



affinisep

Chloramphenicol

Application Note



**Selective Solid Phase Extraction of Chloramphenicol
from Shrimp using AFFINIMIP® SPE Chloramphenicol**

Food testing



Chloramphenicol: a major concern for human health and a challenge in food safety analysis

Chloramphenicol (see Figure 1) is a broad-spectrum antibiotic widely used in the world in the past. Several health problems are related to its use. As a consequence, several countries (e.g. U.S.A, E.U, Canada...) have prohibited its use for food-producing animals including for aquaculture products. As no permitted limit has been established, E.U. has defined a Minimum Required Performance Limits (MRPLs) of 0.3µg/kg for product of animal origin (Commission decision 2003/181/EC).

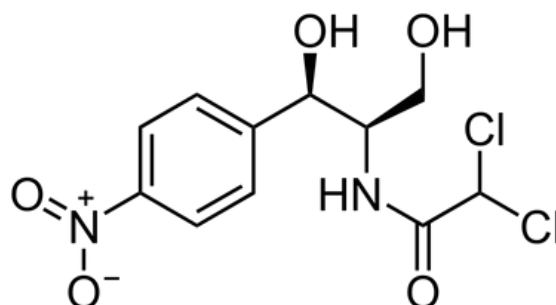


Figure 1. Chemical structure of **Chloramphenicol**

For such a low MRPL threshold, a clean-up step is crucial in order to improve the sensitivity, the reliability and the specificity before analysis. 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.

How to solve this ?

AFFINISEP has developed a new class of intelligent polymers based on molecularly imprinted polymers (AFFINIMIP®) specific to Chloramphenicol used as a powerful technique for cleanup and pre-concentration. **AFFINIMIP® SPE Chloramphenicol** cartridge is a simple, fast, sensitive and selective tool for the extraction of Chloramphenicol from complex matrices.

We demonstrate in this application note that a reliable quantification of Chloramphenicol from Shrimp at low concentrations using **AFFINIMIP® SPE Chloramphenicol** and a single quadrupole mass detection is possible.

In a very complex matrix such as shrimp, we obtained a high recovery yield (> 90%) with a low background, even with UV detection.

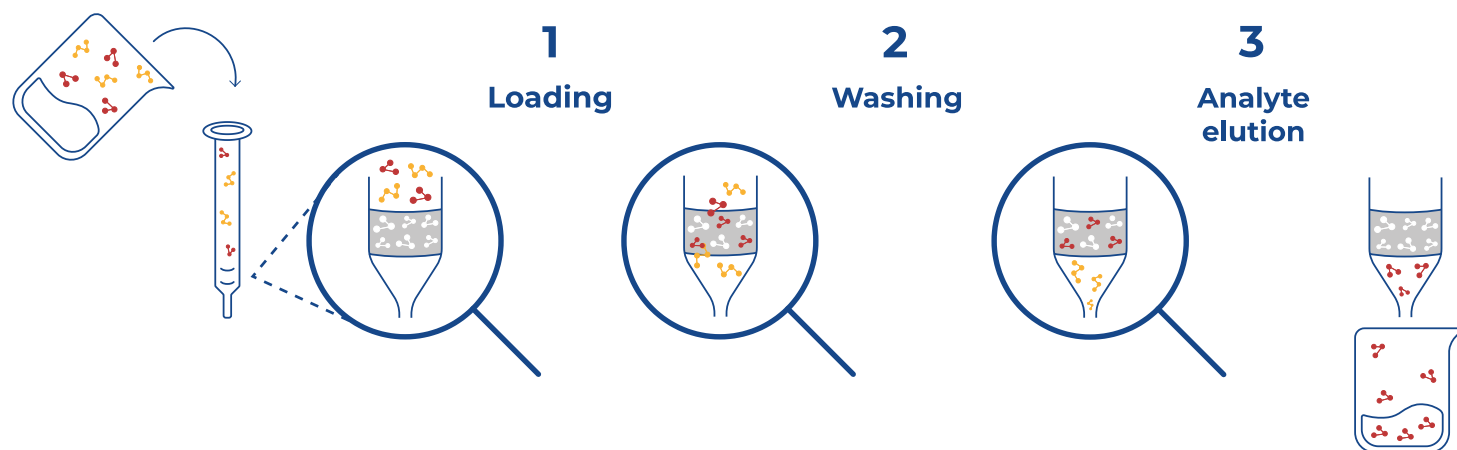


Figure 2. Principle of Solid Phase Extraction (SPE)

Proceeding of the experiment

Sample preparation

5g peeled shrimp were homogenized 2min with a vortex in 20mL of ethyl acetate. Then the solution was filtered on filter paper (25µm). The supernatant was evaporated to dryness and reconstituted in 10mL of Water. This solution was used as the loading solution.

