

Instruction sheet

AFFINIMIP® SPE **Multimyc**o LCMSMS cartridges

CLEAN-UP PROCEDURE OF MYCOTOXINS FROM GRAIN

Users should read all instructions before using this kit.

For laboratory use only

AFFINIMIP® SPE **Multimyco LCMSMS** 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 Solid Phase Extraction of Mycotoxins using Molecularly Imprinted Polymers

1. INTRODUCTION

AFFINIMIP® SPE Multimyco LCMSMS has been developed to extract mycotoxins (Aflatoxins, Zearalenone, Ochratoxin A, HT-2, T-2, Fumonisin, Deoxynivalenol (DON)) from grain products such as wheat or maize.

By using **AFFINIMIP® SPE**, the expected result is a clean-up and a pre-concentration of the sample at trace level suitable for LC-MS/MS detection

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 Multimyco LCMSMS** of this kit contains 100mg of sorbent in 3mL or 6mL cartridges.

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 Multimyco LCMSMS. The extraction procedure described below has been optimized for the extraction of Mycotoxins from grains. For the treatment of another kind of matrix, please contact us to adapt the extraction procedure.**

5. GENERAL INSTRUCTIONS FOR SPE

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 vacuum concentrator to dry the collected samples

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.

Preparation process

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

6. CLEAN-UP PROCEDURE OF MYCOTOXINS FROM GRAIN (INCLUDING DON):

6.1. Preparation of solutions

Extraction solution

- Solution Water/Acetonitrile (ACN) 50/50 (v/v)

In a 100mL-volumetric flask, add 50mL of ACN and complete with water.

Washing solution

- Solution Water/Acetonitrile (ACN) 95/5 (v/v)

In a 100mL-volumetric flask, add 5mL of ACN and complete with water.

- Solution Water-1% Acetic acid (v/v)

In a 100mL-volumetric flask, add 1mL of Acetic acid and complete with water.

Elution solution

- Solution Formic acid/ACN/Methanol (MeOH) 3/48.5/48.5 (v/v/v)

In a 100mL-volumetric flask, add 48.5mL of MeOH, 3mL formic acid and complete with Acetonitrile.

- Solution Formic acid/Ethyl Acetate/ Methanol (MeOH) 3/48.5/48.5 (v/v/v)

In a 100mL-volumetric flask, add 48.5mL of MeOH, 3mL formic acid and complete with Ethyl Acetate.

6.2. Protocol for clean-up:

Preparation of the loading solution:

25g of finely ground cereal are mixed during 2 minutes in a blender with 100mL of extraction solvent (50/50 Acetonitrile/ Water). Some cereals (wheat...) can swell and absorb a large amount of extraction solution, *for this reason, 100mL of additional water/acetonitrile extraction solution can be added to obtain a mixture which is more fluid.*

The extract is filtered through a filter paper (4-7µm).

Then, 2mL of the extract is diluted with 18mL of water. 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 5% Acetonitrile.

Duration of the protocol: 35min

Step (Flow rate)	AFFINIMIP® SPE Multimyco LCMSMS
Equilibration with (1 drops/s)	<ul style="list-style-type: none"> ▪ 3mL ACN ▪ 3mL Water ▪ Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop every 2 seconds)	Up to 6mL of the loading solution
Washing (interferences removal) (1 drop/s)	<ul style="list-style-type: none"> • 6mL Water-1% Acetic acid • 2mL Water/ACN 95/5 (v/v)
Drying :	<p>Apply vacuum 3 to 5 minutes in order to remove remaining water residues to decrease the evaporation time of the elution solution</p> <p><i>This step is not necessary if you don't evaporate elution solution</i></p>
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> • 4mL 3/48.5/48.5 Formic Acid / Methanol / Acetonitrile (v/v/v) OR • 4mL 3/48.5/48.5 Formic Acid / Methanol / Ethyl Acetate (v/v/v)

The elution (E) is diluted to a known volume or evaporated until dryness under nitrogen with a mini-vap evaporator at RT or with a centrifugal evaporator at 45°C. The residue is dissolved in mobile phase for analysis by LC-MS/MS detection. We highly recommend using at least 15% organic solvent and preferably acetonitrile to properly dissolve the residue. We also highly recommend using vortex and ultrasound to properly homogenize your residue with your mobile phase (low recoveries could be observed if the residue is not properly dissolve).

7. CLEAN-UP PROCEDURE OF MYCOTOXINS FROM GRAIN (WITHOUT DON ANALYSIS):

7.1. Preparation of solutions

Extraction solution

- Solution Water/Acetonitrile (ACN) 50/50 (v/v)

In a 100mL-volumetric flask, add 50mL of ACN and complete with water.

Washing solution

- Solution Water/Acetonitrile (ACN) 85/15 (v/v)

In a 100mL-volumetric flask, add 15mL of ACN and complete with water.

Elution solution

- Solution Formic acid/ACN/Methanol (MeOH) 3/48.5/48.5 (v/v/v)

In a 100mL-volumetric flask, add 48.5mL of MeOH, 3mL formic acid and complete with Acetonitrile.

- Solution Formic acid/Ethyl Acetate/ Methanol (MeOH) 3/48.5/48.5 (v/v/v)

In a 100mL-volumetric flask, add 48.5mL of MeOH, 3mL formic acid and complete with Ethyl Acetate.

7.2. Protocol for clean-up:

Preparation of the loading solution:

25g of finely ground cereal are mixed during 2 minutes in a blender with 100mL of extraction solvent (50/50 Acetonitrile/ Water). Some cereals (wheat...) can swell and absorb a large amount of extraction solution, *for this reason, 100mL of additional water/acetonitrile extraction solution can be added to obtain a mixture which is more fluid.*

The extract is filtered through a filter paper (4-7 μ m).

Then, 4mL of the extract is diluted with 16mL of water. After a filtration through a filter paper (4-7 μ m), 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 15% Acetonitrile.

Duration of the protocol: 35min

Step (Flow rate)	AFFINIMIP® SPE Multimyco LCMSMS
Equilibration with (1 drops/s)	<ul style="list-style-type: none"> ▪ 3mL ACN ▪ 3mL water ▪ Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop every 2 seconds)	Up to 6mL of the loading solution
Washing (interferences removal) (1 drop/s)	<ul style="list-style-type: none"> • 6mL water • 4mL Water/ACN 85/15 (v/v)
Drying :	<p>Apply vacuum 3 to 5 minutes in order to remove remaining water residues to decrease the evaporation time of the elution solution</p> <p><i>This step is not necessary if you don't evaporate elution solution</i></p>
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> • 4mL 3/48.5/48.5 Formic Acid / Methanol / Acetonitrile (v/v/v) OR • 4mL 3/48.5/48.5 Formic Acid / Methanol / Ethyl Acetate (v/v/v)

The elution (E) is diluted to a known volume or evaporated until dryness under nitrogen with a mini-vap evaporator at RT or with a centrifugal evaporator at 45°C. The residue is dissolved in mobile phase for analysis by LC-MS/MS detection. We recommend using at least 15% organic solvent (ACN...) to properly dissolve the residue. We also recommend using vortex and ultrasound to properly homogenize your residue with your mobile phase.

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