

# The Selective Extraction of Chloramphenicol Using Molecular Imprinted Polymer (MIP) SPE

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Chloramphenicol is a broad spectrum antibiotic that has recently been determined as a causative agent of aplastic anemia and possible carcinogen in humans. Because of these health concerns, the EU, US and Canada have banned the use of chloramphenicol in food-producing animals and livestock. Because the drug is still widely available in developing countries and no "safe" residue levels have been determined in food, public health concerns still arise. As of today, a "zero" tolerance level has been established for this antibiotic. It is therefore critical to develop a highly selective and sensitive analytical assay to control and monitor chloramphenicol residues in difficult matrices such as food stuffs.

In December 2006, Supelco and MIP Technologies AB, Lund, Sweden, entered into a collaborative agreement in which Supelco will be the exclusive global distributor of MIP Technologies' patent protected line of molecular imprinted polymer (MIPs) SPE cartridges (trademarked SupelMIP) for the simple and fast analysis of trace analytes in complex matrices.

In this article we discuss the selective use of SupelMIP™ SPE for the extraction and analysis of chloramphenicol from milk. This method is compared against a conventional hydrophilic polymer-based SPE method obtained from a peer-reviewed journal.

## What are Molecular Imprinted Polymers?

Molecular imprinted polymers 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, guide the formation of specific

cavities or imprints that are sterically and chemically complementary to the target analyte(s). As illustrated in Figure 1, MIPs are prepared by first mixing a template molecule that consists of the analyte of interest or structural analog of the analyte(s) of interest with one or more functional monomers. 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. 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. Because extraction selectivity is significantly improved,

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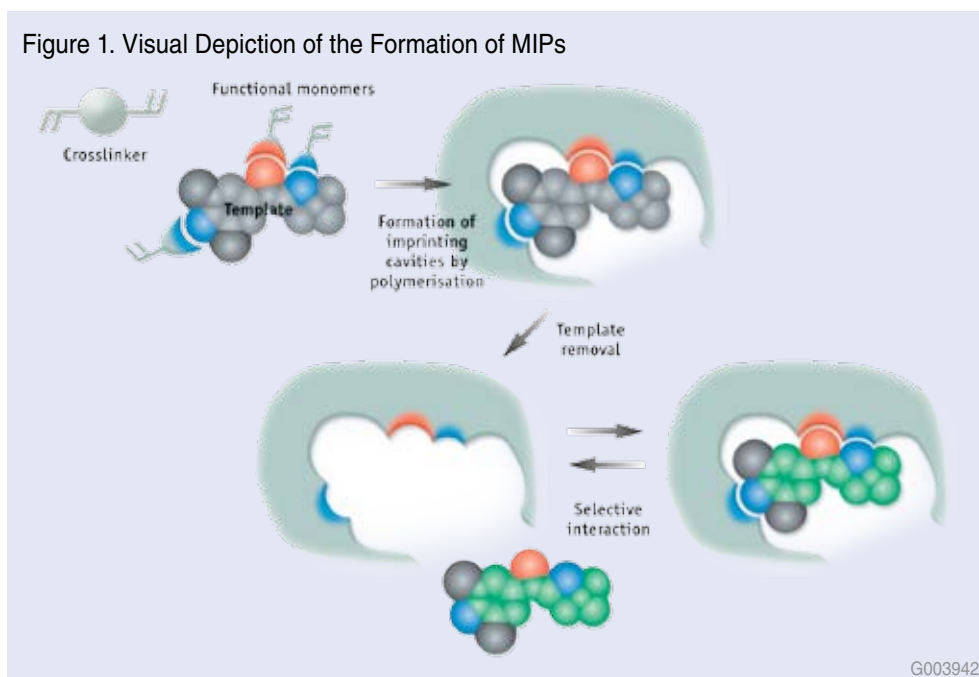


Table 1. Comparison of SupelMIP SPE Method and Conventional Method Using a Hydrophilic Polymer SPE Phase

