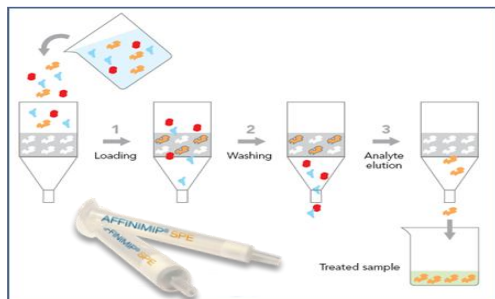


Selective Solid Phase Extraction for Tetracyclines Analysis from pork kidney tissue using AFFINIMIP®SPE



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

Tetracyclines (TCs, see Figure 1) are broad-spectrum antibiotics and are widely used as veterinary medicines and feed additives. These residues can cause toxic or allergic reactions in hypersensitive individuals and also transfer drug-resistant bacteria from food to humans.

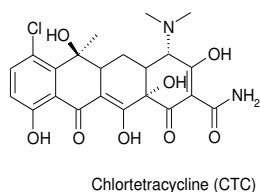
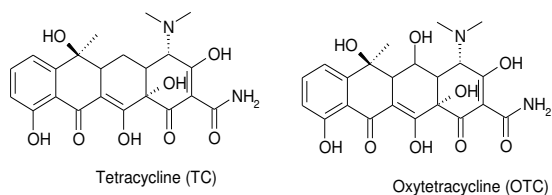


Figure 1. Chemical structure of Tetracyclines

In response to these concerns and to prevent harmful effects of residual antibiotics on the human health, various international health organizations have established the maximum residual limit (MRL) of TCs in animal source foods in their countries. Worldwide maximum residue levels (MRL) for tetracycline antibiotics are 200µg/kg in muscle, 600µg/kg in liver and 1200µg/kg in kidney.

How to solve this?

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

their epimers and Doxycycline) from complex matrices.

In this application note, we demonstrate a reliable quantification of Tetracyclines (TC, OTC and CTC) from pork kidney tissue using AFFINIMIP® SPE Tetracyclines with an LC-UV analysis suitable for routine analysis.

We obtained good recovery yields and repeatability with UV detection proving the efficiency of AFFINIMIP® SPE Tetracyclines cleanup.

Good recoveries in pork kidney tissue

Molecules	R% (n=5)	RSDr (%)
Tetracycline (TC)	85	5,4
Chlortetracycline (OTC)	79	2,8
Oxytetracycline (CTC)	80	4,2

Table 1. Recovery of Tetracyclines after AFFINIMIP® SPE Tetracyclines clean-up of pork kidney tissue spiked at 910µg/kg for TC; 980µg/kg for OTC and 860µg/kg for CTC

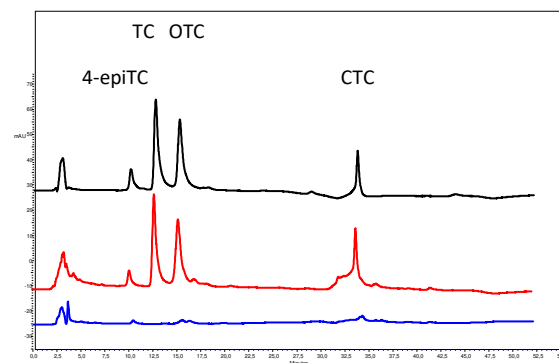


Figure 2. UV Chromatograms (355nm) obtained after clean-up with AFFINIMIP® SPE Tetracyclines of pork kidney tissue (red) or water (black) spiked with 910µg/kg Tetracycline, 980µg/kg Oxytetracycline and 860µg/kg Chlortetracycline as well as pork kidney not spiked (blue)

Experimental conditions

Materials

All reagents and chemicals were ACS grade quality or better. Meat was purchased at a supermarket.

Pretreatment of Meat prior to SPE with AFFINIMIP® SPE Tetracyclines Cartridge

EDTA/Mc Ilvaine's Buffer :

50mL of a 0.1M citric acid solution and 31.25 mL of 0.1M Na₂HPO₄·7H₂O solution were mixed and adjust to pH 4 with a NaOH solution. Then 3g of disodium EDTA were dissolved.

Preparation of loading solution for pork kidney tissue based on AOAC 995.09 method

10g meat were blend during 30 seconds with 40mL of EDTA/Mc Ilvaine's Buffer and stirred during 10min with a magnetic stirrer. The mixture was centrifuged at 2500g for 10 minutes at a temperature below 15°C. The supernatant was collected. This operation was repeated with 40mL of buffer and again with 20mL of buffer. Then, all the supernatants were gathered and centrifuged during 20min at 2500g, filtered on Buchner. Around 10mL 1N NaOH solution were added to the filtrate and adjusted to **pH 6.5** with a NaOH solution (this mixture was the loading solution).

Solid phase extraction (SPE) protocol

The SPE procedure used a 1mL AFFINIMIP® SPE Tetracyclines cartridge or 3mL cartridge. The details of each step are as follow:

- Condition the SPE cartridge with 1mL Acetonitrile (ACN), then with 1mL of deionized Water (2 drops/s)
- Load the loading solution : 4mL to 6mL of kidney loading solution) (0.5 drop/s)
- Wash the cartridge with 1mL of NaHCO₃ 1% in water (1 drop/s)
- Immediately wash the cartridge with 2mL of deionized Water/Acetonitrile (60/40, v/v) (1 drop/s)
- Apply vacuum during 30 sec to 1 minute to remove excess of solvent (only if elution is evaporated)
- Elute Tetracyclines with 2mL of 2% HCOOH Methanol (1 drop/s)

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

We observed that recovery yield is affected by the evaporation condition of the elution solution, especially for Chlortetracycline. We advise to not evaporate until dryness but to keep around 10µL to 30µL inside the vial. So, for LC-MS/MS analyses, we recommend a straightforward injection after dilution of the elution solution.

Analysis

HPLC was performed on a ThermoFinnigan Spectra System with a Thermo Hypersil Gold column (150mm x 2.1mm; 3µm). Separation was done by using a gradient (see Table 3) at a flow rate of 0.2mL/min.

Table 3. Gradient for the analysis of Tetracyclines.

Time (min)	% 10mM Oxalic Acid Water	% 10mM Oxalic Acid ACN	% MeOH
0	90	5	5
20	90	5	5
21	80	10	10
40	80	10	10
41	90	5	5

The detection system was a ThermoFinnigan Spectra System Model UV6000LP set to 355nm. The injection volume was 100µL.

Product reference

• AFFINIMIP® SPE Tetracyclines

Catalog number: FS112-02A for 25 cartridges 1mL
 FS112-03A for 50 cartridges 1mL
 FS112-02 for 25 cartridges 3mL
 FS112-03 for 50 cartridges 3mL

Related product

SPE Adapter & Reservoir kit: kit of 12 reservoirs 15ml and adapters for use with 1, 3 & 6 mL columns

Catalog number: ACC-AR2

For more information or ordering: contact@affinisep.com

Website: www.affinisep.com