Comprehensive solutions to comply regulation on Patulin analysis
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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
- Turnkey implementation of your own analytical laboratory
- AFFINIMIP® SPE Patulin KIT evaluated for a high number of products
- Analyses of your matrices

For more detailed information about our services, please contact us at contact@affinisep.com
COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006
setting maximum levels for certain contaminants in foodstuffs

<table>
<thead>
<tr>
<th>Foodstuffs</th>
<th>Maximum levels for patulin (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.1 Fruit juices, concentrated fruit juices as reconstituted and fruit nectars (14)</td>
<td>50</td>
</tr>
<tr>
<td>2.3.2 Spirit drinks (15), cider and other fermented drinks derived from apples or containing apple juice</td>
<td>50</td>
</tr>
<tr>
<td>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</td>
<td>25</td>
</tr>
<tr>
<td>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)</td>
<td>10</td>
</tr>
<tr>
<td>2.3.5 Baby foods other than processed cereal-based foods for infants and young children (3) (4)</td>
<td>10</td>
</tr>
</tbody>
</table>


(4) The maximum level refers to the products ready to use (marketed as such or after reconstitution as instructed by the manufacturer).


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

<table>
<thead>
<tr>
<th>Level µg/Kg</th>
<th>RSDᵣ %</th>
<th>RSDᵣ %</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>≤30</td>
<td>≤40</td>
<td>50 to 120</td>
</tr>
<tr>
<td>20-50</td>
<td>≤20</td>
<td>≤30</td>
<td>70 to 105</td>
</tr>
<tr>
<td>&gt;50</td>
<td>≤15</td>
<td>≤25</td>
<td>75 to 105</td>
</tr>
</tbody>
</table>
AFFINIMIP® SPE Patulin kit

- SPE cartridge
- Standard solution
- Pectinase

- DETAILED protocole of use
- Analytical method

A READY TO USE AFFINIMIP® SPE Patulin KIT

Certificate of analysis

✓ 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 method.

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

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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 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 day
>>> 20 to 24 samples analyses are easily obtained

Easy to use with SPE automate
See application with the ASPEC automate

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Liquid - Liquid extraction Procedure

7 steps ≈ 1h for 1 sample

10 g sample + 20mL ethyl acetate shake 1min → Organic layer

Aqueous layer + 20mL ethyl acetate shake 1min → Organic layer

Aqueous layer + 20mL ethyl acetate shake 1min → Organic layer

3 Organic layer + 4mL Na₂CO₃ Shake 30 seconds

Ethyl acetate phase

Filtration on filter paper containing 15g Na₂SO₄

Evaporation

Dissolution + filtration + analysis

AFFINIMIP® SPE Patulin Procedure

4 steps ≈ 1h for 6 to 12 samples

2g sample + 2mL Water-Acetic acid Preparation : 5min/sample

Extraction with AFFINIMIP® SPE Patulin 20- 30 min – 6 to 12 samples

Evaporation 2mL eluate (Nitrogen) 20min – 6 to 12 samples

Dissolution + analysis

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

- Adopted by more than 50 customers
- Mainly industrials QC labs
- Within 20 countries
- Used for control of fruits receipt and final products QC
- For various and complex matrices – solid, liquid, viscous, concentrate...
- For baby food
- Tested for all kinds of apple-based products and various other fruits
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

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

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

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DETERMINATION OF PATULIN IN BABY FOOD
APPLE JUICE

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 NaHCO₃ 1% in water
• 2mL Water

Drying by applying vacuum 10 seconds

Washing of interferences (W2)
• 1mL Diethyl Ether

Elution (E)
• 2mL Ethyl Acetate

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

HPLC Analytical method n°1 (description p 35)

RESULTS

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

<table>
<thead>
<tr>
<th>Recoveries % (n=9)</th>
<th>% RSD$_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>97.9</td>
<td>11</td>
</tr>
</tbody>
</table>

Catalog number:
3mL-100mg sorbent for most uses
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges

6mL-200mg sorbent for a DRIED APPLE or higher enrichment with apple juice
FS102-02B -200mg for 25 cartridges
FS102-03B -200mg for 50 cartridges

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DETERMINATION OF PATULIN IN APPLE JUICE

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 NaHCO₃ in Water
- 2mL Water

Drying by applying vacuum 10 seconds

Washing of interferences (W2)
- 1mL Diethyl Ether

Elution (E)
- 2mL Ethyl Acetate

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

HPLC Analytical method nº1 (description p 35)

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

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.

