



The art of making  
sample preparation easier



## PATULIN ANALYSIS

---

Comprehensive solution  
to comply regulation

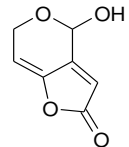


# TABLE OF CONTENTS

<b>Patulin analyses</b>	<b>4</b>
<b>Our solutions</b>	<b>5</b>
<b>Regulation – Limit Patulin Concentration</b>	<b>6</b>
<b>Regulation – performance criteria</b>	<b>7</b>
<b>AFFINIMIP® SPE Patulin kit</b>	<b>8</b>
<b>AFFINIMIP® SPE Patulin kit composition</b>	<b>9</b>
<b>Advantages of using AFFINIMIP® SPE Patulin</b>	<b>10</b>
<b>AFFINIMIP® SPE Patulin Vs alternative method on Apple juice</b>	<b>11</b>
<b>A solution approved by QC labs</b>	<b>12</b>
<b>Quality Control and Proficiency testings for AFFINIMIP®SPE Patulin</b>	<b>13</b>
<b>Application notes</b>	<b>14</b>
<b>Analytical methods for Patulin determination</b>	<b>37</b>
<b>Related products &amp; services</b>	<b>39</b>
<b>SPE ACCESSORIES</b>	<b>40</b>
<b>PERSONALISED SERVICES</b>	<b>41</b>
<b>Ordering information</b>	<b>42</b>
<b>About AFFINISEP</b>	<b>43</b>

# Patulin analyses

Patulin [4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one] is a mycotoxin produced by a variety of molds, particularly *Aspergillus* and *Penicillium* species. It is commonly found in rotting apples, and the amount of patulin in apple products is generally viewed as a measure of the quality of the apples used in production.



Studies have shown that it is genotoxic.

The level of this contaminant is regulated in several foods matrices.

AFFINISEP brings an innovative solution for the analyses of Patulin in all kinds of matrices with **AFFINIMIP®SPE Patulin** KIT, an efficient and simple clean-up and analytical method.

Based on this kit, AFFINISEP also proposes a complete range of services to determine Patulin levels in your product from simple services such as a training or development on SPE methods to more complex with the full implementation of in-house QC lab.

This booklet gathers very useful information on patulin analyses such as regulation reminder, application notes etc.

# Our solutions

Whatever your situation is,  
We can help you!

AFFINISEP brings you a solution as  
**PRODUCTS** or as **SERVICES** at each  
step of the analytical process of  
Patulin determination



Method  
development  
for your own  
matrix



Training on  
analytical  
methods and  
sampling

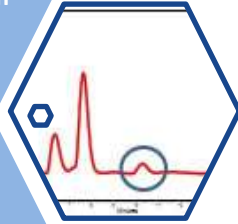


AFFINIMIP®  
SPE Patulin  
KIT evaluated  
for a high  
number of  
products

Turnkey  
implementatio  
n of your own  
analytical  
laboratory



Analyses of  
your matrices



For more detailed information  
about our services, please  
contact us at:  
[contact@affinisep.com](mailto:contact@affinisep.com)

# Regulation – Limit Patulin concentration

- ✓ Patulin: Regulated analyte and methods
- ✓ Ready to use kit
- ✓ Simple & Fast process
- ✓ Tested and approved by QC labs
- ✓ Evaluated within proficiency tests
- ✓ Validated for a high number of matrices

## COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006

setting maximum levels for certain contaminants in foodstuffs

	Foodstuffs	Maximum levels for patulin (µg/kg)
2.3.1	Fruit juices, concentrated fruit juices as reconstituted and fruit nectars (14)	50
2.3.2	Spirit drinks (15), cider and other fermented drinks derived from apples or containing apple juice	50
2.3.3	Solid apple products, including apple compote, apple puree intended for direct consumption with the exception of foodstuffs listed in 2.3.4 and 2.3.5	25
2.3.4	Apple juice and solid apple products, including apple compote and apple puree, for infants and young children (16) and labelled and sold as such (4)	10
2.3.5	Baby foods other than processed cereal-based foods for infants and young children (3) (4)	10

(3) Foodstuffs listed in this category as defined in Commission Directive 96/5/EC of 16 February 1996 on processed cereal-based foods and baby foods for infants and young children (OJ L 49, 28.2.1996, p. 17) as last amended by Directive 2003/13/EC (OJ L 41, 14.2.2003, p. 33).

(4) The maximum level refers to the products ready to use (marketed as such or after reconstitution as instructed by the manufacturer).(14) Foodstuffs listed in this category as defined in Council Directive 2001/112/EC of 20 December 2001 relating to fruit juices and certain similar products intended for human consumption (OJ L 10, 12.1.2002, p. 58).

(14) Foodstuffs listed in this category as defined in Council Directive 2001/112/EC of 20 December 2001 relating to fruit juices and certain similar products intended for human consumption (OJ L 10, 12.1.2002, p. 58).

(15) Foodstuffs listed in this category as defined in Council Regulation (EEC) No 1576/89 of 29 May 1989 laying down general rules on the definition, description and presentation of spirit drinks (OJ L 160, 12.6.1989, p. 1), as last amended by the Protocol concerning the conditions and arrangements for admission of the Republic of Bulgaria and Romania to the European Union.

(16) Infants and young children as defined in Directive 91/321/EEC and Directive 96/5/EC.

# Regulation – Performance criteria

- ✓ **Patulin: Regulated analyte and methods**
- ✓ Ready to use kit
- ✓ Simple & Fast process
- ✓ Tested and approved by QC labs
- ✓ Evaluated within proficiency tests
- ✓ Validated for a high number of matrices

**COMMISSION REGULATION (EC) No 401/2006 of 23 February 2006** laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs:

## 4. METHOD OF ANALYSIS TO BE USED BY THE LABORATORY AND LABORATORY CONTROL REQUIREMENTS

### 4.3. Specific requirements

#### 4.3.1. Performance criteria

Where no specific methods for the determination of mycotoxin levels in foodstuffs are required by Community legislation, laboratories may select any method provided the selected method meets the following criteria:

#### Performance criteria for patulin

Level µg/Kg	RSD <sub>r</sub> %	RSD <sub>R</sub> %	Recovery %
<20	≤30	≤40	50 to 120
20-50	≤20	≤30	70 to 105
>50	≤15	≤25	75 to 105

# AFFINIMIP® SPE Patulin kit

- ✓ Patulin: Regulated analyte and methods
- ✓ Ready to use kit
- ✓ Simple & Fast process
- ✓ Tested and approved by QC labs
- ✓ Evaluated within proficiency tests
- ✓ Validated for a high number of matrices





# AFFINIMIP® SPE Patulin kit composition

## AFFINIMIP® SPE Patulin cartridges



**AFFINIMIP® SPE Patulin** cartridges are **innovative solution for a fast and cost - effective analysis of Patulin**. Based on Molecularly Imprinted Polymers technology, these cartridges selectively interact with Patulin.

### Available formats:

- 3mL open cartridge -100mg
- 6mL open cartridge -200mg

## Patulin Standard solution



Our Patulin standard solution is accurately prepared solutions of Patulin at 100µg/mL in Acetonitrile. These standards can be used for the implementation of the calibration curve as well as for the development of a new analytical methods

**Concentration:** 100µg/ml

**Volume :** 1ml

**Mycotoxin purity :** 98.5%

**Stability :** 1 year

**Storage :** 4°C

## Pectinase enzymatic solution



Pectinase enzyme recommended by the AOAC and validated by AFFINISEP for apple puree pretreatment

The enzymatic solution is also used for cloudy apple juice, dried apple, thick juice or concentrate pretreatment.

**Volume :** 50ml

**Storage :** 4°C

## Custom-made reagents

Custom-made reagents for SPE, extraction and analytical process can be delivered to your facilities.

