

Instruction sheet

AFFINIMIP® SPE Ochratoxin A cartridges

CLEAN-UP PROCEDURE OF Ochratoxin A

Users should read all instructions before using this kit.

For laboratory use only

AFFINIMIP® SPE Ochratoxin A is:

- developed and manufactured by AFFINISEP

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Method for Selective Phase Extraction of Ochratoxin A using Molecularly Imprinted Polymers

1. INTRODUCTION

AFFINIMIP® SPE Ochratoxin A has been developed to selectively extract Ochratoxin A in cereals such as wheat, in beverage such as wine or spices such as pepper or paprika.

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

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 Ochratoxin A contains 100mg of sorbent in a 3mL 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 Ochratoxin A. *The extraction procedure described below has been optimized for the extraction of Ochratoxin A from wheat sample. For the treatment of another matrices, please contact us to adapt the extraction procedure.*

5. RECOMMENDATIONS FOR HPLC ANALYSIS

For the HPLC analysis, the following conditions have been used:

Column: C18 (USP L1), 2.1 x 150mm, spherical silica gel (Type A), particle size: 3µm

Mobile phase: 60-39-1 Methanol - Water - Acetic Acid (v/v/v)

Flow rate: 0.2mL/min

Detection of Ochratoxin A by fluorescence (λ_{ex} : 333 nm, λ_{em} : 460 nm)

For an optimal resolution (Rs), we recommend a retention time of Ochratoxin A higher than 10 minutes.

6. GENERAL INSTRUCTIONS FOR SPE USE

6.1. Materials and equipment required

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

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

6.2. Flow rate

Flow information is given for each step in the protocol. It is important to follow these instructions. Especially during 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.

6.3. Preparation process

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

6.4. Preparation of solutions for the SPE protocol

- Solution 60/40 Hydrochloric acid (pH=1) / Acetonitrile (v/v)

In a 10mL-volumetric flask, add 4mL of Acetonitrile and complete with HCl pH=1 0,1M.

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

In a 10mL-volumetric flask, add 200µL of Acetic Acid and complete with Methanol.

7. CLEAN-UP PROCEDURE OF OCHRATOXIN A FROM WHEAT MATRICES WITH 60/40 ACETONITRILE/WATER EXTRACTION SOLUTION:

1.1. Preparation of solutions

Extraction solvent: Solution 60/40 Acetonitrile/ deionized water (v/v)

In a 100mL-volumetric flask, add 40mL of deionized water and complete with Acetonitrile.

1.2. Preparation of the loading solution

50g of finely ground wheat are mixed during 1 minute in a blender with 100mL of extraction solvent (60/40 Acetonitrile/deionized Water). The extract is filtered through a filter paper (4-7µm). Then, 5mL of the extract is diluted with 5mL of HCl solution pH=1, 0.1M. After a filtration through a filter paper (4-7µm), this solution is used as the loading solution.

Users developing their own extraction method must take into considerations that the composition of the Acetonitrile – Water loading solution must contains a maximum of 30% Acetonitrile. In addition, the pH of the solution must be ≤ 1 .

Step (Flow rate)	AFFINIMIP® SPE Ochratoxin A (100mg/3mL)
Equilibration with (2 drops/s)	<ul style="list-style-type: none"> ▪ 3mL Acetonitrile ▪ 3mL deionized water ▪ Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop/s)	<ul style="list-style-type: none"> • Up to 4mL of the loading solution
Washing of interferents (1 drop/s)	<ul style="list-style-type: none"> • 6mL 60/40 HCl solution pH=1, 0.1M/Acetonitrile (v/v)
Drying	Apply vacuum 3 minutes
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> • 2mL 2/98 Acetic acid/Methanol (v/v)

The elution (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at room temperature (or a centrifugal evaporator). The residue is dissolved in 500 μ L of mobile phase for further analysis. Alternatively, the elution may be diluted to a known volume by addition of water for further analysis.

8. CLEAN-UP PROCEDURE OF OCHRATOXIN A FROM WINE MATRICES:

Preparation of the loading solution

5mL of wine (red or white) are diluted with 5mL of HCl solution (pH=1, 0.1M).

Step (Flow rate)	AFFINIMIP® SPE Ochratoxin A (100mg/3mL)
Equilibration with (2 drops/s)	<ul style="list-style-type: none"> ▪ 3mL Acetonitrile ▪ 3mL deionized water ▪ Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop/s)	<ul style="list-style-type: none"> • 2 to 10mL of the loading solution
Washing of interferents (1 drop/s)	<ul style="list-style-type: none"> • 6mL 60/40 HCl solution pH=1,0.1M/Acetonitrile (v/v)
Drying	Apply vacuum 3 minutes
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> • 2mL 2/98 Acetic acid/Methanol (v/v)

The elution (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at room temperature (or a centrifugal evaporator). The residue is dissolved in 500µL of mobile phase for further analysis. Alternatively, the elution may be diluted to a known volume by addition of water for further analysis.

9. CLEAN-UP PROCEDURE OF OCHRATOXIN A FROM PAPRIKA MATRICES:

9.1. Preparation of solutions

Extraction solvent: Solution NaHCO₃ 1%

In a 100mL-volumetric flask, add 1g of NaHCO₃ and complete with deionized water.

9.2. Preparation of the loading solution

10g of paprika are shaken during 30 minutes with 100mL of extraction solvent (Solution NaHCO₃ 1%). The extract is centrifuged for 30 minutes at 4000 rpm at room temperature then filtered through a filter paper (4-7µm).

25mL of the extract is diluted with 25mL of HCl solution pH=1, 0.1M. After a filtration through a filter paper (4-7µm), this solution is used as the loading solution.

Users developing their own extraction method must take into considerations that the composition of the Acetonitrile – Water loading solution must contains a maximum of 30% Acetonitrile. In addition, the pH of the solution must be ≤1.

Step (Flow rate)	AFFINIMIP® SPE Ochratoxin A (100mg/3mL)
Equilibration with (2 drops/s)	<ul style="list-style-type: none"> ▪ 3mL Acetonitrile ▪ 3mL deionized water ▪ Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop/s)	<ul style="list-style-type: none"> • Up to 20mL of the loading solution
Washing of interferences (1 drop/s)	<ul style="list-style-type: none"> • 6mL 60/40 HCl solution pH=1, 0.1M /Acetonitrile (v/v)
Drying	Apply vacuum 3 minutes
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> • 2mL 2/98 Acetic acid/Methanol (v/v)

The elution (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at room temperature (or a centrifugal evaporator). The residue is dissolved in 500µL of mobile phase for further analysis. Alternatively, the elution may be diluted to a known volume by addition of water for further analysis.

PRODUCTS LIST

AFFINIMIP® SPE Products	Designation	Description
Multimycos10	AFFINIMIP® SPE Multimycos10	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
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