromatography (GC) to detect volatile organic compounds (VOC) in breath. More recently, Michael Philips and his Menassia coworkers focused on determination of breath compounds that are attracting attention in clinical and toxicological analysis. Although breath analysis is of great importance in disease detection, toxicology, and the study of metabolic processes, its use by doctors and clinicians as a diagnostic tool is a lost art.

The classes of VOCs that can be present in exhaled breath include hydrocarbons, alcohols, ketones, aldehydes, esters, and organic sulfides. The determination of VOCs in exhaled air requires the detection of very low concentrations. Hence, the analytical methods employed must include a preconcentration step. The common preconcentration methodologies currently utilized for VOCs are sorption onto an adsorbent and cold trapping.

However, solid phase microextraction (SPME) is a viable alternative to these methods, as will be shown here. SPME has been widely used for the determination of volatile organic compounds in various matrices, including exhaled breath. Compared to other preconcentration techniques, SPME is simple, inexpensive, and solvent-free. It is fully automatable, and no thermal desorption unit or modifications to the GC instrument are necessary. Compatible with all GC systems, SPME can be used by practically every laboratory. The objective of this study was to use SPME with GC-MS analysis to identify volatile biomarkers of lung cancer.

Experimental
Breath samples were collected from ten healthy volunteers and twelve patients with lung cancer. Each participant provided via questionnaire their age, sex, other diseases, medications, smoking habits, and composition of recent meals. Breath samples were collected in 1 L Tedlar bags which were kept at a constant 25 °C. A gas standard containing the compounds of interest was made by vaporizing a liquid mixture of the compounds in a glass bulb. A defined volume of the mixture was transferred into the Tedlar bag prior to sampling. During extraction, the CAR/PDMS SPME fiber was introduced into the bag containing breath sample or gas standards through a septum and exposed for 15 minutes. Ambient air samples were collected for background. External calibration was employed. The fiber desorption and sample analysis (GC-MS) conditions are shown in Figure 1.

Figure 1. GC-MS Analysis of VOC in Breath from Lung Cancer Patient after SPME Using CAR/PDMS Fibers

SPME Conditions
- sample: exhaled breath, 1 L in Tedlar bag
- fiber: Carboxen®/Polymethylsiloxane (CAR/PDMS), 75 μm film (57318)
- holder: manual SPME holder (57330-U)
- extraction: 15 min at 25 °C
- desorption: 1 min at 220 °C

GC-MS Conditions
- inj. temp.: 200 °C
- oven: 40 °C (2 min), 10 °C/min to 140 °C, 5 °C/min to 270 °C (3 min)
- carrier gas: helium, 40 cm/sec, constant
- injection: splitless 1 min, then split at 35:1
- detector: MS, full scan, m/z 15-220, rate 3.46 scans/sec, EI ion source and transfer line temp: 220 °C

1. Ethanol
2. Acetonitrile
3. Acetone
4. 2-Propanol
5. Isoprene
6. Pentane
7. Methacrolein
8. Methyl vinyl ketone
9. Ethyl acetate
10. 2-Methylpentane
11. 3-Methylpentane
12. Hexane
13. Toluene
14. Hexanal
15. Limonene
Results

The linearity, precision, and detection limits for VOCs determination in human breath are presented in Table 1. The relative standard deviation (RSD) was in the range of 3.4% to 9.4%. Linear regression coefficient values ($r^2$) were close to 1. The lowest LOD values obtained for hydrocarbons varied from 0.3 to 0.49 ppb.