The composition of your AFFINIMIP® SPE Patulin kit can be done by selecting the items, you need

# Advantages of using AFFINIMIP® SPE Patulin

- ✓ Patulin: Regulated analyte and methods
- ✓ Ready to use kit
- ✓ **Simple & Fast process**
- ✓ Tested and approved by QC labs
- ✓ Evaluated within proficiency tests
- ✓ Validated for a high number of matrices

## Greater Recoveries

- Minimal sample transfer
- Specific procedure for Patulin analyte (equivalent to IAC column)

## Greater Accuracy

- No cross contamination

## Save time

- Faster Protocol
- Fewer steps

## Lower Cost

- Lower solvent consumption
- Lower reagent consumption
- Less apparatus

## Greater Safety

- Less exposure to toxic agents

## No Emulsion Problems

- Less sample handling
- Fewer steps

## No Transporting of Samples to Lab

- Direct field sampling

## Reduced Harm to Labile Samples

- Minimal evaporation

## Minimal Glass Breakage

- Less glassware used, less to wash

## Manual SPE manifold

10 to 12 SPE could be made in the same time and two series of SPE could be easily made during one days

>>> 20 to 24 samples analyses are easily obtained

## Easy to use with SPE automate

See application with the ASPEC automate

# AFFINIMIP® SPE Patulin

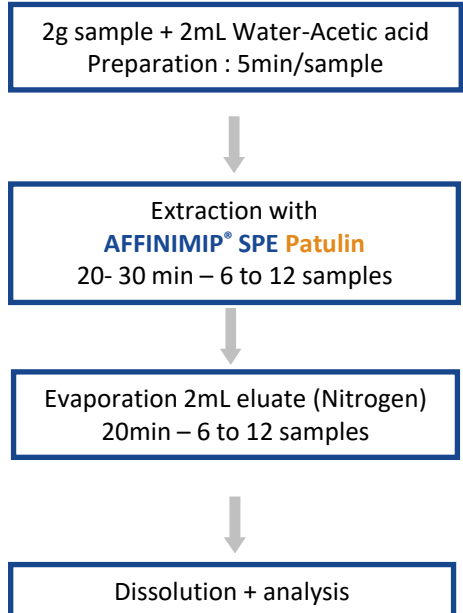
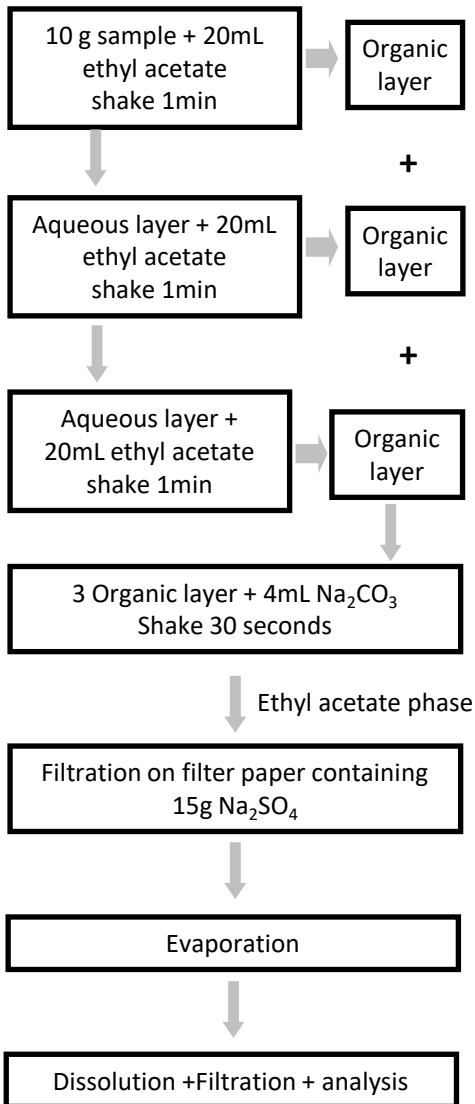
## Vs alternative method on Apple juice

Liquid –Liquid extraction Procedure

AFFINIMIP® SPE Patulin Procedure

7 steps ≈ 1h for 1 sample

4 steps ≈ 1h for 6 to 12 samples

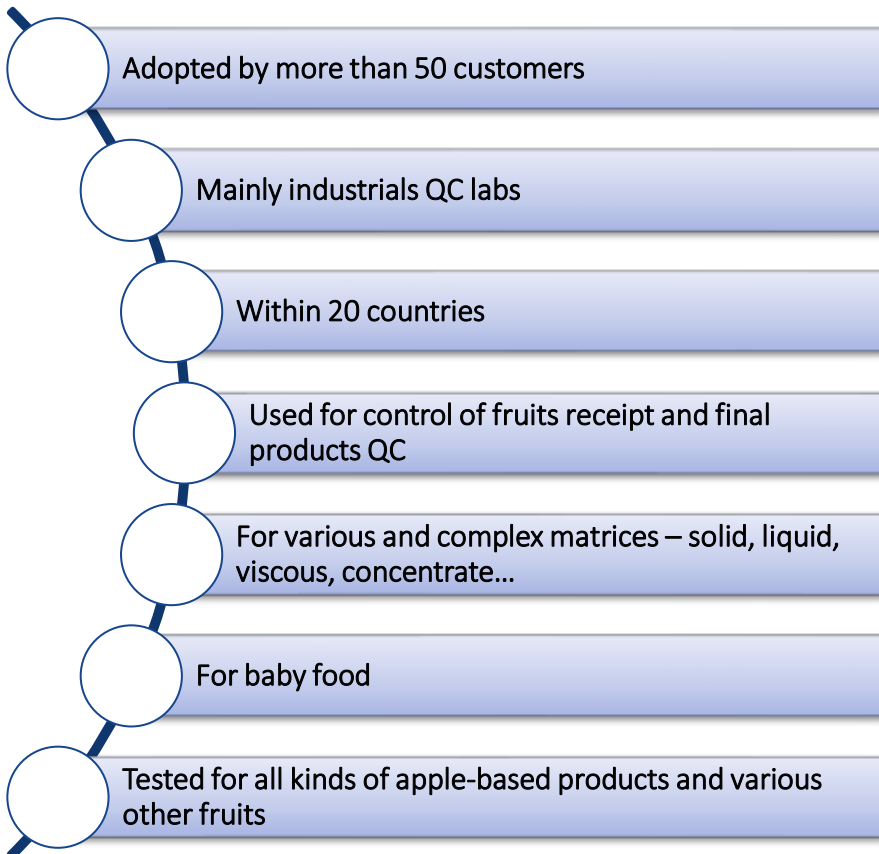


- ✓ Easy process
- ✓ High productivity

Save your time!



## A solution approved by QC labs

- ✓ Patulin: Regulated analyte and methods
- ✓ Ready to use kit
- ✓ Simple & Fast process
- ✓ Tested and approved by QC labs
- ✓ Evaluated within proficiency tests
- ✓ Validated for a high number of matrices



## Quality Control and Proficiency testings for AFFINIMIP®SPE Patulin

- ✓ Patulin: Regulated analyte and methods
- ✓ Ready to use kit
- ✓ Simple & Fast process
- ✓ Tested and approved by QC labs
- ✓ Evaluated within proficiency tests
- ✓ Validated for a high number of matrices

Proficiency test	Test Materiel	Assigned Value µg/kg	AFFINIMIP®SPE results µg/kg	Z- score
				
Test 1645 – October 2011	Apple puree	23.6	24.31	<b>0.1</b>
Test 1646 – March 2012	Cloudy Apple Juice	26.8	27	<b>0.0</b>
Test 1647 – July 2012	Clear Apple Juice	39.3	37.5	<b>-0.2</b>
				
December 2011	Multifruits compote	9	10	<b>0.20</b>
February 2012	Cider	61	65	<b>0.38</b>
April 2012	Stewed apples	39	29	<b>-0.80</b>

**These blind testings confirm that AFFINIMIP®SPE Patulin method always performs clean-up with excellent results.**

# Application notes

- ✓ Patulin: Regulated analyte and methods
- ✓ Ready to use kit
- ✓ Simple & Fast process
- ✓ Tested and approved by QC labs
- ✓ Evaluated within proficiency tests
- ✓ Validated for a high number of matrices

PATULIN ANALYSIS IN BABY FOOD APPLE JUICE - UV	15
PATULIN ANALYSIS IN APPLE JUICE - UV	16
PATULIN ANALYSIS IN APPLE PUREE - UV	17
PATULIN ANALYSIS IN APPLE PUREE (cartridge 6mL-200mg) - UV	18
PATULIN ANALYSIS IN BABY FOOD APPLE PUREE - UV	19
PATULIN ANALYSIS IN APPLE – FRUIT PUREE - UV	20
PATULIN ANALYSIS IN WHOLE APPLE - UV	21
PATULIN ANALYSIS IN CIDER - UV	22
PATULIN ANALYSIS IN ALCOHOL POMMEAU - UV	23
PATULIN ANALYSIS IN LIQUOR - UV	24
PATULIN ANALYSIS IN DRIED APPLE - UV	25
PATULIN ANALYSIS IN TOMATO KETCHUP AND TOMATO POWDER - UV	26
PATULIN ANALYSIS IN BLUEBERRY JUICE - UV	27
PATULIN ANALYSIS IN GRAPEFRUIT CONCENTRATE JUICE - UV	28
PATULIN ANALYSIS IN THICK JUICE BASED ON MANGO - UV	29
PATULIN ANALYSIS IN APPLE JUICE WITH AN AUTOMATE GX-271 ASPEC™ – UV	30
PATULIN ANALYSIS IN PEAR/APPLE PUREE - LC-MS/MS	31
PATULIN ANALYSIS IN APPLE JUICE - LC-MS/MS	32
PATULIN ANALYSIS IN APPLE JUICE WITH AN AUTOMATE ASPEC™Xi -LC-MS/MS(article)	33
PATULIN ANALYSIS IN APPLE PUREE AND FRUIT – APPLE PUREE – UV (article)	34
PATULIN ANALYSIS IN APPLE PUREE, JUICE AND JAM BY UHPLC – UV (article)	35
PATULIN ANALYSIS IN APPLE JUICE – UV	36

## Regulations for apple juice:

Europe (EC 1881/2006) : 50µg/Kg

USA (FDA CPG Sec.510.150) : 50µg/Kg

## Regulations for apple juice for infants and young children:

Europe (EC 1881/2006) : 10µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

Loading solution: 2.5mL apple juice and 2.5mL of water-2% acetic acid are mixed.

### Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL water

#### Loading

- 4mL of loading solution

#### Washing of interferences (W1)

- 1mL NaHCO<sub>3</sub> 1% in water
- 2mL Water

#### Drying by applying vacuum 10 seconds

#### Washing of interferences (W2)

- 1mL Diethyl Ether or MTBE

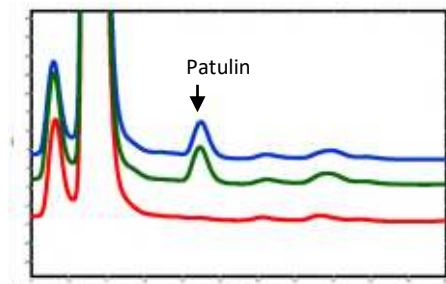
#### Elution (E)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°1 (description p 37)

## RESULTS



Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of an apple juice spiked at 10µg/kg with Patulin (Green and blue) or not spiked (Red)

Recovery of Patulin (n=9) at a contamination level of 10µg/kg in apple Juice after AFFINIMIP® SPE Patulin Clean-up.

Recoveries % (n=9)	% RSD <sub>R</sub>
97.9	11

### Catalog number:

**3mL-100mg sorbent for APPLE JUICE**

FS102-03 for 50 cartridges

**6mL-200mg sorbent for all applications**

FS102-03B -200mg for 50 cartridges

# PATULIN ANALYSIS IN APPLE JUICE - UV

## Regulations for apple juice:

Europe (EC 1881/2006) : 50µg/Kg

USA (FDA CPG Sec.510.150) : 50µg/Kg

## Regulations for apple juice for infants and young children:

Europe (EC 1881/2006) : 10µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

Loading solution: 2.5mL apple juice and 2.5mL of water-2% acetic acid are mixed.

### Purification with a 3mL/100mg **AFFINIMIP® SPE Patulin** cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL water

#### Loading

- 4mL of loading solution

#### Washing of interferences (W1)

- 1mL NaHCO<sub>3</sub> 1% n Water
- 2mL Water

#### Drying by applying vacuum 10 seconds

#### Washing of interferences (W2)

- 1mL Diethyl Ether or MTBE

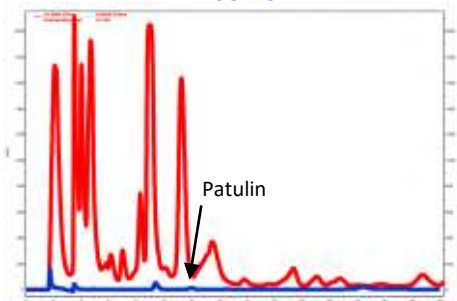
#### Elution (E)

- 2mL Ethyl Acetate

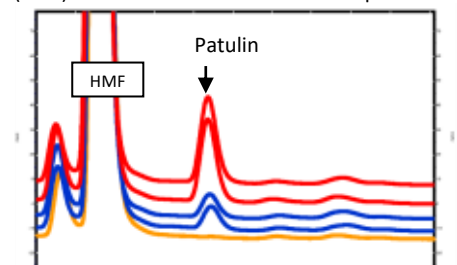
The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°1 (description p 37)

## RESULTS



Chromatograms of apple juice containing 25µg/kg of Patulin before (Red) and after (Blue) **AFFINIMIP® SPE Patulin** Clean-up



Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of an apple juice spiked at 40µg/kg (tested twice, red) or at 10µg/kg (tested twice, blue) with Patulin or not spiked (orange)

Recovery of Patulin in apple juice after **AFFINIMIP® SPE Patulin** Clean-up and relative standard deviation calculated from results generated under reproducibility conditions.

Concentration of Patulin (ng/mL)	Recovery %	% RSD <sub>R</sub>
10	97.9	11 (n=9)
40	90.6	11 (n=41)

### Catalog number:

**3mL-100mg sorbent for APPLE JUICE**

FS102-03 for 50 cartridges

**6mL-200mg sorbent for all applications**

FS102-03B -200mg for 50 cartridges



# PATULIN ANALYSIS IN APPLE PUREE - UV

## Regulations for apple puree:

Europe (EC 1881/2006) : 25µg/Kg

## Regulations for apple juice for infants and young children:

Europe (EC 1881/2006) : 10µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

10g of apple puree, 150µL of a pectinase enzyme solution and 10mL water are mixed. 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.

### Purification with a 3mL/100mg **AFFINIMIP® SPE Patulin** cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL Water

#### Loading

- 5mL of loading solution

#### Washing of interferences (W1)

- 4mL Water -1%Acetic acid
- 1mL NaHCO<sub>3</sub> 1% solution
- 3mL Water

#### Drying by applying vacuum 10 seconds

#### Washing of interferences (W2)

- 500µL Diethyl Ether or MTBE

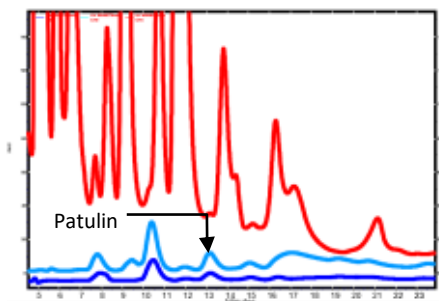
#### Elution (E)

- 2mL Ethyl Acetate

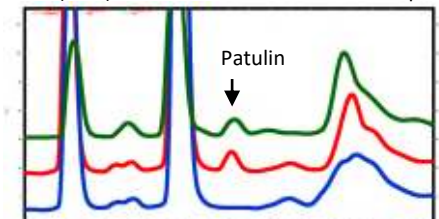
The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms of apple puree containing 40µg/kg or 80µg/kg of Patulin before (Red) and after (Blue) **AFFINIMIP® SPE Patulin** Clean-up



Chromatograms of apple puree containing 0µg/kg (blue) or 20µg/kg (tested twice, green and red) of Patulin after **AFFINIMIP® SPE Patulin** Clean-up

Recovery and repeatability of Patulin (n=3) at a contamination level of 20µg/kg in apple puree after **AFFINIMIP® SPE Patulin** Clean-up.

Concentration of Patulin (µg/kg)	Recoveries % (n=3)	% RSDr
20	84	4.5

### Catalog number:

#### **3mL-100mg sorbent**

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL

Pectinase

REA-001-50mL for 50mL Pectinase solution

# PATULIN ANALYSIS IN APPLE PUREE – CARTRIDGE 6mL - 200mg - UV

A format tailored for the larger liquid volume required for apple puree protocol

## Regulations for apple puree:

Europe (EC 1881/2006) : 25µg/Kg

## Regulations for apple juice for infants and young children:

Europe (EC 1881/2006) : 10µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

10g of apple puree, 150µL of a pectinase enzyme solution and 10mL water are mixed. 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.

**Purification with a 6mL/200mg AFFINIMIP®**

### SPE Patulin cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL Water

#### Loading

- 5mL of loading solution

#### Washing of interferences (W1)

- 4mL Water -1%Acetic acid
- 1mL NaHCO<sub>3</sub> 1% solution
- 3mL Water

#### Drying by applying vacuum 10 seconds

#### Washing of interferences (W2)

- 500µL Diethyl Ether or MTBE

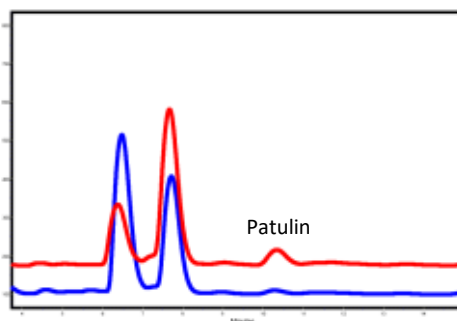
#### Elution (E)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms of apple puree spiked with 20µg/kg of Patulin (Red) and not spiked (blue) after AFFINIMIP® SPE Patulin Clean-up

Recovery and repeatability of Patulin (n=6) at a contamination level of 10µg/kg in apple puree after AFFINIMIP® SPE Patulin Clean-up.

Concentration of Patulin (µg/kg)	Recoveries %	% RSDr
10 (n=6)	90	9
20 (n=3)	92	11

## Catalog number:

### 6mL - 200mg sorbent

FS102-03B-200mg for 50 cartridges

FS102-03BK-200mg for a kit of 50 cartridges + 50mL Pectinase

REA-001-50mL for 50mL Pectinase solution

## Regulations for apple puree:

Europe (EC 1881/2006) : 25µg/Kg

## Regulations for apple puree for infants and young children:

Europe (EC 1881/2006) : 10µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

10g of apple puree, 150µL of a pectinase enzyme solution and 10mL water are mixed. 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.

