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).

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 **

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.

** Results **

** High analyte recovery **

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Loading volume</th>
<th>Mean (µg/kg)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>1mL</td>
<td>38.7</td>
<td>101.7</td>
</tr>
<tr>
<td>38</td>
<td>2mL</td>
<td>36.4</td>
<td>95.8</td>
</tr>
</tbody>
</table>

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

** Figure 2. ** 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 3. 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.

Experimental conditions

Preparation of samples prior to SPE

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.

Solid phase extraction (SPE) protocol

The SPE procedure used a 1mL AFFINIMIP® SPE Chloramphenicol cartridge:

- Conditionning: 2mL Acetonitrile, then 2mL of deionized Water
- Load up to 2mL of the loading solution
- Wash the cartridge with 1mL of deionized Water
- Wash with 1mL of (deionized Water-0.5% Acetic Acid) /Acetonitrile (95/5, v/v)
- Wash with 2mL of 1% Ammonia in water
- Wash with 2mL of (Water - 1% Ammonia) /Acetonitrile (80/20, v/v)
- Apply vacuum during 1 minute
- Wash the cartridge with 250µL diethyl Ether
- Elute Chloramphenicol with 2mL of Methanol and apply vacuum during 10 seconds

The SPE procedure lasted approximately 30 minutes. The elution fraction was then evaporated and dissolved in the mobile phase.

Analysis

HPLC: ThermoFinnigan Surveyor Plus with a Thermo Accucore C18 column (50mm x 2.1mm; 2.5µm).

Injection volume: 20µL.

Separation flow rate: 200µL/min.

Mobile phase: Ammonium Acetate 10mM in water/Methanol (75/25, v/v).

Detection system: ThermoFinnigan MSQ Plus (ESI-), selected ion monitoring (SIM) at m/z = 321.

Product reference

FS110-02A for 25 cartridges 1mL
FS110-03A for 50 cartridges 1mL