Instruction sheet

AFFINIMIP® SPE Patulin cartridges

Format : 200mg/6mL

CLEAN-UP PROCEDURE OF PATULIN

FROM

APPLE JUICE, APPLE PUREE, FRUIT JUICE CONCENTRATE,

THICK FRUIT JUICE AND DRIED APPLE DICE

Users should read all instructions before using this kit.

For laboratory use only

AFFINIMIP® SPE Patulin is developed and manufactured by AFFINISEP

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Version 2.5

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Method for Selective Phase Extraction of Patulin using Molecularly Imprinted Polymers

1. INTRODUCTION

AFFINIMIP® SPE Patulin (200mg) has been developed to selectively extract patulin in dried apple, apple juice and puree, concentrate of fruit juice and thick fruit juice. For apple juice, a higher enrichment of patulin is obtained with a 200mg **AFFINIMIP® SPE Patulin** cartridge by increasing the amount of loading solution.

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 Patulin** of this kit contains 200mg of sorbent in a 6mL 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 Patulin. The extraction procedure described below has been optimized for the extraction of Patulin from matrices done in the introduction. For the treatment of another kind of matrix, please contact us to adapt the extraction procedure.

Patulin is unstable under alkaline conditions (sodium bicarbonate solution) after few minutes.

Patulin could also degrade under dry conditions: Do not allow the cartridge to dry.

Evaporation of elution solution could also be subject of variation of recoveries therefore evaporation should be made carefully and the reconstitution in the solvent has to be made immediately after evaporation.

5. RECOMMENDATIONS FOR HPLC ANALYSIS

For 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: following gradient

Time (min)	% water	% ACN
0	98	2
20	98	2
21	50	50
25	50	50
26	98	2

Flow rate: 0.2mL/min

Detection of patulin by UV at 276nm

6. GENERAL INSTRUCTIONS FOR SPE

6.1. 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 vacuum concentrator to dry the collected samples

6.2. 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.

6.3. Preparation process

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

7. CLEAN-UP PROCEDURE OF PATULIN FROM APPLE JUICE:

7.1. Preparation of solutions

- Solution Water - 0.1% Acetic Acid

  In a 10mL-volumetric flask, add 0.01mL of pure Acetic Acid and complete with water.

- Solution Water - 2% Acetic Acid

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

- Solution $NaHCO_3$ 1% (w/v)

  In a 100mL-volumetric flask, weigh 1g of $NaHCO_3$ and complete with water.

7.2. Protocol for the clean-up of apple juice:

Pre-treatment of cloudy apple juice for the loading solution:

Clear apple juice does not require any pre-treatment before being used as a test portion.

This is different for cloudy apple juice which requires a pre-treatment with a pectinase enzyme solution to clarify it.

So, to 20mL of cloudy apple juice, add 150µL pectinase enzyme solution and mix. Leave solution at room temperature overnight or for 2h at 40°C. Centrifuge at 4500g for 5min. This solution is used as the test portion.

Preparation of the loading solution:

2.5mL of test portion is diluted with 2.5mL of Water - 2% Acetic Acid and mix. This solution is used as the loading solution.

Step (Flow rate)	AFFINIMIP® SPE Patulin (200mg/6mL)
Equilibration with (1 drop/s)	▪ 4mL Acetonitrile ▪ 2mL water ▪ Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop every 2 seconds)	**8 mL** of the loading solution (**Flow rate: 0.5mL/min**)
Washing of interferents (W1) (1 drop/s)	• 2mL $NaHCO_3$ 1% solution • 4mL Water **(Immediately)**
Drying :	**Force the water down into the cartridge and out the bottom (For this step, you can apply vacuum 30 seconds)**
Washing of interferents (W2) (1 drop/s)	• 2mL Diethyl Ether
Elution (E) (1 drop/s)	• 2mL Ethyl Acetate

Then, add 1 drop (~10µL) of pure Acetic Acid in the Elution solution (E) to prevent degradation of Patulin. Afterwards, the elution (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at 40°C (or a centrifugal evaporator). The duration of drying must be as shorter as possible ideally inferior to 20min (a too long evaporation time might result to

lower recovery yield). **Immediately after drying**, add 500µL of 0.1% Acetic Acid in water for further analysis.

8. CLEAN-UP PROCEDURE OF PATULIN FROM APPLE PUREE:

8.1. Recommendations for HPLC analysis

For 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: following gradient

Time (min)	% water	% ACN
0	98	2
20	98	2
21	50	50
25	50	50
26	98	2

Flow rate: 0.2mL/min

Detection of patulin by UV at 276nm

8.2. Preparation of solutions

- Solution Water - 1% Acetic Acid

In a 10mL-volumetric flask, add 0.1mL of pure Acetic acid and complete with water.

- Solution Water - 0.1% Acetic Acid

In a 10mL-volumetric flask, add 0.01mL of pure Acetic acid and complete with water.

- Solution $NaHCO_3$ 1% (w/v)

In a 100mL-volumetric flask, weigh 1g of $NaHCO_3$ and complete with water.

8.3. Protocol for the clean-up of apple puree:

Loading solution

For apple puree, weigh 10g test portion; add 150µL pectinase enzyme solution followed by 10mL water and mix. Leave solution at room temperature overnight or for 2h at 40°C (following the European standard EN14177). Centrifuge at 4500g for 5min and then filter the solution with a 0.2µm filter (Regenerated Cellulose). This solution is used as the loading solution.