### Purification with a 3mL/100mg **AFFINIMIP® SPE Patulin** cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL Water

#### Loading

- 5mL of loading solution

#### Washing of interferences (W1)

- 4mL Water -1%Acetic acid
- 1mL NaHCO<sub>3</sub> 1% solution
- 3mL Water

#### Drying by applying vacuum 10 seconds

#### Washing of interferences (W2)

- 500µL Diethyl Ether or MTBE

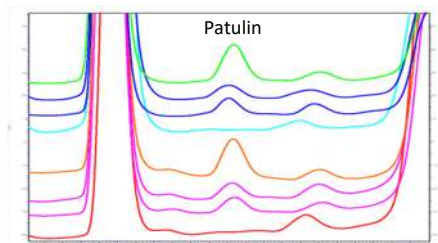
#### Elution (E)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of different apple puree. In the lower part, clean-up of an apple puree from a well-known brand spiked at 25µg/kg (orange), 10µg/kg with Patulin (pink, tested twice) or not spiked (red).

In the top part, clean-up of an apple puree second well known brand spiked at 25µg/kg (green), 10µg/kg with Patulin (blue, tested twice) or not spiked (turquoise).

Recovery and repeatability of Patulin (n=4) at a contamination level of 10µg/kg in apple puree after **AFFINIMIP® SPE Patulin** Clean-up.

Recoveries % (n=4)	% RSD <sub>R</sub>
81.2	2.1

### Catalog number:

#### **3mL-100mg sorbent**

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL

Pectinase

REA-001-50mL for 50mL Pectinase solution

# PATULIN ANALYSIS IN APPLE – FRUIT PUREE - UV

## Regulations for apple puree:

Europe (EC 1881/2006) : 25µg/Kg

## Regulations for apple puree for infants and young children:

Europe (EC 1881/2006) : 10µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

10g of apple puree, 150µL of a pectinase enzyme solution and 10mL water are mixed. 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.

### Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL Water

#### Loading

- 5mL of loading solution

#### Washing of interferences (W1)

- 4mL Water -1%Acetic acid
- 1mL NaHCO<sub>3</sub> 1% solution
- 3mL Water

#### Drying by applying vacuum 10 seconds

#### Washing of interferences (W2)

- 500µL Diethyl Ether or MTBE

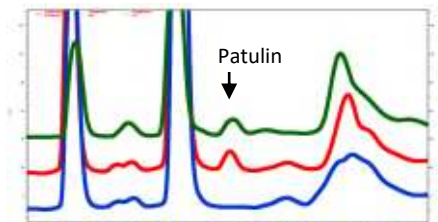
#### Elution (E)

- 2mL Ethyl Acetate

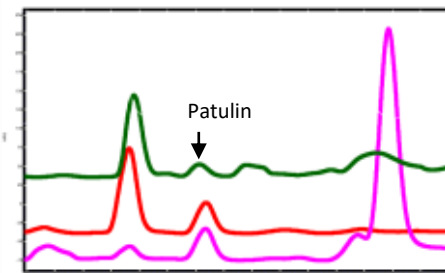
The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms of apple puree containing 0µg/kg (blue) or 20µg/kg (tested twice, green and red) of Patulin after AFFINIMIP® SPE Patulin Clean-up.



Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of different purees.

Recovery and reproducibility of Patulin with different levels of contamination for all tested apple-fruit puree after AFFINIMIP® SPE Patulin Clean-up.

Concentration of Patulin (µg/kg)	Recoveries %	% RSD <sub>R</sub>
10 (n=9)	77.4	8.1
25 (n=8)	90.9	11.4
40 (n=6)	86.0	11.9

### Catalog number:

#### 3mL-100mg sorbent

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL Pectinase

REA-001-50mL for 50mL Pectinase solution

# PATULIN ANALYSIS IN WHOLE APPLE - UV

**Regulations for solid apple products:**  
Europe (EC 1881/2006) : 25µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

#### Preparation with microwave

Whole apple is cut into pieces and put in a microwave for 90s before crushing the pieces. 15g sample and 7.5mL water are mixed with 150µL pectinase solution and put overnight at room temperature or for 2h at 40°C before a filtration with filter 4-7µm to obtain the loading solution.

#### Preparation with a blender

Whole apple is cut into pieces, put in a blender with Water (2:1 Apple: Water) and mix for 1min. 15g sample and 300µL pectinase solution are put overnight at room temperature or for 2h at 40°C before a filtration with filter 4-7µm to obtain the loading solution.

#### Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge

##### Equilibration

- 2mL Acetonitrile
- 1mL Water

##### Loading

- 3mL of loading solution

##### Washing of interferences (W1)

- 3mL Water-2% Acetic Acid

##### Drying by applying vacuum 10 seconds

##### Washing of interferences (W2)

- 250µL Diethyl Ether or MTBE

##### Drying by applying vacuum 10 seconds

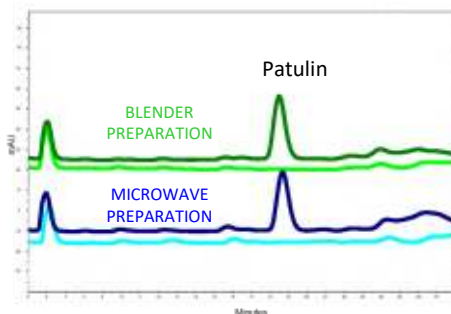
##### Elution (E)

- 1mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of whole apple spiked at 40µg/kg with Patulin (dark colors) or not spiked (light colors).

Recovery yields obtained after **AFFINIMIP® SPE Patulin** Clean-up of spiked whole apple with 40µg/kg of Patulin. Whole apples are prepared according to 2 different methods

Whole apple prepared with blender		Whole apple prepared with microwave	
96	96	95	88

#### Catalog number:

##### **3mL-100mg sorbent**

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL

Pectinase

REA-001-50mL for 50mL Pectinase solution

# PATULIN ANALYSIS IN CIDER - UV

## Regulations for cider:

Europe (EC 1881/2006) : 50µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

The cider is degassed by sonicating sample for 1 hour. Then the degas cider is diluted by 2 with water containing 2% of acetic acid. This solution is mixed and used as the loading solution.

### Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL Water

#### Loading

- 4mL of loading solution

#### Washing of interferences (W1)

- 1mL NaHCO<sub>3</sub> 1% in Water
- 2mL Water

#### Drying by applying vacuum 10 seconds

#### Washing of interferences (W2)

- 500µL Diethyl Ether or MTBE

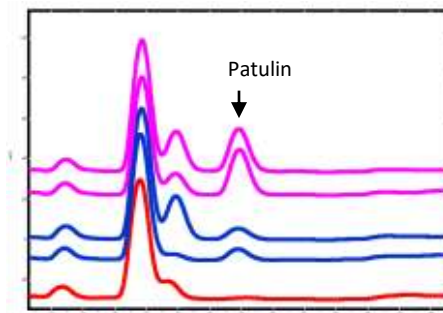
#### Elution (E)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°1 (description p 37)

## RESULTS



Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of a cider spiked at 40µg/kg (tested twice, pink) or at 10µg/kg (tested twice, blue) with Patulin or not spiked (red).

Recovery of Patulin at a contamination level of 10µg/kg and 40µg/kg in cider after AFFINIMIP® SPE Patulin Clean-up and relative standard deviation calculated from results generated under reproducibility conditions.

Concentration of Patulin (ng/mL)	Recoveries %	% RSD <sub>R</sub>
10	87.5 (n=2)	-
40	80.5 (n=5)	7.5

## Catalog number:

### 3mL-100mg sorbent

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL Pectinase

REA-001-50mL for 50mL Pectinase solution

# PATULIN ANALYSIS IN ALCOHOL POMMEAU - UV

## Regulations for apple based beverage :

Europe (EC 1881/2006) : 50µg/Kg

Alcohol Pommeau is a mixture of Calvados and Apple Juice. It contains 17% Alcohol.

## PROTOCOL OF PURIFICATION

Sample preparation

To 1mL of Alcohol Pommeau, add 2mL Water to obtain the loading solution.

Purification with a 3mL/100mg **AFFINIMIP® SPE Patulin** cartridge

### Equilibration

- 2mL Acetonitrile
- 1mL Water

### Loading

- 3mL of loading solution

### Washing of interferences (W1)

- 3mL Water (containing 2% Acetic Acid for AA W1 protocol)

### Drying by applying vacuum 10 seconds

### Washing of interferences (W2)

- 250µL Diethyl Ether or MTBE

### Drying by applying vacuum 10 seconds

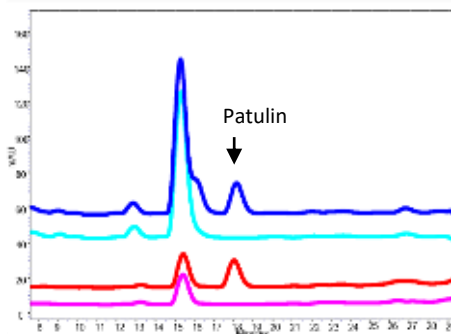
### Elution (E)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of Pommeau spiked at 40µg/L with Patulin (dark blue for Water in W1 and red for Water -AA in W1) or not spiked (light blue and pink). Washing with Acetic acid is more efficient.