SupelMIP SPE - Chloramphenicol Method Sample	Published Chloramphenicol Method Using Conventional Hydrophilic Polymer SPE Phase
<p><b>Pre-Treatment:</b> Whole pasteurized milk (purchased from the local supermarket) was centrifuged for 15 min. at 5k rpm. The aqueous lower layer was spiked with chloramphenicol at the level of 15 ng/mL and 38 ng/mL.</p>	<p><b>Sample Pre-Treatment:</b> 5 mL of milk was spiked with 40 ng chloramphenicol. Proteins were precipitated by the addition of 15 mL 10% trichloroacetic acid in water. The sample was vortexed and heated for 1 hour at 65 °C. After cooling to room temperature, the mixture was centrifuged for 15 min. at 3K rpm. The supernatant was filtered over glass wool, and the filtered was rinsed with 10 mL DI water. The pH of the filtrate was adjusted to pH 5 with 0.1 M sodium acetate.</p>
<p><b>SPE Procedure:</b> SupelMIP SPE – Chloramphenicol, 25 mg/10mL (LRC) (53210-U)</p> <ol style="list-style-type: none"> <li>1. Condition and equilibrate MIP phase with 1 mL methanol followed by 1 mL DI water.</li> <li>2. Apply 1 mL of the pre-treated milk sample to the cartridge.</li> <li>3. Elute interferences using the following wash scheme: <ul style="list-style-type: none"> <li>2 x 1 mL MS-grade water</li> <li>1 mL 5% acetonitrile in 0.5% acetic acid</li> <li>2 x 1 mL MS-grade water</li> <li>1 mL 20% acetonitrile in 1% ammonium hydroxide</li> </ul>           Dry SPE tubes for 15 min. under gentle vacuum            3 x 1 mL dichloromethane            Dry SPE tubes for 1 min. under gentle vacuum         </li> <li>4. Elute chloramphenicol with 2 x 1 mL methanol:acetic acid:MS-grade water (89:1:10, v/v/v)</li> <li>5. Evaporate combined eluate to dryness at 50 °C under nitrogen. Reconstitute 150 µL LC mobile phase prior to LC-MS analysis.</li> </ol>	<p><b>SPE Procedure: Conventional Hydrophilic Polymer SPE, 500 mg/12 mL</b></p> <ol style="list-style-type: none"> <li>1. Condition and equilibrate SPE phase with 3 mL methanol, 4 mL DI water, and 4 mL 10 mM HCl</li> <li>2. Apply the pre-treated milk extract to the cartridge.</li> <li>3. Elute interferences using the following wash scheme: <ul style="list-style-type: none"> <li>4 mL MS-grade water</li> <li>2 mL 5% methanol</li> <li>2 mL 50% methanol</li> </ul> </li> <li>4. Elute chloramphenicol with 2 mL methanol</li> <li>5. Evaporate combined eluate to dryness at 50°C under nitrogen. Reconstitute 0.4 mL DI water</li> </ol> <p>Liquid-liquid Extraction:</p> <ol style="list-style-type: none"> <li>1. Liquid-liquid extract of reconstituted eluate with 0.6 mL acetonitrile:dichloromethane (4:1, v/v).</li> <li>2. Centrifuge at 7k rpm for 5 min. Transfer upper organic layer to a fresh tube.</li> <li>3. Repeat steps 1 &amp; 2 of the LLE procedure two additional times on the lower aqueous layer.</li> <li>4. Combine all organic layers, evaporate to dryness at 60 °C under nitrogen. Reconstitute with 0.2 mL LC mobile phase and filter through a 0.2 µm nylon filter.</li> </ol>

(continued from page 9)

lower background is observed allowing analysts to achieve lower detection limits.

### The Extraction of Chloramphenicol from Milk

In this study, an extraction method using SupelMIP SPE phase was compared against a published method using a conventional hydrophilic polymer SPE phase (1). Table 1 describes the two extraction protocols.