Step (Flow rate)	AFFINIMIP®SPE Patulin (200mg/6mL)
Equilibration with (2 drops/s)	• 2mL Acetonitrile • 2mL water • Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop every 2 s)	• **5 mL** of the loading solution (**Flow rate: 0.5mL/min**)
Washing of interferences (W1) (1 drop/s)	• 4mL Water-1% Acetic Acid • 1mL $NaHCO_3$ 1% solution • 3mL Water **(Immediately)**
Drying :	**Force the water down into the cartridge and out the bottom (For this step, you can apply vacuum 30 seconds)**
Washing of interferences (W2) (1 drop/s)	• 500µL Diethyl Ether
Elution (E) (1 drop/s)	• 2mL Ethyl Acetate

Then, add 1 drop (~10µL) of pure Acetic Acid in the Elution solution (E) to prevent degradation of Patulin. Afterwards, the elution (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at 40°C or RT (or a centrifugal evaporator). The duration of drying must be as shorter as possible ideally inferior to 20min (a too long evaporation time might result to lower recovery yield). **Immediately after drying**, add 500µL of 0.1% Acetic Acid in water for further analysis.

9. CLEAN-UP PROCEDURE OF PATULIN FROM FRUIT JUICE CONCENTRATE AND THICK FRUIT JUICE:

9.4. Recommendations for HPLC analysis

For 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: following gradient

Time (min)	% water	% ACN
0	98	2
20	98	2
21	50	50
25	50	50
26	98	2

Flow rate: 0.2mL/min

Detection of patulin by UV at 276nm

9.5. Preparation of solutions

- Solution Water - 2% Acetic Acid

  In a 10mL-volumetric flask, add 0.2mL of pure Acetic acid and complete with water.

- Solution Water - 0.1% Acetic Acid

  In a 10mL-volumetric flask, add 0.01mL of pure Acetic acid and complete with water.

- Solution $NaHCO_3$ 1% (w/v)

  In a 100mL-volumetric flask, weigh 1g of $NaHCO_3$ and complete with water.

9.6. Protocol for the clean-up of fruit juice concentrate:

2.5g of fruit juice concentrate are mixed with 10mL water and 100µL Pectinase. Leave the solution at room temperature overnight or for 2h at 40°C. Centrifuge at 4000g for 10min and collect the supernatant. Dilute the supernatant by 2 with acetic acid 2% in water. This solution is used as the loading solution.

9.7. Protocol for the clean-up of thick fruit juice:

15mL of thick fruit juice concentrate are mixed with 120µL Pectinase. Leave the solution at room temperature overnight or for 2h at 40°C. Centrifuge at 4000g for 10min and collect the supernatant. Dilute the supernatant by 2 with acetic acid 2% in water. This solution is used as the loading solution.

9.8. Protocol for the clean-up of thick fruit juice and fruit juice concentrate:

Step (Flow rate)	AFFINIMIP®SPE Patulin (200mg/6mL)
Equilibration with (2 drops/s)	• 4mL Acetonitrile • 4mL water • Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop every 2 s)	• **4 to 6 mL** of the loading solution (**Flow rate: 0.5mL/min**)
Washing of interferences (W1) (1 drop/s)	• 2mL $NaHCO_3$ 1% solution • 4mL Water **(Immediately)**
Drying :	**Force the water down into the cartridge and out the bottom (For this step, you can apply vacuum 30 seconds)**
Washing of interferences (W2) (1 drop/s)	• 1mL Diethyl Ether
Elution (E) (1 drop/s)	• 2mL Ethyl Acetate

Then, add 1 drop (~10µL) of pure Acetic Acid in the Elution solution (E) to prevent degradation of Patulin. Afterwards, the elution (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at 40°C or RT (or a centrifugal evaporator). The duration of drying must be as shorter as possible ideally inferior to 20min (a too long evaporation time might result to lower recovery yield). **Immediately after drying**, add 500µL of 0.1% Acetic Acid in water for further analysis.

10. CLEAN-UP PROCEDURE OF PATULIN FROM DRIED APPLE DICES:

10.1. Preparation of solutions

- Solution Water - 0.1% Acetic Acid

In a 10mL-volumetric flask, add 0.01mL of pure Acetic Acid and complete with water.

- Solution Water - 2% Acetic Acid

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

10.2. Protocol for clean-up:

Preparation of the loading solution for dried apple dices:

Weigh 3g of dried apple dices; add 150µL of a pectinase enzyme solution followed by 30mL water and mix. Leave solution at room temperature overnight or for 2h at 40°C. Centrifuge at 4500g for 5min and then filter the solution with a 0.2µm filter. This solution is used as the loading solution.

Step (Flow rate)	AFFINIMIP® SPE Patulin (200mg/6mL)
Equilibration with (1 drop/s)	▪ 4mL Acetonitrile ▪ 2mL water ▪ Do not allow the cartridge to dry during conditioning
Loading (L) (1 drop every 2 seconds)	**10 mL** of the loading solution (**Flow rate: 0.5mL/min**)
Washing of interferents (W1) (1 drop/s)	• 5 mL Water – 2% Acetic acid • 5mL Water
Drying :	**Force the water down into the cartridge and out the bottom (For this step, you can apply vacuum 30 seconds)**
Washing of interferents (W2) (1 drop/s)	• 500µL Diethyl Ether
Elution (E) (1 drop/s)	• 2mL Ethyl Acetate

Then, add 1 drop (~10µL) of pure Acetic Acid in the Elution solution (E) to prevent degradation of Patulin. Afterwards, the elution (E) is evaporated until dryness under nitrogen

with a mini-vap evaporator at 40°C (or a centrifugal evaporator). The duration of drying must be as shorter as possible ideally inferior to 20min (a too long evaporation time might result to lower recovery yield). Immediately after drying, add 500µL of 0.1% Acetic Acid in water for further analysis.

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