Recovery yields obtained for Pommeau after **AFFINIMIP® SPE Patulin** Clean-up. W1 with water or Water -2%Acetic acid

	Water for W1		Water-AA for W1	
<b>Pommeau</b>	101	101	90	93

## Catalog number:

**3mL-100mg sorbent**

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL

Pectinase

REA-001-50mL for 50mL Pectinase solution

# PATULIN ANALYSIS IN LIQUOR – UV

**Regulations for apple based beverage :**  
Europe (EC 1881/2006) : 50µg/Kg

Manzella liquor contains 20% alcohol and 2.1% of concentrated apple juice.

## PROTOCOL OF PURIFICATION

Sample preparation

To 1mL of Manzella Liquor, add 2mL Water to obtain the loading solution.

**Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge**

### Equilibration

- 2mL Acetonitrile
- 1mL Water

### Loading

- 3mL of loading solution

### Washing of interferences (W1)

- 3mL Water (containing 2% Acetic Acid for AA W1 protocol)

### Drying by applying vacuum 10 seconds

### Washing of interferences (W2)

- 250µL Diethyl Ether or MTBE

### Drying by applying vacuum 10 seconds

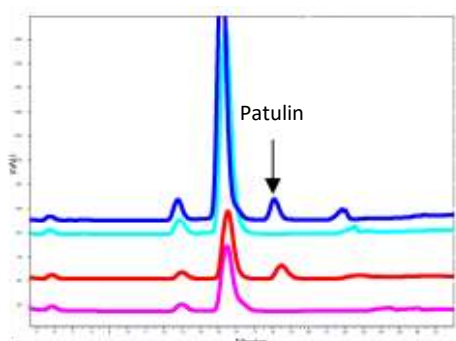
### Elution (E)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of Manzella liquor spiked at 40µg/L with Patulin (dark blue for Water in W1 and red for Water –AA in W1) or not spiked (light blue and pink). Washing with Acetic acid is more efficient.

Recovery yields obtained for Manzella after **AFFINIMIP® SPE Patulin** Clean-up. W1 with water or Water -2%Acetic acid

	Water for W1		Water-AA for W1	
Manzella	102	106	87	90

### Catalog number:

#### **3mL-100mg sorbent**

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL

Pectinase

REA-001-50mL for 50mL Pectinase solution



# PATULIN ANALYSIS IN DRIED APPLE - UV

**Regulations for solid apple products:**  
Europe (EC 1881/2006) : 25µg/Kg

## PROTOCOL OF PURIFICATION

### Sample preparation

3g of dried apple dices, 30mL of water and 150µL of pectinase are mixed and left at room temperature overnight. Then, they are centrifuged at 4500rpm during 5min and filtered with 0.2µm filter to obtain the loading solution.

### Purification with a 6mL/200mg AFFINIMIP® SPE Patulin cartridge

#### Equilibration

- 4mL Acetonitrile
- 2mL Water

#### Loading

- 10mL of loading solution

#### Washing of interferences (W1)

- 5mL Water-2% Acetic Acid
- 5mL Water

#### Drying by applying vacuum 30 seconds

#### Washing of interferences (W2)

- 500µL Diethyl Ether or MTBE

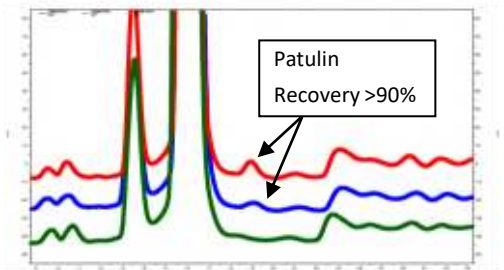
#### Elution (E)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of dried apple dices spiked at 20µg/kg (red) or at 10µg/kg (blue) with Patulin or not spiked (green).

### Catalog number:

**6mL-200mg sorbent for all matrices**  
FS102-03B -200mg for 50 cartridges

# PATULIN ANALYSIS IN TOMATO KETCHUP AND TOMATO POWDER - UV

## PROTOCOL OF PURIFICATION

### Sample preparation

#### Preparation OF TOMATO KETCHUP

10g tomato ketchup and 10mL water are mixed with 150 $\mu$ L pectinase solution and left overnight at RT before a filtration with filter 0.2 $\mu$ m to obtain the loading solution.

#### Preparation OF TOMATO POWDER

10g tomato ketchup and 20mL water are mixed. 10g of the mixture, 10mL water and 150 $\mu$ L pectinase solution are left overnight at RT before a centrifugation at 4500rpm during 5 min. Then the mixture is filtered with filter 0.2 $\mu$ m to obtain the loading solution.

### Purification with a 3mL/100mg AFFINIMIP<sup>®</sup> SPE Patulin cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL Water

#### Loading

- 5mL of loading solution from tomato ketchup or 2mL from tomato powder

#### Washing of interferences (W1)

- 4mL Water-1% Acetic Acid
- 4mL Water

#### Drying by applying vacuum 10 seconds

#### Washing of interferences (W2)

- 500 $\mu$ L Diethyl Ether or MTBE

#### Elution (E)

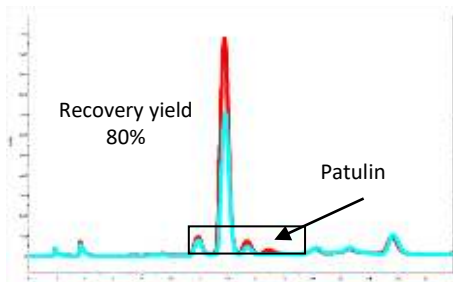
- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n<sup>o</sup>2 (description p 37)

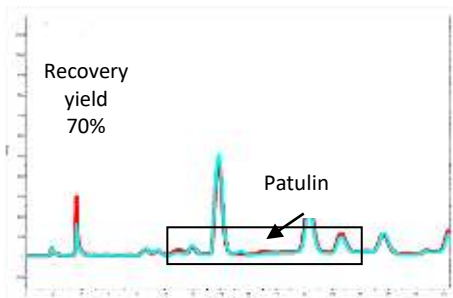
## RESULTS

### TOMATO KETCHUP



Chromatograms obtained after AFFINIMIP<sup>®</sup> SPE Patulin Clean-up of TOMATO KETCHUP spiked at 40 $\mu$ g/kg with Patulin (red) or not spiked (blue).

### TOMATO POWDER



Chromatograms obtained after AFFINIMIP<sup>®</sup> SPE Patulin Clean-up of TOMATO POWDER spiked at 36 $\mu$ g/kg with Patulin (red) or not spiked (blue).

#### Catalog number:

**3mL-100mg sorbent**

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL

Pectinase

REA-001-50mL for 50mL Pectinase solution

## PROTOCOL OF PURIFICATION

### Sample preparation

5mL Blueberry juice is diluted with 5mL water containing 2% of acetic acid to obtain the loading solution.

**Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge**

### Equilibration

- 2mL Acetonitrile
- 1mL Water

### Loading

- 4mL of loading solution

### Washing of interferences (W1)

- 1mL NaHCO<sub>3</sub> 1% in Water
- 2mL Water

### Drying by applying vacuum 10 seconds

### Washing of interferences (W2)

- 500µL Diethyl Ether or MTBE

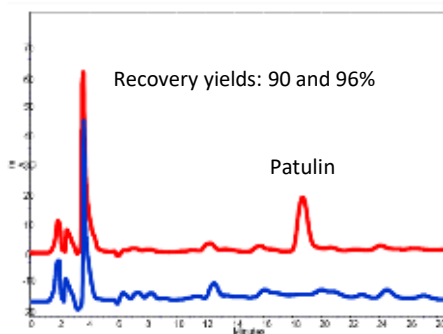
### Elution (E)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS



Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of Blueberry juice spiked at 40µg/L with Patulin (red) or not spiked (blue).

### Catalog number:

#### **3mL-100mg sorbent**

FS102-03 for 50 cartridges

FS102-03K for a kit of 50 cartridges + 50mL Pectinase

REA-001-50mL for 50mL Pectinase solution

# PATULIN ANALYSIS IN GRAPEFRUIT CONCENTRATE JUICE - UV

## PROTOCOL OF PURIFICATION

Preparation of fruit juice concentrate samples  
2.5g of fruit juice concentrate are mixed with 10mL water and 100µL Pectinase. (REA-001-50mL). 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.

**Purification with a 6mL/200mg AFFINIMIP® SPE Patulin cartridge**

### Equilibration

- 4mL Acetonitrile
- 4mL Water

### Loading

- 4 to 6mL of loading solution

### Washing of interferences (W1)

- 2mL NaHCO<sub>3</sub> 1% in Water
- 4mL Water

### Drying by applying vacuum 30 seconds

### Washing of interferences (W2)

- 1mL Diethyl Ether or MTBE

### Elution (E)

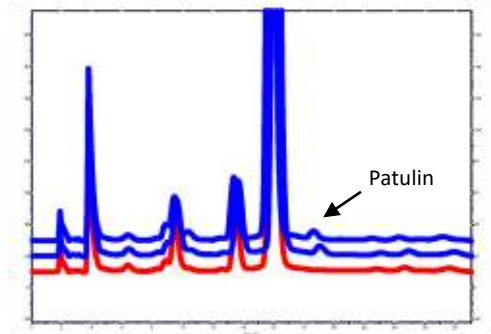
- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS

### CONCENTRATE JUICE



Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of grapefruit juice concentrate spiked at 10µg/kg (blue) with Patulin or not spiked (red).