Table 1. Validation Parameters for Volatile Organic Compounds (VOCs)*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Linear Range (ppb)</th>
<th>$r^2$</th>
<th>%RSD</th>
<th>LOD (ppb)</th>
<th>LOQ (ppb)</th>
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<tr>
<td>Acetone</td>
<td>1.6 - 920.7</td>
<td>0.991</td>
<td>8.9</td>
<td>0.54</td>
<td>1.62</td>
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<tr>
<td>Acetonitrile</td>
<td>2.3 - 234.0</td>
<td>0.996</td>
<td>3.4</td>
<td>0.75</td>
<td>2.25</td>
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<td>Ethyl acetate</td>
<td>1.3 - 136.7</td>
<td>0.995</td>
<td>4.6</td>
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<td>Methyl vinyl ketone</td>
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<td>9.90</td>
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<td>3-Methylpentane</td>
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<td>Ethanol</td>
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<td>4.5</td>
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<td>1.63</td>
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<td>4.5</td>
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<td>6.2</td>
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<td>Hexane</td>
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<td>3.4</td>
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<td>Isopropanol</td>
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<td>0.998</td>
<td>3.7</td>
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<tr>
<td>Pentane</td>
<td>1.5 - 150.0</td>
<td>0.998</td>
<td>5.2</td>
<td>0.49</td>
<td>1.47</td>
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<td>Methacrolein</td>
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<td>0.994</td>
<td>4.8</td>
<td>0.33</td>
<td>0.99</td>
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* Conditions same as Figure 1 except sample is a mixture of VOC standards. n=3. Bolded compounds had statistical significance.

Figure 1 shows a typical GC-MS chromatogram of breath from a lung cancer patient. Analysis of exhaled air from healthy volunteers and cancer patients identified seventeen volatile compounds, mainly hydrocarbons, ketones, aldehydes, and alcohols. Similar compounds were found in both healthy and cancer patients, except furan derivatives which are considered to be markers for tobacco smoking. Statistical tests were applied to distinguish cancer patients from the healthy control group. The VOC concentration data were log-transformed and tested for normality using the Shapiro-Wilks W test, (p<0.05). Full details of the statistical analysis is beyond the scope of this brief report; however, they are available upon request.

Summarizing: Although there was variation between the patients and not all patients exhibited the same biomarker pattern, four compounds stood out statistically from the others: methyl vinyl ketone, 1-propanol, 2-propanol, and o-xylene. These compounds showed statistically higher levels in cancer patients compared to the healthy control group.

Conclusion

This brief report is intended to demonstrate the potential of solid phase microextraction (SPME) as a clinical research tool, in this case toward the extraction from human breath of VOCs associated with lung cancer. By using SPME with GC-MS analysis and applying rigorous statistical methods, we found the VOC profiles between a small set of healthy individuals and those diagnosed with lung cancer were significantly different. These promising findings would necessarily be followed up with studies on larger populations for definitive associations. The SPME-GC-MS method presented here had the requisite linearity and sensitivity, and could be easily adopted by laboratories as an investigational tool into biomarker discovery, among many other applications relevant to the clinical and biochemical fields.

Acknowledgments

This research work was supported by Grant N N 204 026238 (2010-2013).

References


Featured Products

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<tr>
<td>SPME fiber holder for use with manual sampling</td>
<td>57330-U</td>
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<tr>
<td>Capillary GC Column</td>
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</table>

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96-well plates are also available, but were not used in this study.
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**Featured Products**

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</tr>
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<tr>
<td>2 mL amber glass with graduated marking spot, PTFE/silicone with slit</td>
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<td>2 mL amber glass with graduated marking spot</td>
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Determination of Additives in Beverages Using Ascentis® Express Columns

Olga Shimelis, Hugh Cramer, and Michael D. Buchanan
mike.buchanan@sial.com

Introduction

Beverages, such as sodas and energy drinks, can include a number of polar ingredients, which are easily soluble in the water matrix of the drinks. These ingredients include sweeteners (sugars and sugar substitutes), caffeine, vitamin supplements, amino acids, organic acids, and plant extracts. Because the analytes are already in solution, there is no need for extensive sample preparation. Dilution followed by direct injection into an HPLC is typically suitable.

