

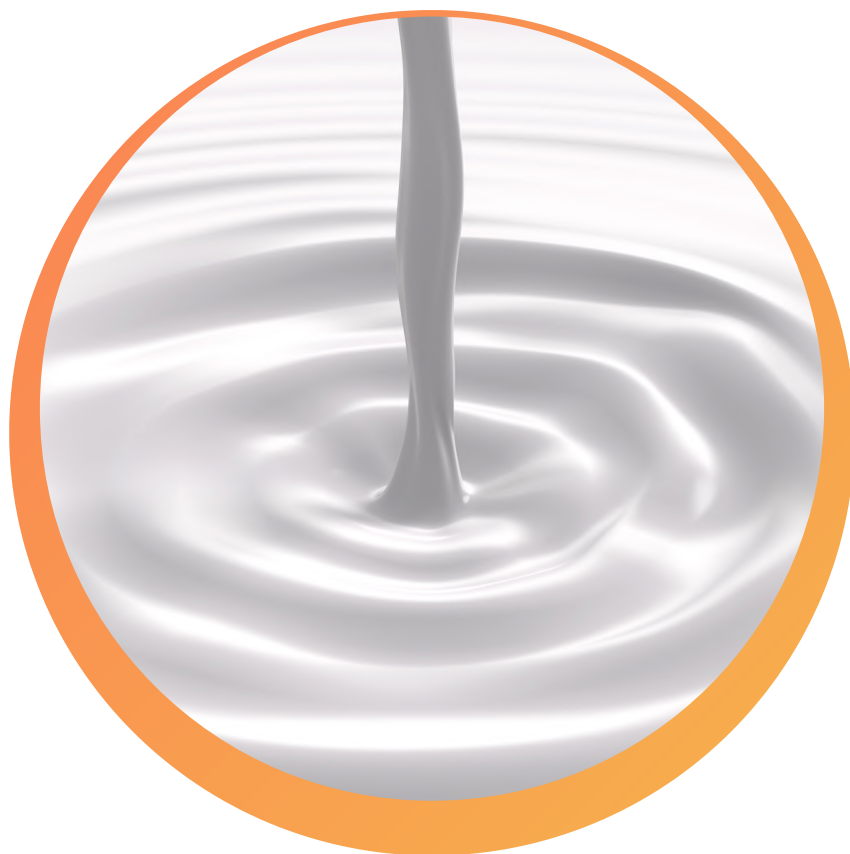


affinisep



Chloramphenicol

Application Note



**Selective Solid Phase Extraction of Chloramphenicol
from milk 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).

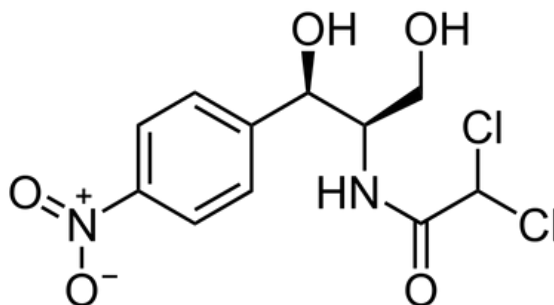


Figure 1. Chemical structure of **Chloramphenicol**

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 milk at low concentrations using **AFFINIMIP® SPE Chloramphenicol and a single quadrupole mass detection is possible.**

In a complex matrix such as milk, we obtained a high recovery yield (89%) with a low background, even with UV detection proving the efficiency of **AFFINIMIP® SPE Chloramphenicol** cleanup.

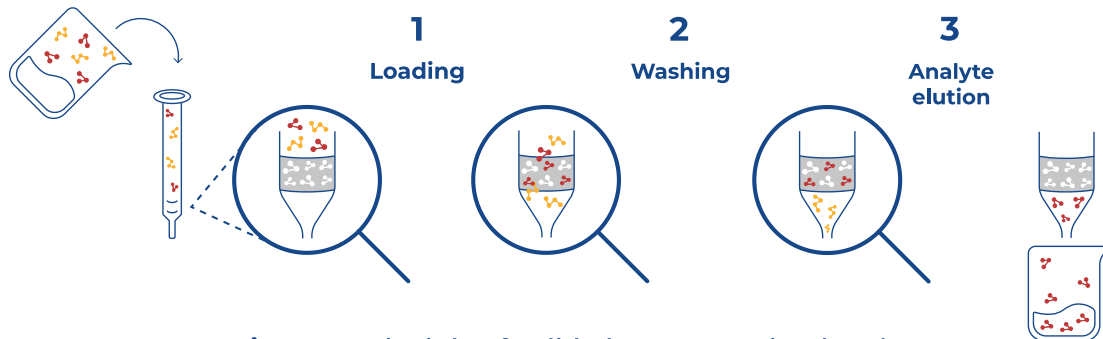


Figure 2. Principle of Solid Phase Extraction (SPE)

Proceeding of the experiment

Sample preparation

Milk is centrifuged for 15 minutes at 5000 rpm. Collect the liquid layer below the upper lipid layer to obtain the loading solution.

