SupelMIP®
Solid Phase Extraction

Molecularly Imprinted Polymers for the Highly Selective Extraction of Trace Analytes from Complex Matrices
What are Molecularly Imprinted Polymers?

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related target compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guides the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte(s).

MIPs are prepared by first mixing a template molecule that consists of a structural analog of the analyte(s) of interest with one or more functional monomers. As illustrated in Figure 1, the monomers form spontaneous complexes around the template. Upon complex formation, cross-linking monomers are then added with a suitable porogen (solvent that aids in the role in pore formation) to drive polymerization. An extensive wash procedure is used to remove the template from the polymer, leaving imprints or binding sites that are sterically and chemically complementary to the template.

How is Selectivity Improved Using SupelMIP SPE?

By careful design of the imprinting site, either by molecular modeling, experimental design, or screening methods, the binding cavities can be engineered to offer multiple interactions with the analyte(s) of interest (Figure 2). Multiple non-covalent interaction points (ion-exchange, reversed-phase with polymer backbone, and hydrogen bonding) between the MIP phase and analyte functional groups allow for stronger and more specific analyte retention. Improved selectivity is then introduced through the use of harsher wash conditions during sample prep methodology (Figure 3). Because extraction selectivity is significantly improved, lower background is observed allowing analysts to achieve lower detection limits relative to other less selective sample prep techniques (Table 1).

Table 1. Relative Selectivity of Various Sample Prep Techniques

<table>
<thead>
<tr>
<th>Non-Selective</th>
<th>Protein Precipitation</th>
<th>Dirty Extracts</th>
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<tr>
<td>Liquid-Liquid Extraction</td>
<td>Hydrophobic Resin SPE</td>
<td>Supported LLE</td>
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<tr>
<td>C18-C2 Silica Based SPE</td>
<td>Mixed-Mode SPE</td>
<td></td>
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<tr>
<td>Highly Selective</td>
<td>SupelMIP SPE</td>
<td>Clean Extracts (Lower LODs/LOQs)</td>
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</table>

1. Condition and equilibrate the cartridge
2. Load Sample
3-4. Apply vigorous wash steps. Analytes of interest will be retained selectively while interferences will be eluted.
5. Elute the analyte of interest
Minimal to No Method Development Required

Sample prep methods are often developed using a variety of schemes such as: referring to published methods of similar/identical applications; implementation of generic methodology; requesting support from a chromatography vendor; screening of techniques, phase chemistries, and method conditions. These approaches are often effective; however, more often than not, a sample prep method can often be frustrating and time consuming.

Unlike many traditional sample prep techniques, SupelMIP is developed and tailored for very specific applications. Therefore, each SupelMIP SPE phase comes with a detailed protocol and analytical technique for its respective application. Figure 4 depicts a typical data/instruction sheet that is included with each SupelMIP SPE phase.

High Stability

SupelMIP SPE consists of highly cross-linked polymers that maintain stability when exposed to a broad range of organic solvents, can withstand high temperatures, and can be used over broad pH ranges, without loss of selectivity. Furthermore, they can be stored at room temperatures for prolonged periods of times. This is extremely advantageous over immunoaffinity based products.

SupelMIP Phases and Methods Available for:

- PAHs in edible oil
- Non-steroidal anti-inflammatory drugs (NSAIDs) in wastewater and other sample matrices
- Nitroimidazoles in milk, eggs, and other food matrices
- Fluoroquinolones in bovine kidney, honey, and milk
- Chloramphenicol in milk, plasma, honey, urine and shrimp/prawns
- NNAL in urine
- TSNAs in urine and tobacco
- β-agonists in tissue, urine and wastewater
- Clenbuterol in urine
- Riboflavin in milk
- Patulin in fruit matrices
- Aminoglycosides in animal tissue, cell culture, and honey
- Bisphenol A from broth or milk-based matrices
Aminoglycosides are a microbial resistant class of antibiotics that are routinely monitored in animal-derived foods. Due to the concern of the impact that these compounds may have on human health and development, many countries have instituted regulatory limits for aminoglycosides.

This study focuses on the extraction and analysis of ten aminoglycosides from porcine tissues using molecular imprinted polymer (MIP) solid phase extraction with LC-MS/MS detection. This study utilizes the unique extraction capabilities of MIPs to successfully quantitate ten aminoglycosides by LC-MS/MS at 100 ng/g (400 ng/g for neomycin) with recoveries ≥ 70%. The SPE cleanup procedure, using SupelMIP SPE-Aminoglycosides, as well as the HPLC analysis, using an Ascentis® Express C18 HPLC column, is described in the condition section of Figure 5. Quantitation was performed using matrix matched calibration standards, ranging from concentrations of 10 ng/mL to 1000 ng/mL.

