



affinisep

Chloramphenicol

Application Note



Selective Solid Phase Extraction of Chloramphenicol from Bovine Urine using AFFINIMIP® SPE Chloramphenicol

Veterinary testing

Introduction

In this application note, we demonstrate a reliable quantification of Chloramphenicol from bovine urine at low concentrations using **AFFINIMIP® SPE Chloramphenicol** and even a single quadrupole mass detection.

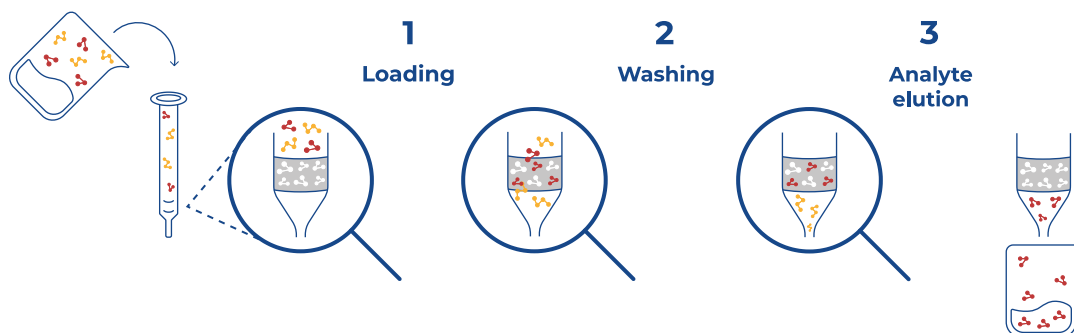


Figure 1. Principle of Solid Phase Extraction (SPE)

Proceeding of the experiment

Sample preparation

10 mL of urine were adjusted at pH 7 with Ammonia 1%. This solution was mixed 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

High analyte recovery

C° (µg/kg)	Mean (µg/kg)	Recoveries %
17.6	16.7	90

Table 1 - Recovery of Chloramphenicol spiked at 17.6µg/kg after **AFFINIMIP® SPE Chloramphenicol** clean-up of 1 mL of Urine.

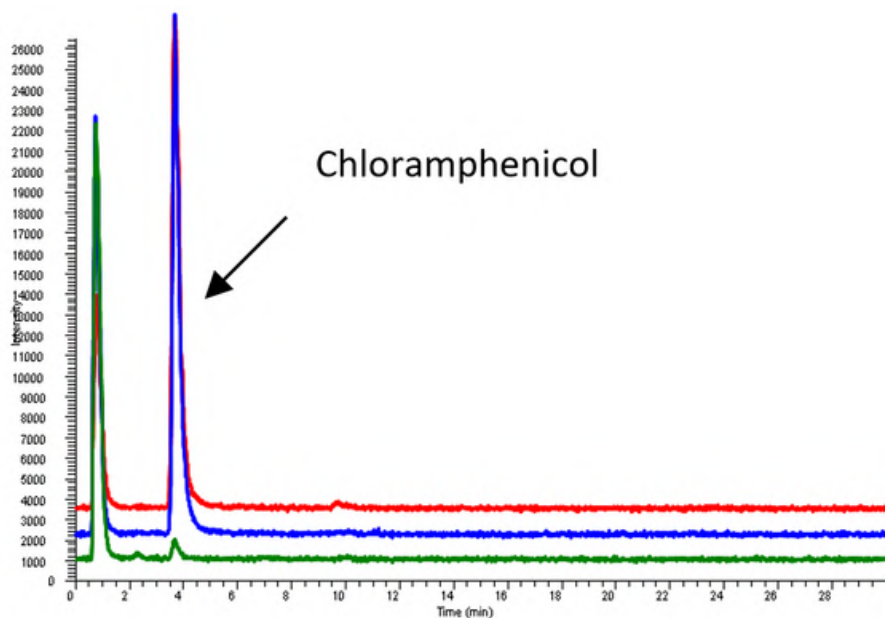


Figure 2. SIM Chromatograms obtained after clean-up with **AFFINIMIP® SPE Chloramphenicol** of 1 mL of Urine spiked with Chloramphenicol at 17.6µg/kg (red and blue) or not spiked (green).

UV chromatograms demonstrate a perfect cleanup

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

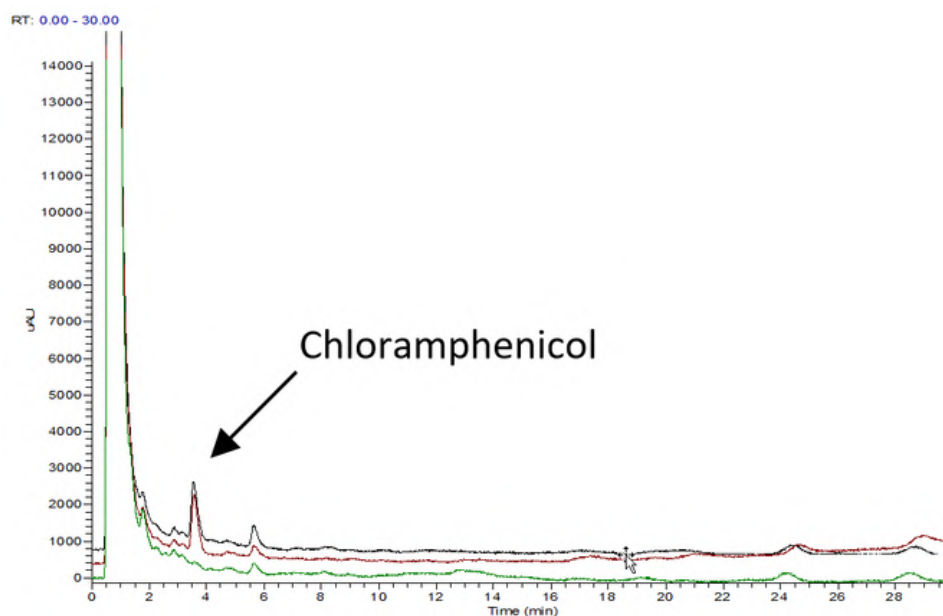


Figure 3. UV Chromatograms of Urine spiked with Chloramphenicol at 17.6 µg/kg (red and black) or not spiked (green) after clean-up with **AFFINIMIP® SPE Chloramphenicol**.

Product reference

- **AFFINIMIP® SPE Chloramphenicol**

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

Other format available

FS110-03 for 50 cartridges 3mL