In this article we present two beverage applications using Ascentis Express HPLC columns. Ascentis Express columns offer faster HPLC on any system. One benefit is their ability to produce the resolution, efficiency, and speed on conventional HPLC systems that is associated with the use of sub-2 micron columns on a UHPLC system, without generating high backpressure. Column chemistries (RP-Amide and HILIC) were selected for this article based on their enhanced performance with polar compounds in comparison to C18.

Analysis of Diet Soda

Artificial sweeteners are often used in drinks in specific combinations to mimic the sweetness of natural sugars. They contain less energy than sugar, and are added to maintain the taste without increasing the caloric value. In addition to sweeteners, sodas can contain preservatives, which are added to inhibit microbial growth and to prolong the shelf-life of the drinks. Because artificial sweeteners and preservatives are both considered additives, they are often regulated. Therefore, their identities and concentrations should be determined. Figure 1 shows the HPLC separation of 3 artificial sweeteners (acesulfame, aspartame, and neotame), two preservatives (benzoic acid and sorbic acid), and caffeine. In this case, the RP-Amide column was used. Amide-functional groups in the RP-Amide column provide different selectivity than alkyl-only phases, such as C18. The selectivity differences are most noticeable with polar compounds, like those in this application. The separation of all major components on the Ascentis Express RP-Amide column was achieved in less than 1 minute using a conventional HPLC system with acceptable backpressure (3200 psi/220 bar).

Analysis of Caffeinated Energy Drink

Energy drinks contain a variety of marketable ingredients, such as sugars, vitamins, and caffeine. Like sodas, they also typically contain preservatives. The resulting chromatogram from the analysis of an energy drink on an Ascentis Express HILIC column is shown in Figure 2. HILIC chromatography is suitable for the retention and separation of hydrophilic compounds based on differences in their polarities, whether these analytes are acidic, basic, charged, or neutral. Both UV and ELSD were used to detect different types of compounds; the ELSD allowed the detection of the non-UV absorbing sugars.

Figure 2. HPLC Analysis of Sugars, Vitamins, Preservatives, and Caffeine in an Energy Drink

- Column: Ascentis Express HILIC, 10 cm x 3.0 mm i.d., 2.7 μm particles (S5970-U)
- Mobile phase: (A) 100 mM ammonium acetate, pH 5.0; (B) water; (C) acetonitrile (9:1:90, A:B:C)
- Flow rate: 0.6 mL/min
- Pressure: 815 psi
- Column temp.: 35 °C
- Detector: UV, 254 nm or ELSD, 55 °C, 3.5 bar nitrogen
- Injection: 2 μL commercial energy drink diluted 1:9 in acetonitrile

1. Caffeine
2. Niacinamide (vitamin B3)
3. Pyridoxine hydrochloride (vitamin B6)
4. Benzoic acid
5. Sorbic acid
6. Riboflavin (vitamin B2)
7. Fructose
8. Glucose
9. Sucrose
10. Taurine

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The conditions used an MS-friendly mobile phase, and yielded rapid analysis (under 2 minutes) and low backpressure (815 psi). Because of these benefits, a HILIC column should always be considered for analysis of beverage components.

Conclusion
These brief examples demonstrate the application of two Ascentis® Express columns for the analysis of beverages. Two column chemistries, RP-Amide and HILIC, both amenable to the analysis of polar compounds, were successfully used to resolve several beverage components, including sugars, vitamins, sweeteners, preservatives, and caffeine. Ascentis Express columns provided rapid separation using conventional HPLC systems without the major increase in backpressure associated with sub-2 micron columns. Selectivity differences between the two columns can be further employed to alter elution order and compound retention.

