APPLICATION
NOTEBOOK FOR
SAMPLING &
SAMPLE PREPARATION

Be selective

Food / Feed QC
Environment
Cosmetics
Pharmaceutical R&D
INTRODUCTION

AFFINISEP offers a comprehensive range of sorbents for the challenging fields of sample preparation, sample clean-up and extraction, from conventional to more sophisticated sorbents. So, for very specific and challenging applications, AFFINISEP has developed AFFINIMIP®SPE products, SPE cartridges based on Molecularly Imprinted Polymers (MIP) which require ready-to-use protocols. AFFINISEP has also developed AttractSPE™ products, SPE cartridges based on classical polymeric sorbents.

In addition, our SPE products experience is continuously enriched with customer interactions and an endless analytical development for new applications. This experience is communicated through Application notes (available on website and via newsletters).

For your convenience, this application notebook will be permanently updated with new protocols and results. Please regularly visit our website www.affinisep.com for the latest version of the Application Notebook.
Moreover, we have as well evaluated our products through interlaboratories proficiency testing such as FAPAS and BIPEA. For more information, please contact us at contact@affinisep.com.

This Application notebook will be an essential tool to address your technical issues.

TECHNICAL SUPPORT

AFFINISEP has fully integrated technologies platform with specialized teams in organic chemistry, polymer chemistry, analytical and bioanalytical chemistry who are at your disposal to help you in your challenges.

At AFFINISEP, we are committed to providing the best technical support possible. Our Technical Support Group is a team of highly qualified M.Sc. and PhD Chemists, who are at your disposal to resolve your problem and to answer to your queries. For technical inquiries, feel free to contact us either by email: tech.support@affinisep.com

We are also very thankful to customer’s feedback about our products, protocols and customer services by email to: contact@affinisep.com
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See our application notebook for more applications and details...
## AVAILABLE FORMATS

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<td><strong>Material:</strong> Polypropylene; glass (6mL)</td>
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<td><strong>Material:</strong> Polypropylene</td>
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For each sorbent, the catalog gives references for the most usual formats

**If you wish other formats, please contact us**

www.affinisep.com
Analysis of MYCOTOXINS
**REGULATIONS FOR UNPROCESSED CEREAL EXCEPT MAIZE:**
Europe (EC 1126/2007) : 100µg/Kg

**REGULATIONS FOR MAIZE:**
Europe (EC 1126/2007) : 350µg/Kg

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**PROTOCOL OF CLEANUP**

**Sample preparation**

**Cleanup with a 3mL/100mg AFFINIMIP® SPE Zearalenone cartridge**

25g of ground cereal-based samples were extracted with 100 mL of acetonitrile/deionized water (75/25, v/v) for 3 min. The extract was filtered through a folded filter paper and 10 mL of the filtrate were diluted with 10 mL of deionized water. Then, this solution was filtered through a filter paper. This solution was used as the loading solution.

- **Equilibration**
  - 3mL Acetonitrile
  - 3mL Water

- **Loading**
  - 12mL of loading solution (eq. 1.5g sample)

- **Washing of interferences (W1)**
  - 3mL 58/2/40 Water/Acetic Acid/ACN

- **Elution (E)**
  - 2mL Methanol – 2% Acetic Acid

The elution fraction was then evaporated and dissolved in water before HPLC analysis.

---

**HPLC Method with Fluorescence detection**

- **Column:** Hypersil Gold C18 - 150mmx 4.6mm
- **Mobile phase:** water/MeOH (40/60, v/v)
- **Flow rate:** 1mL/min
- **Fluorescence detection:** excitation/emission wavelengths: 275 / 450nm
- **Injection volume:** 100µL.

---

**RESULTS**

Chromatogram obtained after Cleanup of Maize (contaminated at 41 µg / kg) with AFFINIMIP® SPE Zearalenone.

Chromatogram obtained after Cleanup of Rice (contaminated at 41 µg / kg) with AFFINIMIP® SPE Zearalenone.

Recoveries of Zearalenone at a contamination level of 41µg / kg after AFFINIMIP® SPE Zearalenone. Clean-up in Maize (n=9)

<table>
<thead>
<tr>
<th>Recoveries %</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>86</td>
<td>8</td>
</tr>
</tbody>
</table>

Catalog number: FS100-02
PROTOCOL OF CLEANUP
Sample preparation

Cleanup with a 3mL/100mg AFFINIMIP® SPE Zearalenone cartridge
25g of ground cereal-based samples were extracted with 100 mL of acetonitrile/deionized water (75/25, v/v) for 3 min. The extract was filtered through a folded filter paper and 10 mL of the filtrate were diluted with 10 mL of deionized water. Then, this solution was filtered through a filter paper.
This solution was used as the loading solution.

Equilibration
3mL Acetonitrile
3mL Water

Loading
12mL of loading solution (eq. 1.5g sample)

Washing of interferences (W1)
3mL 58/2/40 Water/Acetic Acid/ACN

Elution (E)
2mL Methanol – 2% Acetic Acid
The elution fraction was then evaporated and dissolved in water before HPLC analysis.

HPLC Method with Fluorescence detection
Column: Hypersil Gold C18 -150mm x 4.6mm
Mobile phase: water/MeOH (40/60, v/v)
Flow rate: 1mL/min
Fluorescence detection: excitation/emission wavelengths: 275 / 450nm
Injection volume: 100µL.

RESULTS
Chromatogram obtained after Cleanup of Cereal-based babyfood (contaminated at 41µg / kg) AFFINIMIP® SPE Zearalenone (after dilution by 2 of the elution fraction with water).

Chromatograms obtained after Cleanup of Cereal-based babyfood (contaminated at 10µg/kg (blue) or 0µg/kg (red)) with AFFINIMIP® SPE Zearalenone (after evaporation of the elution fraction and dissolution in 1mL of the mobile phase).

Recoveries of Zearalenone at a contamination level of 41µg / kg after AFFINIMIP® SPE Zearalenone . Clean-up in Cereal – based baby food (n=5)

<table>
<thead>
<tr>
<th>Recoveries %</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>3</td>
</tr>
</tbody>
</table>

Catalog number: FS100-02

Regulations for processed cereal based food for baby food:
Europe (EC 1126/2007) : 20µg/Kg
 Regulations for processed cereal based food for baby food:  
Europe (EC 1126/2007) : 20µg/Kg

PROTOCOL OF CLEANUP
Sample preparation
Corn oil is diluted 1/3 in Diethyl Ether to obtain the loading solution.

Cleanup with a 3mL/100mg AFFINIMIP® SPE Zearalenone cartridge

Equilibration
3mL Diethyl Ether

Loading
3mL of loading solution (eq. 1mL of corn oil)

Washing of interferences (W1)
6mL Diethyl ether

Drying 30 seconds

Washing of interferences (W2)
6mL 58/2/40 Water/Acetic Acid/ACN

Elution (E)
4mL Methanol – 2% Acetic Acid

The elution fraction was then evaporated and dissolved in water before HPLC analysis.

HPLC Method with Fluorescence detection same as p 15

<table>
<thead>
<tr>
<th>C° (µg/L)</th>
<th>Mean C° (µg/L)</th>
<th>Recoveries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>230</td>
<td>115</td>
</tr>
<tr>
<td>400</td>
<td>440</td>
<td>110</td>
</tr>
<tr>
<td>600</td>
<td>678</td>
<td>113</td>
</tr>
</tbody>
</table>

Chromatograms obtained after cleanup by AFFINIMIP®SPE Zearalenone of Corn Oil spiked with Zearalenone at 200µg/L (red), 400µg/L (green), 600 µg/L (blue) or not spiked (purple).

Recoveries of Zearalenone in Corn Oil at various contamination levels after AFFINIMIP®SPE Zearalenone cleanup.

Catalog number: FS100-02
**PROTOCOL OF CLEANUP**

Sample preparation

25g of meat are mixed during 2 minutes with 100mL of a solution of deionized water/ acetonitrile (50/50). Then the mixture is filtered through filter paper (4-7µm). This solution was then diluted 10 times with deionized water to obtain the loading solution used for the clean-up protocol.

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

**Equilibration**
- 2mL Acetonitrile
- 2mL Water

**Loading solution**
- Up to 6mL of loading solution

**Washing of interferences**
- 6mL 40/60 Acetonitrile/deionized Water
- Drying 3-5min

**Elution (E)**
- 2mL 2/98 Acetic Acid/Methanol

The elution fraction was then concentrated and diluted to mobile phase before HPLC analysis.

**RESULTS**

**Recovery for ZON > 80%**

* Tested at 960µg/kg

SIM Chromatograms obtained after clean-up of MEAT with AFFINIMIP® SPE ZEARALENONE.

- Blue trace for spiked with 960µg/kg of ZEARALENONE.
- Red trace for the blank sample

**HPLC Method with LC-MS**

HPLC Column: Hypersil gold column (50mm x 2.1mm)

Mobile phase: 73/27 0.1 Formic acid in water / Acetonitrile

Flow rate: 0.2mL/min

Injection volume: 20µL.

Catalog number: FS100-02
OCHRATOXIN A IN CEREALS

**Regulations for unprocessed cereals:**
Europe (EC 1881/2006) : 5µg/Kg
Codex Alimentarius Standard: 5µg/Kg for raw wheat

**PROTOCOL OF CLEANUP**
Sample preparation
50g of finely ground wheat are mixed during 1 minute in a blender with 100mL of extraction solvent (60/40 Acetonitrile/deionized Water). The extract is filtered through a filter paper.
Then, 5mL of the extract is diluted with 5mL of HCl solution pH=1, 0.1M. After a filtration through a filter paper, this solution is used as the loading solution.

**Cleanup with a 3mL/100mg AFFINIMIP® SPE Ochratoxin A cartridge**

**Equilibration**
3mL Acetonitrile
3mL Water

**Loading**
4mL of loading solution (eq. 1g wheat)

**Washing of interferences**
6mL 60/40 HCl solution pH 1, 0.1M/ACN

**Elution (E)**
2mL Methanol – 2% Acetic acid
The elution fraction was then evaporated and dissolved in water before HPLC analysis.

**RESULTS**
Chromatogram obtained after Cleanup of wheat (spiked at 5µg / kg (pink) or not contaminated (orange)) with AFFINIMIP® SPE Ochratoxin A

Recoveries of Ochratoxin A after AFFINIMIP® SPE Ochratoxin A Clean-up in wheat (n=6)

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Recoveries %</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>96.3</td>
<td>7.7</td>
</tr>
</tbody>
</table>

**HPLC Method with Fluorescence detection**
Column: Hypersil Gold C18 column 150mm x 2.1mm
Mobile phase: water/acetic acid/MeOH (39/1/60, v/v)
Flow rate: 0.2mL/min
Fluorescence detection: excitation/emission wavelengths: 333 / 460nm
Injection volume: 20µL.

Catalog number: FS101-02
OCHRATOXIN A IN PAPRIKA

Regulations for paprika:
Europe (EC 594/2012): 30µg/Kg until 31.12.14 then 15µg/Kg

PROTOCOL OF CLEANUP
Sample preparation
10g of paprika are shaken during 30 minutes with 100mL of NaHCO₃ 1% in water. The extract is centrifuged for 30 minutes at 4000 rpm at room temperature then filtered through a filter paper.
25mL of the extract is diluted with 25mL of HCl solution pH=1, 0.1M. After a filtration through a filter paper, this solution is used as the loading solution.

Cleanup with a 3mL/100mg AFFINIMIP® SPE Ochratoxin A cartridge

Equilibration
3mL Acetonitrile
3mL Water

Loading
4mL of loading solution (eq. 1g sample)

Washing of interferences
6mL 60/40 HCl solution pH 1, 0.1M/ACN

Elution (E)
2mL Methanol – 2% Acetic acid
The elution fraction was then evaporated and dissolved in water before HPLC analysis.

HPLC Method with Fluorescence detection same as p 18

RESULTS

Chromatogram obtained after Cleanup of paprika (spiked at 30µg / kg (pink) or not contaminated (orange)) with AFFINIMIP® SPE Ochratoxin A

Recoveries of Ochratoxin A after AFFINIMIP® SPE Ochratoxin A Clean-up in paprika (n=4).

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Recoveries %</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>93.3</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Catalog number: FS101-02
Regulations for wine:
Europe (EC 1881/2006) : 2µg/L

PROTOCOL OF CLEANUP
Sample preparation
10mL of wine is diluted with 10mL of HCl solution pH=1, 0.1M. This solution is used as the loading solution.

Cleanup with a 3mL/100mg AFFINIMIP® SPE Ochratoxin A cartridge

Equilibration
3mL Acetonitrile
3mL Water

Loading
2 to 10mL of loading solution (eq. 1 to 5mL sample)

Washing of interferences
6mL 60/40 HCl solution pH 1, 0.1M/ACN

Elution (E)
2mL Methanol – 2% Acetic acid
The elution fraction was then evaporated and dissolved in water before HPLC analysis.

Recoveries of Ochratoxin A after AFFINIMIP® SPE Ochratoxin A Clean-up in wine (white and red).

<table>
<thead>
<tr>
<th>Matrix</th>
<th>C° (µg/kg)</th>
<th>Recovery %</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>White wine</td>
<td>2</td>
<td>91.3</td>
<td>6.2</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red wine</td>
<td>2</td>
<td>78.8</td>
<td>2.8</td>
</tr>
<tr>
<td>(n=4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HPLC Method with Fluorescence detection same as p 18

RESULTS
Chromatograms obtained after Cleanup of white wine spiked at 2µg/kg (loading with 5mL (blue); loading with 10mL (pink)) and after a loading of 5mL of not contaminated white wine (orange) with AFFINIMIP® SPE Ochratoxin A

Chromatograms obtained after Cleanup of red wine spiked at 2µg / kg (loading with 2mL (orange); loading with 5mL (blue); loading with 10mL (pink)) and after a loading of 2mL of not contaminated red wine (grey) with AFFINIMIP® SPE Ochratoxin A

Catalog number: FS101-02
OCHRATOXIN A IN HUMAN URINE

PROTOCOL OF CLEANUP

Sample preparation
The urine sample was centrifuged for 10 min at 10,000g, followed by filtration (0.45 μm glass microfiber filter). Then, 10 ml of the filtrate was adjusted to pH 2.5 with 0.1 M HCl, and an aliquot of 10 ml of the filtrate was used as the loading solution.

Cleanup with a 3ml/100mg AFFINIMIP® SPE Ochratoxin A cartridge

| Equilibration | 4mL Acetonitrile | 4mL Water |
| Loading | 10mL of loading solution |
| Washing of interferences | 7mL 60/40 HCl solution 0.1M/ACN |
| Elution (E) | 2mL Methanol – 2% Acetic acid |

The elution fraction was then evaporated and dissolved in 1mL of mobile phase before UPLC analysis.

UPLC Fluorescence Method
Column: Acquity UPLC BEH C18 column (100×2.1 mm, 1.7 μm)
Mobile phase: MeOH/Water (70/30, v/v) with 0.5% Acetic acid
Flow rate: 0.2mL/min
Detection: Fluo – λ<sub>exc</sub> 333nm - λ<sub>em</sub> 460nm
Injection volume: 2μL.

LC-MS/MS Method for confirmation
Column: EC-C18 column (50×4.6 mm, 2.7 μm)
Mobile phase: phase: MeOH/Water (70/30, v/v) with 0.5% Acetic acid
Flow rate: 0.3mL/min
Detection: MS/MS – ESI<sup>+</sup> mode MRM
Injection volume: 2μL.

RESULTS

<table>
<thead>
<tr>
<th>C° (μg/kg)</th>
<th>Recovery %</th>
<th>% RSD&lt;sub&gt;n=6&lt;/sub&gt;</th>
<th>% RSD&lt;sub&gt;R&lt;/sub&gt;</th>
<th>n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>98.9</td>
<td>1.8</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>98.8</td>
<td>1.1</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>92.0</td>
<td>0.6</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

Recoveries, repeatability RSD<sub>r</sub>, and reproducibility RSD<sub>R</sub> of Ochratoxin A after AFFINIMIP® SPE Ochratoxin A Clean-up determined by UPLC-Fluo.

Publications

Catalog number: FS101-02
PROTOCOL OF CLEANUP

Sample preparation
20g ginger were extracted by sonication for 20 min with 40 mL of acetonitrile/water (60/40, v/v). The extract was filtered and an aliquot of 20 mL of filtrate were diluted with 20 mL of water and then adjusted to pH 1.0 with 1.0M HCl. After filtered (glass microfiber), 4 mL (1 g equivalent) of the filtrate was used as the loading solution.

Cleanup with a 3mL/100mg AFFINIMIP® SPE Ochratoxin A cartridge

Equilibration
5mL Acetonitrile
5mL Water

Loading
4mL of loading solution

Washing of interferences
5mL 60/40 HCl 0.1M/ACN

Elution (E)
2mL Methanol – 2% Acetic acid
The elution fraction was then evaporated and dissolved in 1mL of mobile phase before UPLC analysis.

UPLC - Fluorescence Method
Column: Acquity UPLC HSS T3 column (50mm 2.1mm, 1.8) at 30°C
Mobile phase: MeOH/0.5% aqueous acetic acid Water (65/35, v/v)
Flow rate: 0.2mL/min
Detection: Fluo – λexc 333nm - λem 460nm
Injection volume: 1µL.

LC-MS/MS Method for confirmation
Column: Capcell Core C18 column (50 mm 2.1 mm, 2.7 mm)
Mobile phase: phase: MeOH/0.5% aqueous acetic acid Water (65/35, v/v)
Flow rate: 0.3mL/min
Detection: MS/MS – ESI+ mode MRM
Injection volume: 2µL.

Regulations for ginger:
Europe: 15µg/L

RESULTS

LOD with signal-to-noise :ratio of 3: 0.09ng/mL
LOQ with signal-to-noise :ratio of 10: 0.30ng/mL

Recoveries, and repetability RSDr of Ochratoxin A after AFFINIMIP® SPE Ochratoxin A Clean-up determined by UPLC-Fluo

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Recovery %</th>
<th>% RSDr n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>92.3</td>
<td>2.8</td>
</tr>
<tr>
<td>15.0</td>
<td>91.1</td>
<td>3.3</td>
</tr>
<tr>
<td>25.0</td>
<td>91.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Repetability RSDr and reproducibility RSDR for the whole procedure including AFFINIMIP® SPE Ochratoxin A Clean-up determined by UPLC-Fluo

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>% RSDr n=6</th>
<th>% RSDR n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>2.5</td>
<td>3.7</td>
</tr>
<tr>
<td>15.0</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>25.0</td>
<td>1.6</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Publications

Data extracted from the article: Molecularly imprinted polymer-based solid phase clean-up for analysis of ochratoxin A in ginger and LC-MS/MS confirmation, Cao J., Zhou S., Kong W., Yang M., Wan L., Yang S., Food control, 33(2), 337-343, 2013.

Catalog number: FS101-02
OCHRATOXIN A IN BEER, RED WINE AND GRAPE JUICE

RESULTS

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Recovery %</th>
<th>% RSDr n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>99.8</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>97.5</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>96.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Beer

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Recovery %</th>
<th>% RSDr n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>93.5</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>101.7</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>99.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Red wine

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Recovery %</th>
<th>% RSDr n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>93.5</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>101.7</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>99.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Grape juice

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Recovery %</th>
<th>% RSDr n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>93.5</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>101.7</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>99.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Publications

Data extracted from the article: Molecularly imprinted polymer-based solid phase clean-up for analysis of ochratoxin A in beer, red wine, and grape juice, Jiliang Cao, Weijun Kong, Shujun Zhou, Lihui Yin, Li Wan, Meihua Yang, J. Sep. Sci., 36(7), 1291-1297, 2013

PROTOCOL OF CLEANUP

Sample preparation

20mL samples and 20 mL of water were filtrated (glass microfiber filter). 20 mL of filtrate were adjusted to pH 1.0 with 1.0 M HCl as loading solutions.

Cleanup with a 3mL/100mg AFFINIMIP® SPE Ochratoxin A cartridge

Equilibration

5mL Acetonitrile
5mL Water

Loading

4mL of loading solution

Washing of interferences

7mL 60/40 HCl 0.1M/ACN

Elution (E)

2mL Methanol – 2% Acetic acid

The elution fraction was then evaporated and dissolved in 1mL of mobile phase before UPLC analysis.

HPLC - Fluorescence Method

Column: Acquity Ultimate XB-C18 (250 mm × 4.6mm, 5 µm) at 30°C

Mobile phase: MeOH/0.5% aqueous acetic acid Water (65/35, v/v)

Flow rate: 1mL/min

Detection: Fluo – λexc 333nm - λem 460nm

Injection volume: 25µL.

LC-MS/MS Method for confirmation

Column: Capcell Core C18 column (50 mm 2.1 mm, 2.7 mm) at 30°C

Mobile phase: phase: MeOH/0.5% aqueous acetic acid Water (65/35, v/v)

Flow rate: 0.3mL/min

Detection: MS/MS – ESI+ mode MRM

Injection volume: 2µL.

Catalog number: FS101-02
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 CLEANUP

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

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

Equilibration
2mL Acetonitrile
1mL water

Loading
4mL of loading solution

Washing of interferences (W1)
1mL NaHCO₃
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 Method
Column: Atlantis T3, 150mm x 2.1mm
Mobile phase: Deionized water/ACN (95/5, v/v) Flow rate: 0.2mL/min
Detection: UV - 276nm
Injection volume: 100µL.

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.

