affinisep

Chloramphenicol

Application Note

Selective Solid Phase Extraction of Chloramphenicol from Honey using AFFINIMIP® SPE Chloramphenicol

Food testing

Introduction

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. 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).

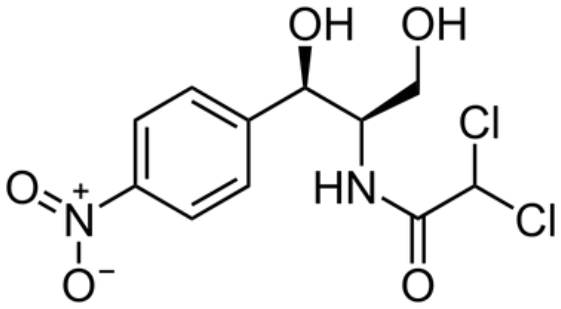

Figure 1. Chemical structure of **Chloramphenicol**

However, due to its broad spectrum of activity and its availability, Chloramphenicol is still used in several countries to treat food-producing animals. Therefore, chloramphenicol analysis is still a current affair.

In addition, food matrices are very complexes and induce ion-suppression phenomena which distort analysis results. 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 Solid Phase Extraction (SPE) of Chloramphenicol from complex matrices.

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

In a very complex matrix such as honey, we obtained a high recovery yield (> 90%) with a low background, even with UV detection. The tests carried out on several kinds of honey demonstrated a good reproducibility, proving the efficiency of **AFFINIMIP® SPE Chloramphenicol** cleanup.

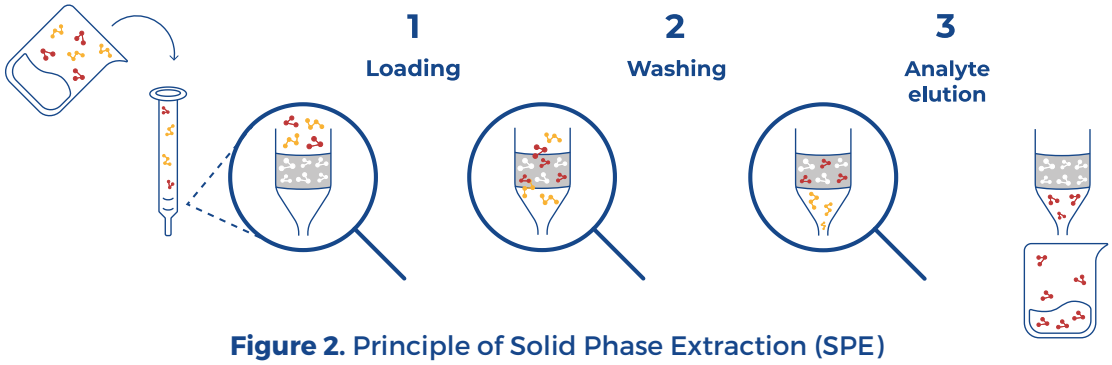

Figure 2. Principle of Solid Phase Extraction (SPE)

Proceeding of the experiment

Sample preparation

10g of honey were dissolved in 10mL of deionized Water. This solution was mixed under magnetic stirring for 10 minutes and 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 mL loading solution (or 10mL for 0.3µg/kg) 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

Clean extracts for Lowered LODs

By using **AFFINIMIP® SPE Chloramphenicol**, the detection of very low Chloramphenicol concentrations can be done without using a MS/MS detector. Indeed, with a single quadrupole mass spectrometer, we managed to detect 0.3µg/kg Chloramphenicol, which is the minimum required performance limits (Figure 3). A much lower limit of detection is expected with a LC-MS/MS.

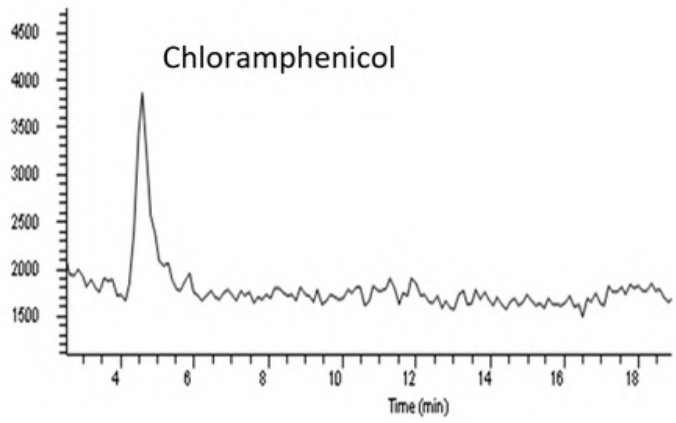

Figure 3. SIM Chromatogram obtained after clean-up with **AFFINIMIP® SPE Chloramphenicol** of 10g of Honey spiked with Chloramphenicol at 0.3µg/kg

To detect such concentrations, up to 10mL loading volume can be deposited on the cartridge. As for a 1 mL loading, the tests performed show a perfect cleanup.