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Analysis of Methylimidazoles in Caramel Colored Carbonated Beverages

Craig Aurand and Michael D. Buchanan
mike.buchanan@sial.com

Introduction
Caramel colorings are used as additives in a broad range of food and beverage products to impart a desired color, but have no nutritional or preservative function. Recently, the potential hazard to humans of ammonia- and ammonia-sulfite-process caramel colorings was raised, because they contain the by-product 4-methylimidazole, which is a potential carcinogen.1 The methylimidazole compounds are difficult to analyze due to their polar nature and low molecular weight. Traditional reversed phase techniques are unsuccessful in retaining these small polar compounds. Therefore, most HPLC methods utilize ion-exchange resins for analysis. Another common method involves GC analysis after the analytes first undergo a derivatization step. The purpose of the work shown in this article was to develop a simple and fast analytical method to determine the levels of 2-methylimidazole and 4-methylimidazole in caramel colored carbonated beverages.

Experimental
A 16 oz individual plastic container of three popular diet colas were obtained. 10 mL aliquots of each sample were then placed into separate 40 mL glass vials and shaken vigorously to expunge the dissolved carbon dioxide. 1 mL aliquots of each degassed sample were then placed directly into separate 2 mL HPLC autosampler vials. Samples were analyzed directly with no further sample treatment. For quantitative purposes, a calibration range was developed for each analyte at levels of 50, 100, 200 and 300 ng/mL. Standards were diluted in acetonitrile. Due to the ionic nature of the methylimidazoles, peak tailing is an issue on silica based materials, and low pH conditions are necessary to help minimize excessive ionic interactions. In this study, the use of the HILIC chromatographic technique was selected due to its ability to retain and separate hydrophilic compounds by differences in polarity, whether the analytes are acidic, basic, charged, or neutral. In fact, HILIC often provides retention and selectivity that reversed phase and normal phase techniques lack. Chromatograms of a calibration standard and the three sample extracts analyzed on an Ascentis® Express HILIC column are shown in Figures 1-4.

Figure 1. Standard, Each Analyte at 200 ng/mL in Acetonitrile

| Column | Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 μm particles (53939-U) |
| Mobile phase | 2 mM ammonium formate (95:5 acetonitrile-water) pH 4.4 titrated with formic acid |
| Flow rate | 0.6 mL/min |
| Pressure | 130 bar |
| Column temp. | 50 °C |
| Detector | MS, ESI(+), m/z 50-800 |
| Injection | 1 μL |

1. 4-Methylimidazole
2. 2-Methylimidazole

Figure 2. Diet Cola 1
Y-axis enlarged to show detail.
Peak IDs and conditions are the same as Figure 1.

Figure 3. Diet Cola 2
Y-axis enlarged to show detail.
Peak IDs and conditions are the same as Figure 1.

Figure 4. Diet Cola 3
Y-axis enlarged to show detail.
Peak IDs and conditions are the same as Figure 1.
Results and Discussion

The Ascentis® Express HILIC phase was shown to be a suitable stationary phase, able to provide sufficient retention, plus good resolution and peak shapes, of 2-methylimidazole and 4-methylimidazole. Even with limited sample preparation (degassing), no co-eluting interference was observed in the elution range of either methylimidazole compound under full scan MS conditions. Interestingly, only 4-methylimidazole was observed in the three tested diet colas, with each having a slightly different concentration. Because each manufacturer uses a unique secret formulation for their cola with a different amount of caramel coloring from the others, it was expected that each would contain a distinctive quantity of methylimidazole.

Conclusion

The use of an Ascentis Express HILIC column offers a unique approach for analysis of the methylimidazole compounds. The direct analysis technique simplifies the sample workflow, and combined with HILIC chromatographic conditions, results in a simple and fast analytical method to determine the levels of 2-methylimidazole and 4-methylimidazole in caramel colored carbonated beverages.

Reference

1. Petition to Bar the Use of Caramel Colorings Produced With Ammonia and Containing the Carcinogens 2-Methylimidazole and 4-Methylimidazole, Center for Science in the Public Interest, February 16, 2011.