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

Catalog number: FS102-02
REGULATIONS FOR APPLE JUICE:
Europe (EC 1881/2006): 50 µg/Kg
USA (FDA CPG Sec.510.150): 50 µg/Kg

REGULATIONS FOR APPLE JUICE FOR INFANTS AND YOUNG CHILDREN:
Europe (EC 1881/2006): 10 µg/Kg

PROTOCOL OF CLEANUP

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

Clean-up with a 3 mL/100 mg AFFINIMIP® SPE Patulin cartridge

Equilibration
- 2 mL Acetonitrile
- 1 mL water

Loading
- 4 mL of loading solution

Washing of interferences (W1)
- 1 mL NaHCO₃ in Water
- 2 mL Water

Drying by applying vacuum 10 seconds

Washing of interferences (W2)
- 1 mL Diethyl Ether

Elution (E)
- 2 mL Ethyl Acetate

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

HPLC Method same as p 24.

RESULTS

Chromatograms of apple juice containing 25 µg/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 40 µg/kg (tested twice, red) or at 10 µg/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>C° of Patulin (ng/mL)</th>
<th>Recovery %</th>
<th>% RSDᵣ</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: FS102-02
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 - 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 at 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: FS102-02
PATULIN IN BABY FOOD APPLE PUREE

**PROTOCOL OF CLEANUP**

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.

Cleanup 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.

**RESULTS**

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

Clean-up of an apple puree from a well-known brand spiked at 25µg/kg (orange), 10µk/kg with Patulin (pink, tested twice) or not spiked (red).

Clean-up of an apple puree second well-known brand spiked at 25µg/kg (green), 10µk/kg with Patulin (dark blue, tested twice) or not spiked (light blue).

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>Recovery % (n=4)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>81.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

**HPLC Method**

Column: Atlantis T3 column, 150mm x 2.1mm

Mobile phase: gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</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.

**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

**Catalog number:** FS102-02
PATULIN IN APPLE PUREE

PROTOCOL OF CLEANUP
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.

Cleanup 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 Method same as p 27

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

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>C° of Patulin (µg/kg)</th>
<th>Rec. %</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: FS102-02B-200mg
**PROTOCOL OF CLEANUP**

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.

**Cleanup 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 Method**

Column: Atlantis T3 column, 150mm x 2.1mm

Mobile phase: gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</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.

**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>C° of Patulin (µg/kg)</th>
<th>Recovery %</th>
<th>% RSDr</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: FS102-02
**PROTOCOL OF CLEANUP**

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.

**Cleanup 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 Method same as p 27**

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

Catalog number: FS102-02
REGULATIONS FOR CIDER:
Europe (EC 1881/2006): 50µg/Kg

PROTOCOL OF CLEANUP
Sample preparation
The cider is degassed by sonication of the sample for 1 hour. Then the degassed cider is diluted by 2 with water containing 2% of acetic acid. This solution is mixed and used as the loading solution.

Cleanup 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.

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>C° of Patulin (ng/mL)</th>
<th>Recoveries %</th>
<th>% RSDᵦ</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>

HPLC METHOD
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.

CATALOG NUMBER: FS102-02
PATULIN IN ALCOHOL POMMEAU AND LIQUOR

Manzella liquor contains 20% alcohol and 2.1% of concentrated apple juice. Alcohol Pommeau is a mixture of Calvados and Apple Juice. It contains 17% Alcohol.

PROTOCOL OF CLEANUP

Sample preparation
To 1mL of Manzella Liquor or Alcohol Pommeau, add 2mL Water to obtain the loading solution.

Cleanup 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 Method same as p 27

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

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 Pommeau and 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>Pommeau</td>
<td>101</td>
<td>106</td>
</tr>
<tr>
<td>Manzella</td>
<td>102</td>
<td>106</td>
</tr>
</tbody>
</table>

Catalog number: FS102-02
**PROTOCOL OF CLEANUP**

**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.

**Cleanup 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 Method same as p 27**

**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%
- 70%

**Catalog number: FS102-02**
PATULIN IN BLUEBERRY JUICE

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

Cleanup 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.

RESULTS
Recovery yields: 90 and 96%

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

HPLC Method same as p 27

Catalog number: FS102-02
AFFINIMIP® SPE Patulin

PATULIN IN CONCENTRATE JUICE AND THICK JUICE

PROTOCOL OF CLEANUP
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.

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.

Cleanup 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 Method same as p 27

RESULTS

THICK JUICE

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.

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: FS102-02B-200mg
PATULIN IN DRIED APPLE

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

**RESULT**

**PROTOCOL OF CLEANUP**
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.

Cleanup 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 Method same as p 27

**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).

Patulin Recovery >90%

Catalog number: FS102-02B-200mg
The regulations for unprocessed corn or durum wheat for food:
Europe (EC 1126/2007) : 1750µg/Kg

RESULTS

Recovery of Deoxynivalenol after AFFINIMIP® SPE Deoxynivalenol clean-up and relative standard deviation (repeatability conditions).

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Detection</th>
<th>Mean µg/kg</th>
<th>R%</th>
<th>%RSDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>UV</td>
<td>623.4</td>
<td>78.0</td>
<td>1.4</td>
</tr>
<tr>
<td>(800µg/kg)</td>
<td></td>
<td></td>
<td></td>
<td>(n=6)</td>
</tr>
<tr>
<td>Corn</td>
<td>MS</td>
<td>642.7</td>
<td>80.3</td>
<td>3.4</td>
</tr>
<tr>
<td>(800µg/kg)</td>
<td></td>
<td></td>
<td></td>
<td>(n=6)</td>
</tr>
<tr>
<td>Wheat</td>
<td>MS</td>
<td>540.0</td>
<td>90.0</td>
<td>9.8</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td>(n=3)</td>
</tr>
</tbody>
</table>

PROTOCOL OF CLEANUP

Sample preparation with EXTRACTION WITH WATER:
20g of cereals were ground in a blender for 1 minute. Then, 80 ml of deionized water were added. This mixture was then ground for 2 additional minutes. After grinding the mixture was placed in a beaker and left stirred under magnetic agitation for 30 minutes. Then the whole mixture was transferred in a centrifuge vial and centrifuged at 2500 rpm for 15 minutes. After centrifugation the supernatant was filtered through filter paper. This solution was then diluted 5 times using deionized water.

Cleanup with a 6mL/100mg AFFINIMIP® SPE Deoxynivalenol cartridge:

- **Equilibration**: 2mL Acetonitrile, 2mL Water
- **Loading**: 6mL of loading solution
- **Washing of interferences (W1)**: 3mL NaHCO₃ 1% in water
- **Drying 30 seconds**
- **Washing of interferences (W2)**: 1mL Diethylether
- **Elution (E)**: 4mL Ethyl Acetate

The elution fraction was then evaporated and dissolved in water -0,1% HCOOH before HPLC analysis.

HPLC Method with MS or UV detection:
Column: Hypersil Gold C18 50mm x 2,1mm
Mobile phase: water with 0,1% formic acid/ACN (95/5, v/v)
Flow rate: 0,2mL/min
MS detection: m/z 265 (ESI-)
UV detection: 220nm
Injection volume: 20µL.

Catalog number: FS117-02B
DEOXYNIVALENOL IN BABYFOOD CEREALS

REGULATIONS FOR CEREAL BASED FOOD FOR BABY FOOD:
Europe (EC 1126/2007) : 200µg/Kg

RESULTS

HPLC Method with MS detection same as the previous page

PROTOCOL OF CLEANUP
Sample preparation
150 ml of deionized water were added to 20g of cereals - based babyfood. This mixture was then placed in a beaker and left stirring under magnetic agitation for 30 minutes.
Then, the whole mixture was centrifuged at 2500 g for 15 minutes. After centrifugation, the supernatant was filtered through filter paper.

Cleanup with a 6mL/100mg AFFINIMIP® SPE Deoxynivalenol cartridge

Equilibration
  2mL Acetonitrile
  2mL Water

Loading
  6mL of loading solution

Washing of interferences (W1)
  3mL NaHCO₃ 1% in water

Drying 30 seconds

Washing of interferences (W2)
  1mL Diethylether

Elution (E)
  4mL Ethyl Acetate

The elution fraction was then evaporated and dissolved in water -0.1% HCOOH before HPLC analysis.

MS chromatograms obtained after water extraction of Deoxynivalenol from cereals - based babyfoods and clean-up with AFFINIMIP® SPE Deoxynivalenol:
• black, red and green spiked with Deoxynivalenol at 150µg/kg
• dark yellow not spiked
• blue, a standard solution of Deoxynivalenol at 200ng/mL is prepared by dilution of a 100µg/mL Deoxynivalenol standard solution (reference : REA-DON-1mL) in mobile phase

Recovery of Deoxynivalenol after AFFINIMIP®SPE Deoxynivalenol clean-up and relative standard deviation calculated from results generated under reproducibility conditions.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>C° µg/kg</th>
<th>Mean µg/kg</th>
<th>R%</th>
<th>%RSD R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babyfood (n=3)</td>
<td>150</td>
<td>136.5</td>
<td>91</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Catalog number: FS117-02B
**Regulations for DON in animal feed:**
Europe (EC 576/2006):
8mg/Kg for cereals and cereals products
12mg/Kg for maize by-products

**PROTOCOL OF CLEANUP**
Sample preparation with **EXTRACTION WITH WATER**
20g of animal feed were ground in a blender for 1 minute. Then, 80 ml of deionized water were added. This mixture was then ground for 2 additional minutes. After grinding the mixture was placed in a beaker and left stirred under magnetic agitation for 30 minutes. Then, the whole mixture was centrifuged at 2500 g for 15 minutes. After centrifugation the supernatant was filtered through filter paper. This solution was then diluted 5 times using deionized water.

**Cleanup with a 6mL/200mg AFFINIMIP®SPE Deoxynivalenol cartridge**
- **Equilibration**
  2mL Acetonitrile
  2mL Water
- **Loading**
  2mL of loading solution
- **Washing of interferences (W1)**
  3mL NaHCO₃ 1% in water
- **Drying 30 seconds**
- **Washing of interferences (W2)**
  1mL Diethylether
- **Elution (E)**
  4mL Ethyl Acetate
The elution fraction was then evaporated and dissolved in water -0,1% HCOOH before HPLC analysis.

**HPLC Method same as p 37**

**RESULTS**

UV chromatograms obtained after WATER extraction of DON from wheat (animal feed) and clean-up with AFFINIMIP®SPE Deoxynivalenol:
- black, red and green spiked with DON at 6mg/kg
- dark yellow not spiked
- blue, a standard solution of DON at 1µg/mL is prepared by dilution of a 100µg/mL Deoxynivalenol standard solution (reference : REA-DON-1mL) in mobile phase

Analysis of Whiskas:
- a. Extraction solution with water
- b. Loading solution
- c. Elution solution

Recovery of Deoxynivalenol after AFFINIMIP®SPE Deoxynivalenol clean-up and relative standard deviation - repeatability conditions (n=3).

<table>
<thead>
<tr>
<th>Feed Matrices</th>
<th>C° mg/kg</th>
<th>Mean mg/kg</th>
<th>R%</th>
<th>%RSDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>6</td>
<td>5.7</td>
<td>94</td>
<td>0.1</td>
</tr>
<tr>
<td>Whiskas</td>
<td>0.8</td>
<td>0.73</td>
<td>91</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Catalog number: FS117-02B-200mg**
DEOXYNIVALENOL IN MEAT

PROTOCOL OF CLEANUP
Sample preparation
25g of meat are mixed during 2 minutes with 100mL of a solution of deionized water/ acetonitrile (50/50). Then the mixture is filtered through filter paper (4-7µm). This solution was then diluted 10 times with deionized water to obtain the loading solution used for the clean-up protocol.

Cleanup with a 6mL/100mg AFFINIMIP® SPE DEOXYNIVALENOL cartridge

Equilibration
2mL Acetonitrile
2mL Water

Loading solution
Up to 6mL of loading solution

Washing of interferences
3mL NaHCO₃ 1% solution

Drying 30s

Washing of interferences
1mL Diethyl Ether

Elution (E)
4mL Ethyl Acetate

The elution fraction was then concentrated and diluted to mobile phase before HPLC analysis.

HPLC Method with MS detection
Column: Hypersil Gold C18 column 50mm x 2,1mm
Mobile phase: water with 0,1% formic acid/ACN (95/5, v/v)
Flow rate: 0,2mL/min
MS detection: m/z 265 (ESI⁻)
Injection volume: 20µL.

RESULTS

Recovery for DON > 90%*
*Tested at 8000µg/kg

UV Chromatograms (220nm) obtained after clean-up of MEAT with AFFINIMIP® SPE DEOXYNIVALENOL.

- Blue trace for spiked with 8000µg/kg of DEOXYNIVALENOL
- Red trace for the blank sample

Catalog number: FS117-02B
DEOXYNIVALENOL, 3-AcetylDON AND 15-AcetylDON IN CEREALS (Hydro-organic extraction)

RESULTS

HPLC Method with MS detection same as p37 except for the mobile for 3-AcDON and 15-AcDON analyses: water with 0.1% formic acid/ACN (90/10, v/v)

PROTOCOL OF CLEANUP
Sample preparation WITH HYDROORGANIC EXTRACTION
20g of cereals were ground in a blender for 1 minute. Then, a solution of deionized water: acetonitrile (50:50) was added. This mixture was then ground for 2 additional minutes. After grinding, the mixture was placed in a beaker and left stirred under magnetic agitation for 30 minutes. Then the mixture was centrifuged at 2500 g for 15 minutes. After centrifugation, the supernatant was filtered through filter paper. This solution was then diluted 10 times using deionized water.

Cleanup with a 6mL/100mg AFFINIMIP® SPE Deoxynivalenol cartridge

- Equilibration
  2mL Acetonitrile
  2mL Water

- Loading
  6mL of loading solution

- Washing of interferences (W1)
  3mL NaHCO₃ 1% in water

- Drying 30 seconds

- Washing of interferences (W2)
  1mL Diethylether

- Elution (E)
  • 4mL Ethyl Acetate

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

Recovery obtained for DON, 3-acetylDON and 15-acetylDON after AFFINIMIP® SPE Deoxynivalenol clean-up of Corn and relative standard deviation - repeatability conditions (n=3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>C° µg/kg</th>
<th>Mean µg/kg</th>
<th>R%</th>
<th>%RSDr</th>
</tr>
</thead>
<tbody>
<tr>
<td>DON</td>
<td>800</td>
<td>653.7</td>
<td>81.7</td>
<td>0.3</td>
</tr>
<tr>
<td>3-AcetylDON</td>
<td>800</td>
<td>601.0</td>
<td>75.1</td>
<td>2.3</td>
</tr>
<tr>
<td>15-AcetylDON</td>
<td>800</td>
<td>641.8</td>
<td>80.2</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Catalog number: FS117-02B

Regulations for unprocessed corn or durum wheat for food:
Europe (EC 1126/2007): 1750µg/Kg
**PROTOCOL OF CLEANUP**

**Sample preparation**

**Cleanup with a 3mL/100mg AFFINIMIP® SPE FumoZON cartridge**

25g of ground samples were extracted with 100 mL of Acetonitrile/Methanol/deionized Water (25/25/50, v/v/v) for 3 min using a blender. The extract was filtered through a folded filter paper and 10 mL of the filtrate were diluted with 10 mL of deionized water. Then, this solution was filtered through a filter paper. This solution was used as the loading solution.

- **Equilibration**
  - 2mL Acetonitrile
  - 2mL Water

- **Loading**
  - 6mL of loading solution

- **Washing of interferences**
  - 6mL 60/40 Water/ACN

- **Elution (E)**
  - 2mL Methanol – 2% Acetic Acid

The elution fraction was then evaporated and dissolved in water before HPLC analysis.

**HPLC Method with MS detection**

- Column: Hypersil Gold C18 column 50mm x 2.1mm
- Mobile phase ZON AND FB1: Water-Formic Acid 0.1%/ACN (73/27)
- Mobile phase FB2: Water-Formic Acid 0.1%/ACN (65/35)
- Flow rate: 0.2mL/min
- MS detection: m/z 722 for Fumonisins B1 (ESI⁺) m/z 706 for Fumonisins B2 (ESI⁺) m/z 317 for Zearalenone (ESI⁺)
- Injection volume: 20µL.

**RESULTS**

Recovery of Zearalenone, Fumonisins B1 and B2 in maize-based baby food after AFFINIMIP® SPE FumoZON clean-up and relative standard deviation calculated from results generated under reproducibility conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C° µg/kg</th>
<th>Mean µg/kg</th>
<th>Recoveries %</th>
<th>% RSDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zearalenone</td>
<td>20</td>
<td>16.9</td>
<td>84.4</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(n=4)</td>
<td></td>
</tr>
<tr>
<td>Fumonisin B1</td>
<td>200</td>
<td>168.6</td>
<td>84.3</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(n=3)</td>
<td></td>
</tr>
<tr>
<td>Fumonisin B2</td>
<td>200</td>
<td>185.6</td>
<td>92.8</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(n=3)</td>
<td></td>
</tr>
</tbody>
</table>

**ION SUPPRESSION EVALUATION**

Ion suppression phenomenon can induce an erroneous quantification. To evaluate the ion-suppression, blank maize-based baby food samples were cleaned up with AFFINIMIP® SPE FumoZON. The SPE extracts were spiked with a mixture of Fumonisins B1 and Zearalenone at 2 different concentrations. The standard calibration curves were compared to the matrix SPE extracts. The use of AFFINIMIP® SPE FumoZON strongly reduces ion-suppression phenomena with a maximum of 15% observed for Fumonisins.

Ion suppression percentage obtained in Maize-based baby food (tested twice).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>C° µg/kg</th>
<th>Ion suppression %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zearalenone</td>
<td>10</td>
<td>1% and 5%</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>50</td>
<td>0% and 5%</td>
</tr>
<tr>
<td>Fumonisin B1</td>
<td>100</td>
<td>8% and 11%</td>
</tr>
<tr>
<td>Fumonisin B1</td>
<td>500</td>
<td>12% and 14%</td>
</tr>
</tbody>
</table>

Catalog number: FS109-02
Regulations for cereal flour:

- **Zearalenone**
  - Europe (EC 1126/2007): 75µg/Kg
  - USA: FDA advisory 2000µg/Kg

- **Fumonisins**
  - Europe (EC 1126/2007): 1000µg/Kg for maize flour

**PROTOCOL OF CLEANUP**

Sample preparation

**Cleanup with a 3mL/100mg AFFINIMIP® SPE FumoZON cartridge**

25g of ground samples were extracted with 100 mL of Acetonitrile/Methanol/deionized Water (25/25/50, v/v/v) for 3 min using a blender. The extract was filtered through a folded filter paper and 10 mL of the filtrate were diluted with 10 mL of deionized water. Then, this solution was filtered through a filter paper. This solution was used as the loading solution.

- **Equilibration**
  - 2mL Acetonitrile
  - 2mL Water

- **Loading**
  - 6mL of loading solution

- **Washing of interferences**
  - 6mL 60/40 Water/ACN

- **Elution (E)**
  - 2mL Methanol – 2% Acetic Acid

The elution fraction was then evaporated and dissolved in water before HPLC analysis.

**RESULTS**

Chromatograms obtained after AFFINIMIP® SPE FumoZON clean-up of a maize flour spiked at 38µg/kg with Zearalenone, 2408µk/kg with Fumonisin B1 and 630µg/kg with Fumonisin B2.

Recovery of Zearalenone, Fumonisins B1 and B2 in maize flour after AFFINIMIP® SPE FumoZON clean-up and relative standard deviation calculated from results generated under reproducibility conditions:

<table>
<thead>
<tr>
<th>Sample</th>
<th>C° µg/kg</th>
<th>Mean µg/kg</th>
<th>Yield %</th>
<th>% RSD R</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZON</td>
<td>38</td>
<td>39.2</td>
<td>103.2</td>
<td>8.5 (n=8)</td>
</tr>
<tr>
<td>Fumonisins B1</td>
<td>2408</td>
<td>2002.2</td>
<td>83.1</td>
<td>10.3 (n=8)</td>
</tr>
<tr>
<td>Fumonisins B1</td>
<td>400</td>
<td>401.0</td>
<td>100.2</td>
<td>- (n=2)</td>
</tr>
<tr>
<td>Fumonisins B2</td>
<td>630</td>
<td>684.6</td>
<td>108.7</td>
<td>11.5 (n=3)</td>
</tr>
</tbody>
</table>

HPLC Method with MS detection same as previous page

Catalog number: FS109-02
**Regulations for cereal flour:**

**Fumonisins**
- Europe (EC 1126/2007): 1000μg/Kg for maize flour
- USA: FDA advisory 2000μg/Kg

**PROTOCOL OF CLEANUP**

**Sample preparation**

Cleanup with a 3mL/100mg AFFINIMIP® SPE FumoZON cartridge

25g of well-ground samples were extracted with 100 mL of Acetonitrile/Methanol/deionized Water (25/25/50, v/v/v) for 30 min using a stirrer. The extract was centrifuged for 10 min at 10,730 g. 10mL of supernatant was diluted with 10mL of water. This solution was used as the loading solution.

**Equilibration**
- 4mL Methanol
- 4mL Water

**Loading**
- 8mL of loading solution

**Washing of interferences**
- 6mL 75/25 Water/ACN

**Elution (E)**
- 4mL Methanol – 2% Formic Acid

The elution fraction was then evaporated and dissolved in water before HPLC analysis.

**HPLC Method with MS/MS detection**

Column: Kinetex 2.6μm, PFP, 100mm x 2.1mm
Mobile phase: Gradient with
- phase A: MeOH/water/AAc (20:79.9:0.1)
- phase B: MeOH/ water/ AAc (79:19.9:0.1).