High analyte recoveries and good repeatability

C° (µg/kg)	Mean (µg/kg)	Recoveries %	% RSDr
16.0	15.4	96.1	3.3

Table 1 - Recovery of Chloramphenicol spiked at 16µg/kg after AFFINIMIP® SPE Chloramphenicol clean-up of 1g of Honey and relative standard deviation calculated from results generated under **repeatability conditions** (n=3)

Good reproducibility

The results illustrated in table 2 and figure 4 show the quality of the cleanup using AFFINIMIP® SPE Chloramphenicol. The tests were carried out with different batches of AFFINIMIP® SPE Chloramphenicol and with 3 kinds of honey.

C° (µg/kg)	Mean (µg/kg)	Recoveries %	% RSD_R
15.7	16.9	108.1	6.5 (n=6)
18.2	16.6	91.4	11.4 (n=12)

Table 2 - Recovery of Chloramphenicol spiked at different concentrations after AFFINIMIP® SPE Chloramphenicol clean-up of 1g of Honey and relative standard deviation calculated from results generated under **reproducibility conditions**.

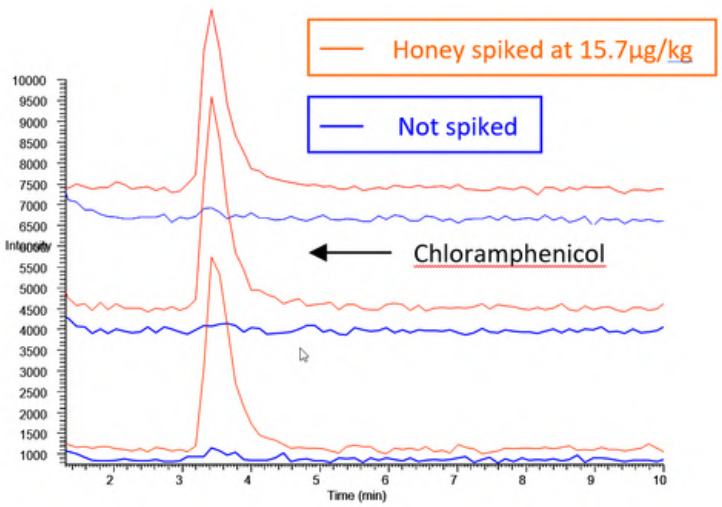

Figure 4. SIM Chromatograms obtained after clean-up with AFFINIMIP® SPE Chloramphenicol of 1g of Honey spiked with Chloramphenicol at 15.7µg/kg (red) or not spiked (blue).

UV chromatograms demonstrate a perfect cleanup and a high capacity

The use of UV detection aims to prove the efficiency of **AFFINIMIP® SPE Chloramphenicol** cleanup. Indeed, the UV chromatogram presented in figure 5 shows a **very low background and no interferents at the retention time of Chloramphenicol.**

The samples were spiked at 2000 µg/Kg of Chloramphenicol to test the capacity of the cartridge. More than 96% recoveries were obtained at this level and saturation of the cartridge was far from being reached.

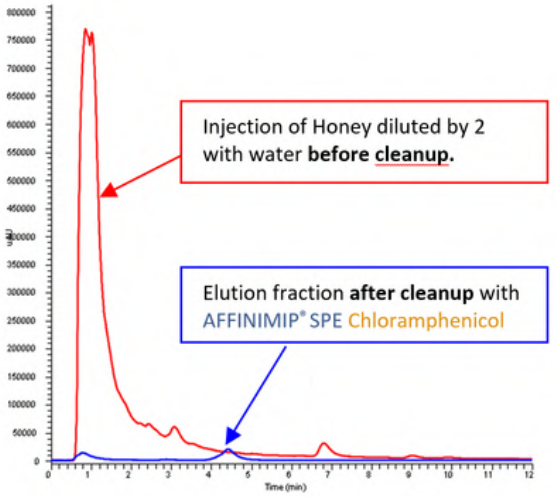

Figure 5. UV Chromatograms of Honey containing 2000 µg/Kg of Chloramphenicol before cleanup (Red) and after cleanup (Blue) with AFFINIMIP® SPE Chloramphenicol.

Product reference

- **AFFINIMIP® SPE Chloramphenicol**

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

Other format available

FS110-03 for 50 cartridges 3mL