<table>
<thead>
<tr>
<th>Concentration of Patulin (ng/mL)</th>
<th>Recoveries %</th>
<th>% RSDₚ</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>97.9</td>
<td>11 (n=9)</td>
</tr>
<tr>
<td>40</td>
<td>90.6</td>
<td>11 (n=41)</td>
</tr>
</tbody>
</table>

Catalog number:
3mL-100mg sorbent for most uses
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
6mL-200mg sorbent for a DRIED APPLE or higher enrichment with apple juice
FS102-02B -200mg for 25 cartridges
FS102-03B -200mg for 50 cartridges

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Determinations of Patulin in Apple Puree

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 NaHCO₃ 1% solution
• 3mL Water

Drying by applying vacuum 10 seconds

Washing of Interferences (W2)
• 500µL Diethyl Ether

Elution (E)
• 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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.

<table>
<thead>
<tr>
<th>Concentration of Patulin (µg/kg)</th>
<th>Recoveries % (n=3)</th>
<th>% RSDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>84</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Catalog number:

3mL-100mg sorbent
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN APPLE PUREE – CARTRIDGE 6mL -200mg

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 NaHCO₃ 1% solution
• 3mL Water

Drying by applying vacuum 10 seconds

Washing of interferences (W2)
• 500µL Diethyl Ether

Elution (E)
• 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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.

<table>
<thead>
<tr>
<th>Concentration of Patulin (µg/kg)</th>
<th>Recoveries %</th>
<th>% RSDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (n=6)</td>
<td>90</td>
<td>9</td>
</tr>
<tr>
<td>20 (n=3)</td>
<td>92</td>
<td>11</td>
</tr>
</tbody>
</table>

Catalog number:
6mL - 200mg sorbent
FS102-02B-200mg for 25 cartridges
FS102-03B-200mg for 50 cartridges
FS102-02BK-200mg for a kit of 25 cartridges + 50mL Pectinase
FS102-03BK-200mg for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN BABY FOOD APPLE PUREE

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 NaHCO₃ 1% solution
• 3mL Water

Drying by applying vacuum 10 seconds

Washing of interferences (W2)
• 500µL Diethyl Ether

Elution (E)
• 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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.

<table>
<thead>
<tr>
<th>Recoveries % (n=4)</th>
<th>% RSD&lt;sub&gt;R&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>81.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Catalog number:
3mL-100mg sorbent
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN APPLE – FRUIT PUREE

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 NaHCO₃ 1% solution
• 3mL Water

Drying by applying vacuum 10 seconds

Washing of interferences (W2)
• 500µL Diethyl Ether

Elution (E)
• 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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.

<table>
<thead>
<tr>
<th>Concentration of Patulin (µg/kg)</th>
<th>Recoveries %</th>
<th>% RSD _R</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (n=9)</td>
<td>77.4</td>
<td>8.1</td>
</tr>
<tr>
<td>25 (n=8)</td>
<td>90.9</td>
<td>11.4</td>
</tr>
<tr>
<td>40 (n=6)</td>
<td>86.0</td>
<td>11.9</td>
</tr>
</tbody>
</table>

Catalog number:
3mL-100mg sorbent
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution

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DETERMINATION OF PATULIN IN WHOLE APPLE

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 1 min. 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

Drying by applying vacuum 10 seconds

Elution (E)
- 1mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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

<table>
<thead>
<tr>
<th>Whole apple prepared with blender</th>
<th>Whole apple prepared with microwave</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>95</td>
<td>88</td>
</tr>
</tbody>
</table>

CATALOG NUMBER:

3mL-100mg sorbent

FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN CIDER

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 NaHCO₃ 1% in Water
• 2mL Water

Drying by applying vacuum 10 seconds

Washing of interferences (W2)
• 500µL Diethyl Ether

Elution (E)
• 2mL Ethyl Acetate
The elution fraction was then evaporated and dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°1 (description p 35)

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.

<table>
<thead>
<tr>
<th>Concentration of Patulin (ng/mL)</th>
<th>Recoveries %</th>
<th>% RSDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>87.5 (n=2)</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>80.5 (n=5)</td>
<td>7.5</td>
</tr>
</tbody>
</table>

CATALOG NUMBER:
3mL-100mg sorbent
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN ALCOHOL POMMEAU

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

Drying by applying vacuum 10 seconds

Elution (E)
- 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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

<table>
<thead>
<tr>
<th></th>
<th>Water for W1</th>
<th>Water-AA for W1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pommeau</td>
<td>101</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>101</td>
<td>93</td>
</tr>
</tbody>
</table>

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

Catalog number:
3mL-100mg sorbent
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN LIQUOR

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
Drying by applying vacuum 10 seconds
Elution (E)
- 2mL Ethyl Acetate
The elution fraction was then evaporated and dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 35)

