

# Instruction sheet

## **AFFINIMIP®SPE Patulin** cartridges

**Format: 3mL**

### **CLEAN-UP PROCEDURE OF PATULIN**

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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## Method for Selective Phase Extraction of Patulin using Molecularly Imprinted Polymers

### 1. INTRODUCTION

**AFFINIMIP® SPE Patulin** has been developed to selectively extract patulin in apple products such as apple juice and apple puree.

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

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

**The evaporation of elution solution is a very sensitive step which affect the recovery yields therefore evaporation should be made carefully and the reconstitution in the solvent has to be made immediately after evaporation. It is important to evaluate the loss of Patulin during this evaporation step by spiking a blank elution sample with a known concentration of Patulin.**

**We recommend to use a reference sample to evaluate this loss for each serie of Patulin analysis to obtain the most accurate result.**

## 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
- Pectinase enzyme solution (typical activity 1400U/g) for apple puree analysis

### 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<sub>3</sub> 1% (w/v)

In a 100mL-volumetric flask, weigh 1g of NaHCO<sub>3</sub> and complete with water.

## 7.2. Preparation of the loading solution

### 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.

## 7.3. Protocol for clean-up of apple juice:

### Duration of the protocol: 20min

Step (Flow rate)	AFFINIMIP® SPE Patulin (100mg/3mL)
Equilibration with (1 drops/s)	<ul style="list-style-type: none"> <li>▪ 2mL Acetonitrile</li> <li>▪ 1mL water</li> <li>▪ Do not allow the cartridge to dry during conditioning</li> </ul>
Loading (L) (1 drop every 2 seconds)	<b>2x2 mL of the loading solution (Flow rate: 0.5mL/min)</b>
Washing of interferents (W1) (1 drop/s)	<ul style="list-style-type: none"> <li>• 1mL NaHCO<sub>3</sub> 1% solution</li> <li>• 2mL Water (<b>Immediately</b>)</li> </ul>
Drying :	<b>Force the water down into the cartridge and out the bottom (For this step, you can apply vacuum 10 seconds)</b>
Washing of interferents (W2) (1 drop/s)	<ul style="list-style-type: none"> <li>• 1mL Diethyl Ether</li> </ul>
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> <li>• 2mL Ethyl Acetate</li> </ul>

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.

## 8. CLEAN-UP PROCEDURE OF PATULIN FROM CIDER:

### 8.1. 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.

### 8.2. Preparation of the loading solution for cider

The cider has been degassed by sonicating sample for 1 hour. 2.5mL of degassed cider is diluted with 2.5mL of Water - 2% Acetic Acid. This solution is mixed and used as the loading solution.

### 8.3. Protocol for clean-up of cider: Main protocol

AFFINISEP advises to use this protocol for the clean-up of cider. However, in case of too many interferences in the elution fraction, a more eluting protocol is also described.

**Duration of the protocol: 20min**

<b>Step (Flow rate)</b>	<b>AFFINIMIP® SPE Patulin (100mg/3mL)</b>
Equilibration with (1 drops/s)	<ul style="list-style-type: none"> <li>▪ 2mL Acetonitrile</li> <li>▪ 1mL water</li> <li>▪ Do not allow the cartridge to dry during conditioning</li> </ul>
Loading (L) (1 drop every 2 seconds)	<b>2x2 mL</b> of the loading solution ( <b>Flow rate: 0.5mL/min</b> )
Washing of interferents (W1) (1 drop/s)	<ul style="list-style-type: none"> <li>• 2mL Water</li> </ul>
Drying :	<b>Force the water down into the cartridge and out the bottom (For this step, you can apply vacuum 20 seconds)</b>
Washing of interferents (W2) (1 drop/s)	<ul style="list-style-type: none"> <li>• 250µL Diethyl Ether</li> </ul>
Drying :	<b>As the volume of solvent is very low, Force the solvent down into the cartridge and out the bottom (For this step, you can apply vacuum 10 seconds)</b>
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> <li>• 2mL Ethyl Acetate</li> </ul>

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.

#### 8.4. Protocol for clean-up of cider: More eluting protocol

**Duration of the protocol: 20min**

<b>Step (Flow rate)</b>	<b>AFFINIMIP® SPE Patulin (100mg/3mL)</b>
Equilibration with (1 drops/s)	<ul style="list-style-type: none"> <li>▪ 2mL Acetonitrile</li> <li>▪ 1mL water</li> <li>▪ Do not allow the cartridge to dry during conditioning</li> </ul>
Loading (L) (1 drop every 2 seconds)	<b>2x2 mL</b> of the loading solution ( <b>Flow rate: 0.5mL/min</b> )
Washing of interferents (W1) (1 drop/s)	<ul style="list-style-type: none"> <li>• 1mL NaHCO<sub>3</sub> 1% solution</li> <li>• 2mL Water (<b>Immediately</b>)</li> </ul>
Drying :	<b>Force the water down into the cartridge and out the bottom (For this step, you can apply vacuum 20 seconds)</b>
Washing of interferents (W2) (1 drop/s)	<ul style="list-style-type: none"> <li>• 500µL Diethyl Ether</li> </ul>
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> <li>• 2mL Ethyl Acetate</li> </ul>

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 APPLE PUREE:**

### 9.1. 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<sub>3</sub> 1% (w/v)

In a 100mL-volumetric flask, weigh 1g of NaHCO<sub>3</sub> and complete with water.

### 9.2. Preparation of the loading solution from apple puree

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.

### 9.3. Protocol for apple puree

Step (Flow rate)	AFFINIMIP®SPE Patulin (100mg/3mL)
Equilibration with (2 drops/s)	<ul style="list-style-type: none"> <li>• 2mL Acetonitrile</li> <li>• 1mL water</li> <li>• Do not allow the cartridge to dry during conditioning</li> </ul>
Loading (L) (1 drop every 2 s)	<ul style="list-style-type: none"> <li>• <b>2x2.5 mL</b> of the loading solution <b>(Flow rate: 0.5mL/min)</b></li> </ul>
Washing of interferences (W1) (1 drop/s)	<ul style="list-style-type: none"> <li>• 2x2mL Water-1% Acetic Acid</li> <li>• 1mL NaHCO<sub>3</sub> 1% solution</li> <li>• 3mL Water (<b>Immediately</b>)</li> </ul>
Drying :	<b>Force the water down into the cartridge and out the bottom (For this step, you can apply vacuum 10 seconds)</b>
Washing of interferences (W2) (1 drop/s)	<ul style="list-style-type: none"> <li>• 500µL Diethyl Ether</li> </ul>
Elution (E) (1 drop/s)	<ul style="list-style-type: none"> <li>• 2mL Ethyl Acetate</li> </ul>

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.

#### **For more information:**

For more information on our products & services, please visit [www.polyintell.com](http://www.polyintell.com)