### Catalog number:

#### **6mL-200mg sorbent**

FS102-03B-200mg for 50 cartridges

FS102-03KB-200mg for a kit of 50 cartridges  
+ 50mL Pectinase

REA-001-50mL for 50mL Pectinase solution

## PROTOCOL OF PURIFICATION

### Preparation of thick fruit juice samples

15mL of thick fruit juice are mixed with 120 $\mu$ L Pectinase (REA-001-50mL). 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.

### Purification with a 6mL/200mg AFFINIMIP® SPE Patulin cartridge

#### Equilibration

- 4mL Acetonitrile
- 4mL Water

#### Loading

- 4 to 6mL of loading solution

#### Washing of interferences (W1)

- 2mL NaHCO<sub>3</sub> 1% in Water
- 4mL Water

#### Drying by applying vacuum 30 seconds

#### Washing of interferences (W2)

- 1mL Diethyl Ether or MTBE

#### Elution (E)

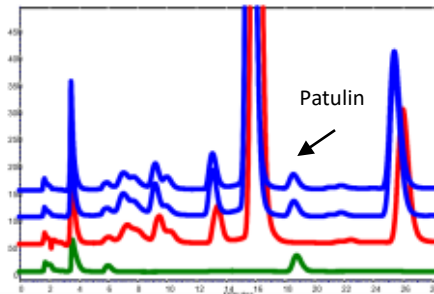
- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 37)

## RESULTS

### THICK JUICE



Chromatograms obtained after AFFINIMIP® SPE Patulin clean-up of apple mango juice spiked at 20 $\mu$ g/kg (blue) with Patulin or not spiked (red). In green, Patulin solution at 50ng/mL, prepared by dilution of a 100 $\mu$ g/mL Patulin standard solution (REA-PAT-1mL) in mobile phase.

#### Catalog number:

#### 6mL-200mg sorbent

FS102-03B-200mg for 50 cartridges

FS102-03KB-200mg for a kit of 50 cartridges + 50mL Pectinase

REA-001-50mL for 50mL Pectinase solution

# PATULIN ANALYSIS IN APPLE JUICE WITH AN AUTOMATE GX-271 ASPEC™ - UV



Gilson GX-271 ASPEC™ System with 406 syringe pump

## PROTOCOL OF PURIFICATION

### Sample preparation

Loading solution: 2.5mL apple juice and 2.5mL of water-2% acetic acid are mixed.

**Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge (35min)**

#### Equilibration (1mL/min)

- 2mL Acetonitrile
- 1mL water

#### Loading (0.5mL/min)

- 4mL of loading solution

#### Washing of interferences (W1) (1mL/min)

- 1mL NaHCO<sub>3</sub> 1% in Water
- 2mL Water

#### Drying by air push of 1000uL

#### Washing of interferences (W2)(1mL/min)

- 500µL Diethyl Ether or MTBE

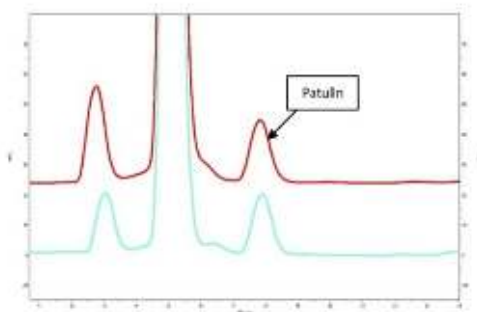
#### Elution (E) (0.8mL/min)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°1 (description p 37)

## RESULTS



Chromatograms of apple juice containing 40µg/kg of Patulin tested twice (Red and Blue) after **AFFINIMIP® SPE Patulin** Clean-up using a Gilson GX-271 ASPEC™

Recovery of Patulin in apple juice after **AFFINIMIP® SPE Patulin** Clean-up using a Gilson GX-271 ASPEC™

Concentration of Patulin (ng/mL)	Recoveries %
40 (n=2)	80

#### Catalog number:

**3mL-100mg sorbent for apple juice**

FS102-03 for 50 cartridges

**6mL-200mg sorbent for all applications**

FS102-03B -200mg for 50 cartridges

# PATULIN ANALYSIS IN PEAR/APPLE PUREE - LC-MS/MS

## PROTOCOL OF PURIFICATION

### Sample preparation

Mix 30g of puree (A mixture of pear and apple puree (50/50)), 30 mL of ultrapure water and 900µL of pectinase. Leave the solution overnight at RT for 2 hours at 40°C. Centrifuge 10 minutes at 4000 RPM and filter the supernatant through 11µm paper filter to form the loading solution.

### Purification with a 6mL/200mg AFFINIMIP® SPE

#### Patulin cartridge

##### Equilibration (1mL/min)

- 2mL Acetonitrile
- 2mL water

##### Loading (0.5mL/min)

- 6mL of loading solution

##### Washing of interferences (2mL/min)

- 1mL water - 1% Acetic acid
- 1mL NaHCO<sub>3</sub> 1% in Water
- 2mL Water (immediately)

##### Elution (E)

- 3mL Acetonitrile

Elutions are then homogenized, diluted by 5 with water 0.1% Acetic acid, and analyzed.

*Note: An evaporation can also be processed in order to reach concentrations lower than 1 µg/Kg. See application note of Patulin in apple juice (LC-UV) for optimized protocol.*

Analytical Method by LC-MS/MS

Analysis by HPLC – MS/MS (QTRAP 4000)

Column: SilactHPLC LC-Patulin 150\*2.1 mm (3µm)

Column temperature: 30°C

Flow rate: 0,2mL/min

Injection volume: 20µL

HPLC gradient for the analysis.

Time (min)	% water	% Acetonitrile
0	100	0
2	100	0
10	80	20
12	80	20
13	20	80
16	20	80
17	100	0
25	100	0

### MRM transitions for the analysis

Analyte	Q1	Q3	DP (V)	EP (V)	CE (V)	CXP (V)
Patulin Q	153	109	-45	-10	-14	-7
Patulin q	153	81	-45	-10	-18	-1

### Mass parameters:

Ion source: ESI Negative

Curtain gas: 40

Collision gas: Medium

IonSpray voltage: -3000 V

Source temperature: 550°C

GS1: 50

GS2: 50

## RESULTS

Recovery at a concentration of 10µg/Kg

Analyte	Recovery (%)	RSDr (%) (n=4)
Patulin	97	6

✓ Shorter cleanup – No evaporation required

✓ Excellent Recovery (> 80%)

✓ Good repeatability (RSDr < 10%)

### Catalog number:

#### 6mL - 200mg sorbent

FS102-03B-200mg for 50 cartridges

FS102-03BK-200mg for a kit of 50 cartridges

+ 50mL Pectinase

REA-001-50mL for 50mL Pectinase solution

#### HPLC column

LC-Pat-150.2.1 for SilactHPLC LC-Patulin 150x2.1mm (3µm)

# PATULIN ANALYSIS IN APPLE JUICE - LC-MS/MS

## PROTOCOL OF PURIFICATION

### Sample preparation

Clear apple juice was diluted by two with ultrapure water – 2% Acetic acid to form the loading solution.

**Purification with a 3mL/100mg AFFINIMIP® SPE**

**Patulin cartridge**

### Equilibration (1mL/min)

- 2mL Acetonitrile
- 1mL water

### Loading (0.5mL/min)

- 4mL of loading solution

### Washing of interferences (W1) (2mL/min)

- 1mL NaHCO<sub>3</sub> 1% in Water
- 2mL Water (immediately)

### Elution (E)

- 2mL Acetonitrile

Elutions are then homogenized, diluted by 5 with water 0.1% Acetic acid, and analyzed.

*Note: An evaporation can also be processed in order to reach concentrations lower than 1 µg/Kg. See application note of Patulin in apple juice (LC-UV) for optimized protocol.*

Analytical Method by LC-MS/MS

Analysis by HPLC – MS/MS (QTRAP 4000)

Column: SilactHPLC LC-Patulin 150\*2.1 mm (3µm)

Column temperature: 30°C

Flow rate: 0,2mL/min

Injection volume: 20µL

HPLC gradient for the analysis.