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

<table>
<thead>
<tr>
<th></th>
<th>Water for W1</th>
<th>Water-AA for W1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manzella</td>
<td>102</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>90</td>
</tr>
</tbody>
</table>

Catalog number:
3mL-100mg sorbent
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN DRIED APPLE

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

Elution (E)
• 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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 a DRIED APPLE or higher enrichment with apple juice
FS102-02B -200mg for 25 cartridges
FS102-03B -200mg for 50 cartridges
DETERMINATION OF PATULIN IN TOMATO KETCHUP AND TOMATO POWDER

PROTOCOL OF PURIFICATION

Sample preparation

Preparation of TOMATO KETCHUP
10g tomato ketchup and 10mL water are mixed with 150µL pectinase solution and left overnight at RT before a filtration with filter 0.2µm to obtain the loading solution.

Preparation of TOMATO POWDER
10g tomato ketchup and 20mL water are mixed. 10g of the mixture, 10mL water and 150µL pectinase solution are left overnight at RT before a centrifugation at 4500rpm during 5 min. Then the mixture is filtered with filter 0.2µm to obtain the loading solution.

Purification with a 3mL/100mg AFFINIMIP® 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)
• 500µL Diethyl Ether
Elution (E)
• 2mL Ethyl Acetate
The elution fraction was then evaporated and dissolved in water containing 0.1% acetic acid before HPLC analysis.

HPLC Analytical method n°2 (description p 35)

RESULTS

TOMATO KETCHUP

Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of TOMATO KETCHUP spiked at 40µg/kg with Patulin (red) or not spiked (blue).

TOMATO POWDER

Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of TOMATO POWDER spiked at 36µg/kg with Patulin (red) or not spiked (blue).

Patulin Recovery yield 80%
Patulin Recovery yield 70%

Catalog number:
3mL-100mg sorbent
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution

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DETERMINATION OF PATULIN IN BLUEBERRY JUICE

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 NaHCO₃ 1% in Water
- 2mL Water

Drying by applying vacuum 10 seconds

Washing of interferences (W2)
- 500µL Diethyl Ether

Elution (E)
- 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
FS102-02K for a kit of 25 cartridges + 50mL Pectinase
FS102-03K for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN GRAPEFRUIT CONCENTRATE JUICE

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 NaHCO₃ 1% in Water
• 4mL Water

Drying by applying vacuum 30 seconds

Washing of interferences (W2)
• 1mL Diethyl Ether

Elution (E)
• 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

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
FS102-02B-200mg for 25 cartridges
FS102-03B-200mg for 50 cartridges
FS102-02KB-200mg for a kit of 25 cartridges + 50mL Pectinase
FS102-03KB-200mg for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN THICK JUICE BASED ON MANGO

PROTOCOL OF PURIFICATION

Preparation of thick fruit juice samples
15mL of thick fruit juice are mixed with 120µL 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 NaHCO₃ 1% in Water
• 4mL Water

Drying by applying vacuum 30 seconds

Washing of interferences (W2)
• 1mL Diethyl Ether

Elution (E)
• 2mL Ethyl Acetate

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

HPLC Analytical method n°2 (description p 35)

RESULTS

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

Catalog number:
6mL-200mg sorbent
FS102-02B-200mg for 25 cartridges
FS102-03B-200mg for 50 cartridges
FS102-02KB-200mg for a kit of 25 cartridges + 50mL Pectinase
FS102-03KB-200mg for a kit of 50 cartridges + 50mL Pectinase
REA-001-50mL for 50mL Pectinase solution
DETERMINATION OF PATULIN IN APPLE JUICE
WITH AN AUTOMATE GX-271 ASPEC™

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 NaHCO₃ 1% in Water
- 2mL Water

Drying by air push of 1000uL

Washing of interferences (W2)(1mL/min)
- 500µL Diethyl Ether

Elution (E) (0.8mL/min)
- 2mL Ethyl Acetate

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

HPLC Analytical method n°1 (description p 35)

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™

<table>
<thead>
<tr>
<th>Concentration of Patulin (ng/mL)</th>
<th>Recoveries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 (n=2)</td>
<td>80</td>
</tr>
</tbody>
</table>

Catalog number:
3mL-100mg sorbent for most uses
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
6mL-200mg sorbent for a DRIED APPLE or higher enrichment with apple juice
FS102-02B -200mg for 25 cartridges
FS102-03B -200mg for 50 cartridges
DETERMINATION OF PATULIN IN APPLE JUICE WITH AN AUTOMATE ASPECTM XLI AND LC-MS/MS

