

# Instruction sheet

## CLEAN-UP PROCEDURE OF ZERANOL RESIDUES

### FROM URINE AND PLASMA

## AFFINIMIP® SPE **Zeranol Residues** cartridges

Users should read all instructions before using this kit.

***For research use only (RUO)***

**AFFINIMIP® SPE **Zeranol Residues** is:**

- **Developed and manufactured by AFFINISEP**

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## Method for Selective Phase Extraction of Zeranol Residues using Molecularly Imprinted Polymers

### 1 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® selectivity comes from the technology of molecularly imprinted polymer (MIP) used during the synthesis.

**AFFINIMIP® SPE Zeranol residues** has been developed to selectively extract Zeranol residues compounds such as Zeranol, Zearalenone,  $\beta$ -Zearalanol,  $\alpha$  and  $\beta$ -Zearalenol, Zearalanone (resorcylic acid lactone compounds) in urine, plasma and animal tissues.

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

### 2 PRODUCT INFORMATION

#### Description of the kit

Each solid phase extraction (SPE) cartridge **AFFINIMIP® SPE Zeranol Residues** contains 100mg of sorbent in a 3mL cartridge.

#### Information and storage

Storage: Room temperature.

Each cartridge has a single use.

### 3 PRECAUTIONS FOR USE

**SPE methods developed for C18 or other sorbents are not appropriate for AFFINIMIP® SPE Zeranol Residues. The extraction procedure described below has been optimized for the extraction of Zeranol Residues from urine and plasma.**

***For the treatment of another matrix, please contact us to adapt the extraction procedure.***

## 4 GENERAL INSTRUCTIONS FOR SPE

### 4.1 Materials and equipment required

In addition to standard laboratory equipment, the following material is required for the use of AFFINIMIP® SPE cartridges:

- SPE automate or SPE vacuum Manifold
- Nitrogen Mini-vap evaporator or a vacuum concentrator to dry the collected sample
- Acetonitrile (ACN)
- Methanol (MeOH)
- Distilled or deionized water
- Acetic acid glacial

### 4.2 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.

### 4.3 Preparation process

For the MIP preparation, a template is required. Zeranol Residues analogues were used instead of Zeranol residues to prevent a false positive signal in case of bleeding.

## 5 CLEAN-UP PROCEDURE OF ZERANOL RESIDUES FROM URINE:

### 5.1 Preparation of solutions

- Solution 35/1/64 Acetonitrile/Acetic Acid/ deionized water (v/v/v)

In a 20mL-volumetric flask, add 7mL of Acetonitrile, 0.2mL Acetic Acid and complete with deionized water.

- Solution 2/98 Acetic Acid/Methanol (v/v)

In a 10mL-volumetric flask, add 0.2mL of Acetic Acid and complete with Methanol.

- Sodium acetate buffer 0.2M pH=5

In a 100mL-volumetric flask, add 1.64g of sodium acetate and complete with Water. Adjust the pH to obtain a solution with pH=5.

## **5.2 Preparation of the loading solution from urine : Enzymatic hydrolysis for deconjugation of zeranol residues**

For 5mL of urine; add 5mL of acetate buffer (0.2M) pH=5 and 40μL of Helix Pomatia enzyme and mix. Enzymatic deconjugation was carried out overnight at 37°C. This solution is used as the loading solution.

## **5.3 Protocol for clean-up**

<b>Step</b>	<b>AFFINIMIP® SPE Zeranol Residues (100mg/3mL)</b>
Equilibration with (1 drop/s)	<ul style="list-style-type: none"> <li>▪ 2 mL Acetonitrile (ACN)</li> <li>▪ 2 mL deionized water</li> </ul> <p>Do not allow the cartridge to dry during conditioning</p>
Loading (L) (1 drop every 2 seconds)	<ul style="list-style-type: none"> <li>• <b>3mL of the loading solution</b></li> </ul>
Washing of interferents (1 drop/s)	<ul style="list-style-type: none"> <li>• 6mL Water</li> <li>• 3mL 35/1/64 Acetonitrile/Acetic Acid/ deionized water (v/v/v)</li> </ul>
Elution (E) (1 drop /s)	<ul style="list-style-type: none"> <li>• 2mL 2/98 Acetic Acid/Methanol (v/v)</li> </ul>

The elution fraction (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at room temperature (or a centrifugal evaporator). The residue is dissolved in 0.5mL of mobile phase for further analysis. Alternatively, the elution may be diluted to a known volume by addition of water for further analysis (duration time: around 25min).

## 6 CLEAN-UP PROCEDURE OF ZERANOL RESIDUES FROM PLASMA:

### 6.1 Preparation of the loading solution

In a 20mL-volumetric flask: add 4mL of plasma and completed with 16mL of ultrapure water

### 6.2 Preparation of solutions

- Solution 35/65 Acetonitrile/ deionized water (v/v/v)

In a 20mL-volumetric flask, add 7mL of Acetonitrile and complete with deionized water.

- Solution 2/98 Acetic Acid/Methanol (v/v)

In a 10mL-volumetric flask, add 0.2mL of Acetic Acid and complete with Methanol.

### 6.3 Protocol for clean-up of Zeranol residues from plasma

Step	AFFINIMIP® SPE Zeranol residues (100mg/3mL)
Equilibration with (1 drop / s)	<ul style="list-style-type: none"> <li>2 mL Acetonitrile (ACN)</li> <li>2 mL deionized water</li> </ul> <p>Do not allow the cartridge to dry during conditioning</p>
Loading (L) (1 drop every 2 seconds)	<ul style="list-style-type: none"> <li><b>Up to 9mL of the loading solution</b></li> </ul>
Washing of interferents	<ul style="list-style-type: none"> <li>6mL ultrapure water</li> <li>3mL of (65/35) ultrapure water/ Acetonitrile (v/v)</li> </ul>
Drying (1 drop / s)	<ul style="list-style-type: none"> <li>Apply vacuum or nitrogen flow through cartridge during <b>30 seconds</b></li> </ul>
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> <li><b>2mL 2/98 Acetic Acid/Methanol (v/v)</b></li> </ul>

The elution fraction (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at room temperature (or a centrifugal evaporator). The residue is dissolved in 0.5mL of mobile phase for further analysis. Alternatively, the elution may be diluted to a known volume by addition of water for further analysis (duration time around 35min).

## PRODUCTS LIST

AFFINIMIP® SPE Products	Designation	Description
Multimycoto10	AFFINIMIP® SPE Multimycoto10	selective SPE cartridges 3mL for ZON, OTA, HT-2, T-2, Aflatoxins and Fumonisin
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 Fumonisin 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
Metanephries	AFFINIMIP® SPE Metanephries	selective SPE cartridges for Metanephries
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](http://www.polyintell.com)