

Selective sample preparation for Chloramphenicol from shrimp using SupelMIP SPE

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Introduction to Molecularly Imprinted Polymers

MIPs allow for selective extraction of low levels of target compounds in the presence of a mixture of potentially interfering matrix components. Compared to biological receptors, MIP polymer recognition systems have the advantages of superior chemical and mechanical stability and compatible with most solvents, pressures and pH conditions. The MIP materials can be engineered for an almost unlimited variety of small molecules, such as natural products, pharmaceuticals, peptides etc.

By engineering both the binding site and the polymer backbone a wide range of optimized extraction phases can be produced. The MIPs extract the targeted analytes with a high degree of selectivity if the template used to form them is designed to form strong interactions with the functional monomers. By taking into account the specific interaction mechanisms, the analytical SPE method can be optimised for each individual MIP material. The principle of the selective interaction mechanism is outlined in Figure 1 and the general SPE procedure is described in Figure 2.

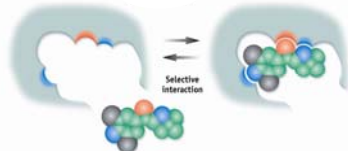


Figure 1. The Molecularly Imprinted Cavity
 The MIP materials consist of highly cross-linked polymer phases that have pre-determined selectivity for a single analyte or a group of structurally related analytes. The MIP selective binding site has key recognition elements placed in sterically locked positions.

Selective SPE by MIPs

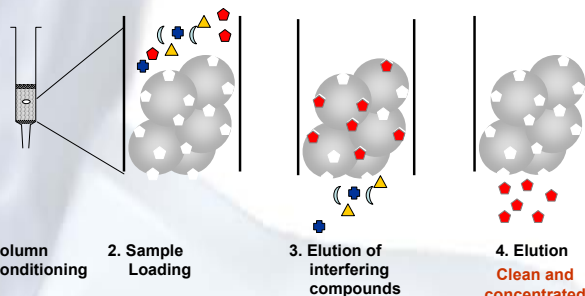


Figure 2. The Figure shows a typical MIP SPE procedure.

Background

In food, veterinary, doping, and environmental monitoring sensitive methods for determination of pharmaceutical drugs are required. 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, USA 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.

Methods

In this study, a SPE method using SupelMIP SPE Chloramphenicol was evaluated for extraction of Chloramphenicol from shrimp. The SupelMIP SPE extraction method was compared against a recommended method using a conventional hydrophilic polymer SPE phase³. Table 1 describes the SupelMIP SPE Chloramphenicol extraction protocol. Extracts were analyzed via LC-MS. Details of the analytical method are shown in Table 1. The SupelMIP Chloramphenicol material has previously been described by Mohamed et al¹ and Boyd et al.² In this work we discuss the selective use of SupelMIP SPE for the extraction and analysis of Chloramphenicol from shrimp.

SupelMIP SPE Chloramphenicol

Condition cartridge Note: recommended flow rate ~0.5 mL/min.	<ul style="list-style-type: none"> • 1 mL methanol • 1 mL DI water
Load Note: recommended flow rate ~0.5 mL/min.	Apply 2 mL sample (aq) to the cartridge.
Wash The maximum flow rate during the wash steps should not be greater than 0.5 mL/min.	<ul style="list-style-type: none"> • 2 x 1 mL DI water • 1 mL 5 % acetonitrile / 95% acetic acid (0.5%v/v aq) • 2 x 1 mL 1 % (v/v) ammonia (aq) • 1 mL 20 % acetonitrile / 80 % ammonia (1 %v/v aq) * <ul style="list-style-type: none"> • Apply full vacuum through cartridge to remove residual solvent from cartridge.
	• 3 x 1 mL dichloromethane
	• Apply full vacuum through cartridge to remove residual solvent from cartridge.
Analyte elution Note: recommended flow rate ~0.2 mL/min.	Elute chloramphenicol with 2 x 1 mL 10 % methanol/90 % dichloromethane (v/v) **

Evaporate to dryness and reconstitute in 150 µL in LC mobile phase (30% Acetonitrile / 70% 10mM Ammonium Acetate) prior to analysis.