Gradient: 0–4 min, 80% A; 10–25 min, 45% A; 30–40 min, 100% B; and 45–60 min 80% A.

Flow rate: 0.15mL/min
Injection volume: 25μL.

**RESULTS**

Calibration for spiked samples (corn flour and corn grits) to a final concentration of 100, 300 and 500μg/kg of each FB1, FB2 and FB3.

Linearity with $R^2 > 0.99$

LOQ set at 25μg/kg

Accuracy of the method ranged from 95 to 100% for the studied fumonisins

**Analysis of 49 cereals (42 maize-based and seven wheat-based products)**

Chromatograms obtained after AFFINIMIP® SPE FumoZON Clean-up of corn flour and corn grits

<table>
<thead>
<tr>
<th>Sample</th>
<th>CO</th>
<th>FB1</th>
<th>FB2</th>
<th>FB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>R%</td>
<td>100</td>
<td>111.6</td>
<td>92.0</td>
<td>99.0</td>
</tr>
<tr>
<td>RSD&lt;sub&gt;R&lt;/sub&gt;</td>
<td>100</td>
<td>15.4</td>
<td>19.7</td>
<td>14.2</td>
</tr>
<tr>
<td>R%</td>
<td>300</td>
<td>103.3</td>
<td>101.3</td>
<td>108.8</td>
</tr>
<tr>
<td>RSD&lt;sub&gt;R&lt;/sub&gt;</td>
<td>300</td>
<td>11.9</td>
<td>15.9</td>
<td>12.3</td>
</tr>
<tr>
<td>R%</td>
<td>500</td>
<td>101.8</td>
<td>91.3</td>
<td>98.1</td>
</tr>
<tr>
<td>RSD&lt;sub&gt;R&lt;/sub&gt;</td>
<td>500</td>
<td>6.0</td>
<td>12.7</td>
<td>7.5</td>
</tr>
</tbody>
</table>

**Publications**


Catalog number: FS109-02
Affinity-based SPE sorbents have been developed to be selective in extracting the target analytes like molecularly imprinted polymer (MIP) and immunoaffinity sorbent. Immunoaffinity columns (IAC) are biological sorbents based on the use of antibodies that are specific to the target analytes. Molecularly imprinted polymer is a synthetic material with artificially generated three-dimensional network able to specifically rebind a target molecule. Based on molecularly imprinted polymers, AFFINISEP’s AFFINIMIP® SPE cartridges have the advantages to be highly selective and specific. Contrary to IAC, AFFINIMIP® SPE cartridges are chemically and thermally stable, compatible with all solvents as well as cost effective.

### PROPERTIES OF MIP AND IAC

<table>
<thead>
<tr>
<th>Feature</th>
<th>IAC</th>
<th>AFFINIMIP® SPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selectivity</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Capacity</td>
<td>6µmol/g</td>
<td>10-100µmol/g</td>
</tr>
<tr>
<td>Analyte recognition in water</td>
<td>Good</td>
<td>Variable</td>
</tr>
<tr>
<td>Analyte recognition in Organics</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Stability</td>
<td>Poor</td>
<td>Very High</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>Variable</td>
<td>Good</td>
</tr>
<tr>
<td>Cost</td>
<td>Expensive</td>
<td>Inexpensive</td>
</tr>
</tbody>
</table>

Compared to IAC, AFFINIMIP® SPE provides:
- Easier and faster protocol
- Lower dilution
- Easier automatisation

(Cf. Automated method for the selective SPE of Ochratoxin A from wheat Using Molecularly Imprinted Polymer; Gilson Application Notes Handbook 2011; volume 1 Issue 4)

### PROTOCOL: Zearalenone (ZON) from maize flour

<table>
<thead>
<tr>
<th>Step</th>
<th>Vicam IAC</th>
<th>AFFINIMIP® SPE ZON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction of target analyte</td>
<td>25g sample in 100mL 90/10 Methanol/water Blender 3 minutes + filtration</td>
<td>25g sample in 100mL 75/25 ACN/water Blender 3 minutes + filtration</td>
</tr>
<tr>
<td>Preparation loading solution</td>
<td>4mL extract + 96mL water</td>
<td>10mL extract + 10mL Water</td>
</tr>
<tr>
<td>Loading</td>
<td>100mL Loading solution</td>
<td>8mL Loading solution</td>
</tr>
<tr>
<td>Washing</td>
<td>20mL Water</td>
<td>4mL 2/58/40 Acetic acid / water / ACN</td>
</tr>
<tr>
<td>Elution</td>
<td>1.5mL Methanol</td>
<td>2mL 98/2 Methanol/Acetic acid</td>
</tr>
<tr>
<td>Protocol time</td>
<td>55min</td>
<td>30min</td>
</tr>
</tbody>
</table>

### PROTOCOL: Ochratoxin A (OTA) from wheat flour

<table>
<thead>
<tr>
<th>Step</th>
<th>Vicam IAC</th>
<th>AFFINIMIP® SPE OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction of target analyte</td>
<td>50g sample in 100mL 60/40 ACN/water Blender 1 minute + filtration</td>
<td>10mL extract + 10mL HCl 0.1M pH=1</td>
</tr>
<tr>
<td>Preparation loading solution</td>
<td>10mL extract + 40mL PBS</td>
<td>10mL Loading solution</td>
</tr>
<tr>
<td>Loading</td>
<td>10mL Loading solution</td>
<td>4mL Loading solution</td>
</tr>
<tr>
<td>Washing</td>
<td>10mL PBS 10mL Water</td>
<td>7mL 60/40 HCl 0.1M pH=1/ACN</td>
</tr>
<tr>
<td>Elution</td>
<td>1.5mL Methanol</td>
<td>2mL 98/2 Methanol/Acetic acid</td>
</tr>
<tr>
<td>Protocol time</td>
<td>30min</td>
<td>20min</td>
</tr>
</tbody>
</table>
Figure 1. Chromatogram of Maize sample spiked with Zearalenone at 85 µg/kg obtained after cleanup by AFFINIMIP® SPE Zearalenone (red) or Vicam IAC (blue).

Figure 2. Chromatogram of wheat sample spiked with Ochratoxin A obtained after cleanup by AFFINIMIP® SPE Zearalenone (red, spiked at 10ng/g) or Vicam IAC (blue, spiked at 6ng/g).

Figure 3. Recovery of Ochratoxin A or Zearalenone obtained after cleanup by AFFINIMIP® SPE or Vicam IAC.

Figure 4. Comparison of capacity between AFFINIMIP® SPE Zearalenone (red) and Vicam IAC (blue).

Figure 5. Comparison of capacity between AFFINIMIP® SPE OTA (red) and Vicam IAC (blue).
Analysis of
ENDOCRINE DISRUPTING
COMPOUNDS
ESTROGENS IN PLASMA

**PROTOCOL OF CLEANUP**

Sample preparation
2mL serum samples spiked with 40pg 17β-Estradiol-d3. Then 2mL of Acetate buffer (0.8M, pH 6.8) and 100µL β-glucuronidase were added. Hydrolysis performed overnight at 37°C and samples centrifuged at 4000 rpm for 10min. Upper layer was used as loading solution.

**Cleanup with a 3mL/100mg AFFINIMIP® SPE Estrogens cartridge**

**Equilibration**
- 3mL Methanol
- 3mL Acetonitrile
- 3mL Water

**Loading solution from sample preparation**

**Washing of interferences**
- 3mL Water
- 3mL Water/Acetonitrile (60/40)

**Elution (E)**
- 3mL Methanol

The elution fraction was then evaporated and estrogens were derivatised 40min at 60°C with BSTFA before GC-MS/MS analysis.

**RESULTS**

MRM chromatograms from GC-MS/MS analysis of fortified calves’ plasma samples at 0, 10, 40 and 100 pg.mL⁻¹ with 17α-estradiol, 17β-estradiol and estrone. Chromatograms obtained after a clean-up with AFFINIMIP® SPE Estrogens (Courtesy of Emmanuelle Bichon - LABERCA)

**GC-MS/MS Analysis**

Column: RTX-1614 Resteck 15m x 0.25mm x 0.10µm

Gradient temperature: 80 to 320°C (15°C/min)

Data extracted from ‘Quantification of estrogens at ppt levels in bovine plasma by Molecularly Imprinted Solid Phase Extraction and GC-MS/MS analysis’, Emmanuelle Bichon et al. (LABERCA) Poster session, HTSP-2 and HTC 2012

**Regulations for Estrogens:**
Europe (EC directive) : 40pg/mL of plasma or serum of bovine animals

**Catalog number:** FS104-02
SYNTHETIC AND NATURAL ESTROGENS IN RIVER WATER

PROTOCOL OF PURIFICATION

Sample preparation
100 mL of river water were filtered through 0.45 µm cellulose filter to obtain the loading solution.

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

Equilibration
5mL Acetonitrile
5mL Water

Loading solution from sample preparation

Washing of interferents
4mL Water/Acetonitrile (80/20)
2mL Water

Drying under vacuum during 5min

Washing of interferents
2mL Acetonitrile
2mL Methanol/Acetonitrile (5/95)

Elution (E)
3mL Methanol

The elution fraction was then evaporated and reconstituted in 500µL of UHPLC.

RESULTS

SRM Chromatograms of Estrogens extracted from 100 mL river water spiked at 100 ng L⁻¹ (Courtesy of P. Lucci, University of Barcelona, SPAIN)

Recorvery yield in river water

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone (E1)</td>
<td>89</td>
</tr>
<tr>
<td>17α-Estradiol (α-E2)</td>
<td>101</td>
</tr>
<tr>
<td>17β-Estradiol (β-E2)</td>
<td>93</td>
</tr>
<tr>
<td>Estriol (E3)</td>
<td>82</td>
</tr>
<tr>
<td>17α- Ethynilestradiol (EE2)</td>
<td>100</td>
</tr>
</tbody>
</table>

Publications

Data extracted from Solid-phase extraction using molecularly imprinted polymer for selective extraction of natural and synthetic estrogens from aqueous samples, Paolo Lucci, Oscar Núñez, M.T. Galceran, Journal of Chromatography A, 1218(30), 4828-4833, 2011
Molecules analyzed: Estradiol, estrone, estriol, 17α-Estradiol (α-E2)

PROTOCOL OF PURIFICATION
Sample preparation
40 mL of coelomic fluid were spiked with the internal standard estradiol-d2 to the final concentration of 10 ng ml⁻¹ and centrifuged to obtain the loading solution.

Purification with a 3mL/100mg AFFINIMIP® SPE Estrogens cartridge
Equilibration
• 3mL Acetonitrile
• 3mL Water

Loading solution from sample preparation
Washing of interferents
• 3mL water
• 3mL Water/Acetonitrile (60/40)

Elution (E)
3mL Methanol
The elution fraction was evaporated until dryness under nitrogen before derivatization with 100 µl of dansyl chloride (1mg ml⁻¹ in acetone) and 100 µl of 0,1 M sodium bicarbonate in water, heated at 60°C for 3 minutes. The derivatized extract was reconstituted in 1mL of methanol:water (70:30 v/v).

LC-MS/MS Analysis
Column: Synergi Hydro RP (150mmx2.0 mm, 4µm)
Column Temperature: 30°C
Injection volume: 10µL
Flow rate: 0.3mL/min
Detection: LC-MS/MS ESI+
Mobile phase: gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Methanol</th>
<th>% Water-0.1% acid formic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>14</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Publications
Data extracted from

Catalog number: FS104-02
ESTROGENS IN WATER BY GC-MS/MS

PROTOCOL OF PURIFICATION

Sample preparation
100mL of tap water spiked with 17β-E2-d₃ to a final concentration of 75ng/L was the loading solution.

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

**Equilibration**
- 3mL Acetonitrile
- 3mL Water

**Loading solution from sample preparation**

**Washing of interferents**
- 3mL water
- 3mL Water/Acetonitrile (60/40)

**Elution (E)**
- 3mL Methanol

The elution fraction was then evaporated to dryness under a stream of nitrogen. Residues was treated with 10 μL of a mixture containing BSTFA +1 % TMCS and 8 μL of pyridine (dried with solid KOH). After a vortex stirring, derivatisation was performed for 30 min at 55 °C. The derivatives were cooled to room temperature, 2-μL aliquots of the recovery standard (pyrene-d10) were added to each vial and the samples were subjected to GC-MS analysis.

**GC-MS/MS Analysis**

Column: Rtx-5 fused silica capillary columns (30 m, 0.25-mm ID, 0.25-μm film thickness)
Gas carrier: Helium at a flow 1.2mL/min
Injection temperature: 50 to 300 °C at 100 °C/min, held at 300 °C for 10 min
GC-MS transfer line temperature: 280°C
Temperature program: 100°C during 2min; 10°C/min to 265°C; 265°C during 2min ; 10°C/min to 300°C; 300°C during 3 min ; 20°C/min to 310°C; 310°C during 3min
Injection volume: 5μL
Detector: GC-MS/MS EI+ mode
Detection mode: Selected reaction monitoring (SRM)

RESULTS

Method validation for 17β-E2 and 17α-EE2 by GC-MS/MS

<table>
<thead>
<tr>
<th></th>
<th>17β-E2</th>
<th>17α-EE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range, ng/ L</td>
<td>0.08-80.0</td>
<td>0.08-80.0</td>
</tr>
<tr>
<td>Linearity (R²)</td>
<td>0.995</td>
<td>0.9998</td>
</tr>
<tr>
<td>m-LOQ, ng/L</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Spiking level ng/L (n=5)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Recovery %</td>
<td>111</td>
<td>104</td>
</tr>
<tr>
<td>Precision (n=5)</td>
<td>6.2</td>
<td>6.8</td>
</tr>
<tr>
<td>Spiking level ng/L (n=5)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Recovery %</td>
<td>108</td>
<td>110</td>
</tr>
<tr>
<td>Precision (n=5)</td>
<td>9.7</td>
<td>15.3</td>
</tr>
</tbody>
</table>

Publications

Data extracted from **Determination of steroidal oestrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS)**, D. Zacs, I. Perkons, V. Bartkevics, *Environ Monit Assess* 188, 433, 2016.

Catalog number: FS104-02

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ESTROGENS IN WATER BY GC-HRMS

PROTOCOL OF PURIFICATION

Sample preparation
100 mL of tap water spiked with 17β-E2-d₃ to a final concentration of 75 ng/L was the loading solution.

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

Equilibration
- 3 mL Acetonitrile
- 3 mL Water

Loading solution from sample preparation

Washing of interferents
- 3 mL water
- 3 mL Water/Acetonitrile (60/40)

Elution (E)
3 mL Methanol

The elution fraction was then evaporated to dryness under a stream of nitrogen. Residues were treated with 10 µL of a mixture containing BSTFA +1 % TMCS and 8 µL of pyridine (dried with solid KOH). After a vortex stirring, derivatisation was performed for 30 min at 55 °C. The derivatives were cooled to room temperature, 2-µL aliquots of the recovery standard (pyrene-d10) were added to each vial and the samples were subjected to GC-MS analysis.

RESULTS

Method validation for 17β-E2 and 17α-EE2 by GC-MS/MS

<table>
<thead>
<tr>
<th></th>
<th>17β-E2</th>
<th>17α-EE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range, ng/L</td>
<td>0.08-80.0</td>
<td>0.08-80.0</td>
</tr>
<tr>
<td>Linearity (R²)</td>
<td>0.9990</td>
<td>0.9990</td>
</tr>
<tr>
<td>m-LOQ, ng/L</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Spiking level ng/L</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery %</td>
<td>113</td>
<td>111</td>
</tr>
<tr>
<td>Precision (n=5)</td>
<td>4.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Spiking level ng/L</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>(n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery %</td>
<td>99</td>
<td>106</td>
</tr>
<tr>
<td>Precision (n=5)</td>
<td>4.3</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Publications

Data extracted from Determination of steroidal oestrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS), D. Zacs, I. Perkons, V. Bartkevics, Environ Monit Assess 188, 433, 2016.

Catalog number: FS104-02
The analysis of 13 analytes: estrone (E1), 17α-estradiol (α-E2), 17β-estradiol (β-E2), estriol (E3), 17α-ethinylestradiol (EE2), diethylstilbestrol (DES), bisphenol A (BPA), bisphenol S (BPS), 4-n-octylphenol (OP), 4-n-nonylphenol (NP), coumestrol (COU), genistein (GEN), and enterolactone (ENT) was performed by using AFFINIMIP® SPE PHENOLICS prior UHPLC–MS/MS analysis.

### RESULTS

Validation parameters in ultra-pure water: recoveries, reproducibility, reproducibility and quantification limits

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Recovery (%)(n=2)</th>
<th>RSD, % n=10 1µg/L</th>
<th>LOQ (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>107.6</td>
<td>10.8</td>
<td>0.07</td>
</tr>
<tr>
<td>α-E2</td>
<td>124.2</td>
<td>7.9</td>
<td>0.30</td>
</tr>
<tr>
<td>E3</td>
<td>121.6</td>
<td>60.2</td>
<td>2.7</td>
</tr>
<tr>
<td>β-E2</td>
<td>107.4</td>
<td>7.3</td>
<td>0.20</td>
</tr>
<tr>
<td>EE2</td>
<td>106.7</td>
<td>6.6</td>
<td>0.10</td>
</tr>
<tr>
<td>OP</td>
<td>51.8</td>
<td>Nd</td>
<td>0.07</td>
</tr>
<tr>
<td>NP</td>
<td>45.1</td>
<td>Nd</td>
<td>0.15</td>
</tr>
<tr>
<td>BPA</td>
<td>104.6</td>
<td>9.8</td>
<td>0.04</td>
</tr>
<tr>
<td>BPS</td>
<td>129.6</td>
<td>36.2</td>
<td>0.07</td>
</tr>
<tr>
<td>COU</td>
<td>235.7</td>
<td>15.9</td>
<td>0.10</td>
</tr>
<tr>
<td>ENT</td>
<td>253.1</td>
<td>73.1</td>
<td>0.10</td>
</tr>
<tr>
<td>GEN</td>
<td>208.4</td>
<td>47.7</td>
<td>0.25</td>
</tr>
<tr>
<td>DES</td>
<td>109.5</td>
<td>38.7</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### Publications

**RESULTS**

Chromatograms of Infant Formula containing 1µg/L of Bisphenol A before clean-up (Red) and after clean-up (Blue) with AFFINIMIP® SPE Bisphenols.

Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of 15mL of Infant Formula spiked with Bisphenol A at 2µg/L (tested twice, blue) or at 1µg/L (tested twice, red) or not spiked (pink).

Recovery of Bisphenol A in 15mL of infant formula after AFFINIMIP® SPE Bisphenols clean-up and relative standard deviation calculated from results generated - under reproducibility conditions % RSDR

<table>
<thead>
<tr>
<th>C° (µg/L)</th>
<th>Mean (µg/L)</th>
<th>Recoveries %</th>
<th>% RSDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>84.4</td>
<td>7.4</td>
</tr>
<tr>
<td>2.0</td>
<td>1.7</td>
<td>85.8</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Catalog number: FS106-02

---

**PROTOCOL OF CLEANUP**

Sample preparation

Cleanup with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge

**Equilibration**
- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
- Up to 15mL of infant formula (pH adjusted to 5-6)

**Washing of interferences**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 30 seconds**

**Elution (E)**
- 3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with Fluorescence detection**

Column: Hypersil Gold C18 column 150mm x 4.6mm

Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>35</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min

Fluorescence detection: excitation/emission wavelengths: 230 / 315nm

Injection volume: 50µL.
**PROTOCOL OF CLEANUP**

Sample preparation

4.4g powdered infant milk was reconstituted in 30 mL of water and warmed up at ~ 50°C during 20 seconds using microwaves. Then 20 mL of acetonitrile were added to 20 mL of warm milk and centrifuged at 4000 rpm during 10 minutes. The supernatant was collected and filtered on filter paper (4-7µm). This extract was diluted 1:1 with water to form the loading solution.

**Cleanup with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge**

**Equilibration**

- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**

Up to 40mL of infant formula (pH adjusted to 5-6)

**Washing of interferences**

- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 30 seconds**

**Elution (E)**

- 3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**RESULTS**

Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of equivalent at 10mL of Infant Formula spiked with Bisphenol A at 4.3µg/L (tested twice, red) or at 2.1µg/L (tested twice, blue) or not spiked (pink).

Recovery of Bisphenol A spiked at different concentrations after 3mL/100mg AFFINIMIP® SPE Bisphenols clean-up of 40mL of loading solution (equivalent to 10mL of reconstituted Infant milk) and relative standard deviation calculated from results generated under repeatability conditions.

<table>
<thead>
<tr>
<th>C° of BPA in reconstituted milk (µg/L)</th>
<th>Mean concentration (µg/L)</th>
<th>Recovery %</th>
<th>RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>2.3 (n=5)</td>
<td>108</td>
<td>8.7</td>
</tr>
<tr>
<td>4.3</td>
<td>4.0 (n=4)</td>
<td>95</td>
<td>3.7</td>
</tr>
</tbody>
</table>

**HPLC Method with Fluorescence detection same as p 54**
**AFFINIMIP® SPE Bisphenols**

**BISPHENOL A IN CANNED FOOD (Liquid form)**

**RESULTS**

Chromatograms after clean-up with **AFFINIMIP® SPE Bisphenols** of 10mL liquid form of canned Peas and carrots spiked with Bisphenol A at 1µg/L (tested twice, blue) or not spiked (green).