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Improve Pigment Removal and Decrease Matrix Interferences for Pesticide Determination in Oranges Using Supel QuE Z-Sep/C18 QuEChERS Sorbent

Olga Shimelis, Emily Barrey, and Jennifer Claus
jenner.claus@sial.com

Introduction
Although pesticides have proven to be invaluable in crop control, the same toxicity, stability, and mobility that enables them to effectively kill insects causes detrimental effects in humans and the environment. Worldwide pesticide use in modern agriculture has led to the manifestation of pesticide residues in the majority of fruits and vegetables. Because of the deleterious effects associated with pesticides, authorities have applied maximum residue limits (MRLs) on many types of produce for environmental and consumer health protection. Implementation of these MRLs has increased pesticide residue analysis globally.

The current importance of pesticide residue analysis has caused scientists to seek robust, inexpensive, and straightforward methods that produce quality results, for an extensive range of analytes and matrices at low detection limits. The development of the Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) sample preparation approach by Anastassiades and Lehotay, a dispersive SPE method utilizing bulk SPE material for sample cleanup, provides a method fitting the aforementioned criteria. The comparison of Supel™ QuE Z-Sep/C18, PSA/C18, and PSA QuEChERS sorbents, in terms of color removal and analyte recovery, is described herein for the cleanup of oranges prior to pesticide analysis.

Experimental
An orange was homogenized with the rind, and 4 replicate samples of 10 grams were weighed into separate 50 mL centrifuge tubes. Three samples were spiked at 50 ng/g and the fourth was left unspiked. Ten mL of acetonitrile was added to each tube and the samples were shaken for 1 minute. Contents of citrate extraction tubes were added to each sample and the tubes were shaken immediately for an additional minute. The tubes were then centrifuged at 3200 rpm for 5 minutes. Separate 0.7 mL aliquots of the acetonitrile layer from each sample were then transferred into separate cleanup tubes, containing Z-Sep (zirconia-coated silica) in combination with C18 was shown to enhance sample cleanup for complex matrices. The comparison of Supel™ QuE Z-Sep/C18, PSA/C18, and PSA QuEChERS sorbents, in terms of color removal and analyte recovery, is described herein for the cleanup of oranges prior to pesticide analysis.

Results and Discussion
The chromatogram in Figure 1 illustrates the LC-MS/MS analysis of 38 pesticides extracted from a spiked orange sample following sample cleanup with Z-Sep/C18 sorbent. In this experiment.
use of an Ascentis® Express C18 column yielded the necessary peak efficiencies to obtain sufficient resolution and elution of all 38 analytes in under 10 minutes, minimizing analysis time and increasing throughput. The visual comparison of extracts shown in Figure 2 illustrates that more thorough removal of color is achieved with Z-Sep/C18 sorbent, as opposed to PSA sorbent. In addition to reducing matrix interferences, the improved cleanup by Z-Sep/C18 sorbent can decrease column and instrument fouling, leading to an extended HPLC column life while reducing instrument downtime.

The bar chart in Figure 3 provides a visual comparison of selected analyte recovery after using Z-Sep/C18, PSA/C18, and PSA sorbents. Overall, the three methods produced similar recovery values for the majority of the pesticides tested. The Z-Sep/C18 sorbent provided recoveries superior for two of the pesticides tested, anilazine and naled, that were not detectable with PSA/C18 or PSA sorbents due to matrix effects. For a few pesticides, sethoxidim, clethodim, and edifenphos, matrix effects showed increased ion suppression in samples cleaned with PSA sorbent. In addition, carbophenothion exhibited more matrix enhancement with the PSA sorbent than with the Z-Sep/C18 sorbent.

Conclusion
Comparison of color removal and analyte recovery for pesticide residue analysis in orange extracts after the extracts underwent cleanup with Z-Sep/C18, PSA/C18, or PSA QuEChERS sorbent was performed. For a number of the pesticides analyzed, results showed the Z-Sep/C18 sorbent was superior to that of PSA/C18 and PSA sorbents. Results for the remaining pesticides were similar to those observed with both PSA/C18 and PSA sorbents, indicating that Z-Sep does not adversely bind any of the tested analytes. Decreased matrix effects were also observed with Z-Sep/C18 sorbent as compared to PSA/C18 and PSA sorbents. Additionally, Z-Sep/C18 sorbent provided improved color removal over PSA sorbent. These observations support the fact that Supel™-QuE Z-Sep/C18 has the potential to be a direct replacement for C18 and PSA phases in current methods.