Analytical Method

Column:	Ascents C18 (100mm X 2.1mm, 3µm)
Mobile phase:	10 mM ammonium acetate (pH 6.6) acetonitrile (70:30) 0.2 mL/min.
Flow rate:	0.2 mL/min.
Temperature:	22 °C
Run time:	Isocratic: 5 mins.
Injection Volume:	25 µL
Detection:	MS/MS, MRM transitions
MRM transitions:	Quantification (321.00/152.00) Identification (321.00/257.00) I.S. (326.00/157.1)
Ion mode:	ESI Negative
Ion source:	TurboSpray
Ion spray voltage:	2000 V
source temperature:	500 °C
Ion source gas 1:	70 psi
Ion source gas 2:	40 psi
Curtain gas:	10 psi
DP:	200 V
Dwell time:	150 msec

Table 1. Left; SupelMIP SPE method for extraction of Chloramphenicol from Shrimp. The samples were pre-treated using Ethyl acetate extraction followed by vortex (2 min.) and a filter (5.5 µm). Samples were evaporated to dryness and redissolved in DI water. Right; analytical method used for analysis of the extracts.

Results

In this study the SupelMIP SPE Chloramphenicol method for extraction of Chloramphenicol from shrimp was evaluated. The SupelMIP SPE method was compared to a conventional hydrophilic polymer SPE phase³. In table 2 recoveries are presented showing higher recoveries and precision using the SupelMIP method. Figure 3 shows MRM chromatograms of spiked samples revealing an increased peak intensity and reduced signal to noise using the SupelMIP method. In figure 4 Q1 chromatograms show that the SupelMIP method gives very clean extracts with a low level of interfering contaminants. Limit of detection was determined to be 7 ng/kg.

Increased precision and increased recoveries

Method	SupelMIP SPE Chloramphenicol	Hydrophilic polymer SPE
Absolute recovery (%)	75.5 ± 6.9	25.5 ± 35.6
Recovery (%)	96.8 ± 5.2	75.6 ± 11.7

Table 2. Recoveries in extracts using the SupelMIP SPE Chloramphenicol method respectively the hydrophilic polymer method. The SupelMIP shows high recoveries and precision.

Increased peak intensity and increased signal to noise

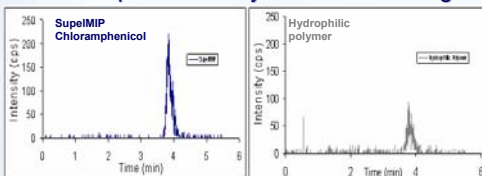


Figure 3. Chromatogram, MRM 321/152. Comparison of shrimp sample extracts spiked with 100 ng/kg CAP cleaned-up using SupelMIP material resp. a hydrophilic polymer. CAP Retention time 3.8 min. The intensity is significantly higher for the chloramphenicol peak in the SupelMIP chromatogram than in the chromatogram of the extract from the hydrophilic polymer.

Clean extracts for lowered LODs

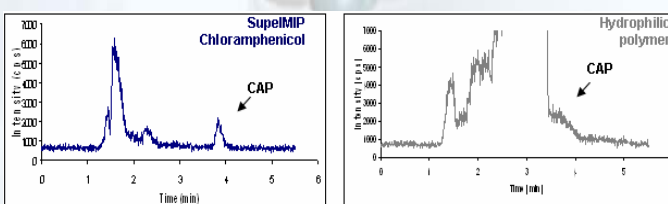


Figure 4. Chromatogram, Q1 SIM 321 amu, of the Shrimp extracts spiked with 200 ng/kg and clean-up using the SupelMIP CAP resp. the hydrophilic polymer. CAP retention time 3.8 min. The SupelMIP chromatogram shows a cleanly resolved chloramphenicol peak with a very low amount of interferences.

Conclusion

The origin of MIP selective binding is chemically and structurally well defined key recognition sites. Due to the stability of the MIP material and the high selectivity MIPs are well suited for removal of contaminants from complex matrices and even in the presence of a mixture of potentially interfering contaminants. In this study the SupelMIP SPE Chloramphenicol approach provided a significant increase in selectivity to Chloramphenicol relative to the described conventional hydrophilic polymer SPE method. The SupelMIP method was robust with a high precision (5 %).

The recoveries were determined to be above 90 %. The extracts were cleaner and contained lowered amounts of interferences. Limit of detection for the method was determined to 7 ng/kg. This SPE method is particularly advantageous where trace detection limits and routine analysis are required. Applications of SupelMIP SPE Chloramphenicol are available in urine, plasma, milk as well as shrimp tissue.

- ### Features of SupelMIPs
- Simple and rapid sample preparation
 - Lowered quantification limits
 - High reproducibility and repeatability
 - Minimized ion suppression
 - Lower risk of false positives
 - Robust materials

References

- Mohamed R, Richo-Pajot J, Gremaud E, Mottier P, Yilmaz E, Tabet JC and Guy P, 2007. Advantages of molecularly imprinted polymers LC-ESI-MS/MS for the selective extraction and quantification of Chloramphenicol in milk-based matrices. Comparison between a classical sample preparation, Anal.Chem. (2007); 79(24); 9557-9565.
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