Recovery of Bisphenol A after **AFFINIMIP® SPE Bisphenols** clean-up of 10mL of canned peas and carrots (liquid) spiked at 1µg/L and relative standard deviation calculated from results generated
- under repeatability conditions (n=4).

<table>
<thead>
<tr>
<th>C° (µg/L)</th>
<th>Mean (µg/L)</th>
<th>Recoveries %</th>
<th>% RSD R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.05</td>
<td>105.1</td>
<td>5</td>
</tr>
</tbody>
</table>

- under reproducibility conditions (n=4).

<table>
<thead>
<tr>
<th>C° (µg/L)</th>
<th>Mean (µg/L)</th>
<th>Recoveries %</th>
<th>% RSD R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.04</td>
<td>104.3</td>
<td>10</td>
</tr>
</tbody>
</table>

More information in the application note on our website

**Regulations for Bisphenol A:**
Europe (directive 2011/8/EU) : Specific migration limit in food from packaging of 0.6mg/kg

**PROTOCOL OF CLEANUP**

Cleanup with a 3mL or 6mL/100mg **AFFINIMIP® SPE Bisphenols** cartridge

**Equilibration**
3mL Methanol -2% Acetic Acid
3mL Acetonitrile
3mL Water

**Loading**
10mL liquid from canned food after filter paper filtration (pH adjusted to 5-6)

**Washing of interferences**
9mL Water
6mL Water/Acetonitrile (60/40)

**Drying 30 seconds**

**Elution (E)**
3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with Fluorescence detection same as p 54**
**BISPHENOL A IN CANNED FOOD (Vegetable)**

**Regulations for Bisphenol A:**
Europe (directive 2011/8/EU): Specific migration limit in food from packaging of 0.6mg/kg

---

**PROTOCOL OF CLEANUP**

Sample preparation
150g of drained canned peas - carrots and 200mL of Water /ACN (50/50) are blended during 2 min and centrifuged during 10 min at 4000 rpm. The supernatant solution is collected, filtered (4-7 µm) and diluted ½ with water to give the loading solution. (pH adjusted to 5-6)

Cleanup with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge

**Equilibration**
- 3mL Methanol - 2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
- 20mL loading solution

**Washing of interferences**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying**
- 30 seconds

**Elution (E)**
- 3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with Fluorescence detection same as p 54**

---

**RESULTS**

Chromatograms after clean-up with AFFINIMIP® SPE Bisphenols of 20mL loading solution of extract of canned Peas- carrots spiked with Bisphenol A at 2µg/L (tested twice, blue and red) or not spiked (green).

**Recovery yield: 97-99%**

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Catalog number: FS106-02
BISPHENOL A IN BEER

**Regulations for Bisphenol A:**
Europe (directive 2011/8/EU) : Specific migration limit in food from packaging of 0.6mg/kg

**PROTOCp OF CLEANUP**
Sample preparation
The beer is degassed by sonication for 1 hour. (pH adjusted to 5-6)

Clean up with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge

**Equilibration**
- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
- 10mL of degassed beer

**Washing of interferences**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 30 seconds**

**Elution (E)**
- 3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with Fluorescence detection same as p 55**

**RESULTS**

Injection 50µL of beer before treatment
Bisphenol A after treatment of 10mL of Beer

Chromatograms of beer containing 1µg/L of Bisphenol A before (Red) and after (Blue) AFFINIMIP® SPE Bisphenols clean-up.

Chromatograms obtained after AFFINIMIP® SPE Bisphenols clean-up of 10mL of beer spiked at 2µg/L (tested 3 times, orange) or at 1µg/L (tested 3 times, blue) with Bisphenol A or not spiked (red)

Recovery of Bisphenol A in spiked beer after AFFINIMIP® SPE Bisphenols clean-up and relative standard deviation calculated from results generated under reproducibility conditions (% RSDR).

<table>
<thead>
<tr>
<th>C° (µg/L)</th>
<th>Mean µg/L</th>
<th>Recoveries %</th>
<th>% RSDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>99.3</td>
<td>8.9</td>
</tr>
<tr>
<td>2.0</td>
<td>1.8</td>
<td>90.6</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Catalog number: FS106-02
BISPHENOL A IN RED/WHITE WINES

REGULATIONS FOR BISPENOL A:
Europe (directive 2011/8/EU) : Specific migration limit in food from packaging of 0.6mg/kg

PROTOCOL OF CLEANUP

Cleanup with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge

Equilibration
3mL Methanol -2% Acetic Acid
3mL Acetonitrile
3mL Water

Loading
Up to 10mL of wine (pH adjusted to 5-6)

Washing of interferences
9mL Water
6mL Water/Acetonitrile (60/40)

Drying 1 minute

Elution (E)
3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

RESULTS

Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of 10mL of white wine spiked with Bisphenol A at 2µg/kg (tested three times, blue) or not spiked (red). The white wine naturally contained 2µg/kg of BPA.

Recovery of Bisphenol A spiked at 2µg/kg after AFFINIMIP® SPE Bisphenols clean-up of 6mL of red wine or 10mL of white wine.

<table>
<thead>
<tr>
<th>Matrice Spiked at 2µg/kg</th>
<th>Mean C° (µg/kg)</th>
<th>Recoveries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red wine 1</td>
<td>1.93 (n=2)</td>
<td>96.6</td>
</tr>
<tr>
<td>Red wine 2</td>
<td>2.13 (n=2)</td>
<td>106.5</td>
</tr>
<tr>
<td>Red wine 3</td>
<td>1.66 (n=2)</td>
<td>83.0</td>
</tr>
<tr>
<td>White wine</td>
<td>1.60 (n=3)</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Catalog number: FS106-02

HPLC Method with Fluorescence detection same as p 54
**PROTOCOL OF CLEAUNP**

Cleanup with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenol cartridge

**Equilibration**
- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
- 6mL of Cola drinks after 30min degassing with ultrasounds (pH adjusted to 5-6)

**Washing of interferences**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 3 minute**

**Elution (E)**
- 3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**RESULTS**

Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of 10mL of white wine spiked with Bisphenol A at 2µg/kg (tested three times, blue) or not spiked (red). The white wine naturally contained 2µg/kg of BPA

Recovery of Bisphenol A spiked at 5µg/kg after AFFINIMIP® SPE Bisphenols clean-up of 6mL of Cola drinks

<table>
<thead>
<tr>
<th>Mean concentration (µg/kg)</th>
<th>Recoveries %</th>
<th>RSDr %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.93 (n=2)</td>
<td>96.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**HPLC Method with Fluorescence detection same as p 54**

Catalog number: FS106-02
BISPHENOL A AND BADGE IN MILK

PROTOCOL OF CLEANUP

Cleanup with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge

Equilibration
3mL Methanol -2% Formic Acid
3mL Acetonitrile
3mL Water

Loading
9mL of Milk (pH adjusted to 5-6)

Washing of interferences
9mL Water
6mL Water/Acetonitrile (60/40)

Drying 3 minute

Elution (E)
3mL Methanol (E1)
3mL Acetonitrile (E2)

The elution fractions E1 and E2 were gathered, evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with Fluorescence detection same as p 54

RESULTS

Fluorescence chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of 9mL of milk spiked with 10µg/kg Bisphenol A and 10µg/kg BADGE (tested twice, blue) or not spiked (red).

Recovery of Bisphenol A and BADGE spiked at 10ng/mL after AFFINIMIP® SPE Bisphenols clean-up of 9mL of milk.

<table>
<thead>
<tr>
<th>Matrice Spiked at 10ng/mL</th>
<th>Mean concentration (µg/kg)</th>
<th>Recoveries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>10.85</td>
<td>108.5</td>
</tr>
<tr>
<td>BADGE</td>
<td>7.5</td>
<td>75</td>
</tr>
</tbody>
</table>

Catalog number: FS106-02
**TOTAL BISPHENOL A IN HUMAN URINE**

**PROTOCOL OF CLEANUP**

Sample preparation
3mL urine sample, 1mL of sodium acetate buffer 0.1M at pH 5.0 and 20μL of β-glucuronidase/sulfatase *Helix pomatia* enzyme solution at 1.0mg/mL in the same buffer were mixed thoroughly by vortex. The enzymatic reaction was carried out for 2h at 37°C to obtain the loading solution.

Cleanup with a 6mL/100mg AFFINIMIP® SPE Bisphenols glass cartridge

**Equilibration**
- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading solution**
- Up to 12mL of loading solution (Equivalent to around 9mL of urine)

**Washing of interferences**
- 4mL Water
- 4mL Water/Acetonitrile (60/40)

**Elution (E)**
- 3mL Methanol

The elution fraction was then concentrated and diluted to 1mL before HPLC analysis.

**RESULTS**

Mean percentage recoveries of Bisphenol A spiked at different concentrations in 3mL of urine after AFFINIMIP® SPE Bisphenols clean-up:

<table>
<thead>
<tr>
<th>C° (ng/mL)</th>
<th>1</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recoveries %</td>
<td>102.6</td>
<td>94.7</td>
<td>97.6</td>
</tr>
</tbody>
</table>

**Publications**

By courtesy of Nadia Diano, Dept. of Experimental Medicine, Second University of Naples (Italy)

More details in the following article


Catalog number: FS106-02G
The analysis of BPA (derivatized with TMS) was performed by **GC-MS/MS**, SRM mode after a clean-up protocol using **AFFINIMIP® SPE Bisphenols** of various solid and liquid complex food matrices (illustration here for salmon and milk).

**RESULTS**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon</td>
<td>0.67µg/Kg BPA</td>
</tr>
<tr>
<td>Milk</td>
<td>0.13µg/Kg BPA</td>
</tr>
</tbody>
</table>

**Publications**

Data extracted from the poster *Utilisation de la spectrométrie de masse pour le dosage du Bisphénol A dans les matières alimentaires, Emmanuelle Bichon et al. (LABERCA), Poster for SMAP 2011, Avignon (France)*

Catalog number: FS106-02
A report of the French Health Agency (ANSES) on **assessment of the health risks associated with bisphenol A** (BPA) was published on 9 April 2013. Quantitative analysis of Bisphenol A in all liquid or solid food matrices were carried out by using AFFINIMIP® SPE Bisphenols (Analyses carried out by LABERCA and described in Annex 12 of Annexes of the report p132 (in french)).


**Example of tested food:**
- Cereals for breakfast, muesli, cornflakes
- Bread, toast, brioche, pastries, sweet and salted biscuits, cookies, pasta...
- Cereals: rice, wheat...
- Cheese: camembert, cantal...
- Milk (skimmed, concentrated ...), Yoghurt, cream, butter
- Oils, eggs
- Fish: cooked fish, fried breaded fish, canned atun, steamed and smoked salmon, hake...
- Seafood: crustacean, oysters, mussel, shrimp...
- Vegetable: salad, tomatoes, radish, onion, soja, carrots, cauliflower, zucchini, peas, spinash....
- Cooked food such as paella, couscous
- Meat: roasted meat, lamb, pork, duck, beef, sheep, turkey, poultry
- Delicatessen: Raw and cooked ham, foie gras, paté, sausage, bacon, chipolatas, merguez...
- Fruits and dried fruits: almonds, peach, orange, compote....
- Drink water, apple juice, soda...
- Coffee, chocolate, cacao...
The analysis of seven bisphenol analogues in beverage and canned food samples was performed by using AFFINIMIP® SPE Bisphenols prior LC–MS analysis. Bisphenol analogs tested: BPS, BPF, BPA, BPB, BPAF, tetrachlorobisphenol A (TCBPA), TBBPA.

Matrices: beverage and canned food (soda, tea drink, juice, red wine, vegetable, fish and meat)

**PROTOCOL OF PURIFICATION**

**Sample preparation for beverage**
10mL beverage is degassed or centrifuged 9000g during 5min.

**Sample preparation for canned food**
1g of canned food is extracted with 5mL acetonitrile with sonication during 20min and centrifugation 9000g for 5min. Fat is removed with 5mL Hexane by LLE. The acetonitrile layer is concentrated to 1mL and diluted with water to 10mL.

**Purification with a 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge**

**Equilibration**
- 5mL Methanol -2% Acetic Acid
- 5mL Acetonitrile
- 5mL Water

**Loading**
- Loading solution

**Washing of interferents**
- 6mL Water
- 3mL Water/Acetonitrile (60/40)

**Drying 30 min**

**Washing of interferents**
- 2mL Acetonitrile
- 2mL Methanol/Acetonitrile (10/90)

**Elution (E)**
- 4mL Methanol containing 2% Formic Acid

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**RESULTS FOR CANNED FISH**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Conc (ng/mL)</th>
<th>Recovery (%)</th>
<th>LOQ (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPS</td>
<td>0.1</td>
<td>73</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>BPF</td>
<td>1</td>
<td>78</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>BPA</td>
<td>0.5</td>
<td>81</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>BPB</td>
<td>1</td>
<td>79</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>BPAF</td>
<td>0.1</td>
<td>81</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>TCBPA</td>
<td>0.5</td>
<td>72</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>TBBPA</td>
<td>1</td>
<td>57</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>61</td>
<td></td>
</tr>
</tbody>
</table>

**Publications**

Data extracted from the article *Molecularly imprinted solid phase extraction for the selective extraction of bisphenol analogues in beverages and canned food*, Y. Yang et al., *J. Agric. Food Chem.*, 2014, 62 (46), pp 11130–11137

Catalog number: FS106-02B
ONIRIS – LABERCA describes an accurate and sensitive method of determination of 18 Bisphenol analogues in human breast milk by GC-MS/MS. By using AFFINIMIP® SPE Bisphenols in the sample preparation protocol, LABERCA analyzes FREE and TOTAL bisphenol analogues with recovery yields higher than 90% for all analogues.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Recovery (%) Spiked at 0.1ng</th>
<th>Recovery (%) Spiked at 1ng</th>
<th>Recovery (%) Spiked at 10ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphenol A</td>
<td>97</td>
<td>94</td>
<td>105</td>
</tr>
<tr>
<td>Bisphenol B</td>
<td>96</td>
<td>99</td>
<td>102</td>
</tr>
<tr>
<td>Bisphenol AP</td>
<td>100</td>
<td>90</td>
<td>92</td>
</tr>
<tr>
<td>Bisphenol AF</td>
<td>100</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>Bisphenol BP</td>
<td>108</td>
<td>109</td>
<td>99</td>
</tr>
<tr>
<td>Bisphenol C</td>
<td>92</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>Bisphenol CI2</td>
<td>102</td>
<td>101</td>
<td>93</td>
</tr>
<tr>
<td>Bisphenol E</td>
<td>96</td>
<td>94</td>
<td>102</td>
</tr>
<tr>
<td>Bisphenol PH</td>
<td>94</td>
<td>93</td>
<td>102</td>
</tr>
<tr>
<td>Bisphenol S</td>
<td>100</td>
<td>99</td>
<td>93</td>
</tr>
<tr>
<td>Bisphenol F</td>
<td>103</td>
<td>109</td>
<td>104</td>
</tr>
<tr>
<td>DHDPE</td>
<td>104</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>Bisphenol FL</td>
<td>103</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Bisphenol Z</td>
<td>100</td>
<td>97</td>
<td>103</td>
</tr>
<tr>
<td>Biphenyl-4,4’-diol</td>
<td>109</td>
<td>103</td>
<td>104</td>
</tr>
<tr>
<td>Bisphenol M</td>
<td>96</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>Bisphenol P</td>
<td>97</td>
<td>92</td>
<td>99</td>
</tr>
<tr>
<td>Bis-2(hydroxyphenyl)methane</td>
<td>108</td>
<td>103</td>
<td>109</td>
</tr>
</tbody>
</table>

**Publications**

Data extracted from the article


Catalog number: FS106-02
The metabolic effects induced by feed contaminated with a lower or a higher concentration of nonylphenol (NP), 4-tert-octylphenol (t-OP) or bisphenol A (BPA), three environmental endocrine disruptors, were assessed in juvenile sea bream liver.

The extraction of NP, t-OP and BPA in water and feed was performed by using AFFINIMIP® SPE Bisphenols prior LC/ESI-QTRAP-MS/MS analysis.

**PROTOCOL OF PURIFICATION**

Sample preparation for feed
1g of homogenized feed and 5mL water/Acetonitrile 50/50 were shaken for 10min then centrifuged at 1267g for 10min. The supernatant was collected and the extraction on feed was repeated. Then 2mL supernatant and 50µL solution NaCl 20% were mixed with 4mL ethyl acetate, vortexed and centrifuged at 1267g for 5 min. The upper layer was evaporated under nitrogen and diluted with 2mL Water/Acetonitrile (50/50) and 1mL water to form the loading solution.

Purification with a 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge

**Equilibration**
- 5mL Methanol -2% Acetic Acid
- 5mL Acetonitrile
- 5mL Water

**Loading**
- Loading solution

**Washing of interferents**
- 10mL Water
- 6mL Water/Acetonitrile (60/40)

**Elution (E)**
- 3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**Publications**

Catalog number: FS106-02B
AFFINIMIP® SPE Bisphenols

DETERMINATION OF BPA, BPB, BPF, BADGE AND BFDGE IN CANNED ENERGY DRINKS

The analyses of 5 bisphenol analogues in canned energy drinks was performed by using AFFINIMIP® SPE Bisphenols prior UPLC - Fluorescence analysis.

Bisphenol analogs tested: BPF, BPA, BPB, BADGE, BFDGE.

**PROTOCOL OF PURIFICATION**

Sample preparation for beverage

20mL of energy drinks is degassed for 60min in an ultrasonic bath. Then 5mL of solution plus 1mL 0.2M aqueous ammonium acetate were vortexed for 30s. Adjust pH at 4 to form the loading solution.

**Purification with a 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge (glass cartridge)**

**Equilibration**
- 3mL Methanol - 2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
Loading solution

**Washing of interferences**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 30s**

**Elution (E)**
- 3mL Methanol
- 3mL Acetonitrile

The elution fractions were gathered, evaporated and dissolved in methanol before UPLC-FLD analysis.

**VALIDATION WITH CANNED ENERGY DRINKS**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Conc (ng/mL)</th>
<th>Recovery (%) (n=6)</th>
<th>RSD % n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>2.0</td>
<td>58</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>52</td>
<td>8.6</td>
</tr>
<tr>
<td>BPB</td>
<td>2.0</td>
<td>93</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>78</td>
<td>7.7</td>
</tr>
<tr>
<td>BPF</td>
<td>2.0</td>
<td>82</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>89</td>
<td>9.0</td>
</tr>
<tr>
<td>BADGE</td>
<td>2.0</td>
<td>88</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>94</td>
<td>8.1</td>
</tr>
<tr>
<td>BFDGE</td>
<td>2.0</td>
<td>87</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>91</td>
<td>7.0</td>
</tr>
</tbody>
</table>

LOQ = 0.50 ng/mL LOD = 0.15 ng/mL

**UPLC Method with Fluorescence detection**

Column: Ascensis Express RP-Amide 75mm x 4.6mm

Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>0.5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>5.5</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>8.5</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>10.5</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Flow rate: 0.5mL/min

Fluorescence detection: excitation/emission wavelengths: 275 / 305nm
Injection volume: 5µL.

**Publications**

Data extracted from the article **Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection**, P. Gallo *et al.*, *Food Chemistry* 220 (2017) 406–412

Catalog number: FS106-02G
**PARABENS IN COSMETIC PRODUCTS**

**PROTOCOL OF CLEANUP**

Sample preparation

1g of lotion was mixed 1 minute with 1mL of H2SO4 2M and 50mL of 90/10 Ethanol/Water. The mixture was heated during 5 min at 60°C. Then the solution is filtered on filter paper (4-7µm). This extract was diluted by 3 with water. The solution was spiked with methylparaben to simulate a concentration of paraben in the lotion at 0.2%, 0.4% and 0.8%.

Cleanup with a 3mL/100mg **AFFINIMIP® SPE Phenolics** cartridge

**Equilibration**
- 3mL Acetonitrile
- 3mL Water

**Loading**
- Up to 5mL of loading solution

**Washing of interferences**
- 3mL Water / Acetonitrile (75/25 v/v)

**Elution (E)**
- 3mL Methanol
  - The elution fraction was diluted by 2 with water prior to analysis.

**HPLC-UV Method**

Column: Thermo Hypersil gold, 150mm x 2.1mm

Mobile phase: 60/40 (v/v) Water/Methanol

Flow rate: 0.2mL/min

Detection: UV - 254nm

Injection volume: 20µL.

**RESULTS**

Chromatograms of a cream containing 0.2% of methylparaben before clean-up (blue) and after clean-up (Red) with **AFFINIMIP® SPE Phenolics**.

Chromatograms obtained after clean-up with **AFFINIMIP® SPE Phenolics** of a cream (without parabens) spiked with different concentrations of methylparaben.

**Recovery yields and reproducibility after **AFFINIMIP® SPE Phenolics** Clean-up.**

<table>
<thead>
<tr>
<th>Recoveries % (n=6)</th>
<th>RSD_R %</th>
</tr>
</thead>
<tbody>
<tr>
<td>101.1</td>
<td>8</td>
</tr>
</tbody>
</table>

Catalog number: FS103-02
Analysis of
ANTIBIOTICS
AND
DRUG RESIDUES
CHLORAMPHENICOL IN HONEY

REGULATIONS FOR CHLORAMPHENICOL IN RESIDUES IN FOOD OF ANIMAL ORIGIN:
Europe 2003/181/EC prohibited with a minimum required performance limits of 0.3µg/Kg

PROTOCOL OF CLEANUP
Sample preparation
10g of honey and 10mL Water were mixed under magnetic stirring during 10 minutes and used as the loading solution.