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<td>Kanamycin</td>
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<td>Dihydrostreptomycin</td>
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<td>Apramycin</td>
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</tbody>
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1. Spectinomycin
2. Hygromycin B
3. Streptomycin
4. Dihydrostreptomycin
5. Amikacin
6. Kanamycin
7. Apramycin
8. Tobramycin
9. Gentamicin C1
10. Neomycin

Figure 5. LC-MS/MS Analysis of Aminoglycosides after SupelMIP SPE Cleanup

- Sample/matrix: 3 mL of pork extract (For additional information regarding this application, refer to an article from Supelco Reporter 32.2 available at sigma-aldrich.com/supelmip)
- SPE tube/cartridge: SupelMIP SPE – Aminoglycosides, 50 mg/3 mL (52777-U)
- Conditioning: 1 mL of methanol, then 1 mL of 50 mM potassium phosphate in water (pH = 7.8)
- Sample addition: 3 mL of pork extract
- Washing: 3 mL of water, followed by drying with slight vacuum for 10 seconds
- Washing: 1 mL of 50:50 dichloromethane:methanol (v/v), followed by drying with slight vacuum for 10 seconds
- Elution: 1 mL of 1% formic acid containing 5 mM heptafluorobutyric acid (HFBA) in 80:20 water:acetonitrile (v/v)
- Eluate post-treatment: thoroughly mix via vortex agitation, and transfer to polypropylene HPLC vials
- Column: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm (53823-U)
- Mobile phase: (A) 5mM heptafluorobutyric in water; (B) 5 mM heptafluorobutyric in acetonitrile
- Gradient: 20 to 90% B in 3.0 min; held at 90% B for 1 min; 90 to 20% B in 0.1 min; held at 20% B for 5.9 min
- Flow rate: 0.4 mL/min
- Temp.: 40 °C
- Injection: 10 µL
- Det.: MS/MS, ESI(+), MRM

(a) Matrix Matched Standard
(b) Pork Muscle Blank
(c) Pork Muscle Spiked with 100 ng/g of Aminoglycosides
Application: Low Level Quantification of Patulin in Organic Apple Puree for Infants

Patulin is a mycotoxin produced by a number of fungal species. One species, Penicillium Expansum, is the primary source of patulin contamination in food products.\(^1\) P. Expansum occurs most often in apples and is commonly present in apple juice, and purees.\(^2\) Because studies have shown that patulin possesses immunotoxic and genotoxic properties, regulatory limits for patulin have been set in 50 countries, including the USA and the European Union.

Historically, analytical methods for patulin have employed liquid-liquid extraction (LLE) followed by HPLC separation with UV detection at 276 nm.\(^1,3\) Researchers have highlighted problems with these methodologies, including:

- Tedious sample preparation associated with liquid-liquid extraction
- Patulin instability in alkaline conditions resulting from sodium carbonate cleanup
- The requirement of extra cleanup or chromatographic method development to prevent the coelution of patulin and interfering matrix component 5-hydroxymethylfurfural (HMF).\(^1,4\)

Therefore, a quick, simple and robust sample preparation method for patulin analysis is needed.

The application outlined in Figure 7 demonstrates the effectiveness of the SupelMIP SPE – Patulin cartridge when used to concentrate low levels of patulin in an apple puree matrix. The application uses a Titan™ C18 UHPLC column for the analysis of patulin in a product marketed for consumption by infants. (Please note, this application can be adapted to a variety of apple-containing products.)

As seen in Figure 7, chromatographic analysis demonstrates that no direct matrix interference with patulin analysis was observed. Unlike LLE procedures, the cleanup procedure using SupelMIP SPE – Patulin successfully removed HMF and other common interfering components from the final extract. Sodium bicarbonate was necessarily used for an effective SPE cleanup, so the final extract was acidified to stabilize patulin for analysis. A highly concentrated patulin sample was produced, and patulin was easily detected by LC-UV analysis in apple puree at concentrations of 10 ng/g.
This study successfully demonstrates the use of SupelMIP SPE – Patulin for the cleanup and concentration of patulin from apple puree. By selectively extracting patulin with the use of molecularly imprinted polymers and effectively removing interfering matrix compounds during the SPE washing procedure, UHPLC-UV analysis of patulin was made possible. The average recovery of patulin from the spiked apple puree sample was calculated at 70.2% with a relative standard deviation (RSD) of 2.7% (n=3). No patulin was detected in the unspiked infant organic apple puree sample.