### Improved Selectivity and Recovery Using SupelMIP SPE

Upon sample extraction using the two procedures described in Table 1, resulting extracts were analyzed via LC-MS. Recovery was determined for each protocol against a calibration curve (data not shown) using external standards. An average chloramphenicol recovery of 84% (n=4) was obtained using the SupelMIP method and 79% (n=2) for the hydrophilic polymer SPE method. However, a pronounced difference in selectivity was determined between the two extraction methods. In Figure 2, we see that signal/noise ratio for the hydrophilic polymer SPE method was double that of the SupelMIP ion-chromatograms (320-323 m/z range); and blank milk samples processed using the SupelMIP were free of interfering responses in the elution area of

chloramphenicol. In Figure 3, a significantly cleaner mass spectra is observed for the SupelMIP SPE extract relative to the conventional hydrophilic polymer extract. Also, unlike the conventional hydrophilic polymer method that required an extensive sample pre-treatment involving a protein precipitation step, an SPE cleanup procedure, and three LLE steps, the SupelMIP method only required a simple sample pre-treatment followed by a single SPE cleanup step.

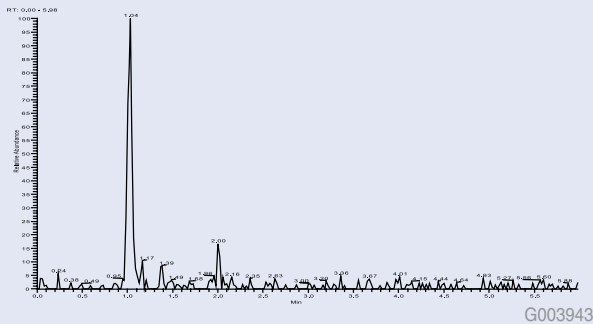
### Conclusion

In this report, we discussed the utility of molecular imprinted polymer SPE technology for the extraction of chloramphenicol from milk. Because selectivity is introduced during the development of the MIP phase itself, it allows for a binding site that is sterically and chemically complementary to the target analyte(s). The multiple interactions that take place between the imprint binding site and analyte(s) of interest offer strong interactions enabling the use of harsher wash conditions during the SPE process. For chloramphenicol, the SupelMIP SPE approach provided simpler methodology and significant increases in selectivity relative to the described conventional hydrophilic polymer SPE method. Both points are particularly advantageous where trace detection limits and routine analysis are required.

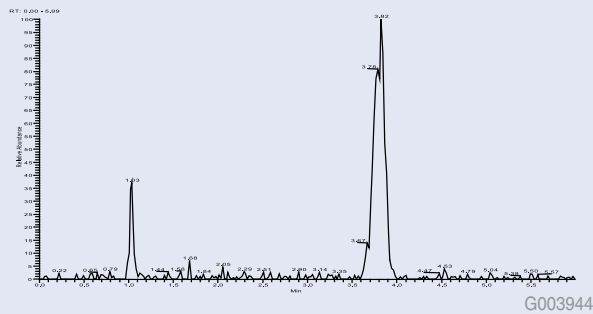
## Figure 2. Chloramphenicol Spiked Milk Samples Extracted on SupelMIP SPE vs. Conventional Hydrophilic Polymer SPE

column: Ascentis C18, 2.1 mm x 10 cm I.D., 3  $\mu$ m particles (581301-U)  
 instrument: Jasco HPLC interfaced with a ThermoFinnigan Advantage ion trap mass spectrometer via an electrospray ionization source  
 mobile phase: 100 mM ammonium acetate (pH unadjusted):  
 MS-grade water:acetonitrile (10:60:30)  
 temp.: 35  $^{\circ}$ C  
 flow rate: 0.2 mL/min., split to MS  
 det.: MS, ESI(-) (320-323 m/z range)  
 injection: 5  $\mu$ L