Cleanup with a 1mL/50mg AFFINIMIP® SPE Chloramphenicol cartridge

Equilibration
2mL Acetonitrile
2mL Water

Loading
1mL of loading solution for 15µg/kg (or 10mL for 0.3µg/Kg)

Washing of interferences (W1)
1mL Water
1mL (Water - 0.5% AA)/ACN (95/5)
2mL of Ammonia (1%) in Water
2mL (Water-1% Ammonia)/ACN (80/20)

Drying 1 min
Washing of interferences (W2)
0.25mL Diethyl ether

Elution (E)
2mL Methanol
The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

UV Chromatograms obtained after clean-up with AFFINIMIP® SPE Chloramphenicol of 1g of Honey spiked with Chloramphenicol at 15.7µg/kg (red) or not spiked (blue).

SIM Chromatograms obtained after clean-up with AFFINIMIP® SPE Chloramphenicol of 1g of Honey spiked with Chloramphenicol at 15.7µg/kg (red) or not spiked (blue).

Recovery of Chloramphenicol spiked at 16µg/kg after AFFINIMIP® SPE Chloramphenicol clean-up of 1g of Honey and relative standard deviation calculated from results generated under reproducibility conditions (% RSDR).

```
<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Mean (µg/kg)</th>
<th>Recoveries %</th>
<th>% RSDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.7</td>
<td>16.9</td>
<td>108.1</td>
<td>6.5</td>
</tr>
<tr>
<td>18.2</td>
<td>16.6</td>
<td>91.4</td>
<td>11.4</td>
</tr>
</tbody>
</table>
```

Catalog number: FS110-02A

HPLC Method with MS detection same as p 73
CHLORAMPHENICOL IN BOVINE URINE

PROTOCOL OF CLEANUP
Sample preparation
10 mL of urine were adjusted at pH 7 with Ammonia 1%. This solution was mixed and used as the loading solution.

Cleanup with a 1mL/50mg AFFINIMIP® SPE Chloramphenicol cartridge

Equilibration
2mL Acetonitrile
2mL Water

Loading
1mL of loading solution

Washing of interferences (W1)
1mL (Water - 0.5% Acetic Acid)/Acetonitrile (95/5)
2mL of Ammonia (1%) in Water
2mL (Water-1% Ammonia)/Acetonitrile (80/20)

Drying 1 min

Washing of interferences (W2)
0.25mL Diethyl ether

Elution (E)
2mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with MS detection
Column: Thermo Accucore C18 column 50mm x 2.1mm
Mobile phase: Ammonium acetate (10mM) in water /Methanol (75/25)
flow rate: 0.2mL/min
MS detection: m/z 321 (ESI-)
Injection volume: 20µL.

RESULTS

Regulations for Chloramphenicol in residues in food of animal origin:
Europe (2003/181/EC) : prohibited with a Minimum Required Performance Limits of 0.3µg/Kg
USA FDA: prohibited

SIM Chromatograms obtained after clean-up with AFFINIMIP® SPE Chloramphenicol of 1 mL of Urine spiked with Chloramphenicol at 17.6µg/kg (red and blue) or not spiked (green).

UV Chromatograms of Urine spiked with Chloramphenicol at 17.6 µg/kg (red and black) or not spiked (green) after clean-up with AFFINIMIP® SPE Chloramphenicol

Recovery of Chloramphenicol spiked at 17.6µg/kg after AFFINIMIP® SPE Chloramphenicol clean-up of 1 mL of Urine.

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Mean (µg/kg)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.6</td>
<td>16.7</td>
<td>90</td>
</tr>
</tbody>
</table>

Catalog number: FS110-02A
CHLORAMPHENICOL IN SHRIMP

PROTOCOL OF CLEANUP
Sample preparation
5g peeled shrimp were homogenized 2min with a vortex in 20mL of ethyl acetate. Then the solution was filtered on filter paper (25µm). The supernatant was evaporated to dryness and reconstituted in 10mL of Water to obtain the loading solution.

Cleanup with a 1mL/50mg AFFINIMIP® SPE Chloramphenicol cartridge

Equilibration
2mL Acetonitrile
2mL Water

Loading
1 or 2mL of loading solution

Washing of interferences (W1)
1mL Water
1mL (Water - 0.5% Acetic Acid)/Acetonitrile (95/5)
2mL of Ammonia (1%) in Water
2mL (Water-1% Ammonia)/Acetonitrile (80/20)

Drying 1 min

Washing of interferences (W2)
0.25mL Diethyl ether

Elution (E)
2mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with MS detection same as p 73

RESULTS

SIM Chromatograms obtained after clean-up with AFFINIMIP® SPE Chloramphenicol of Shrimp spiked with Chloramphenicol at 38µg/kg. Loading of 1mL (spiked in green and not spiked in black) and of 2mL (spiked in red and not spiked in blue)

UV: VERY LOW BACKGROUND

UV Chromatograms of the same solutions

Recovery of Chloramphenicol spiked at 38µg/kg after AFFINIMIP® SPE Chloramphenicol clean-up of Shrimp.

<table>
<thead>
<tr>
<th>C° (µg/kg)</th>
<th>Loading volume</th>
<th>Mean (µg/kg)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>1mL</td>
<td>38.7</td>
<td>101.7</td>
</tr>
<tr>
<td>38</td>
<td>2mL</td>
<td>36.4</td>
<td>95.8</td>
</tr>
</tbody>
</table>

Catalog number: FS110-02A

Regulations for Chloramphenicol in residues in food of animal origin:
Europe (2003/181/EC): prohibited with a Minimum Required Performance Limits of 0.3µg/Kg
USA FDA: prohibited
AMPHETAMINES IN HUMAN URINE

Example of Regulations:
France: prohibited cut-off limit of 1µg/mL in urine and 50ng/mL of blood
Virginia (USA): 100ng/mL of blood

PROTOCOL OF CLEANUP
Sample preparation
Human urine is diluted by 2 with an ammonium acetate buffer (13mM, pH 8.5). The pH of the diluted urine is adjusted with NH₃ or CH₃COOH at pH 8.5.

Cleanup with a 3mL AFFINIMIP® SPE Amphetamines cartridge
Equilibration
1mL Acetonitrile
2mL Water
Loading
5mL of diluted urine
Washing of interferences (W1)
3mL Water
3mL Water/Acetonitrile (60/40)
Drying 30 seconds
Elution (E)
1.5mL Methanol – 2% Formic acid
The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

Capacity: different concentrations of Amphetamine in urine were applied on AFFINIMIP® SPE Amphetamines cartridge (25mg) to measure the capacity of the product.

<table>
<thead>
<tr>
<th>Quantity loaded µg</th>
<th>Quantity obtained in the elution fraction µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.90</td>
</tr>
<tr>
<td>2.5</td>
<td>2.41</td>
</tr>
<tr>
<td>5.0</td>
<td>3.51</td>
</tr>
</tbody>
</table>

HPLC Method with MS detection same as p 76

RESULTS

Mass Chromatogram (SIM) obtained after AFFINIMIP® SPE Amphetamines clean-up of a human urine sample spiked at 20ng/mL with Amphetamine and its derivatives.

Recovery of Amphetamines in human urine spiked at 20ng/mL after AFFINIMIP® SPE Amphetamines clean-up and relative standard deviation calculated from results generated under reproducibility conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ng/mL</th>
<th>R %</th>
<th>% RSD_R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>17.5</td>
<td>87.5</td>
<td>8.9 (n=8)</td>
</tr>
<tr>
<td>MDA</td>
<td>18.6</td>
<td>93.1</td>
<td>9.6 (n=8)</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>18.6</td>
<td>93.2</td>
<td>9.2 (n=8)</td>
</tr>
<tr>
<td>MDMA</td>
<td>21.1</td>
<td>105.4</td>
<td>1.5 (n=4)</td>
</tr>
<tr>
<td>MDEA</td>
<td>20.3</td>
<td>101.7</td>
<td>12.4 (n=8)</td>
</tr>
</tbody>
</table>

Catalog number: DG102-02
PROTOCOL OF CLEANUP

Sample preparation
Human serum is diluted by 5 with an ammonium acetate buffer (13mM, pH 8.5). The pH of the diluted urine is adjusted with NH₃ or CH₃COOH at pH 8.5.

Cleanup with a 3mL AFFINIMIP® SPE Amphetamines cartridge

Equilibration
1mL Acetonitrile
2mL Water

Loading
2.5mL of diluted serum

Washing of interferences (W1)
3mL Water
3mL Water/Acetonitrile (60/40)

Drying 30 seconds

Elution (E)
1.5mL Methanol – 2% Formic acid

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with MS detection
Column: Syncronis Aq column 150mm x 2.1mm
Mobile phase: gradient profile with A (Water – Ammonium Acetate 10mM) and B (Acetonitrile – Ammonium Acetate 1mM)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>12.1</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

flow rate: 0.4mL/min
MS detection (ESI⁺) : m/z 136 (Amphetamine); 180 (MDA); 150 (Methamphetamine); 194 (MDMA); 208 (MDEA)
Injection volume: 20µL.

RESULTS

Mass Chromatogram (SIM) obtained after AFFINIMIP® SPE Amphetamines clean-up of a human serum sample spiked at 100ng/mL with Amphetamine and its derivatives.

Recovery of Amphetamines in human serum spiked at 100ng/mL after AFFINIMIP® SPE Amphetamines clean-up and relative standard deviation calculated from results generated under reproducibility conditions (n=4).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ng/mL</th>
<th>Recovery %</th>
<th>% RSDᵣ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>87.9</td>
<td>87.9</td>
<td>5.0</td>
</tr>
<tr>
<td>MDA</td>
<td>94.4</td>
<td>94.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>90.7</td>
<td>90.7</td>
<td>2.2</td>
</tr>
<tr>
<td>MDMA</td>
<td>106.2</td>
<td>106.2</td>
<td>2.5</td>
</tr>
<tr>
<td>MDEA</td>
<td>111.0</td>
<td>111.0</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Catalog number: DG102-02
PROTOCOL OF CLEANUP

Sample preparation for Milk
Mix 1.5mL of Milk with 6mL of EDTA/Mc Ilvaine’s Buffer and centrifuge at 4000rpm for 10 min at a temperature below 15°C. Collect the supernatant and adjust to pH 6-7 with a NaOH solution (this mixture was the loading solution).

Sample Preparation for Salmon based on AOAC 995.09 method
Blend 10g Salmon with 40mL of EDTA/Mc Ilvaine’s Buffer during 30 s and stir during 10min with a magnetic stirrer. Centrifuge the mixture at 2500g for 10 min at a temperature < 15°C. Collect the supernatant. Repeat this operation with 40mL buffer and again with 20mL of buffer. Then, gather all the supernatants and centrifuge during 20min at 2500g, filter on Buchner. Add 1N NaOH solution to the filtrate and adjust to pH 6-7 (this mixture was the loading solution).

Cleanup with a 1mL/10mg AFFINIMIP® SPE Tetracyclines cartridge
Equilibration
- 1mL Acetonitrile
- 1mL Water

Loading
Loading solution (7.5mL)

Washing of interferences
- 1mL Water
- 2mL Water/Acetonitrile (60/40)

Drying 3 minutes

Elution (E)
- 2mL Methanol with 2% Formic acid
The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with UV detection same as p 78

UV Chromatograms (355nm) obtained after clean-up with AFFINIMIP® SPE Tetracyclines of 1.5mL of Milk spiked with Tetracycline, Chlortetracycline and 4-epoxytetracycline (4-epiOTC) at 50µg/L (blue) or not spiked (red) or of 1.5mL of water spiked with Tetracycline, Chlortetracycline and 4-epoxytetracycline at 50µg/L (pink)

Recovery of Tetracyclines after AFFINIMIP® SPE Tetracyclines clean-up of Salmon or milk spiked at 50 or 100µg/L and relative standard deviation calculated from results generated under repeatability conditions (n=3).

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Mean (µg/L)</th>
<th>Milk R (%)</th>
<th>% RSD</th>
<th>Salmon R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>49.6</td>
<td>99.4</td>
<td>4.9</td>
<td>113</td>
</tr>
<tr>
<td>OTC</td>
<td>45.6</td>
<td>91.3</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>CTC</td>
<td>37.2</td>
<td>74.4</td>
<td>6.3</td>
<td>74</td>
</tr>
<tr>
<td>4-epiTC</td>
<td>47.9</td>
<td>95.9</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>4-epiCTC</td>
<td>108.4</td>
<td>108.4</td>
<td>15.0</td>
<td>97</td>
</tr>
<tr>
<td>4-epiOTC</td>
<td>43.7</td>
<td>87.4</td>
<td>9.1</td>
<td>71</td>
</tr>
<tr>
<td>DOX</td>
<td>43.8</td>
<td>88.0</td>
<td>2.9</td>
<td>89</td>
</tr>
</tbody>
</table>

Recovery of Tetracyclines after AFFINIMIP® SPE Tetracyclines clean-up of Salmon or milk spiked at 50 or 100µg/L and relative standard deviation calculated from results generated under repeatability conditions (n=3).

Catalog number: FS112-02A
**PROTOCOL OF CLEANUP**

**Preparation of loading solution for Meat based on AOAC 995.09 method**

10g meat were blend during 30 seconds with 40mL of EDTA/Mc Ilvaine’s Buffer and stirred during 10min with a magnetic stirrer. The mixture was centrifuged at 2500g for 10 minutes at a temperature below 15°C. The supernatant was collected. This operation was repeated with 40mL of buffer and again with 20mL of buffer. Then, all the supernatants were gathered and centrifuged during 20min at 2500g, filtered on Buchner. Adjust the filtrate to pH 6 -7 with a NaOH solution (this mixture was the loading solution).

**Cleanup with a 1mL/10mg AFFINIMIP® SPE Tetracyclines cartridge**

**Equilibration**
- 1mL Acetonitrile
- 1mL Water

**Loading**
- 5mL Loading solution

**Washing of interferences**
- 1mL Water
- 2mL Water/Acetonitrile (60/40)

**Drying 1 minute (only if elution is evaporated)**

**Elution (E)**
- 2mL Methanol with 2% Formic acid

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with UV detection**

Column: Hypersil Gold C18 column 150mm x 2.1mm, 3µm

Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% 10mM Oxalic Acid</th>
<th>% 10mM Oxalic Acid ACN</th>
<th>% MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>90</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>21</td>
<td>80</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>40</td>
<td>80</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>41</td>
<td>90</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Flow rate: 0.2mL/min
UV detection: 355nm
Injection volume: 100µL.

**RESULTS**

UV Chromatograms (355nm) obtained after clean-up with AFFINIMIP® SPE Tetracyclines of meat spiked with Tetracycline, Chlortetracycline, 4-epichlortetracycline (4-epiCTC) and 4-epoxytetracycline (4-epiOTC) at 50µg/L (red), not spiked (pink) or of water spiked (green)

Recovery of Tetracyclines after AFFINIMIP® SPE Tetracyclines clean-up of Meat spiked at 200µg/kg (4-epiCTC at 400µg/kg)

<table>
<thead>
<tr>
<th>Molecules</th>
<th>R% (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>98</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>70</td>
</tr>
<tr>
<td>4-epichlortetracycline</td>
<td>74</td>
</tr>
<tr>
<td>4-epoxytetracycline</td>
<td>91</td>
</tr>
</tbody>
</table>

Catalog number: FS112-02A
**TETRACYCLINES IN PORK KIDNEY TISSUS**

**PROTOCOL OF CLEANUP**

Preparation of loading solution for pork kidney tissue based on AOAC 995.09 method

10g meat were blend during 30 seconds with 40mL of EDTA/Mc Ilvaine’s Buffer and stirred during 10min with a magnetic stirrer. The mixture was centrifuged at 2500g for 10 minutes at a temperature below 15°C. The supernatant was collected. This operation was repeated with 40mL of buffer and again with 20mL of buffer. Then, all the supernatants were gathered and centrifuged during 20min at 2500g, filtered on Buchner. Around 10mL 1N NaOH solution were added to the filtrate and adjusted to pH 6.5 with a NaOH solution (this mixture was the loading solution).

**Cleanup with a 1mL/10mg AFFINIMIP® SPE Tetracyclines cartridge**

**Equilibration**
1mL Acetonitrile
1mL Water

**Loading**
4 to 5mL Loading solution

**Washing of interferences**
Wash the cartridge with 1mL of NaHCO3 1% in water
Immediately wash the cartridge with 2mL of deionized Water/Acetonitrile (60/40, v/v)

**Drying 1 minute (only if elution is evaporated)**

**Elution (E)**
2mL Methanol with 2% Formic acid

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**RESULTS**

UV Chromatograms (355nm) obtained after clean-up with AFFINIMIP® SPE Tetracyclines pork kidney tissue (red) or water (black) spiked with 910µg/kg Tetracycline, 980µg/kg Oxytetracycline and 860µg/kg Chlortetracycline as well as pork kidney not spiked (blue)

Recovery and repetability of Tetracyclines after AFFINIMIP® SPE Tetracyclines clean-up of Pork kidney tissue at 910µg/kg for TC; 980µg/kg for OTC and 860µg/kg for CTC

<table>
<thead>
<tr>
<th>Molecules</th>
<th>R% (n=5)</th>
<th>RSDr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline (TC)</td>
<td>85</td>
<td>5,4</td>
</tr>
<tr>
<td>Chlortetracycline (OTC)</td>
<td>79</td>
<td>2,8</td>
</tr>
<tr>
<td>Oxytetracycline (CTC)</td>
<td>80</td>
<td>4,2</td>
</tr>
</tbody>
</table>

Catalog number: FS112-02A
**.Protocol of Purification for Meat and Milk**

Sample preparation for meat

2g meat are mixed during 10min with 10mL extraction buffer (10 mM KH2PO4, 0.4 mm EDTA, 2% trichloroacetic acid). Then centrifuge during 10 min at 4 000 rpm, and collect the supernatant.

Repeat two times. Adjust the pH 7.5 ~ 8.0 with 5 M NaOH (0.3 ~ 0.4 mL) to obtain the loading solution.

Sample preparation for Milk

Mix 5mL milk in 300µL 50% trichloroacetic acid during 10min. Then centrifuge during 15min at 5000rpm and collect the supernatant. Add 300µL 50% trichloroacetic acid to the supernatant and centrifuge again 15sec.

Adjust the pH 7.5 ~ 8.0 with 1M NaOH to obtain the loading solution.

**Purification with a 6mL/200mg AttractSPE™ HLB cartridge**

**Equilibration**

5mL Methanol

5mL Water

**Loading**

Loading solution

**Washing**

5mL water

**Drying 5 min**

**Elution (E)**

2x 3mL 100mM Heptafluorobutyric acid (HFBA) in Acetonitrile–Methanol (2+1, v/v)

Evaporate under nitrogen at 50°C

Reconstitute with 1mL 20mM HFBA solution before analysis.

Detection LC-MS/MS

---

<table>
<thead>
<tr>
<th>Antibacterial Aminoglycosides on Milk or Meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>streptomycin (STR) Dihydrostreptomycin (DHS)</td>
</tr>
<tr>
<td>hygromycin B (HB)</td>
</tr>
<tr>
<td>kanamycin (KM)</td>
</tr>
<tr>
<td>apramycin (APM)</td>
</tr>
<tr>
<td>destomycin A (DA)</td>
</tr>
<tr>
<td>amikacin (AK)</td>
</tr>
<tr>
<td>Paromomycin (PM)</td>
</tr>
<tr>
<td>Tobramycin</td>
</tr>
</tbody>
</table>

---

**Catalog number: HLB-50.S.6.200**
MULTI-RESIDUE DETERMINATION OF SEVERAL FAMILIES OF ANTIBIOTICS IN KIDNEY

**PROTOCOL OF PURIFICATION**
Sample preparation: Vortex 1 g of kidney with 10mL of McIlvaine/EDTA buffer during 1min. Shake for 15min and ultrasonic for 5 min. Centrifuge at 3800g for 10min at 5°C. Filter and collect supernatant.
Repeat extraction with 3mL buffer solution.
Combine the supernatants to obtain the loading solution.

**Purification with a 6mL/200mg AttractSPE™ HLB cartridge**

- **Equilibration**
  - 5mL Methanol
  - 5mL Water

- **Loading**
  - Loading solution

- **Washing**
  - 6mL water/methanol (95/5, v/v)

- **Drying 10 min**

- **Elution (E)**
  - 6mL Methanol

Evaporate under nitroger at 40°C and reconstituted with 1mL water/acetonitrile (90/10, v/v). Vortex, centrifuge and filter.

Analysis: LC-MS/MS

73 substances measured from drug families:
Quinolones
Macrolides
Lincosamides
Sulfonamides
Penicillins
Cephalosporine
Pleuromutilins
Diamino pyrimidine derivatives

Catalog number: HLB-50.S.6.200

**MULTI-RESIDUE DETERMINATION OF NSAID IN MUSCLE TISSUE**

**PROTOCOL OF PURIFICATION**
Sample preparation: Shake 2g of homogenized meat with 10mL ACN for 2min. Centrifuge during 5min at 5500rpm and evaporate the supernatant at 60°C under nitrogen. Reconstitute with 100µL Methanol–900µL Water to obtain the loading solution.

**Purification with a 3mL/60mg AttractSPE™ HLB cartridge**

- **Equilibration**
  - 1mL Methanol
  - 1mL Water

- **Loading**
  - All the loading solution

- **Washing of interferences**
  - 1mL Methanol/Water (5/95)

- **Drying under vaccuum**

- **Elution (E)**
  - 1mL Methanol
  - 1mL Hexane with 10% Acetic acid

The elution fraction was then evaporated at 60°C under nitrogen and reconstituted before HPLC analysis.