In conclusion, the use of SupelMIP SPE – Patulin for sample cleanup eliminates the aforementioned problems associated with liquid-liquid extraction and provides a fast, easy and reliable sample preparation method for patulin analysis in apple matrices.

References
Application: Reduced Ion-Suppression for the Analysis of Tobacco Specific Nitrosamines

Tobacco Specific Nitrosamines (TSNAs) are highly carcinogenic and derived solely from tobacco products. They are generated from the fermentation, curing, and burning of tobacco. For example, NNAL is a valuable biomarker in human urine to determine exposure to second-hand smoke. Because TSNAs are often found in very low concentrations in difficult biological matrixes, a highly selective and sensitive assay is required for sample preparation and analysis.

SupelMIP SPE – NNAL is designed for the extraction of NNAL, and SupelMIP SPE – TSNA is a class selective phase developed for the extraction of four different tobacco specific nitrosamines: NNK, NNN, NAB, and NAT.

Figures 8 and 9 depict LC-MS-MS chromatograms (MRM) of SupelMIP extracts of human urine spiked with 1 ng/mL NNAL and 25 pg/mL TSNAs, respectively.

**SupelMIP SPE – NNAL**:  
- Consistent low background  
- Achieve LODs of 5 pg/mL  
- Recoveries greater than 90%  
- Achieve LOQs of 13 pg/mL

For additional information regarding this application, refer to an article from Supelco Reporter 25.3 available at sigma-aldrich.com/supelmip

**SupelMIP SPE – TSNA**:  
- Consistent low background  
- Achieve LODs of 2.5 pg/mL  
- Recoveries greater than 90%  
- Achieve LOQs of 4 pg/mL

For additional information regarding this application, refer to an article from Supelco Reporter 25.5 available at sigma-aldrich.com/supelmip
Ion-suppression or ion-enhancement is caused by one or more interfering components/species that co-elute with the analyte(s) of interest during LC-MS analysis. These co-eluting species can affect droplet formation or ionize concurrently resulting in an erroneous decrease (suppression) or increase (enhancement) in signal response. Ion-suppression often leads to poor assay reproducibility, accuracy, and sensitivity, and such deleterious effects are often most notable at the lower limits of quantitation.

In order to achieve adequate lower limits of quantitation when conducting trace analysis of analytes in complex matrices such as biological fluids, it is absolutely critical to procure adequate selectivity during sample preparation. By virtue of molecularly imprinted polymer technology, SupelMIP SPE offers the necessary selectivity and sample cleanup required for achieving ever-decreasing detection limits that are challenging analysts today.

Blank urine samples were extracted with SupelMIP SPE – NNAL and the resulting SPE (post-SPE) eluate was spiked with NNAL and analyzed via LC-MS-MS. The resulting chromatogram response (peak area) levels generated were compared against external standards (prepared in buffer). The results (Figure 10) show that ion-suppression was nominal (<4% signal suppression) for the SupelMIP SPE – NNAL urine extract (post-SPE spike) relative to the external standard calibration curve.

In another study, blank urine samples were extracted with SupelMIP SPE Beta-agonists and conventional hydrophilic polymer SPE phases and the resulting SPE eluate was spiked (post-SPE) with metaproterenol at the levels of 0.5, 1, and 5 ng/mL, respectively. Figure 11 compares the response levels and linear relation of known spike concentrations vs. calculated concentrations determined from the signal responses obtained from blank urine extracts spiked post-extraction using both the SupelMIP SPE – Beta-agonist method and conventional polymer SPE method. Increased levels of ion-suppression were observed for the polymer SPE protocol relative to the SupelMIP procedure.

Figure 10. Response Comparison of NNAL Calibration Curve Generated from SupelMIP SPE – NNAL Urine Extract (post-SPE spike) vs. External Standards

Figure 11. Known Spike Concentration vs. Determined Concentration for SupelMIP SPE Beta-agonist and Polymer SPE for Metaproterenol from Urine (Post-Extraction Spike)
Application: Reduced Background and Improved Recoveries for the Extraction of PAHs from Olive Oil

Polynuclear aromatic hydrocarbons (PAHs), some of which are known to be carcinogenic in nature, reside in the environment as a result of petroleum processing and incomplete combustion of fossil fuels.1

This study compares SupelMIP SPE-PAHs and two competitor silica gel SPE cartridges in terms of matrix removal and analyte recovery for the extraction of select polycyclic aromatic hydrocarbons (PAHs) from olive oil. Multiple replicates of both unspiked and spiked (20 ng/g with PAHs) oil samples were processed for each SPE cleanup technique. Extracts were then analyzed by GC-MS (SIM mode). Quantitation was performed against a 5-point calibration curve (1-20 ng/mL) prepared in unspiked olive oil extract with napththalene-d₈, fluoranthene-d₁₀, perylene-d₁₂ internal standards, spiked at 10 ng/mL.