References
7. EN15662:2008, Foods of Plant Origin - Determination of Pesticide Residues using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Cleanup by Dispersive SPE - QuEChERS Method.

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<tbody>
<tr>
<td>Supel-QuE QuEChERS Products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate extraction tube</td>
<td>50</td>
<td>55227-U</td>
</tr>
<tr>
<td>Z-Sep/C18 cleanup tube, 2 mL</td>
<td>100</td>
<td>55284-U</td>
</tr>
<tr>
<td>PSA/C18 cleanup tube, 2 mL</td>
<td>100</td>
<td>55288-U</td>
</tr>
<tr>
<td>PSA cleanup tube, 2 mL</td>
<td>100</td>
<td>55287-U</td>
</tr>
<tr>
<td>Empty centrifuge tube, 50 mL</td>
<td>50</td>
<td>55248-U</td>
</tr>
<tr>
<td>Ascentis Express HPLC Column (2.7 μm particles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18, 5 cm x 2.1 mm</td>
<td>1</td>
<td>53822-U</td>
</tr>
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</table>

Related Products

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>Acetonitrile, LC-MS CHROMASOLV®, &gt;99.9%</td>
<td>34967</td>
</tr>
<tr>
<td>Water, LC-MS CHROMASOLV</td>
<td>39253</td>
</tr>
</tbody>
</table>

Related Information

To learn more about Sigma-Aldrich® solutions for food analysts, visit sigma-aldrich.com/food
Removal of Detergents from Biological Product Matrices

J. Patrick Myers  
pat.myers@sial.com

Most modern biopharmaceutical research and manufacturing requires the use of one or more detergents. Detergents are amphipathic molecules having both hydrophilic and hydrophobic properties. These unique properties are used to alter the interactions between biological molecules in aqueous solutions.

Detergents are organized into broad classes based on the nature of the polar, hydrophilic part of the molecule. The classes include ionic (further broken down into negatively charged anionic and positively charged cationic), nonionic (uncharged), and zwitterionic (having both positively and negatively charged groups but with a net charge of zero). Under the proper conditions, detergents form micelles in water.

A micelle is a three dimensional structure formed when the concentration of a detergent increases in water beyond the amount that can form a monolayer on the surface. Micelles are typically spheres in which the hydrophobic part of the detergent molecules associate with each other and the hydrophilic parts of the molecules contact the water. The number of detergent molecules per micelle (aggregation number) and the range of detergent concentration above which micelles form (called the critical micelle concentration, CMC) are properties specific to each particular detergent. The critical micelle temperature (CMT) is the lowest temperature at which micelles can form. Below the CMT, detergent molecules exist in a crystalline form in solution and are not effective. Above the CMT, detergents form micelles and become effective in disrupting lipid-lipid interactions.

The molecules that make up biological membranes are also amphipathic. They are made up of polar “heads” attached to lipophilic, hydrophobic, “tails.” Stable membranes are formed when the “tails” of the molecules associate with each other in a bilayer sheet-like arrangement leaving the polar “heads” to contact the polar, aqueous milieu on both sides of the membrane. Additional molecules including proteins and cholesterol embedded in the lipid bi-layer are important to the flexibility and function of the membrane. The formation of detergent micelles is important because it allows the dispersion of water-insoluble, hydrophobic compounds into aqueous solutions. It also allows the disruption of biological membranes and extraction of proteins. The disintegration of membranes occurs by disrupting the lipid-lipid and lipid-protein interactions in the membrane.

