Instruction sheet

AFFINIMIP® SPE Tetracyclines cartridges

CLEAN-UP PROCEDURE

Users should read all instructions before using this kit.

For laboratory use only

AFFINIMIP® SPE Tetracyclines is developed and manufactured by AFFINISEP

E-mail: contact@affinisep.com

Technical support: tech.support@affinisep.com

www.polyintell.com

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Method for Selective Phase Extraction of Tetracyclines using Molecularly Imprinted Polymers

1. INTRODUCTION

AFFINIMIP® SPE Tetracyclines has been developed to selectively extract Tetracyclines in water and food matrices, such as infant formula.

By using **AFFINIMIP® SPE**, the expected result is a clean-up and a pre-concentration of the sample at trace level.

2. PRINCIPLE OF AFFINIMIP® SPE

AFFINIMIP® SPE is a solid phase obtained by a polymerisation process to create a three-dimensional network that recognizes the shape and functional group positions of a template molecule. The **AFFINIMIP® SPE** selectivity comes from the technology of molecularly imprinted polymer (MIP) used during the synthesis.

3. PRODUCT INFORMATION

Description of the kit

Each solid phase extraction (SPE) cartridge **AFFINIMIP® SPE Tetracyclines** of this kit contains 10mg of sorbent in a 1mL cartridge.

Information and storage

Storage: Room temperature.

Each cartridge has a single use.

4. PRECAUTIONS FOR USE

SPE methods developed for C18 or other sorbents are not appropriate for AFFINIMIP® SPE Tetracyclines. The extraction procedure described below has been optimized for the extraction of Tetracyclines from water or milk. For the treatment of another kind of matrix, please contact us to adapt the extraction procedure.

5. GENERAL INSTRUCTIONS FOR SPE

5.1. Equipments required

In addition to standard laboratory materials, the following equipments are required for the use of **AFFINIMIP® SPE** cartridges:

- SPE vacuum manifold
- Nitrogen Mini-vap evaporator or centrifugal concentrator to dry the collected samples

5.2. Flow rate

It is very important to follow the flow rate given in the protocol.
Most especially for the loading, if the sample flow rate is too high, components may not interact sufficiently with the sorbent and the analyte recovery yield will be lower.

5.3. Preparation process

For the preparation of the MIP, a template is required. Tetracyclines analogues were used instead of Tetracyclines to prevent false positive signals in case of bleeding.

6. CLEAN-UP PROCEDURE OF TETRACYCLINES FROM MILK, FISH OR WATER MATRICES:

6.1. Preparation of solutions

- Solution 60/40 Water/Acetonitrile (v/v)

In a 10mL-volumetric flask, add 4mL of Acetonitrile and complete with ultrapure water.

- Solution Methanol-2% Formic acid

In a 100mL-volumetric flask, add 2mL of Formic acid and complete with Methanol.

6.2. Loading solution for Milk

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.

Precipitation :

1.5mL of Milk were 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 10 with a NaOH solution (**this mixture is the loading solution for milk**).

6.3. Loading solution for Salmon

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 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 10 with a NaOH solution (**this mixture was the loading solution for salmon**).

6.4. Protocol for clean-up:

Step (Flow rate)	AFFINIMIP® SPE Tetracyclines (10mg/1mL)
Equilibration with (2 drops/s)	▪ 1mL Acetonitrile ▪ 1mL Water ▪ Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop every 2 seconds)	• **All the Milk loading solution** **or** • **1-3mL water** **or** • **10mL of Salmon loading solution**
Washing of interferents (1 drop/s)	• 1 mL ultrapure Water • 2 mL 60/40 Water/Acetonitrile
Drying :	**Force the washing solution down into the cartridge and out the bottom (For this step, you can apply vacuum 3 minutes)** *This step is not necessary if you don't evaporate elution solution*
Elution (E) (1 drop/s)	• 2mL Methanol -2% formic acid

The elution (E) can be diluted to a know volume before analysis or can be evaporated until dryness under nitrogen with a mini-vap evaporator at RT (time evaporation <25min). Immediately after drying, the residue is dissolved in mobile phase for further analysis.

Please note: We observed that recovery yield is affected by the evaporation step of the elution solution. A significant decrease is observed when the evaporation time is long or the mobile phase is added a long time after the drying, especially for Chlortetracycline.

PRODUCTS LIST

AFFINIMIP® SPE Products	Designation	Description
Multimyco10	AFFINIMIP® SPE Multimyco10	selective SPE cartridges 3mL for ZON, OTA, HT-2, T-2, Aflatoxins and Fumonisins
Zearalenone	AFFINIMIP® SPE Zearalenone	selective SPE cartridges 3mL for ZON
Ochratoxin A	AFFINIMIP® SPE Ochratoxin A	selective SPE cartridges 3mL for OTA
Patulin	AFFINIMIP® SPE Patulin	selective SPE cartridges for Patulin
	AFFINIMIP® SPE Patulin & Pectinase kit	kit of selective SPE cartridges for Patulin + 50mL pectinase enzyme solution
Deoxynivalenol	AFFINIMIP® SPE Deoxynivalenol	selective SPE cartridges 6mL for DON
Phenolics	AFFINIMIP® SPE Phenolics	selective SPE cartridges for Phenolic compounds
Estrogens	AFFINIMIP® SPE Estrogens	selective SPE cartridges for Estrogens
Zeranol Residues	AFFINIMIP® SPE Zeranol Residues	selective SPE cartridges for Zeranol Residues
Bisphenol A	AFFINIMIP® SPE Bisphenol A	selective (PP or Glass) SPE cartridges for Bisphenol A
FumoZON	AFFINIMIP® SPE FumoZON	selective SPE cartridges for Fumonisins and Zearalenone
Chloramphenicol	AFFINIMIP® SPE Chloramphenicol	selective SPE cartridges for Chloramphenicol
Tamoxifen	AFFINIMIP® SPE Tamoxifen	selective SPE cartridges for Tamoxifen
Catecholamines	AFFINIMIP® SPE Catecholamines	selective SPE cartridges for Catecholamines
	AFFINIMIP® SPE Catecholamines	selective SPE cartridges for Catecholamines
Metanephrines	AFFINIMIP® SPE Metanephrines	selective SPE cartridges for Metanephrines
Amphetamines	AFFINIMIP® SPE Amphetamines	selective SPE cartridges for Amphetamines
PECTINASE	Pectinase solution	50 mL pectinase enzyme solution
AttractSPE™ Products	**Designation**	**Description**
W/O	AttractSPE™ W/O	HLB SPE cartridges sorbent
SCX	AttractSPE™ SCX	Strong Cation Exchange SPE cartridges sorbent
WCX	AttractSPE™ WCX	Weak Cation Exchange SPE cartridges sorbent
SAX	AttractSPE™ SAX	Strong Anion Exchange SPE cartridges sorbent
WAX	AttractSPE™ WAX	Weak Anion Exchange SPE cartridges sorbent
DVB	AttractSPE™ DVB	Reversed Phase Copolymer SPE cartridges sorbent
Anionic & Cationic AttractSPE polymeric cartridges	AttractSPE™ KIT	Kit of 10 cartridges of each sorbent (SAX, WAX, WCX, SCX)

For more information:

For more information on our products & services, please visit www.polyintell.com