Purification with a 1 mL AFFINIMIP® SPE Chloramphenicol cartridge (~30min)

EQUILIBRATION

1. 2mL Acetonitrile
2. 2mL ultrapure Water

LOADING

1 or 2 mL loading solution at a rate of 1 mL/min

WASHING

1. 1 mL ultrapure Water
2. 1 mL of (0.5% Acetic Acid in water) / Acetonitrile (95/5, v/v)
3. 2 mL 1% NH₃ (in water)
4. 2mL of (1% NH₃ in water) / Acetonitrile (80/20, v/v)
5. Dry cartridge for 1 minute under full vacuum
6. 250µL diethyl ether
7. Dry cartridge for 10 seconds under full vacuum

ELUTION

2 mL methanol (then apply a light vacuum to gather the remaining methanol)

The elution fraction was then evaporated and dissolved in the mobile phase.



Conditions of analysis

HPLC was performed on a ThermoFinnigan Surveyor Plus with a Thermo Accucore C18 column (50mm x 2.1mm; 2.5µm). The injection volume was 20µL. Separation was carried out at a flow rate of 200µL/min using a mobile phase of Ammonium Acetate 10mM in water/Methanol (75/25, v/v). The detection system was a ThermoFinnigan MSQ Plus with an electrospray source (ESI) in negative mode.

The quantification was done in selected ion monitoring (SIM) at m/z = 321.

Results

High analyte recovery

C° (µg/kg)	Loading volume	Mean (µg/kg)	Recoveries %
38	1mL	38.7	101.7
38	2mL	36.4	95.8

Table 1 - Recovery of Chloramphenicol spiked at 38µg/kg after **AFFINIMIP® SPE Chloramphenicol** clean-up of Shrimp.

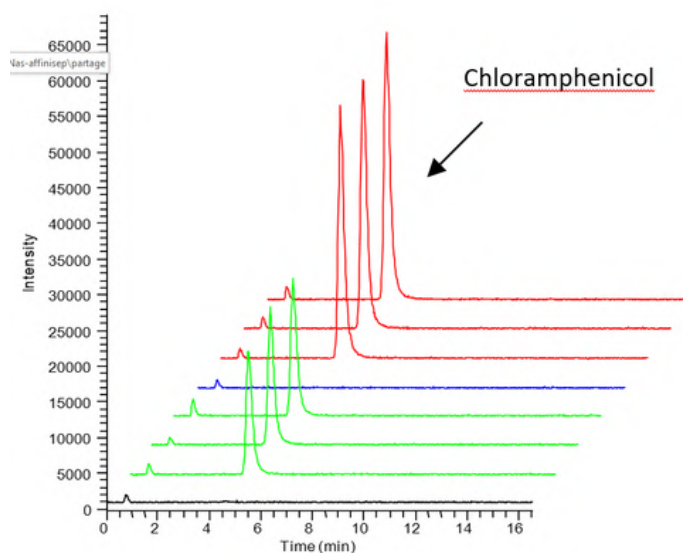


Figure 3. SIM Chromatograms obtained after clean-up with **AFFINIMIP® SPE Chloramphenicol** of shrimp spiked with Chloramphenicol at 38µg/kg. For 1mL loading: green and not spiked: black. For 2mL loading: red and not spiked: blue

UV chromatograms demonstrate a perfect cleanup

The UV chromatograms presented in figure 3 show a **very low background at the retention time of Chloramphenicol**

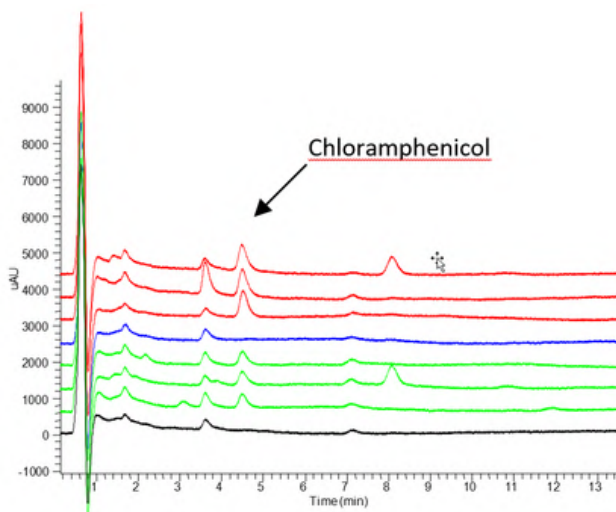


Figure 4. UV Chromatograms obtained after clean-up with **AFFINIMIP® SPE Chloramphenicol** of shrimp spiked with Chloramphenicol at 38µg/kg. For 1mL loading: green and not spiked: black. For 2mL loading: red and not spiked: blue.

Product reference

- **AFFINIMIP® SPE Chloramphenicol**

Catalog number: **FS110-03A** for 50 cartridges 1mL

Other format available

FS110-03 for 50 cartridges 3mL