In biological research and manufacture of biopharmaceutical products, detergents are used for many purposes including stabilizing proteins and nucleic acids during electrophoresis, dissolving membrane proteins, prevention of nonspecific binding in affinity purification and immunoassay procedures, control of protein crystalization and disruption of cell membranes to release membrane bound and intracellular proteins.

In biopharmaceutical production, the detergent must be removed from the product in the downstream processing activities. Methods from removing detergents include gel filtration, size exclusion, dialysis and hydrophobic adsorption chromatography. Data presented in Tables 1 and 2 demonstrate that pre-packed, sterile, endotoxin-free Porozorb™ cartridges can also be used to effectively remove detergents and other nonpolar, hydrophobic materials from biological preparations.

Porozorb cartridges are disposable and available in sizes appropriate for analytical to process scale purification schemes.

The Amberlite™ XAD-4 resin used in Porozorb™ cartridges is a proven technology that is highly effective in removing various detergents from cell culture media for biopharmaceutical applications such as vaccine production. Table 1 shows the capacity of various Porozorb cartridges for several common detergents used in biopharmaceutical applications. Table 2 demonstrates the time required to reduce a 0.1% concentration of one of those detergents, Triton® X-100, in a 10 liter solution to below detectable levels.

Table 1. Capacity of Porozorb Cartridges for Various Detergents

<table>
<thead>
<tr>
<th>Porozorb Cartridge</th>
<th>Bed Volume (mL)</th>
<th>ID (cm)</th>
<th>Length (cm)</th>
<th>Triton X-100 (g)/cartridge</th>
<th>SDS (g)/cartridge</th>
<th>CHAPS (g)/cartridge</th>
</tr>
</thead>
<tbody>
<tr>
<td>254</td>
<td>250</td>
<td>8</td>
<td>6.55</td>
<td>44</td>
<td>125</td>
<td>78</td>
</tr>
<tr>
<td>1004</td>
<td>1000</td>
<td>8</td>
<td>26.2</td>
<td>168</td>
<td>462</td>
<td>288</td>
</tr>
<tr>
<td>4004</td>
<td>4000</td>
<td>12.71</td>
<td>42.04</td>
<td>504</td>
<td>1400</td>
<td>873.6</td>
</tr>
</tbody>
</table>

* Dried to less than 1% moisture.

Table 2. The time required to reduce detergent in 10 liters of 0.1% Triton X-100 solution to below detection level and breakthrough volumes. Detergent detected by monitoring the absorbance at 275 nm, with the lowest detectable limit (0.05 = A275) indicating a positive result.

<table>
<thead>
<tr>
<th>Flow Rate 3.6 liter/hour</th>
<th>Flow Rate 10.3 liter/hour</th>
<th>Flow Rate 22.8 liter/hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed Volume (liter)</td>
<td>Breakthrough Volume (liter)</td>
<td>Recirculation Time (hour)</td>
</tr>
<tr>
<td>250</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>1000</td>
<td>none</td>
<td>9</td>
</tr>
<tr>
<td>4000</td>
<td>none</td>
<td>7</td>
</tr>
</tbody>
</table>
The Amberlite™ XAD-4 resin contained in the Porozorb™ cartridge is cleaned without the use of organics and tested for residues (FCR 21: 173.65) and organics. Each cartridge is packaged separately, and is accompanied by a Certificate of Analysis for sterility and endotoxicity. Sterility testing follows USP XXIII guidelines. Endotoxicity is tested using a validated LAL kit, with any detectable level of endotoxin (0.03 EU/mL) indicating a positive test. Porozorb cartridges can also be packed with a variety of other resins and media to meet your specific application needs. In addition, we can provide bulk materials and/or smaller pre-packed devices for method development through our custom services.
Explore the solutions within.

Discover complete application solutions for unique separation challenges.

Solutions within means digging deep to find the right solution, and never sacrificing on quality. Whether in-stock or highly customized, proprietary or ordinary, our products reflect consistent, leading-edge Supelco performance, and technical expertise.

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