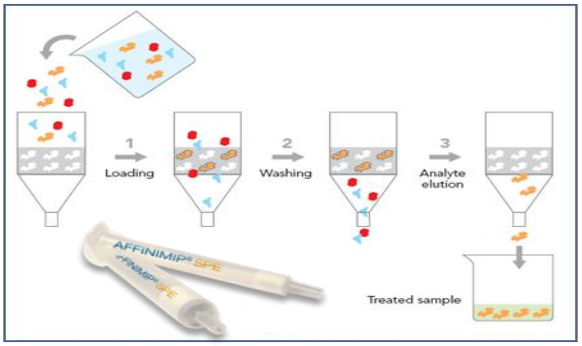
Selective Solid Phase Extraction for Tetracyclines, their Epimers and Doxycycline Analysis from animal source foods using AFFINIMIP® SPE



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

Tetracyclines (TCs, see Figure 1) and their 4-epimers 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.

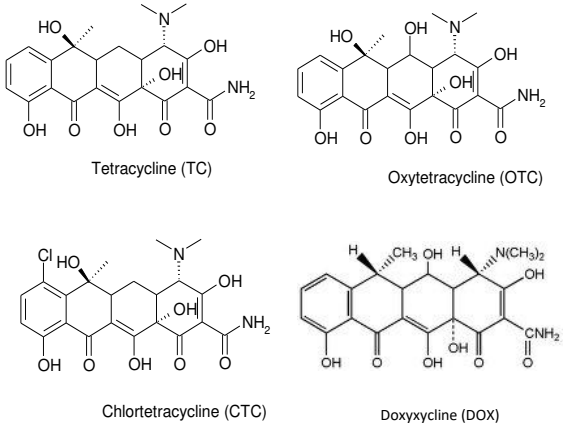


Figure 1. Chemical structure of Tetracyclines and Doxyxycline

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 100ppb (µg/L) in milk and fish.

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, their epimers and Doxycycline from milk and salmon using AFFINIMIP® SPE Tetracyclines based on an LC-UV analysis.

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

Good recoveries and repeatability in Milk

Molecules	Mean (µg/L)	Milk		Salmon
		R %	% RSDr	R%
Tetracycline	49.6	99.4	4.9	113
Oxytetracycline	45.6	91.3	7.1	-
Chlortetracycline	37.2	74.4	6.3	74
4-epitetracycline (4-epiTC)	47.9	95.9	5.1	-
4-epichlortetracycline	108.4	108.4	15.0	97
4-epioxytetracycline	43.7	87.4	9.1	71
Doxycycline (DOX)	43.8	88.0	2.9	89

Table 1. Recovery of Tetracyclines after AFFINIMIP® SPE Tetracyclines clean-up of Salmon or milk spiked at 50 or 100µg/L and relative standard deviation calculated from results generated under **repeatability conditions** (n=3).

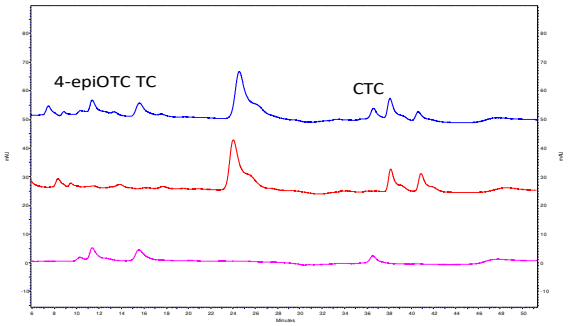


Figure 2. UV Chromatograms (355nm) obtained after clean-up with AFFINIMIP® SPE Tetracyclines of 1.5mL of Milk spiked with Tetracycline, Chlortetracycline and 4-epioxytetracycline (4-epiOTC) at 50µg/L (blue) or not spiked (red) or of 1.5mL of water spiked with Tetracycline, Chlortetracycline and 4-epioxytetracycline at 50µg/L (pink)

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Experimental conditions

Materials

All reagents and chemicals were ACS grade quality or better. Different kinds of milk were purchased at a supermarket.

Pretreatment of Milk and Salmon 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 $Na_2HPO_4.7H_2O$ solution were mixed and adjust to pH 4 with a NaOH solution. Then 3.03g of disodium EDTA were dissolved.

Preparation of loading solution for milk:
1.5mL of Milk was mixed with 6mL of EDTA/Mc Ilvaine's Buffer and the mixture was centrifuged at 4000rpm for 10 minutes at a temperature below 15°C. The supernatant was collected and 750µL of a 1N NaOH solution was added and the solution was then adjusted to pH 6.5 with a NaOH solution (this mixture was the loading solution).

Preparation of loading solution for Salmon based on AOAC 995.09 method
10g Salmon 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. 750µL 1N NaOH solution were added to the filtrate and adjusted to pH 6.5 (this mixture was the loading solution).

Solid phase extraction (SPE) protocol

The SPE procedure used a 1mL AFFINIMIP® SPE Tetracyclines 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 (~7.55mL of milk loading solution or ~10mL of salmon loading solution) (0.5 drop/s)
- Wash the cartridge with 1mL of deionized Water (1 drop/s)
- Wash the cartridge with 2mL of deionized Water/Acetonitrile (60/40, v/v) (1 drop/s)
- Apply vacuum during 3 minutes
- Elute Tetracyclines with 2mL of 2% HCOOH Methanol (1 drop/s)

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

Analysis

HPLC was performed on a ThermoFinnigan Spectra System with a Thermo Hypersil Gold column (150mm x 2.1mm; 3µm). Separation was accomplished 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

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

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