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 NaHCO3 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

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>11.01</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>28</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>28.01</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>LoD (µg/kg)</th>
<th>Determination limit (µg/kg)</th>
<th>Average recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10</td>
<td>81</td>
</tr>
</tbody>
</table>

Publications
Data extracted from the article

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

Catalog number:
3mL-100mg sorbent for most uses
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
6mL-200mg sorbent for a DRIED APPLE or higher enrichment with apple juice
FS102-02B -200mg for 25 cartridges
FS102-03B -200mg for 50 cartridges
DETERMINATION OF PATULIN IN APPLE PUREE AND FRUIT – APPLE PUREE

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 Acetonitril
- 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

RESULTS

Method validation on 5 - 80µg/kg

Mean value recovery and RSD_r (repeatability) for patulin in apple puree

<table>
<thead>
<tr>
<th>Spiking level (µg/kg)</th>
<th>Average recovery (%)</th>
<th>RSD_r (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (n=6)</td>
<td>93.14</td>
<td>2.66</td>
</tr>
<tr>
<td>10 (n=6)</td>
<td>90.27</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Mean value recovery and RSD_r (repeatability) for patulin in apple puree

<table>
<thead>
<tr>
<th>Spiking level (µg/kg)</th>
<th>Mean value conc (µg/kg)</th>
<th>RSD_r (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (n=6)</td>
<td>10.44 ± 1.36</td>
<td>1.87</td>
</tr>
<tr>
<td>40 (n=6)</td>
<td>40.69 ± 2.55</td>
<td>2.73</td>
</tr>
</tbody>
</table>

Mean value recovery and RSD_R (repeatability) for patulin in apple puree

<table>
<thead>
<tr>
<th>Spiking level (µg/kg)</th>
<th>Mean value conc (µg/kg)</th>
<th>RSD_R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 (n=6)</td>
<td>20.52 ± 1.78</td>
<td>5.41</td>
</tr>
</tbody>
</table>

Catalog number:
3mL-100mg sorbent for most uses
FS102-02 for 25 cartridges
FS102-03 for 50 cartridges
6mL-200mg sorbent for a DRIED APPLE or higher enrichment with apple juice
FS102-02B -200mg for 25 cartridges
FS102-03B -200mg for 50 cartridges

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DETERMINATION OF PATULIN IN APPLE PUREE, JUICE AND JAM BY UHPLC

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%NaHCO₃ 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, (repeatability) for patulin in apple juice

<table>
<thead>
<tr>
<th>Spiking level (µg/kg)</th>
<th>Average recovery (%)</th>
<th>RSDr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (n=3)</td>
<td>77</td>
<td>4.1</td>
</tr>
<tr>
<td>30 (n=3)</td>
<td>83</td>
<td>6.5</td>
</tr>
<tr>
<td>50 (n=3)</td>
<td>90</td>
<td>4.4</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% H2O -1% Acetic Acid</th>
<th>% Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>0.5</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>16.15</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

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 NaHCO₃ 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, (repeatability) for patulin in apple puree and jam

<table>
<thead>
<tr>
<th>Spiking level (µg/kg)</th>
<th>Apple puree</th>
<th>Jam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. Rec. (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>10 (n=3)</td>
<td>95</td>
<td>11.8</td>
</tr>
<tr>
<td>15 (n=3)</td>
<td>83</td>
<td>8.7</td>
</tr>
<tr>
<td>25 (n=3)</td>
<td>90</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Publications
Data extracted from the article
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 NaHCO₃ in Water
- 2mL Water

Drying by applying vacuum 10 seconds

Elution (E) (1mL/min)
- 2mL Methyl Acetate

The elution fraction was then evaporated and 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.
For these application notes, the concentration of Patulin has been done according to two analytical HPLC conditions.

**Analytical Method n° 1**
Column: Atlantis T3 column, 150mm x 2.1mm  
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: Atlantis T3 column, 150mm x 2.1mm  
Mobile phase: gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>25</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>26</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

Flow rate: 0.2mL/min  
Detection: UV - 276nm  
Injection volume: 100µL.
RELATED PRODUCTS & SERVICES
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.
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.