Analyse: LC/MS/MS

Regulations - MRL for NSAID in bovive muscle:
Carprofen 500µg/kg
Flunixin 20µg/kg
Tolfenamic acid 50µg/kg
Meloxicam 20µg/kg

Salicylic acid
Phenylbutazone
Flunixin
Tolfenamic acid
Meloxicam
Desoximethasome (IS)
Ketoprofen

Catalog number: HLB-50.S.3.60
PENICILLIN BASED ANTIBACTERIALS ON MUSCLES

PROTOCOL OF PURIFICATION FOR MUSCLES
Sample preparation for muscle:
2g muscle samples are mixed in a 50mL centrifuge tube with
10mL 0.1 M sodium phosphate buffer (pH 4.5) and then
homogenized. Add 2.5 mL 0.17 M sulfuric acid, 2.5 mL 5%
sodium tungstate and mix it well. Centrifuge at 5,000rpm
during 15 min.
The supernatant was adjusted at pH 8.1 - 8.5 with 5M NaOH,
centrifuged at 5,000rpm during 15 min and the supernatant is collected and mix with 10mL
NaCl (20%) to obtain the loading solution.

Purification with a 6mL/200mg AttractSPE™ HLB cartridge

**Equilibration**
- 5mL Methanol
- 5mL Water
- 5mL 2% NaCl

**Loading**
Loading solution

**Washing**
- 5mL 2% NaCl
- 5mL 25mM PBS (pH 9,0)

**Drying 5 min**

**Elution (E)**
2x 3mL Acetonitrile

Evaporate under nitrogen at 40°C and reconstituted before analysis.
Detection LC-MS/MS

ERYTHROMYCIN AND CLINDAMYCIN

PROTOCOL OF PURIFICATION
Sample preparation
Mix 2g of crushed samples with 6mL of 2% acetic acid
solution in centrifuge tube and then centrifuge at 1200g for
10 min to form the loading solution.

Purification with a 6mL/200mg AttractSPE™ HLB cartridge

**Equilibration**
- 5mL Methanol
- 5mL Water

**Loading**
Loading solution

**Washing**
- 5mL distilled water

**Elution (E)**
6mL Methanol
Detection LC-MS

Ampicillin,
Amoxicillin
Penicillin G or
benzypenicillin
Penicillin V
Oxacillin
Nafcillin,
Cloxacillin
Dicloxacillin

Catalog number: HLB-50.S.6.200

Erythromycin

Catalog number: HLB-50.S.6.200

Regulation
EC 37/2010
Erythromycin
200kg/kg muscle
### PROTOCOL OF PURIFICATION

Sample preparation

**Step 1:** Mix 5 g homogenized sample with 100µL of internal standard (Quinoxaline-2-carboxylic acid-D4) and 10mL solution of 10% Methanol in water containing 5% metaphosphoric acid in a 50 mL centrifuge tube and shake, then centrifuge at 4500rpm for 10 min at 30°C.

**Step 2:** Collect the supernatant in a 50mL centrifuge tube. Repeat the step 1 with 10mL solution of 10% Methanol in water containing 5% metaphosphoric acid and centrifuge at 5000rpm for 20 min at 30°C.

**Sept 3:** Combine the supernatants (~20mL)

**Step 4:** Mix vigorously with 10mL Ethyl Acetate for 15min then centrifuge at 5000rpm for 10 min at 30°C and collect the supernatant.

**Step 5:** repeat step 4, combine the supernatants, concentrate them at 60°C under nitrogen. The residue is dissolved in 5mL HCl 0.1M.

### Purification with a 3mL/60mg AttractSPE™ SAX cartridge

**Equilibration**
- 3mL Methanol
- 3mL Water

**Loading**
- Loading solution

**Washing**
- 3mL water

**Drying for 5min**

**Elution (E)**
- 3mL Methanol - 0.1M HCl (90-10 v/v)

This eluate was dried at 60 °C under nitrogen

Analysis: LC-MS/MS

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**Catalog number:** SAX-50.5.3.60
MULTI-CLASS METHOD OF ANTIBIOTICS IN MILK

PROTOCOL OF PURIFICATION
Sample preparation: Mix 1mL Milk and 1mL Acetonitrile in a PP centrifuge tube. Vortex for 10-15s and centrifuge at 4000rpm (4°C) for 10min. Collect the supernatant (avoiding any visible fat layer) and add to a glass test tube containing 9mL 0.1% Formic acid. Vortex the tube during 10 s to obtain the loading solution.

Purification with a 3mL/60mg AttractSPE™ HLB cartridge
Equilibration
3mL Acetonitrile
3mL water with 0.1% formic acid/Acetonitrile (95/5, v/v)
Loading
Loading solution
Washing
2x2mL 0.1% formic acid
Drying 30s
Elution (E)
2.5mL Acetonitrile/Methanol 70/30
Analysis: LC-MS/MS

VANCOMYCIN IN FISH

PROTOCOL OF PURIFICATION
Sample preparation:
Step 1: Mix 5 g homogenized sample with a 15mL solution of 20% ACN in water in a 50 mL centrifuge tube and shake for 20 min, then centrifuge at 7 600rpm for 10 min.
Step 2: Collect the supernatant in a 50mL centrifuge tube. Repeat the step 1 with 10mL solution of 20% ACN in water. Combine the supernatants (~25mL) and mix vigorously with 10mL Hexane for 10min then centrifuge at 7 600rpm for 10 min and remove Hexane to obtain the loading solution.

Purification with a 3mL/60mg AttractSPE™ SCX cartridge
Equilibration
3mL Methanol
3mL Water- 0.1% Formic acid
Loading
3mL Loading solution
Washing
3mL water
Elution (E)
3mL Methanol with 3% Ammonium hydroxide
This eluate was dried at 50 °C under nitrogen and reconstituted in 1mL water and filtered at 0.2µm.
Analysis: LC-MS/MS

25 substances measured from Fluoroquinolones Beta lactam Sulfonamide Macrolides

Catalog number: HLB-50.S.3.60

Same method as FDA Lab information bulletin LIB# 4443, Susan B. Clark, Joseph M. Storey, Sherri B. Turnipseed

Catalog number: SCX-50.S.3.60
GLUCOCORTICOIDS

PROTOCOL OF PURIFICATION
Sample preparation
Mix 2 g of the sample and 10 mL of acetate buffer solution (3 M, pH 4.6) in a 50 mL centrifuge tubes and homogenize for about 2 minutes. Make an enzymatic hydrolysis by adding 50 µL Helix pomatia β-Glucuronidase/Arylsulfatase for 1 h in an oven at 60 °C. After cooling at RT, add 8 mL CAN and centrifuge at 4500 rpm for 10 min. Repeat the above steps. Collect the supernatants and concentrate under nitrogen at 50 °C. Dissolve the residue in 1 mL Ethanol and add 5 mL of distilled water to obtain the loading solution.

**Purification with a 6 mL/500 mg AttractSPE™ HLB cartridge**

**Equilibration**
- 5 mL Methanol
- 5 mL Water

**Loading**
- Loading solution

**Washing**
- 5 mL acetone/distilled water (2/8, v/v)
- 5 mL n-hexane

**Drying 2 min**

**Elution (E)**
- 6 mL Ethyl Acetate

Evaporate under nitrogen at 50°C and reconstituted before analysis with 1 mL mobile phase. Centrifuge at -4 °C, 15000 rpm during 15 min. The clear supernatant is filtered 0.2 µm nylon filter and analyzed by LC-MS/MS after the filtration

**Detection** LC-MS/MS

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SULFONAMIDES IN MILK

PROTOCOL OF PURIFICATION

**Purification with a 3 mL/60 mg AttractSPE™ SCX cartridge**

**Equilibration**
- 2 mL Methanol
- 2 mL Water

**Loading**
- 5 mL Milk

**Washing of interferences**
- 2 mL Methanol/Water (5/95)
- 1 mL 0.5 M HCl
- 2 mL Methanol/Water (20/80)

**Elution (E)**
- 2.5 mL Ammonium bicarbonate/Methanol (10/90)

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**Analyse** LC/MS/MS

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Cortisone, Corticosterone, Aldosterone, Betamethasone, Dexamethasone, Flumethasone, Prednisone, Prednisolone, Methylprednisolone

Catalog number: HLB-50.S.6.500

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Regulations for Sulfonamides:

- Sulfadimethoxine 0.01 ppm in milk (U.S. FDA 21 CFR 556.640)
- Sulfathiazole 0.01 ppm in milk (U.S. FDA 21 CFR 556.650)
- EC 37/2010 100 µg/kg Milk

Catalog number: SCX-25.S.3.60
MULTI-CLASS METHOD OF ANTIBIOTICS IN DISTILLER GRAINS

**PROTOCOL OF PURIFICATION**
Sample preparation
Mix 5mL Distillers grains with 20mL 1,5mM EDTA and 20mL 1% Trichloroacetic acid in water in a 50mL PP centrifuge tube. Shake for 15min and centrifuge at 4000rpm for 10min. Collect the supernatant and dilute it with 150mL water. Repeat extraction with 30mL methanol and centrifuge and combine supernatants. Dilute the supernatants to 200mL with water to obtain the loading solution.

**Purification with a 6mL/150mg AttractSPE™ HLB cartridge**
- **Equilibration**
  - 3mL Methanol
  - 3mL water with Trichloroacetic acid (pH~4)
- **Loading**
  - 10mL Loading solution
- **Drying under vacuum for 5min**
- **Washing**
  - 5mL water
- **Drying under vacuum for 5min**
- **Elution (E)**
  - 2.5mL Methanol
  - Evaporate eluate to about 1mL under nitrogen at 35°C.
- **Analysis**: LC-MS/MS

Analyses of 13 antibiotics
Ampicillin, bacitracin A, erythromycin, tylosin, clarithromycin, penicillin G, virginiamycin M1 and monensin

Catalog number: HLB-50.S.6.150

Same method as FDA Lab information bulletin LIB# 4438
David N. Heller G.K. Hemakanthi de Alwis

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**PRAZIQUANTEL AND TIAMULIN**

**PROTOCOL OF PURIFICATION**
Sample preparation
Mix 2g of pulverized samples with 6mL of 2% ammonium hydroxide solution in centrifuge tube and then centrifuge at 1200g for 10 min to form the loading solution.

**Purification with a 6mL/200mg AttractSPE™ HLB cartridge**
- **Equilibration**
  - 5mL Methanol
  - 5mL Water
- **Loading**
  - Loading solution
- **Washing**
  - 5mL distilled water
- **Elution (E)**
  - 6mL Methanol
- **Analysis**: LC-MS

Catalog number: HLB-50.S.6.200
PROTOCOL OF PURIFICATION
Sample preparation
Mix 2g of homogenized samples with 5mL of 10% trichloroacetic acid solution in a 50mL centrifuge tube and then centrifuge at 3000g for 10 min. Collect the supernatant and make a 1:1 mix with a 4% phosphoric acid to form the loading solution.

Purification with a 3mL/60mg AttractSPE™ SCX cartridge

Equilibration
- 2mL Methanol
- 2mL Water

Loading
- Loading solution

Washing
- 2mL 2% Formic acid
- 2mL Methanol

Elution (E)
- 4mL Methanol with 5% Ammonium hydroxide

This eluate was concentrated at 50 °C under nitrogen and reconstituted with 200µL 0.1% formic acid containing 10% acetonitrile. The solution was centrifuged at 12 000 G for 10 minutes and the supernatant was filtered with a membrane filter.

Analysis: LC-UV (260nm)

VALNEMULIN AND TIAMULIN IN FISH

PROTOCOL OF PURIFICATION
Sample preparation
Step 1: Mix 1 g homogenized sample with a 10mL solution of 40-60 (v:v) ACN-0,01M HCl in a 50 mL centrifuge tube and shake at 300rpm for 15 min, then centrifuge at 10 000rpm for 10 min.
Step 2: Collect the supernatant in a 50mL centrifuge tube. Repeat the step 1 with the lower layer of the first centrifuge tube.
Combine the supernatants (~20mL) and mix vigorously with 20mL Hexane and remove Hexane to obtain the loading solution.

Purification with a 3mL/60mg AttractSPE™ SCX cartridge

Equilibration
- 3mL Methanol
- 3mL Water

Loading
- 3mL Loading solution

Washing
- 3mL 40-60 (v:v) ACN-0,01M HCl

Drying 1min

Elution (E)
- 3mL Methanol with 5% Ammonium hydroxide

This eluate was dried at 40 °C under nitrogen and reconstituted in the mobile phase.

B. Assay conditions
Analysis: LC-MS/MS

Catalog number: SCX-50.S.3.60
PHARMACEUTICALS AND PERSONAL CARE PRODUCTS (GROUP 1 COMPOUNDS) OF EPA METHOD 1694

**PROTOCOL OF PURIFICATION**
Sample preparation
Filtrate 1L solution and adjust the pH to 2 while stirring the water. Add 500mg Na₂ EDTA and mix. Equilibrate during 1-2h to obtain the loading solution.

**Purification with a 6mL/200mg AttractSPE™ HLB cartridge**

**Equilibration**
- 20mL Methanol
- 6mL Water
- 6mL Water pH 2

**Loading**
1L of loading solution, pH 2

**Washing**
10mL water

**Drying 5 min**

**Elution (E)**
6mL Methanol or 6mL Methanol – Acetonitrile (50/50)

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**EPA methods 1694**
- Acetaminophen
- Ormetoprim
- Caffeine
- Sarafloxacin
- Carbadox
- Sulfachloropyridine
- Cefotaxime
- azine
- Ciprofloxacin
- Sulfadiazine
- Clinafloxacin
- Sulfamethazine
- Codeine
- Sulfamethoxazole
- Cotinine
- Sulfamethizole
- 1,7-Dimethylxanthine
- Sulfanilamide
- Enrofloxacin
- Sulfathiazole
- Lincomycin
- Thiabendazole
- Lomefloxacin
- Trimethoprim
- Norfloxacin
- AttractSPE™ HLB

**Catalog number:** HLB-50.S.6.200

**MISCELLANEOUS DRUGS IN WASTEWATER**

**PROTOCOL OF PURIFICATION**
Sample preparation
Filtrate 500mL to 1L of wastewater with 0.45µm glass fiber to form the loading solution.

**Purification with a 3mL/60mg AttractSPE™ HLB cartridge**

**Equilibration**
- 3mL Ethyl Acetate
- 3mL Methanol
- 3mL Water

**Loading**
Loading solution (15mL/min)

**Washing**
3mL Methanol/ water (5/95, v/v)
3mL n-hexane

**Elution (E)**
3x1mL Ethyl Acetate
Evaporate under nitrogen and reconstituted with 0.5mL Methanol.
Analysis: LC-DAD-Fluorescence

**Catalog number:** HLB-50.S.3.60

- Caffeine
- Acetaminophen
- Diclofenac
- Ibuprofen
- Ketoprofen
- Naproxen
- Carbamazepine

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Analysis of
PESTICIDES
Efficient clean-up and enrichment

**PROTOCOL OF PURIFICATION**
Sample preparation for compost
5g of compost sample and 100mL water are shaked during 60 minutes. Centrifuge at 3000g for 10 min and then filter the solution with a 4-7µm filter. This solution is used as the loading solution.

**Purification with a 3mL/60mg **AFFINIMIP® SPE Picolinic Herbicides** cartridge**

Equilibration
- 2mL Acetonitrile
- 1mL Water

Loading
- 3mL of loading solution

Washing of interferences (W1)
- 1mL Water

Drying by applying vacuum 1 min

Washing of interferences (W2)
- 1mL Acetonitrile

Elution (E)
- 3mL 98/2 Ethyl acetate / Trifluoroacetic acid

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**RESULTS**
UV chromatogram of compost or water spiked with Aminopyralid, Picloram and Clopyralid after AFFINIMIP® SPE Picolinic Herbicides clean-up

Recovery and repeatability of Picloram, Aminopyralid and Clopyralid in compost (n=3) and after AFFINIMIP® SPE Picolinic Herbicides Clean-up.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Recoveries % for Water</th>
<th>Recoveries % for Compost</th>
<th>% RSDr compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminopyralid</td>
<td>95</td>
<td>84</td>
<td>3</td>
</tr>
<tr>
<td>Clopyralid</td>
<td>109</td>
<td>120</td>
<td>4</td>
</tr>
<tr>
<td>Picloram</td>
<td>88</td>
<td>89</td>
<td>3</td>
</tr>
</tbody>
</table>

Catalog number: FS115-02
Efficient clean-up and enrichment

Also tested with up to 20% Methanol for percolation and washing

**PROTOCOL OF PURIFICATION**

Purification with a 3mL AFFINIMIP® SPE Glyphosate – AMPA cartridge

**Equilibration**
- 6mL Water

**Loading**
- 3 to 500mL Water

**Washing of interferences (W1)**
- 3mL Water

**Elution (E)**
- 4mL HCl solution (100mM)

**RESULTS**

Recovery of glyphosate and AMPA after AFFINIMIP® SPE Glyphosate – AMPA clean-up of mineral water spiked at 25µg/mL. Loading volume 3mL

Analysis done by CE without derivatization

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Recoveries % for Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>85</td>
</tr>
<tr>
<td>AMPA</td>
<td>87</td>
</tr>
</tbody>
</table>

**CE analysis (no derivatization)**

Column: fused-silica capillary of 60.2 cm (effective length, 50 cm) x 50 µm ID at 25°C

Mobile phase: 7.5 mM phthalic acid - 51.3 mM histidine running buffer (pH 6.5, ionic strength of 21.8 mM, buffer capacity 25 mM L⁻¹ pH⁻¹) containing 1 mM CTAB

Voltage: +25kV

Detection UV-DAD (240nm)

**Poster:**

Extraction SPE basée sur un polymère à empreintes moléculaires pour l’extraction du glyphosate et de son métabolite (AMPA) dans des eaux souterraines, by BRGM and ICOA (ANR project Origami), AFSEP 2014 Paris.

New Selective SPE Clean-Up Method Based on Molecularly Imprinted Polymers for Glyphosate and AMPA Analysis with and without Derivatization for Water, Food and Feed, Pittcon 2015 New Orleans.

Catalog number: FS113-02
MIP performance not affected by physico chemical properties of Water

Physico chemical properties of tested waters
Salt concentrations (mg/L) and pH of analyzed solutions

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Na</th>
<th>Mg</th>
<th>K</th>
<th>HCO3</th>
<th>Cl</th>
<th>NO3</th>
<th>SO4</th>
<th>Fe</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundwater</td>
<td>15,7</td>
<td>11,3</td>
<td>4,9</td>
<td>1,3</td>
<td>76</td>
<td>9,7</td>
<td>&lt;0,5</td>
<td>1,2</td>
<td>7,5</td>
<td>7,1</td>
</tr>
<tr>
<td>Groundwater</td>
<td>22,3</td>
<td>105,7</td>
<td>17</td>
<td>4,7</td>
<td>136</td>
<td>159</td>
<td>8,9</td>
<td>15,8</td>
<td>0,17</td>
<td>6,4</td>
</tr>
<tr>
<td>Groundwater</td>
<td>104,1</td>
<td>13,9</td>
<td>6,9</td>
<td>1,8</td>
<td>203</td>
<td>28,1</td>
<td>113,7</td>
<td>33</td>
<td>7,1</td>
<td></td>
</tr>
<tr>
<td>Geothermal water</td>
<td>799</td>
<td>5163,5</td>
<td>189,5</td>
<td>71,9</td>
<td>9759,7</td>
<td>702,2</td>
<td>3,2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral water</td>
<td>80</td>
<td>6,5</td>
<td>24</td>
<td>1</td>
<td>360</td>
<td>3,8</td>
<td>3,7</td>
<td>12,6</td>
<td>7,2</td>
<td></td>
</tr>
</tbody>
</table>

MIP performance for tested waters
Above five waters spiked at various concentrations with AMPA and Glyphosate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration range</th>
<th>Average Recoveries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>100 to 750ng/L</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>AMPA</td>
<td>100 to 750ng/L</td>
<td>&gt;75%</td>
</tr>
</tbody>
</table>

Method UPLC – MS/MS
Column: Acquity UPLC HSS T3 (2.1mm x 100mm, 1,8µm)  
Mobile phase:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>2</th>
<th>7</th>
<th>7.5</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Water/ Ammonium Acetate 5mM</td>
<td>90</td>
<td>90</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% ACN</td>
<td>10</td>
<td>10</td>
<td>50</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

flow rate: 0.2mL/min  
MS detection: m/z 321 (ESI')  
Injection volume: 20µL.