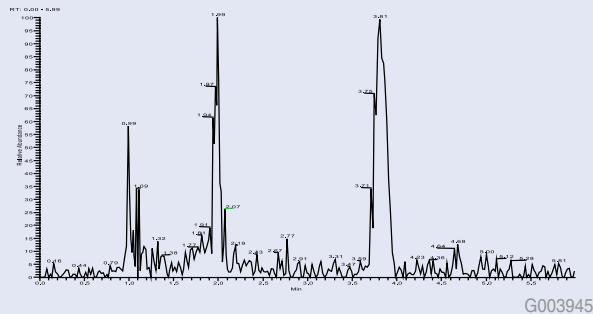
### SupelMIP SPE Extract of Blank Milk



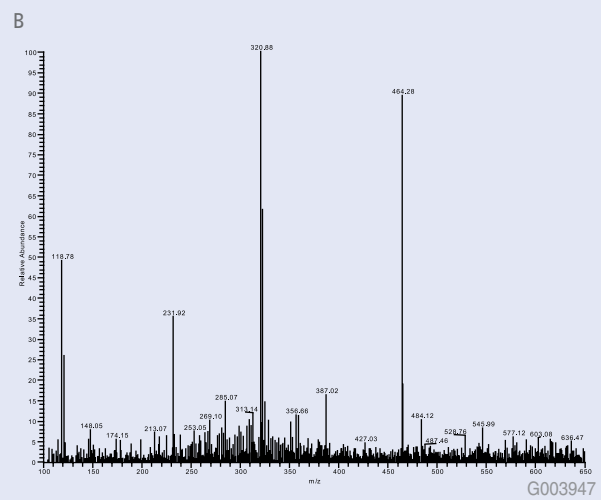
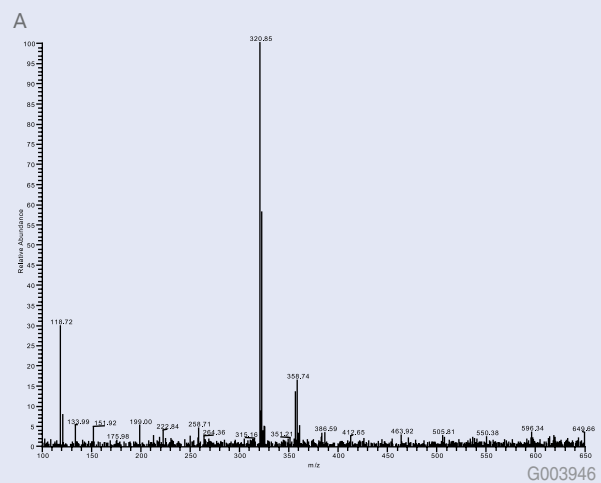
### SupelMIP SPE Extract of Chloramphenicol Spiked Milk



### Conventional SPE Extract of Chloramphenicol Spiked Milk



## Figure 3. Mass Spectrum of Full Ion-chromatograms (3.65-4.00 min.) of the SupelMIP SPE Extract (A) and the Hydrophilic Polymer SPE Extract (B)



### Reference

1. P.A. Guy et al. in J. Chromatogr. A 1054 (2004) 365-371

### Related Products

SupelMIP SPE Cartridges	Sorbent Mass (Mg)	Cartridge Volume (mL)	Cartridges per Box	Cat. No.
Clenbuterol	25	10	50	53201-U
Beta-agonists (class selective)	25	10	50	53202-U
Beta-agonists (class selective)	25	3	50	53225-U
Beta-blockers (class selective)	25	10	50	53218-U
Beta-blockers (class selective)	25	3	50	53213-U
Full Beta Receptor (Beta-agonists and Beta-blockers)	25	10	50	53223-U
Full Beta Receptor (Beta-agonists and Beta-blockers)	25	3	50	53224-U
Chloramphenicol	25	10	50	53210-U
Chloramphenicol	25	3	50	53209-U
NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25	10	50	53206-U
NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25	3	50	53203-U
TSNAs (4 different Tobacco specific Nitrosamines: NNK, NNN, NAB, NAT)	25	10	50	53221-U
TSNAs (4 different Tobacco specific Nitrosamines: NNK, NNN, NAB, NAT)	25	3	50	53222-U
Riboflavin (vitamin B2)	25	10	50	53207-U
Triazines (class selective)	25	10	50	53208-U