**PERSONALISED SERVICES**

**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
# AFFINIMIP SPE and Reactive – Product list

<table>
<thead>
<tr>
<th>Designation</th>
<th>Definition</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><strong>AFFINIMIP® SPE Patulin</strong></td>
<td>50 Selective SPE cartridges for Patulin - 3mL – 100mg</td>
<td>FS102-03</td>
</tr>
<tr>
<td></td>
<td>50 Selective SPE cartridges for Patulin - 6mL – 200mg</td>
<td>FS102-03B-200mg</td>
</tr>
<tr>
<td><strong>AFFINIMIP® SPE Patulin &amp; Pectinase kit</strong></td>
<td>Kit of 50 selective SPE cartridges for Patulin (3mL-100mg) + 50mL Pectinase enzyme solution</td>
<td>FS102-03K</td>
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<tr>
<td></td>
<td>Kit of 50 selective SPE cartridges for Patulin (6mL-200mg) + 50mL Pectinase enzyme solution</td>
<td>FS102-03KB-200mg</td>
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<tr>
<td><strong>AFFINIMIP® SPE Patulin &amp; Standard kit</strong></td>
<td>Kit of 50 selective SPE cartridges for Patulin (3mL-100mg) + 1mL Standard solution 100µg/mL</td>
<td>FS102-03KS</td>
</tr>
<tr>
<td></td>
<td>Kit of 50 selective SPE cartridges for Patulin (6mL-200mg) + 1mL Standard solution 100µg/mL</td>
<td>FS102-03KBS-200mg</td>
</tr>
<tr>
<td><strong>AFFINIMIP® SPE Patulin &amp; Standard &amp; Pectinase kit</strong></td>
<td>Kit of 25 selective SPE cartridges for Patulin (3mL-100mg) + 1mL Standard solution 100µg/mL + 50mL Pectinase enzyme solution</td>
<td>FS102-02KSP</td>
</tr>
<tr>
<td></td>
<td>Kit of 25 selective SPE cartridges for Patulin (6mL-200mg) + 1mL Standard solution 100µg/mL + 50mL Pectinase enzyme solution</td>
<td>FS102-02KBSP-200mg</td>
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<tr>
<td>Pectinase</td>
<td>50 mL Pectinase enzyme solution</td>
<td>REA-001-50mL</td>
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<tr>
<td>Standard solution</td>
<td>1mL Patulin standard solution 100µg/mL</td>
<td>REA-PAT-1ML</td>
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## SPE ACCESSORIES – Product list

<table>
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<tr>
<th>SPE Accessories</th>
<th>Designation</th>
<th>Definition</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Manifold</td>
<td>SPE Vacuum Manifold</td>
<td>12-port model</td>
<td>ACC-MAN1</td>
</tr>
<tr>
<td>Manifold</td>
<td>SPE Vacuum Manifold</td>
<td>24-port model</td>
<td>ACC-MAN2</td>
</tr>
<tr>
<td>SPE Adapter &amp; Reservoir kit</td>
<td>SPE Adapter &amp; Reservoir kit</td>
<td>Kit of 12 reservoirs 60ml and adapters for use with 1,3 &amp; 6 mL cartridges</td>
<td>ACC-AR1</td>
</tr>
<tr>
<td>Mini-Vap</td>
<td>Mini Evaporator/Concentrator</td>
<td>6 port Mini-Vap Evaporator/Concentrator for use with 1 to 250mL containers</td>
<td>ACC-VAP1</td>
</tr>
<tr>
<td>Mini PUMP</td>
<td>Mini vacuum pump</td>
<td>Laboport diaphragm vacuum mini pump, 5.5L/min</td>
<td>ACC-PUMP</td>
</tr>
<tr>
<td>Vacuum pump trap</td>
<td>SPE Vacuum pump trap kit</td>
<td>1L trap kit</td>
<td>ACC-TRAP</td>
</tr>
</tbody>
</table>
About AFFINISEP
AFFINISEP is a worldwide expert in purification and sample preparation applications as well as for the design and the development of intelligent polymers with Molecularly Imprinted Polymers (MIP).
AFFINISEP is dedicated to the development of analytical applications in various fields such as water, biological fluids, food and feed analysis with a complete set of products and services for sample preparation.
Our mission is to develop and market innovative products of high value to customers by a practical contribution to their work. By offering you a most comprehensive range of solid phase extraction products:
- AFFINIMIP® SPE products based on molecularly imprinted polymers,
- AttractSPE™ a range of polymeric phases
- SilactSPE™ Silica based products, associated reagents,
- QuEChERS
- small equipment,
the 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, pharmaceutical R&D and quality control, clinical diagnosis, environment and doping.

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.

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

❖ By mail:
AFFINISEP
Pole d’innovation des couronnes
Bd Sonopa – Rue Aristide Briand
76650 Petit Couronne, FRANCE

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❖ On-line by E-commerce: To save your time and a quicker delivery,
❖ order via our web site www.affinisep.com

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Stay up-to-date with the news on AFFINISEP’s latest innovations by subscribing to our newsletter, delivering new information straight to your inbox.