Same protocols than previous page

Acknowledgment: French ANR project ORIGAMI (ANR ECOTECH 2011 ORIGAMI 11 ECOT 003)

Catalog number: FS113-02
16 PESTICIDES FROM GROUNDWATER

PROTOCOL OF PURIFICATION
Purification with a 6mL/200mg AttractSPE™ HLB cartridge

Equilibration
• 10mL Dichloromethane
• 10mL Acetonitrile
• 10mL Water

Loading
1L Water sample

Washing of interferences
• 5mL Water/Methanol 95/5

Elution (E)
5mL Acetonitrile
5mL Methanol

Analyse HPLC

Desysopropylatrazine,
Desethylatrazine, Aldocarb,
Simazine, Carbofuran,
Metalaxyl, Atrazine, 2, 4-D,
Metazachlor, Dicloran,
Phenmedipham, Linuron,
Iprodione, Procymidone,
Fenitrothion, Vinclozolin

Catalog number: HLB-50.S.6.200

PESTICIDES FROM SURFACE WATER

PROTOCOL OF PURIFICATION
Purification with a 6mL/200mg AttractSPE™ HLB cartridge

Equilibration
• 3mL Methanol/Acetonitrile 50/50
• 6mL Water

Loading
1L Water sample (+0.5g NaCl)

Drying

Elution (E)
3mL Acetonitrile/Methanol 50/50

Analyse HPLC

Desethylsimazin, 2, 6-Dichlorbenzamid,
Ethidimuron, Chloridazon,
Desethylatrazin,
Desethylsebuthylazin,
Bromacil, Simazin,
Metribuzin,
Desethylterbuthylazin,
Metabenzthiazuron,
Chlortoluon, Atrazine, Diuron,
Isoproturon, Mazaclor,
Terbumeton, Sebuthylazin,
Propazin, Dimefuron,
Terbuthylazin, Triadimenol,
Epoxiconazol, Terbutryn,
Metolachlor, propiconazol,
Kresoxim-methyl

Catalog number: HLB-50.S.6.200
**ACETAMIDE HERBICIDES IN DRINKING WATER**

**PROTOCOL OF PURIFICATION**

Purification with a 3mL/60mg AttractSPE™ HLB cartridge

**Equilibration**
- 3mL Methanol
- 2mL Water

**Loading**
- 150mL Water sample

**Washing of interferences**
- 1mL Water

**Elution (E)**
- 1mL Methanol

**Analyse HPLC**

Metolachlor metabolite, 2-Chloro 2, 6 diethylacetanilide, 2,6 Diethylaniline, Alachlor, Metolachlor

Catalog number: HLB-50.S.3.60

**HERBICIDES IN DRINKING WATER**

**PROTOCOL OF PURIFICATION**

Purification with a 3mL/60mg AttractSPE™ HLB cartridge

**Equilibration**
- 3mL Methanol
- 6mL Water

**Loading**
- 75mL Water sample

**Washing of interferences**
- 1mL Water

**Elution (E)**
- 1mL Methanol

**Analyse HPLC**

Desispropylatrazine, Hydroxyatrazine, Desethylatrazine, Simazine, Cyanazine, Atrazine

Catalog number: HLB-50.S.3.60
Analyses of
OTHER RESIDUES AND
MISCELLANEOUS
NNAL IN URINE

**PROTOCOL OF CLEANUP**

Cleanup with a AFFINIMIP® SPE NNAL cartridge

**Equilibration**
- 2mL Toluene
- 2mL 10% MeOH-CH2Cl2
- 3mL CH2Cl2
- Dry
- 1mL CH2Cl2
- 1mL MeOH
- 1mL Water

**Loading**
- 2mL Urine or Water

**Washing of interferences**
- 2mL Water
- Dry 10min
- 1mL Toluene
- 1mL Toluene : CH2Cl2 9:1
- 1mL Toluene : CH2Cl2 4:1
- Dry 2min

**Elution of phenolic compounds**
- 2mL 10% MeOH-CH2Cl2

Recovery for urine 112%

**LC-MS chromatogram of urine spiked with NNAL (spiked at 100ng/mL) after AFFINIMIP®SPE NNAL clean-up**

**HPLC Method with LC-MS/MS detection**

Column: Syncronis aQ column 150mm x 2.1mm

Mobile phase: Water – 0.1% Formic Acid

flow rate: 0.2mL/min

MS detection: m/z 322 (ESI+)

Injection volume: 20µL.

Catalog number: DG103-02
MELAMINE IN FOOD

PROTOCOL OF PURIFICATION
Sample preparation: Add 5mL water and 5mL Acetonitrile to 1g pulverized sample. Shake thoroughly for 30 min and centrifuge for 10 minutes at 2600 rpm. The supernatant is filtered through 0.45µm membrane filter to obtain the loading solution.

Purification with a 6mL/150mg AttractSPE™ SCX cartridge

Equilibration
- 5mL Acetonitrile
- 5mL 4% Formic acid in Water

Loading
- 3mL 4% Formic acid in Water
- 2mL of loading solution

Washing of interferences
- 5mL Acetonitrile
- 5mL 0.2% diethyamine in Acetonitrile

Elution (E)
- 4mL 2% diethylamine in Acetonitrile
The elution fraction was filtered and then evaporated under nitrogen at 50°C and dissolved in the mobile phase before HPLC analysis.

Analyse LC-MS/MS

POLYCYCLIC AROMATIC HYDROCARBONS FROM DRINKING WATER

PROTOCOL OF PURIFICATION
Purification with a 6mL/200mg AttractSPE™ HLB cartridge

Equilibration
- 5mL Dichloromethane
- 5mL Methanol
- 5mL Water

Loading
- 500mL Water sample

Washing of interferences
- 6mL Water

Elution (E)
- 8mL Dichloromethane

Analyse HPLC

Naphtalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibeno(g, h, l)perylen, Benzo(a)pyrene, Benzoperylene, Indenopyrene

Catalog number: SCX-25.5.6.150

Catalog number: HLB-50.5.6.200
**PROTOCOL OF CLEANUP**

Sample preparation
Edible oil is diluted by 10 with cyclohexane. This solution is used as the loading solution.

**Cleanup with a 3mL AFFINIMIP® SPE PAHs cartridge**

**Loading**
- 1mL of loading solution

**Washing of interferences (W1)**
- 3mL Cyclohexane

**Elution (E)**
- 3mL Ethyl acetate

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**RESULTS**

Recoveries of PAHs in cyclohexane and Olive oil after AFFINIMIP® SPE PAHs Clean-up

<table>
<thead>
<tr>
<th>PAHs</th>
<th>Yield cyclohexane</th>
<th>Yield Olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>B[a]A</td>
<td>101%</td>
<td>108%</td>
</tr>
<tr>
<td>B[a]P</td>
<td>83%</td>
<td>120%</td>
</tr>
<tr>
<td>B[b]F</td>
<td>91%</td>
<td>111%</td>
</tr>
<tr>
<td>CHR</td>
<td>91%</td>
<td>72%</td>
</tr>
</tbody>
</table>

**Regulations for B[a]P in oil and fats:**
Europe (EC 208/2005) : 2µg/Kg

---

**UV chromatogram of B[a]A (30ng/mL) in cyclohexane (red) and olive oil (blue) after Clean-up by AFFINIMIP® SPE PAHs.**

**Catalog number: FS119-02**

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PROTOCOL OF CLEANUP

1g of soil spiked with 0.2mg/kg of hydroxylated PAH was extracted by microwave assisted extraction (MAE) with 25mL acetonitrile at 120°C for 30min to form the loading solution. **Cleanup with a 3mL/100mg AFFINIMIP® SPE Phenolics cartridge**

**Equilibration**
- 4mL Toluene
- 2x4mL Acetonitrile

**Loading**
- 25mL of loading solution

**Washing of interferences**
- 4mL Acetonitrile

**Drying**

**Elution (E)**
- 6mL Methanol – 2 Acetic acid

RESULTS

Recoveries of OH-PAHs after clean-up with AFFINIMIP® SPE Phenolics for a linear range of 0.002-0.050 µg/mL

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean Recovery (%) (mean ± SD, n=5)</th>
<th>LOD (µg/g)</th>
<th>LOQ (µg/g)</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-OHNaph</td>
<td>79 ± 5</td>
<td>0.003</td>
<td>0.010</td>
<td>7</td>
</tr>
<tr>
<td>2-OHFlu</td>
<td>93 ± 6</td>
<td>0.003</td>
<td>0.011</td>
<td>9</td>
</tr>
<tr>
<td>9-OHPhe</td>
<td>89 ± 2</td>
<td>0.007</td>
<td>0.023</td>
<td>2</td>
</tr>
<tr>
<td>1-OHPyr</td>
<td>68 ± 6</td>
<td>0.014</td>
<td>0.044</td>
<td>8</td>
</tr>
</tbody>
</table>

HPLC-Fluorescence Method

Column: Envirosep PP C18, 150mm x 4.6mmx5µm

Mobile phase:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Water</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min
Injection volume: 20µL.

Publications

Data extracted from the article

Catalog number: FS103-02
Recovery and RSD of some native dioxins and hydroxylated dioxins analyzed in the publication

**PROTOCOL OF CLEANUP**

Cleanup with a 3mL/100mg AFFINIMIP® SPE Phenolics cartridge

**Equilibration**
- 6mL Methanol – 2% Acetic acid
- 6mL Methanol
- 6mL Dichloromethane

**Loading**
- Loading solution based on dichloromethane

**Washing of interferences**
- 20mL Dichloromethane (elution of neutral compounds)

**Elution of phenolic compounds**
- 20mL dichloromethane – 10% formic acid

**Publications**

Data extracted from the article

Simultaneous separation of chlorinated/brominated dioxins, polychlorinated biphenyls, polybrominated diphenylethers and their methoxylated derivatives from hydroxylated analogues on molecularly imprinted polymers prior to gas/liquid chromatography and mass spectrometry, M. Roszko, K. Szymczyk, R. Jędrzejczak, *Talanta* 144, 171-183, 2015..

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Recovery %</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDF</td>
<td>73</td>
<td>14.7</td>
</tr>
<tr>
<td>2,3,7,8-TCDD</td>
<td>81</td>
<td>17.5</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDD</td>
<td>86</td>
<td>15.6</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDF</td>
<td>88</td>
<td>15.4</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDD</td>
<td>78</td>
<td>9.6</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HeCDF</td>
<td>74</td>
<td>13.3</td>
</tr>
<tr>
<td>CB28</td>
<td>79</td>
<td>12.7</td>
</tr>
<tr>
<td>CB 52</td>
<td>82</td>
<td>14.7</td>
</tr>
<tr>
<td>2,3,7,8-TBDD</td>
<td>76</td>
<td>12.7</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpBDD</td>
<td>77</td>
<td>15.8</td>
</tr>
<tr>
<td>5-MeOBDE99</td>
<td>82</td>
<td>13.9</td>
</tr>
<tr>
<td>4-MeOCB101</td>
<td>86</td>
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<tr>
<td>BDE12</td>
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<td>16.1</td>
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<tr>
<td>BDE25</td>
<td>82</td>
<td>15.9</td>
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<tr>
<td>BDE35</td>
<td>80</td>
<td>13.4</td>
</tr>
<tr>
<td>BDE118</td>
<td>85</td>
<td>11.7</td>
</tr>
<tr>
<td>4-OH-CB19</td>
<td>64</td>
<td>17.8</td>
</tr>
<tr>
<td>4-OH-CB50</td>
<td>75</td>
<td>16.0</td>
</tr>
<tr>
<td>4-OH-CB106</td>
<td>72</td>
<td>14.5</td>
</tr>
<tr>
<td>4-OH-CB159</td>
<td>80</td>
<td>12.3</td>
</tr>
<tr>
<td>4-OH-CB172</td>
<td>74</td>
<td>12.3</td>
</tr>
<tr>
<td>3-OH-BDE28</td>
<td>74</td>
<td>11.6</td>
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<tr>
<td>3-OH-BDE47</td>
<td>80</td>
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<tr>
<td>6-OH-BDE137</td>
<td>82</td>
<td>14.7</td>
</tr>
<tr>
<td>6-OH-BDE-180</td>
<td>73</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Catalog number: FS103-02
**PROTOCOL OF CLEANUP**

Sample preparation
The plasma or serum is diluted by 5 with water. This solution is used as the loading solution.

**Cleanup with a 1mL AFFINIMIP® SPE Metanephrines cartridge**

- **Equilibration**
  - 1mL of phosphate buffer pH 7
  - 2mL Water

- **Loading**
  - 1.5mL of loading solution

- **Washing of interferences (W1)**
  - 1mL Water
  - 500µL Water/Methanol (60/40)

- **Drying 10 seconds**

- **Washing of interferences (W2)**
  - 500µL Methanol

- **Elution (E)**
  - 1mL Methanol – 5% Acetic acid

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**RESULTS**

Recoveries of MN and NMN at a contamination level of 500nM in rabbit plasma after AFFINIMIP® SPE Metanephrines Clean-up and relative standard deviation calculated from results generated under reproducibility conditions (Analysis by LC-MS).

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Recovery %</th>
<th>% RSD_R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metanephrine</td>
<td>79.4</td>
<td>6.3</td>
</tr>
<tr>
<td>Normetanephrine</td>
<td>109</td>
<td>11</td>
</tr>
</tbody>
</table>

Catalog number: DG101-02A

**Analysis by LC-MS/MS:** Total Ion Current of a calf serum after Cleanup by AFFINIMIP® SPE Metanephrines. The sample naturally contained Metanephrine. **Concentration of MN found:** 30nM. In parallel, an SPE was performed on a protocol developed for the analysis of MN using WCX cartridges: the concentration obtained was 7nM for the same sample.

**WCX**

**AFFINIMIP® SPE**

**WCX Recovery : 33%**

**AFFINIMIP SPE Recovery: 101%**

**Analysis by LC-MS/MS:** Selected ion monitoring of Normetanephrine (m/z 180). Chromatograms obtained after Cleanup by AFFINIMIP® SPE Metanephrines or by WCX of a calf serum spiked at 27nM with Normetanephrine.

**HPLC Method with LC-MS/MS detection**

- Column: Syncronis aQ column 150mm x 2.1mm
- Mobile phase: Water – 0.1% Formic Acid
- Flow rate: 0.2mL/min
- MS detection: m/z 322 (ESI+)
- Injection volume: 20µL.
PROTOCOL OF CLEANUP
Artificial urine and Urine were diluted 9 times with Acetic acid – ammonia buffer (pH 7, 25 mmol/L)

Cleanup with a 1mL AFFINIMIP® SPE Metanephrines cartridge

Equilibration
- 4mL CAN
- 4mL Acetic acid – ammonia buffer (pH 7, 25mmol/L)

Loading
2mL of urine

Washing of interferences (W1)
- 10mL Acetic acid – ammonia buffer (pH 7, 25mmol/L)
- 4mL Acetonitrile

Elution (E)
4mL Methanol – 1% Acetic acid

The elution fraction was then evaporated and dissolved in 0.5mL Methanol – water (90/10) with 100µmol/L H₃PO₄ before HPLC analysis.

CE Method with UV detection
Column: uncoated fused-silica capillary of 60.2 cm (effective length, 50 cm) x 50 µm at 25°C
Mobile phase: H₃PO₄-LiOH (pH 4, ionic strength of 80 mmol/L) containing HP-β-CD (10 mmol/L)
Voltage: +25kV or +30kV
Detection: UV at 214nm
Injection volume: 20µL.

RESULTS

Linearity studied between 50-500nmol/L R² = 0.9958 for 3-methoxytyramine (3-MT)
R² = 0.9999 for 5-Hydroxytryptamine (5-HT)

Recoveries of neurotransmitters and LOQ after AFFINIMIP® SPE Metanephrines Clean-up

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Recovery %</th>
<th>LOQ nmol/L</th>
</tr>
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<tbody>
<tr>
<td>3-MT</td>
<td>80-100</td>
<td>11</td>
</tr>
<tr>
<td>5-HT</td>
<td>80-100</td>
<td>5</td>
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<tr>
<td>Dopamine</td>
<td>40</td>
<td>46</td>
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</table>

Publications


Catalog number: DG101-02A
GUAIACOL IN RED/WHITE WINE

PROTOCOL OF CLEANUP

Cleanup with a 3mL/100mg AFFINIMIP® SPE Phenolics cartridge

<table>
<thead>
<tr>
<th>Equilibration</th>
<th>Loading</th>
<th>Washing of interferences</th>
<th>Elution (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 3mL Acetonitrile</td>
<td>• Up to 2mL of red or white wine</td>
<td>• 3mL Water / Acetonitrile (80/20 v/v)</td>
<td>• 2mL Methanol</td>
</tr>
</tbody>
</table>

Recovery yields and reproducibility evaluated with 3 cartridges and 3 different batches of AFFINIMIP® SPE Phenolics by matrix (n=9)

<table>
<thead>
<tr>
<th></th>
<th>C° (µM)</th>
<th>Rec. %</th>
<th>RSDr %</th>
</tr>
</thead>
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<tr>
<td>Red wine 1</td>
<td>0.1</td>
<td>88.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Red wine 2</td>
<td>0.1</td>
<td>93.1</td>
<td>3.7</td>
</tr>
<tr>
<td>White wine 1</td>
<td>0.02</td>
<td>96.8</td>
<td>1.7</td>
</tr>
<tr>
<td>White wine 2</td>
<td>0.02</td>
<td>93.5</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Chromatograms obtained after clean-up with AFFINIMIP® SPE Phenolics of red wine spiked with Guaïacol (0.1µM) (red) or not spiked (blue).

Chromatograms obtained before (red) and after (blue) clean-up with AFFINIMIP® SPE Phenolics of red wine spiked with Guaïacol (0.1µM)

HPLC-UV Method

Column: Thermo Hypersil gold, 150mm x 4.6mm
Mobile phase: 15/85 (v/v) Acetonitrile Water
Flow rate: 1mL/min
Detection: UV - 272nm
Injection volume: 100µL.

RESULTS

HPLC-UV Method

Column: Thermo Hypersil gold, 150mm x 4.6mm
Mobile phase: 15/85 (v/v) Acetonitrile Water
Flow rate: 1mL/min
Detection: UV - 272nm
Injection volume: 100µL.

General structure of Guaïacol

Catalog number: FS103-02
PROTOCOL OF CLEANUP
Sample preparation

25g of turkey was mixed with 200mL of 74.5/25/0.5 ACN/H2O/H3PO4 or Ethanol-0.5% H3PO4 using a blender during 3 minutes. After, the mixture was mixed during 30 minutes with magnetic stirrer. The mixture was filtered on filter paper (4-7µm). Then the mixture was diluted by 2 with water.

Cleanup with a 3mL/100mg AFFINIMIP® SPE Phenolics cartridge

Equilibration
• 3mL Acetonitrile
• 3mL Water

Loading
• Up to 80mL of loading solution

Washing of interferences
• 3mL Water / Acetonitrile (60/40 v/v)

Elution (E)
• 2mL Methanol -1% H3PO4

RESULTS

HPLC-UV Method
Column: Thermo Hypersil gold, 150mm x 4.6mm
Mobile phase: 65/35 (v/v) ACN/Water-0.5% H3PO4
Flow rate: 1mL/min
Detection: UV - 230nm
Injection volume: 5µL.

Chromatogram of a turkey containing 50ppm of Carnosic acid after clean-up with AFFINIMIP® SPE Phenolics. Extraction of the turkey with Ethanol-0.5% H3PO4

Recovery yields obtained by both extraction solvent after AFFINIMIP® SPE Phenolics Clean-up.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Recoveries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>74.5/25/0.5 ACN/H2O/H3PO4</td>
<td>&gt;85%</td>
</tr>
<tr>
<td>Ethanol-0.5% H3PO4</td>
<td>&gt;80%</td>
</tr>
</tbody>
</table>

Catalog number: FS103-02
PROTOCOL OF PURIFICATION
Sample preparation

Crude, Yucca juice concentrate (300 mL, 38° Brix) was repeatedly extracted with 200 mL of ethanol (100%) three times. Ethanolic phases were pulled and concentrated to dryness. The powder obtained was repeatedly washed with 200 mL of ethyl acetate and 200 mL of hexane under reflux for 30 min yielding a brown viscous residue. Subsequently, the residue was partitioned between water phase and 1-butanol phase. Following concentration of the organic phase under vacuum, a brown powder (BP_7.5 g) was obtained. The obtained powder (5 g) was fractionated using column chromatography (FPX-68; 600 g; 1.5 L of water; 1.5 L of water/ethanol 7:3; 1.5 L water/ethanol 3:7). The water/ethanol 7:3 v/v fraction was concentrated in a freeze dryer and yielded 1.2 g of powder.

Cleanup with a 3mL/100mg AFFINIMIP® SPE Phenolics cartridge

**Equilibration**
- Water
- Methanol

**Loading**
- Loading solution

**Washing of interferences**
- Water

**Elution (E)**
- Water / Acetonitrile (10/90 v/v)

Pure saponins were obtained by freeze-drying and used to prepare the Yucca Saponins Standard

HPLC-ELSD Method
Column: Atlantis T3, 150mm x 3.0mm, 3µm
Mobile phase: gradient - A: 0.1% formic acid - water
B 0.1% formic acid in acetonitrile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>35</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

Flow rate: 0.6mL/min
Detection: ELSD with flow 1.20

Publications
Data extracted from the article

Catalog number: FS103-02
ARTIFICIAL SWEETENERS IN WATER

**PROTOCOL OF PURIFICATION**

**Purification with a 6mL/200mg** *AttractSPE™ HLB* cartridge

**Equilibration**
- 5mL Methanol
- 5mL Water pH~2

**Loading**
- 50mL of water (adjusted to pH ~2)

**Washing**
- 10mL distilled water pH ~2

**Drying 30min**

**Elution (E)**
- 2mL Methanol

Analysis: LC-MS/MS

Acesulfame, Aspartame, Cyclamate, Neohesperidine dihydrochalcone, Saccharin, Sucralose

Catalog number: HLB-50.S.6.200

Cocaïne and main metabolites in waste water

**PROTOCOL OF PURIFICATION**

Waste water was adjusted to pH 2 with 37%HCl and filtered to form the loading solution.