Purification with a 1mL or 3mL **AFFINIMIP® SPE Chloramphenicol** cartridge (~30min)

EQUILIBRATION

1. 2mL Acetonitrile
2. 2mL ultrapure Water

LOADING

1mL (for 1mL cartridge) or 3 mL (for 3mL cartridge) of loading solution at a rate of 1 mL/min

WASHING

1. 2mL ultrapure Water
2. 1 mL of (0.5% Acetic Acid in water) / Acetonitrile (95/5, v/v)
3. 2mL of (1% NH₃ in water) / Acetonitrile (80/20, v/v)
4. Dry cartridge for 1 minute under full vacuum
5. 250µL diethyl ether
6. 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; 2.5 μ m). The injection volume was 20 μ L. Separation was carried out at a flow rate of 200 μ L/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

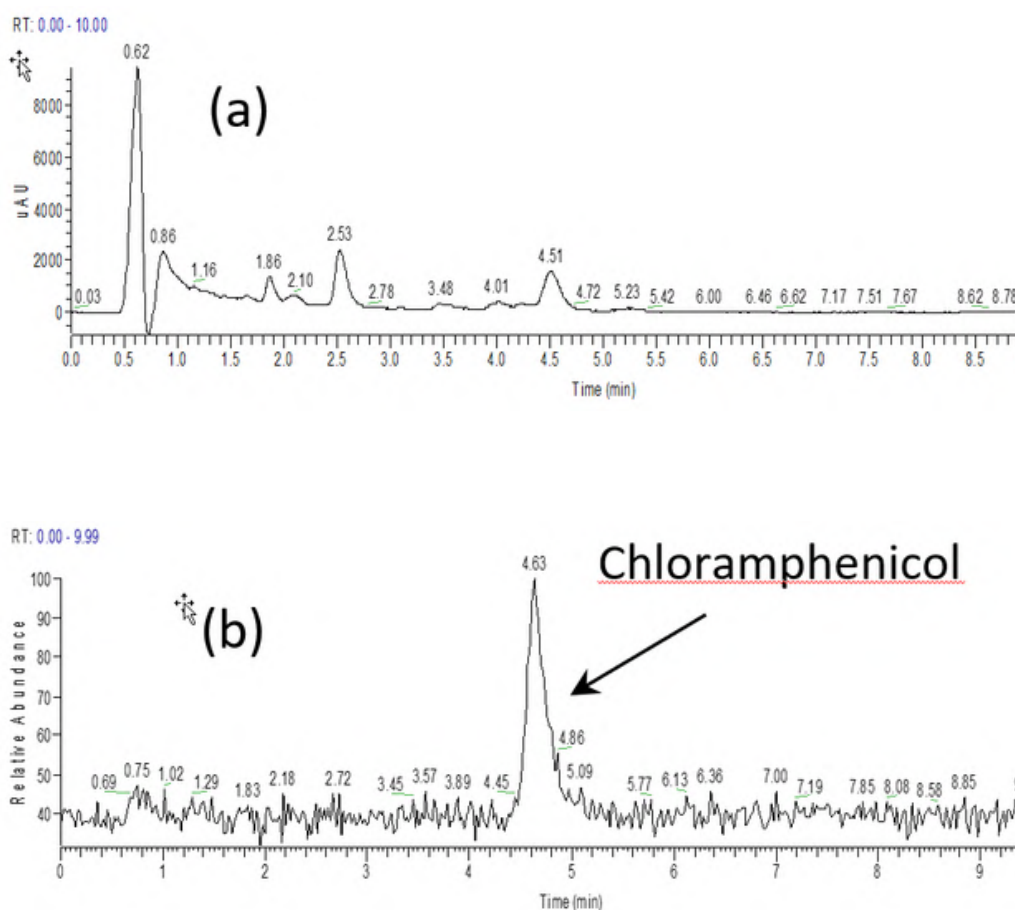


Figure 3. (a) UV (278nm) and (b) SIM ($m/z = 321$) Chromatograms obtained after clean-up with **AFFINIMIP® SPE Chloramphenicol**

C° (µg/kg)	Recoveries %	% RSDr
22,0	89	2,6

Table 1. Recovery of Chloramphenicol spiked at 22µg/kg after **AFFINIMIP® SPE Chloramphenicol** clean-up of 3mL of milk and relative standard deviation calculated from results generated under repeatability conditions (n=5).

Product reference

- **AFFINIMIP® SPE Chloramphenicol**

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

- **AFFINIMIP® SPE Chloramphenicol**

Catalog number: **FS110-03** for 50 cartridges 3mL