Figure 12 shows the GC-MS (full scan) chromatograms of the extracts after cleanup with SupelMIP SPE-PAHs, Competitor A silica gel SPE, and Competitor B silica gel SPE, respectively. The chromatograms illustrate that SupelMIP SPE-PAHs cleanup produces much lower background than the silica gel SPE cleanup.

As illustrated in Figure 13, the SupelMIP SPE-PAHs samples produced better overall analyte recoveries than those cleaned with the silica SPE cartridges. The reproducibility for all PAHs tested, exhibited by percent relative standard deviation (%RSD), fell within an acceptable range of less than 20% for the SupelMIP SPE-PAHs cleaned samples. Also, SupelMIP SPE-PAHs produced better overall recoveries and removed more problematic matrix interferences than the silica gel SPE cartridges, while maintaining good reproducibility. Thus, the SupelMIP SPE-PAHs provides suitable matrix removal for rugged GC-MS analysis of PAHs in olive oil.

Reference

Figure 12. GC-MS Full Scan Chromatograms of Olive Oil Extract (same y axis)

-sample/matrix: 0.5 g oil diluted to 1 mL in cyclohexane
-SPE tube/cartridge: SupelMIP SPE – PAHs, 50 mg/3 mL (52773-U)
-conditioning: 1 mL cyclohexane
-sample addition: 0.5 g oil diluted to 1 mL in cyclohexane
-washing: 1 mL cyclohexane
-elution: 3 x 1 mL ethyl acetate
-eluate post-treatment: Evaporate to less than 1 mL (not dryness) under nitrogen at 40 °C. Adjust final volume to 1 mL with ethyl acetate.

Figure 13. Analyte Recovery of PAHs from Olive Oil Extract (n=3)
Frequently Asked Questions (FAQs):

1. How is sample preparation improved using molecularly imprinted polymer SPE technology?
Because MIPs are tailor-made for individual analytes and analyte classes, analyte retention strength is increased significantly allowing for powerful wash steps within the SPE procedure. This allows for highly selective and simple extractions resulting in lower detection limits and improved MS compatibility (reduced ion-supression). Each SupelMIP phase also comes with a detailed application specific protocol simplifying the method development process which in turn saves time and cost.

2. Are sample packs available?
Yes. Sample packs are available and can be obtained through the SupelMIP website: sigma-aldrich.com/supelmip
Alternatively, you can also request a sample pack by calling or emailing your local Sigma-Aldrich office and connecting with technical service.

3. There is no MIP phase for my application? How do I develop a MIP protocol for my application?
Within the SupelMIP website, sigma-aldrich.com/supelmip, there is a survey where you can describe your application and needs for MIP based SPE product/procedure. Scientists will evaluate your application through a short feasibility stage. If your application is prioritized to move through feasibility, the next stages will be development and optimization.

4. Are process scale MIP products available through Supelco?
No. Process scale MIP products are not available through Supelco.

5. Can we use existing or traditional SPE protocols with SupelMIP SPE technology?
No. Existing protocols cannot be used. Every SupelMIP SPE includes a detailed extraction protocol that is analyte and matrix specific. This protocol needs to be used in order to achieve optimal retention during sample load, maximum interference removal during sample wash, and high recoveries during elution.

6. What dimensions are available for SupelMIP SPE?
Currently, our standard product consists of 25 mg, 50 mg, and 100 mg bed weights packed in 3 mL and 10 mL LRC (large reservoir cartridges) SPE tubes. The phases can be custom packed in all other SPE hardware that Supelco offers (other SPE tube dimensions, glass SPE tubes, 96-well plates, etc.).

**Ordering Information**

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<th>Cartridge Volume (mL)</th>
<th>Cartridge per Box</th>
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For a complete SupelMIP product listing, and to request a SupelMIP sample pack, visit sigma-aldrich.com/supelmip
Publications and Literature References

Analysis of Analytes – The use of MIPs in solid-phase extraction increases efficiency and improves detection limits, Widstrand C, Bjork H, Yilmaz E, Chemical Technology, June 2006

Selective Extractions by Molecular Imprinted Polymers (MIPs), Widstrand C, Yilmaz E, Boyd B, Rees A, 2006 Feb, thecolumn.eu.com


The Selective Extraction of Fluoroquinilones in Veterinary Samples using Molecularly Imprinted Polymer SPE, Supelco Tech Report, id. T408177


To learn more about SupelMIP SPE and to download additional literature, visit sigma-aldrich.com/supelmip