Time (min)	% water	% Acetonitrile
0	100	0
2	100	0
10	80	20
12	80	20
13	20	80
16	20	80
17	100	0
25	100	0

MRM transitions for the analysis

Analyte	Q1	Q3	DP (V)	EP (V)	CE (V)	CXP (V)
Patulin Q	153	109	-45	-10	-14	-7
Patulin q	153	81	-45	-10	-18	-1

### Mass parameters:

Ion source: ESI Negative

Curtain gas: 40

Collision gas: Medium

IonSpray voltage: -3000 V

Source temperature: 550°C

GS1: 50

GS2: 50

## RESULTS

Recovery at a concentration of 10µg/Kg

Analyte	Recovery (%)	RSDr (%) (n=4)
Patulin	81	6

✓ Shorter cleanup – No evaporation required

✓ Excellent Recovery (> 80%)

✓ Good repeatability (RSDr < 10%)

### Catalog number:

**3mL-100mg sorbent for apple juice**

FS102-03 for 50 cartridges

**HPLC column**

LC-Pat-150.2.1 for SilactHPLC LC-Patulin 150x2.1mm (3µm)



# PATULIN ANALYSIS IN APPLE JUICE WITH AN AUTOMATE ASPEC™ XLI - LC-MS/MS (article)

## PROTOCOL OF PURIFICATION

### Sample preparation

Loading solution: 10mL clear apple juice and 10mL of water-2% acetic acid are mixed. After 10 minutes of centrifugation at 8000 rpm at RT, the mixture is filtered. Then centrifuged at 10 000rpm at RT and 5mL of the supernatant is used as loading solution.

### Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge and the ASPEC automate

#### Equilibration (1mL/min)

- 2mL Acetonitrile
- 1mL water

#### Loading (0.5mL/min)

- 4mL of loading solution

#### Washing of interferences (W1) (2mL/min)

- 1mL NaHCO<sub>3</sub> 1% in Water
- 2mL Water

#### Elution (E)

- 2mL Acetonitrile at 0.8mL/min
- 1mL Acetonitrile at 4mL/min

The elution fraction was received in a test tube containing 0.5mL water containing 0.1% acetic acid.

Analytical Method by LC-MS/MS

Column: Gemini C18 column, 150mm x 2.0mm, 3µm

Temperature Oven: 35°C

Mobile phase: gradient

Time (min)	% water	% Acetonitrile
0	98	2
11	98	2
11.01	5	95
28	5	95
28.01	98	2
40	98	2

Flow rate: 0.2mL/min

Detection: LC-MS/MS ESI- MRM mode

Injection volume: 25µL.

## RESULTS

Method validation on 5 - 50µg/kg

FAPAS proficiency test 1651 (in 2013) with a Z-score of 1.6

Limit of detection and average recovery for patulin determination

LoD (µg/kg)	Determination limit (µg/kg)	Average recovery (%)
8	10	81

### Publications

Data extracted from the article

**Automatisierte Anwendung von Affinimip - SPE-Säulen bei der Bestimmung von Patulin in Apfelsaft**, Maria Barricelli, Deutsche Lebensmittel-Rundschau : *DLR ; Analytik, Forschung, Prozesse, Recht* Vol. 110, No. 7 (2014), p. 310-315 (in german)

Maria Barricelli is responsible of the mycotoxin area of the Landeslabor Berlin-Brandenburg

### Catalog number:

**3mL-100mg sorbent for apple juice**

FS102-03 for 50 cartridges

**6mL-200mg sorbent for all applications**

FS102-03B -200mg for 50 cartridges

# PATULIN ANALYSIS IN APPLE PUREE AND FRUIT – APPLE PUREE – UV (article)

## PROTOCOL OF PURIFICATION

Sample preparation  
10g of apple puree, 150µL of a pectinase enzyme solution and 10mL water are mixed. Leave solution at room temperature overnight or for 2h at 40°C. Centrifuge at 6000g for 25min at 5°C. 5mL of the supernatant This solution is used as the loading solution.

**Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge**

### Equilibration

- 2mL Acetonitrile
- 1mL Water

### Loading

- 5mL of loading solution

### Washing of interferences (W1)

- 4mL Water -1%Acetic acid
- 4mL Water

### Drying by applying vacuum 10 seconds

### Washing of interferences (W2)

- 500µL Diethyl Ether

### Elution (E)

- 2mL Acetonitrile – 0.1% Acetic acid

The elution fraction was then evaporated and dissolved in acetonitrile – water 90/10 – pH 4 before HPLC analysis.

Analytical method by LC-UV

Column: Hypersil Gold C18 column, 150mm x 4mm, 5µm

Mobile phase: water acetonitrile (85:15)

Flow rate: 1mL/min

Detection: UV-DAD at 276nm

Injection volume: 25µL.

## Publications

Data extracted from the article

**Assessment of Patulin Content in Apple Puree and Apple and Fruit Puree by High Performance Liquid Chromatography**, M. Catana, L. Catana, E. Iorga, M. Negoita, V. Ionescu, N. Belc, D. Efstatiade, H. Y. Aboul-Enein, *J. Envir. Sci. Eng. A* 5, 371-381, 2016. doi:10.17265/2162-5298/2016.07.005 (FREE ACCESS)

## RESULTS

Method validation on 5 - 80µg/kg

Mean value recovery and RSD<sub>r</sub> (repeatability) for patulin in apple puree

Spiking level (µg/kg)	Average recovery (%)	RSD <sub>r</sub> (%)
5 (n=6)	93,14	2.66
10 (n=6)	90,27	0.86

Mean value recovery and RSD<sub>r</sub> (repeatability) for patulin in apple puree

Spiking level (µg/kg)	Mean value conc (µg/kg)	RSD <sub>r</sub> (%)
10 (n=6)	10.44 ± 1.36	1.87
40 (n=6)	40.69 ± 2.55	2.73

Mean value recovery and RSD<sub>R</sub> (reproducibility) for patulin in apple puree

Spiking level (µg/kg)	Mean value conc (µg/kg)	RSD <sub>R</sub> (%)
20 (n=6)	20.52 ± 1.78	5.41

### Catalog number:

**3mL-100mg sorbent for apple juice**

FS102-03 for 50 cartridges

**6mL-200mg sorbent for all applications**

FS102-03B -200mg for 50 cartridges

# PATULIN ANALYSIS IN APPLE PUREE, JUICE AND JAM BY UHPLC – UV (article)

## PROTOCOL OF PURIFICATION FOR APPLE JUICE

### Sample preparation

Loading solution: 2.5mL apple juice and 2.5mL of water-2% acetic acid are mixed.

**Purification with a 3mL/100mg AFFINIMIP® SPE**

### Patulin cartridge

#### Equilibration

- 2mL Acetonitrile
- 1mL water

#### Loading

- 2x2mL of loading solution

#### Washing of interferences (W1)

- 1mL 1%NaHCO<sub>3</sub> 1% in Water
- 2mL Water

#### Drying by applying vacuum 30 seconds

#### Washing of interferences (W2)

- 1mL Diethyl Ether

#### Elution (E)

- 2x2mL Ethyl Acetate

The elution fraction was then evaporated and reconstituted before HPLC analysis.

Mean value recovery and RSD<sub>r</sub> (repeatability) for patulin in apple juice

Spiking level (µg/kg)	Average recovery (%)	RSD <sub>r</sub> (%)
10 (n=3)	77	4.1
30 (n=3)	83	6.5
50 (n=3)	90	4.4

Analytical method by UHPLC

Column: Poroshell 120 EC-C18 (4.6x150mm, 2.7µm )

Oven Temperature :35°C

Mobile phase: water acetonitrile (85:15) for apple juice or the gradient for apple puree and jam

Time (min)	% H2O -1% AAC	% Acetonitrile
0	98	2
0.5	98	2
10	80	20
12	80	20
15	50	50
16	50	50
16.15	98	2
20	98	2

Flow rate: 0.45mL/min

Detection: UV

Injection volume: 5µL.

## PROTOCOL OF PURIFICATION FOR APPLE PUREE AND JAM

### Sample preparation

10g of apple puree or jam, 150µL of a pectinase enzyme solution and 10mL water are mixed. Leave solution at 2h at 40°C. Centrifuge at 5000g for 5min and then filter the solution with a 0.2µm filter. This solution is used as the loading solution.

**Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge**

#### Equilibration

- 2mL Acetonitrile
- 1mL Water

#### Loading

- 2x2.5mL of loading solution

#### Washing of interferences (W1)

- 2x2mL Water -1%Acetic acid
- 1mL NaHCO<sub>3</sub> 1% solution
- 3mL Water

#### Drying by applying vacuum 30 seconds

#### Washing of interferences (W2)

- 500µL Diethyl Ether

#### Elution (E)

- 2x2mL Ethyl Acetate

The elution fraction was then evaporated and dissolved in water containing 0.1% acetic acid before HPLC analysis.

Mean value recovery and RSD<sub>r</sub> (repeatability) for patulin in apple puree and jam

Spiking level (µg/kg)	Apple puree		Jam	
	Av. Rec. (%)	RSD <sub>r</sub> (%)	Av. Rec. (%)	RSD <sub>r</sub> (%)
10 (n=3)	95	11.8	94	7.8
15 (n=3)	83	8.7	90	8.1
25 (n=3)	90	5.2	96	8.5

## Publications

Data extracted from the article

**Selective solid-phase extraction using a molecularly imprinted polymer for the analysis of patulin in apple-based foods**, Lucci, P., Moret, S., Bettin, S. and Conte, L., *J. Sep. Science.*, 40(2),458–465, (2017)

## PROTOCOL OF PURIFICATION

### Sample preparation

Loading solution: 2mL apple juice and 2mL of water-2% acetic acid are mixed.

### Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge

#### Equilibration (1mL/min)

- 2mL Acetonitrile
- 1mL water

#### Loading (0.5mL/min)

- 4mL of loading solution

#### Washing of interferences (W1) (1mL/min)

- 1mL NaHCO<sub>3</sub> in Water
- 2mL Water

#### Drying by applying vacuum 10 seconds

#### Elution (E) (1mL/min)

- 2mL Ethyl Acetate

The elution fraction was then evaporated under nitrogen stream and immediately dissolved in water containing 0.1% acetic acid before HPLC analysis.

Analytical method by LC-UV  
Column: LiChroCART 250-4.6 Purospher STAR RP-18e (5µm) column  
Oven temperature : 40°C  
Mobile phase: 0.1% formic acid + 8% acetonitrile  
Flow rate: 1mL/min  
Detection: UV-DAD at 276nm  
Injection volume: 50µL.

### Publications

Data extracted from the article **Analysis of Patulin in apple juice using the Chromaster HPLC System with PDA detector**, Application note C-12125 of March 2014 developed by VWR and published in the Chromjournal of March 2015.

#### Catalog number:

**3mL-100mg sorbent for apple juice**

FS102-03 for 50 cartridges

**6mL-200mg sorbent for all applications**

FS102-03B -200mg for 50 cartridges

For these application notes, the concentration of Patulin has been done according to two analytical HPLC conditions.

## Analytical Method n° 1

Column: SilactHPLC LC-Patulin 150x2.1mm (3µm)

Mobile phase: Deionized water/ACN (95/5, v/v) Flow rate: 0.2mL/min

Detection: UV - 276nm

Injection volume: 100µL.

## Analytical Method n° 2

Column: SilactHPLC LC-Patulin 150x2.1mm (3µm)

Mobile phase: gradient

Time (min)	% water/Acetonitrile 98/2 (v/v)	% Acetonitrile
0	100	0
20	100	0
21	50	50
25	50	50
26	100	0
40	100	0

Flow rate: 0.2mL/min

Detection: UV - 276nm

Injection volume: 100µL.

Catalog number:

**HPLC column**

LC-Pat-150.2.1 for SilactHPLC LC-Patulin  
150x2.1mm (3µm)





# RELATED PRODUCTS & SERVICES

# SPE ACCESSORIES

AFFINISEP proposes the complete set of equipments required to carry out SPE experiments:

## Manifold



### ACC-MAN1

### ACC-MAN2

Like all chromatography techniques, Use of SPE cartridges needs a precise control of flow rate for maintaining reproducible extractions. Solid Phase Extraction Vacuum Manifold allows you to control the flow and to process up to 12 (12-port version) or 24 (24-port version) AFFINIMIP® SPE samples simultaneously, to gain significantly time during sample preparation steps.

## SPE Adapter & Reservoir kit



### ACC-AR1

Tube adapters serve to pile one SPE tube on top of another to provide different selectivities. A larger empty syringe barrel can be stacked on top of a smaller SPE tube to act as a larger load reservoir. Or, they can serve as an adapter for positive pressure methods (e.g. from a syringe or air/ N2 line).

## Mini-Vap



### ACC-VAP1

The 6-Port Mini-Vap concentrator/evaporator processes six vials at one time. The Mini-Vap includes a needle valve for fine metering of air or nitrogen drying gas.

## Mini PUMP



### ACC-PUMP

Mini diaphragm vacuum pump for solid phase extraction experiments

- 5.5L/min
- ~120 torr vacuum
- Oil-free
- portable

## Vacuum pump trap

### ACC-TRAP

SPE Vacuum pump trap kit

Installed between the manifold and the vacuum pump, it collects all liquids that are aspirated preventing contamination of the vacuum pump with a capacity of 1L.



# PERSONALISED SERVICES

**AFFINISEP offers full services to help you in your need to determine Patulin level in your facilities**

As leader in Patulin solution provider, AFFINISEP can support you to implement of the patulin analyses in your facilities.



## QC method for YOUR matrices

- **We elaborate the sample preparation protocol for your matrices**
- Simple and fast method
- Comply with your needs and specifications
- Duration: Few days

## Training

- **Training to carry out your own analyses in your QC lab**
- Theoretical or/and practical training
- Duration : Few days

## Turnkey QC lab

- **Complete implementation of turnkey Patulin QC lab in your facilities**
- Including all equipments for Patulin determination and training
- According to your needs and specifications

# Ordering information

## AFFINIMIP SPE and Reactive – Product list

Designation	Definition	Reference
<b>AFFINIMIP® SPE Patulin</b>	50 Selective SPE cartridges for Patulin - 3mL – 100mg	FS102-03
	50 Selective SPE cartridges for Patulin - 6mL – 200mg	FS102-03B-200mg
<b>AFFINIMIP® SPE Patulin &amp; Pectinase kit</b>	Kit of 50 selective SPE cartridges for Patulin (3mL-100mg) + 50mL Pectinase enzyme solution	FS102-03K
	Kit of 50 selective SPE cartridges for Patulin (6mL-200mg) + 50mL Pectinase enzyme solution	FS102-03KB-200mg
<b>AFFINIMIP® SPE Patulin &amp; Standard kit</b>	Kit of 50 selective SPE cartridges for Patulin (3mL-100mg) + 1mL Standard solution 100µg/mL	FS102-03KS
	Kit of 50 selective SPE cartridges for Patulin (6mL-200mg) + 1mL Standard solution 100µg/mL	FS102-03KBS-200mg
<b>AFFINIMIP® SPE Patulin &amp; Standard &amp; Pectinase kit</b>	Kit of 25 selective SPE cartridges for Patulin (3mL-100mg) + 1mL Standard solution 100µg/mL+ 50mL Pectinase enzyme solution	FS102-02KSP
	Kit of 25 selective SPE cartridges for Patulin (6mL-200mg) + 1mL Standard solution 100µg/mL+ 50mL Pectinase enzyme solution	FS102-02KBSP-200mg
<b>Pectinase</b>	50 mL Pectinase enzyme solution	REA-001-50mL
<b>Standard solution</b>	1mL Patulin standard solution 100µg/mL in acetonitrile	REA-PAT-1ML

## SPE ACCESSORIES – Product list


SPE Accessories	Designation	Definition	Reference
<b>Manifold</b>	<b>SPE Vacuum Manifold</b>	12-port model	ACC-MAN2
<b>SPE Adapter &amp; Reservoir kit</b>	<b>SPE Adapter &amp; Reservoir kit</b>	Kit of 12 reservoirs 60ml and adapters for use with 1,3 & 6 mL cartridges	ACC-AR1
<b>Mini-Vap</b>	<b>Mini Evaporator/Concentrator</b>	6 port Mini-Vap Evaporator/Concentrator for use with 1 to 250mL containers	ACC-VAP1
<b>Mini PUMP</b>	<b>Mini vacuum pump</b>	Laboport diaphragm vacuum mini pump, 5.5L/min	ACC-PUMP
<b>Vacuum pump trap</b>	<b>SPE Vacuum pump trap kit</b>	1L trap kit	ACC-TRAP




THE ART OF MAKING SAMPLE PREPARATION EASIER

## ABOUT


Affinisep is a **worldwide expert in sample preparation applications**. Dedicated to the development of analytical applications in various fields such as water monitoring, food quality control and bioanalysis, Affinisep offers a complete set of products for passive sampling and sample preparation.

 **Brands**

AFFINIMIP®  
AttractSPE®  
SilactSPE™...

 **Applications**

Sample Preparation  
Passive Sampling  
Filtration  
Microextraction of  
peptides/proteins

 **Matrices**

Food, Feed, Soil,  
Oil, Water,  
Biological fluids,  
Proteolytic digest

**Analytical chemists can find any solution for sample preparation, selective extraction and sample clean-up needs in various sectors: food and feed safety and quality, life science and quality control, clinical diagnosis, environment and doping.**

**In addition, proteomics users can find a complete set of microextraction products for protein/peptides fractionation or desalting.**

Furthermore, by exploiting our library of innovative polymers and our know-how in chromatography and solid phase extraction, we have a strong capacity to adapt these polymers to meet any specific requirements and to solve unsatisfied purification and extraction needs.

Numerous documents related to our products (Application notebooks, publication references, posters, catalog for different applications...) can be found on our website **[www.affinisep.com](http://www.affinisep.com)**.



# affinisep

## ORDERING INFORMATION

For any order, please choose one of the following ways:

- **On our website :** [www.affinisep.com](http://www.affinisep.com)

Find all our references and benefit from a **FAST** and **SIMPLE** process!

- **By email:** [contact@affinisep.com](mailto:contact@affinisep.com)

### Information about new products or new matrices:

- **Newsletters**

Stay up-to-date on AFFINISEP's latest innovations by subscribing to our newsletter, delivering new information straight to your inbox! Register on our website.