**Purification with a 6mL/500mg** *AttractSPE™ HLB* cartridge

**Equilibration**
- 3mL Methanol
- 3mL Water pH~2

**Loading**
- 100mL Waste water pH ~6

**Washing**
- 3mL Methanol/Water (5/95)

**Drying 15min**

**Elution (E)**
- 2x4mL Methanol

Analysis: LC-MS/MS

Cocaine, benzoylecgonine and eegonine methyl ester

Catalog number: HLB-50.S.6.500
**MELAMINE IN MILK**

**PROTOCOL OF PURIFICATION**
Sample preparation
Add 4mL water to 5g liquid infant formula or 1g dry infant formula. Shake during 10-20min with 20mL 50/50 ACN/Water and centrifuge for 10min at 3400 rpm.
The supernatant is the loading solution.

**Purification with a 6mL/150mg** AttractSPE™ SCX cartridge

**Equilibration**
- 5mL 0.1M NaOH in Acetonitrile
- 5mL 0.1M HCl in Acetonitrile
- 5mL Acetonitrile
- 5mL 4% Formic acid in Water

**Loading**
3mL 4% Formic acid in Water
2mL of loading solution

**Washing of Interferences**
- 5mL Acetonitrile
- 5mL 0.2% diethyamine in Acetonitrile

**Elution (E)**
4mL 2% diethyamine in Acetonitrile

The elution fraction was filtered and then evaporated under nitrogen and dissolved in the mobile phase before HPLC analysis.

**Analyse** LC-MS/MS

**Regulations for Melamine:**
Codex alimentarius 35th CAC session (July 2012):
Maximum limit 0.15mg/kg for liquid infant milk

**Catalog number:** SCX-25.S.6.150

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**CYANURIC ACID IN MILK**

**PROTOCOL OF PURIFICATION**
Sample preparation
Add 4mL water to 5g liquid infant formula or 1g dry infant formula. Shake during 10-20min with 20mL 50/50 ACN/Water and centrifuge for 10min at 3400 rpm.
The supernatant is the loading solution.

**Purification with a 6mL/150mg** AttractSPE™ SAX cartridge

**Equilibration**
- 5mL 0.1M HCl in Acetonitrile
- 5mL 0.1M NaOH in Acetonitrile
- 5mL Acetonitrile
- 5mL 5% NH₄OH in Water

**Loading**
3mL 5% NH₄OH in Water
2mL of loading solution

**Washing of Interferences**
- 5mL Acetonitrile

**Elution (E)**
2mL 4% Formic acid in Acetonitrile

The elution fraction was filtered and then evaporated under nitrogen and dissolved in the mobile phase before HPLC analysis.

**Analyse** LC-MS/MS

**Catalog number:** SAX-25.S.6.150
STEROIDS IN COW URINE

**Sample preparation**
Five milliliters of urine were thawed at room temperature and submitted to an enzymatic deconjugation step using β-glucuronidase from E. Coli at 37 °C overnight. Samples were then centrifuged at 1200×g (5 °C) for at least 10 min.

1 mL of sodium acetate buffer 0.1M at pH 5.0 and 20 µL of β-glucuronidase/sulfatase Helix pomatia enzyme solution at 1.0 mg/mL in the same buffer were mixed thoroughly by vortex. The enzymatic reaction was carried out for 2 h at 37°C to obtain the loading solution.

**Purification with a 3mL/100mg AFFINIMIP® SPE Estrogens cartridge**
- **Equilibration**
  - 5mL Acetonitrile
  - 5mL Water
- **Loading solution**
  - loading solution
- **Washing of interferences**
  - 5mL Water/Acetonitrile (90/10)
  - 5mL Water/Acetonitrile (80/20)
- **Elution (E)**
  - 3mL Methanol

---

**RESULTS**

Total Ion Chromatogram acquired in scan mode (GC-MS) : 2 µL injected of the eluted fraction from SPE-MIP after clean-up with AFFINIMIP® SPE Estrogens

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**Catalog number:** FS104-02
RADIOTRACERS
PURIFICATION
Aromatic nucleophilic substitution is widely used to synthesize fluorous radiotracers. Due to the short lifetime of $^{18}$F radiotracers, the purification is a key step of the synthesis. It must be fast and effective to ensure a high radiochemical purity. This application note shows the effectiveness of the purification method for FBA using a AFFINIMIP® SPE $^{18}$F Aromatic Nucleophilic Substitution cartridge.

Fluoration of 4-Trimethylammoniumbenzaldehyde Tosylate in DMSO. Chromatograms obtained before (black) and after AFFINIMIP® SPE $^{18}$F Aromatic Nucleophilic Substitution Clean-up (E1 : red) and after AttractSPE™ HLB Clean-up (E2 : blue)

**RESULTS**

Recovery of more than 95 % of the fluorous radiotracer was obtained without any contamination of other identified compounds (phenolic and dimethyaminobenzylic compounds, precursor and fluoride). More than 95 % of Kryptofix 2.2.2 was also eliminated. (MS control)

---

**PROTOCOL OF PURIFICATION**

At 95°C were mixed 5 mg of ammonium salt, 6.7 mg of Kryptofix 2.2.2 and 1 mg of potassium fluoride in 400 µL of DMSO. After 15 minutes and the cooling of the reaction mixture, 2 mL of water were added to obtain the loading solution (L).

**Cleanup with a AFFINIMIP® SPE $^{18}$F Aromatic Nucleophilic Substitution cartridge**

**Equilibration**
5mL Acetonitrile

**Loading**
Loading solution

**Washing of interferences**
5mL of 80-20 Water-ACN

**Drying 30s**

**Elution (E)**

- 1-2mL ACN

Catalog number: RP100-01
Aromatic nucleophilic substitution is widely used to synthesize fluorous radiotracers. Due to the short lifetime of $^{18}$F radiotracers, the purification is a key step of the synthesis. It must be fast and effective to ensure a high radiochemical purity. This application note shows the effectiveness of the purification method for Ethyl 4-Fluorobenzoate radiotracers using an AFFINIMIP® SPE $^{18}$F Aromatic Nucleophilic Substitution cartridge.

Fluoration of Ethyl 4-Trimethylammoniumbenzoate iodide in DMSO. Chromatograms obtained before (black) and after AFFINIMIP® SPE $^{18}$F Aromatic Nucleophilic Substitution Clean-up ($E_1$ : red) and after AttractSPE™ HLB Clean-up ($E_2$ : blue)

**RESULTS**

Recovery of more than 95 % of the fluorous radiotracer was obtained without any contamination of other identified compounds (phenolic and dimethylaminobenzylcompounds, precursor and fluoride). More than 95 % of Kryptofix 2.2.2 was also eliminated. (MS control)

**PROTOCOL OF PURIFICATION**

At 95°C were mixed 5 mg of ammonium salt, 6.7 mg of Kryptofix 2.2.2 and 1 mg of potassium fluoride in 400 µL of ACN. After 15 minutes and the cooling of the reaction mixture, 2 mL of water were added to obtain the loading solution.

**Cleanup with a AFFINIMIP® SPE $^{18}$F Aromatic Nucleophilic Substitution cartridge**

- **Equilibration**
  - 5mL Acetonitrile
- **Loading**
  - Loading solution
- **Washing of interferences**
  - 5mL of 80-20 Water-ACN
- **Drying 30s**
- **Elution (E)**
  - 1-2mL ACN

Catalog number: RP100-01
Use of AttractSPE™ HLB cartridge to get the radiotracer in Ethanol

Having the radiotracer in Ethanol at the end of the radiosynthesis can be realized with an AttractSPE™ HLB cartridge. This procedure must be fast and effective to ensure a high radiochemical purity.

Ethyl 4-Fluorobenzoate and 4-Fluorobenzaldehyde were respectively previously obtained in an acetonitrile solution noted E1.

PROTOCOL OF PURIFICATION

Cleanup with a AttractSPE™ HLB reversible cartridge

Equilibration
2mL Ethanol
2mL Water

Loading
Load with the acetonitrile elution E1 diluted with 15mL water

Drying 30 s

Elution
Elute the fluororous radiotracer with 1-2mL of Ethanol until dryness (E2)

HPLC-Fluorescence Method
Column: Hypersil Gold column 50mm x 2.1mm, 1.9 µm

Mobile phase:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% (0.1 % HCOOH Water)</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
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<td>32</td>
<td>70</td>
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<tr>
<td>33</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>53</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Flow rate: 0.2mL/min
Injection volume: 10µL – UV 235nm

RESULTS

Recovery of more than 95 % of the fluorous radiotracer was obtained

Figure 1. Obtention of 4-Fluorobenzaldehyde in Ethanol. Chromatograms obtained before (red) and after AttractSPE™ HLB Clean-up (E2 : blue)

Figure 2. Obtention of Ethyl 4-Fluorobenzoate in Ethanol. Chromatograms obtained before (red) and after AttractSPE™ HLB Clean-up (E2 : blue)

Conclusion
The use of AttractSPE™ HLB allows to get the radiotracer in a minimum of Ethanol.
Passive Sampling with POCIS
Passive sampling enables the monitoring of contaminants in water (surface water, groundwater, coastal water...) for a long period (days or weeks). An average of the concentration of this contaminant is measured.

For hydrophilic organic compounds, the Polar Organic Chemical Integrative Sampler (POCIS) is designed to provide the time weighted average (TWA) concentration of chemicals during the sampling period.

The POCIS consists of a solid sorbent contained between two microporous membranes. The sorbent collects the contaminant in water. Each sorbent may have a retention for specific contaminant or a family of contaminant.

AFFINIMIP® POCIS Glyphosate

AFFINIMIP® POCIS Glyphosate enables the sampling of Glyphosate and AMPA in water (Groundwater, geothermal, mineral...). Then the powder is collected in an empty SPE column for the extraction of Glyphosate and AMPA.

PROTOCOL OF EXTRACTION
Extraction of collected Glyphosate and AMPA from AFFINIMIP® POCIS Glyphosate with a SPE

Washing of interferences (optional)
Water

Extraction of the analytes (E)
HCl solution (100mM)

The extraction solution is then evaporated and reconstituted with water prior analysis.

RESULTS
Laboratory sampling rates estimation for AMPA and glyphosate using the AFFINIMIP® POCIS Glyphosate

Sampling rates: 130mL/day/200mg AFFINIMIP® POCIS Glyphosate in agreement with other pesticides in classical POCIS.

Mineral water (pH = 7) fortified at 500ng/L of AMPA and glyphosate. Concentrations kept constant during whole experiment.
Pesticides concentration in the tank, temperature, TOC and conductivity monitored during the experimental period to verify the stability of physico-chemical conditions in water.

Publications
Data extracted Laboratory calibration of a POCIS-like sampler based on molecularly imprinted polymers for glyphosate and AMPA sampling in water, C. Berho, B. Claude, E. Coisy, A. Togola, S. Bayouhdh, P. Morin, L. Amalric, Anal Bioanal Chem, 409, 2029, 2017

Catalog number: POCIS-GLY.90.55.A.1
PRODUCT LIST

- Food / Feed Safety
- Environment
- Cosmetics
- Pharmaceutical R&D
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<td>AFFINIMIP® SPE Patulin &amp; Pectinase kit</td>
<td>Kit of 3mL cartridges for Patulin + 50mL Pectinase enzyme solution</td>
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<td>AFFINIMIP® SPE Patulin &amp; Pectinase kit</td>
<td>Kit of 6mL - 200mg cartridges for Patulin in dried apple + 50mL Pectinase enzyme solution</td>
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<td>AFFINIMIP® SPE Deoxynivalenol</td>
<td>6mL -100mg for Deoxynivalenol in food and babyfood</td>
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<td>50 mL Pectinase enzyme solution</td>
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<td>FS106-02</td>
<td>FS106-03</td>
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<tr>
<td></td>
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<td>6mL for Bisphenols (PP)</td>
<td>FS106-02B</td>
<td>FS106-03B</td>
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<td>6mL for Bisphenols (Glass)</td>
<td>FS106-02G</td>
<td>FS106-03G</td>
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<tr>
<td>Estrogens</td>
<td>AFFINIMIP® SPE Estrogens</td>
<td>1mL for Estrogens</td>
<td>FS104-02A</td>
<td>FS104-03A</td>
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<td>3mL for Estrogens</td>
<td>FS104-02</td>
<td>FS104-03</td>
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<td>96 well plate for estrogens – 1/pk</td>
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<td>FS104-1.96W</td>
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<td>Catechol amines</td>
<td>AFFINIMIP® SPE Catecholamines</td>
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<td>DG100-02</td>
<td>DG100-03</td>
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<td>1mL for Catecholamines</td>
<td>DG100-02A</td>
<td>DG100-03A</td>
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<tr>
<td>Metanephrines</td>
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<td>3mL for Metanephrines</td>
<td>DG101-02</td>
<td>DG101-03</td>
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<td>1mL for Metanephrines</td>
<td>DG101-02A</td>
<td>DG101-03A</td>
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<tr>
<td>Picloram, Aminopyralid, Clopyralid</td>
<td>AFFINIMIP® SPE Picolinic Herbicides</td>
<td>3mL for Picolinic acid based herbicides</td>
<td>FS115-02</td>
<td>FS115-03</td>
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<td>3mL for Glyphosate and AMPA</td>
<td>FS113-02</td>
<td>FS113-03</td>
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<td>6mL for Glyphosate and AMPA</td>
<td>FS113-02B</td>
<td>FS113-03B</td>
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<td>Glyphosate, AMPA</td>
<td>AFFINIMIP® SPE Glyphosate -AMPA</td>
<td>3mL for NNAL</td>
<td>DG103-02</td>
<td>DG103-03</td>
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<td>NNAL</td>
<td>AFFINIMIP® SPE NNAL</td>
<td>3mL for NNAL</td>
<td>DG103-02</td>
<td>DG103-03</td>
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<td>Amphetamines</td>
<td>AFFINIMIP® SPE Amphetamines</td>
<td>3mL for Amphetamines derivatives</td>
<td>DG102-02</td>
<td>DG102-03</td>
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<tr>
<td>Chloram-phenicol</td>
<td>AFFINIMIP® SPE Chloramphenicol</td>
<td>1mL for Chloramphenicol</td>
<td>FS110-02A</td>
<td>FS110-03A</td>
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<td>3mL for Chloramphenicol</td>
<td>FS110-02</td>
<td>FS110-03</td>
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<td>Tamoxifen</td>
<td>AFFINIMIP® SPE Tamoxifen</td>
<td>3mL for Tamoxifen</td>
<td>PH101-02</td>
<td>PH101-03</td>
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<tr>
<td>Tetracyclines</td>
<td>AFFINIMIP® SPE Tetracyclines</td>
<td>1mL for Tetracyclines</td>
<td>FS112-02A</td>
<td>FS112-03A</td>
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<td>3mL for Tetracyclines</td>
<td>FS112-02</td>
<td>FS112-03</td>
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<tr>
<td>Zeranol Residues</td>
<td>AFFINIMIP® SPE Zeranol Residues</td>
<td>3mL for Zeranol Residues</td>
<td>FS105-02</td>
<td>FS105-03</td>
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<td>Phenolics</td>
<td>AFFINIMIP® SPE Phenolics</td>
<td>3mL for Phenolic compounds</td>
<td>FS103-02</td>
<td>FS103-03</td>
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<td>PAHs</td>
<td>AFFINIMIP® SPE PAHs</td>
<td>3mL for PAHs</td>
<td>FS119-02</td>
<td>FS119-03</td>
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<td>Product</td>
<td>Description</td>
<td>Reference</td>
<td>Number of cartridges</td>
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<tr>
<td>Reversible cartridges (2mL)</td>
<td>SPE cartridges compatible with automates for purification of radiotracers issued from Aromatic Nucleophilic Substitution</td>
<td>RP100-01</td>
<td>10</td>
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<tr>
<td></td>
<td>SPE cartridges compatible with automates for purification of radiotracers issued from Aromatic Nucleophilic Substitution</td>
<td>RP100-02</td>
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<tr>
<td>Reversible cartridges (0.7mL)</td>
<td>SPE cartridges compatible with automates for purification of radiotracers issued from Aromatic Nucleophilic Substitution</td>
<td>RP100A-01</td>
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<td>SPE cartridges compatible with automates for purification of radiotracers issued from Aromatic Nucleophilic Substitution</td>
<td>RP100A-02</td>
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<tr>
<td>Format, amount</td>
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<td>AttractSPE™ HLB</td>
<td>AttractSPE™ SCX</td>
<td>AttractSPE™ WCX</td>
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<td>---------------</td>
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</tr>
<tr>
<td>1mL, 30mg</td>
<td>100</td>
<td>HLB-100.S.1.30</td>
<td>SCX-100.S.1.30</td>
<td>WXC-100.S.1.30</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>HLB-100.S.3.60</td>
<td>SCX-100.S.3.60</td>
<td>WXC-100.S.3.60</td>
</tr>
<tr>
<td>20mL, 1g</td>
<td>25</td>
<td>HLB-25.S.20.1g</td>
<td>SCX-25.S.20.1g</td>
<td>WXC-25.S.20.1g</td>
</tr>
<tr>
<td>96 wells Plate, 30mg</td>
<td>1</td>
<td>HLB-1.96W.30</td>
<td>SCX-1.96W.30</td>
<td>WXC-1.96W.30</td>
</tr>
<tr>
<td>Cartridges format, Sorbent amount</td>
<td>#/box</td>
<td>AttractSPE™ SAX-HCO3</td>
<td>AttractSPE™ PS-H</td>
<td>AttractSPE™ PS-Ag</td>
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<td>-----------------</td>
</tr>
<tr>
<td>1mL</td>
<td>100</td>
<td>Sax-HCO3-100.S.1.30</td>
<td>PSAg-100.S.1.30</td>
<td>PSBa-100.S.1.30</td>
</tr>
<tr>
<td>3mL, 60mg</td>
<td>25</td>
<td>Sax-HCO3-25.S.3.60</td>
<td>PSH-25.S.3.60</td>
<td>PSAg-25.S.3.60</td>
</tr>
<tr>
<td>96 wells Plate</td>
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<td>Sax-HCO3-1.96W.30</td>
<td>PSH-1.96W.30</td>
<td></td>
</tr>
<tr>
<td>Reversible 0.7mL, 30mg</td>
<td>25</td>
<td>Sax-HCO3-25.REV.1.F</td>
<td>PSH-25.R.EV.1.F</td>
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</tr>
<tr>
<td>Reversible 0.7mL, 100mg</td>
<td>25</td>
<td>Sax-HCO3-25.REV.2.F</td>
<td>PSH-25.R.EV.2.F</td>
<td>PSAg-25.R.EV.1.F For 400mg</td>
</tr>
<tr>
<td>Reversible 2mL, 800mg</td>
<td>25</td>
<td>Sax-HCO3-100.S.1.30</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>50</td>
<td>Sax-HCO3-25.S.3.60</td>
<td>PSH-25.S.3.60</td>
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### AttractSPE™ Carbon based SPE - Product list

<table>
<thead>
<tr>
<th>Product</th>
<th>Vol</th>
<th>Sorbent</th>
<th>25 cartridges/box</th>
<th>50 cartridges/box</th>
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</thead>
<tbody>
<tr>
<td>AttractSPE™ Carbon</td>
<td>6mL</td>
<td>500mg</td>
<td>Carb-25.5.6.500</td>
<td>Carb-50.5.6.500</td>
</tr>
<tr>
<td>AttractSPE™ Carbon/PSA</td>
<td>3mL</td>
<td>250mg/250mg</td>
<td>CarbPSA-25.5.3.250.250</td>
<td>CarbPSA-50.5.3.250.250</td>
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<tr>
<td></td>
<td>6mL</td>
<td>500mg/500mg</td>
<td>CarbPSA-25.5.6.500.500</td>
<td>CarbPSA-50.5.6.500.500</td>
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<td>AttractSPE™ Carbon/Amine</td>
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<td>500mg/500mg</td>
<td>CarbNH2-25.5.6.500.500</td>
<td>CarbNH2-50.5.6.500.500</td>
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### AttractSPE™ LipRem

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<th>Cartridges amount format, Sorbent</th>
<th>#/box</th>
<th>AttractSPE™ LipRem</th>
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<tbody>
<tr>
<td>1mL, 20mg</td>
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<td>LipRem-100.5.1.20</td>
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<tr>
<td>3mL, 60mg</td>
<td>25</td>
<td>LipRem-25.5.3.50</td>
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<td>LipRem-50.5.3.50</td>
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<tr>
<td>6mL, 100mg</td>
<td>25</td>
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<td></td>
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<td>LipRem-50.5.6.100</td>
</tr>
<tr>
<td>96 wells Plate</td>
<td>1</td>
<td>LipRem-1.96W.20</td>
</tr>
<tr>
<td>Reversible 0.7mL, 100mg</td>
<td>25</td>
<td>LipRem-1.REV.1.F</td>
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<td></td>
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<td>LipRem-1.REV.1.F</td>
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# SilactSPE™ PRODUCT LIST

<table>
<thead>
<tr>
<th>Cartridges format, Sorbent amount</th>
<th>Non polar sorbents</th>
<th>Polar sorbents</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SilactSPE™ C18</td>
<td>SilactSPE™ C8</td>
</tr>
<tr>
<td>1mL, 50mg</td>
<td>100</td>
<td>C18-100.S.1.50</td>
</tr>
<tr>
<td>1mL, 100mg</td>
<td>100</td>
<td>C18-100.S.1.100</td>
</tr>
<tr>
<td>6mL, 1g</td>
<td>50</td>
<td>C18-50.S.6.1g</td>
</tr>
<tr>
<td>6mL, 2g</td>
<td>50</td>
<td>C18-50.S.6.2g</td>
</tr>
<tr>
<td>12mL, 2g</td>
<td>20</td>
<td>C18-20.S.12.2g</td>
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</table>

For other formats, please contact us

www.affinisep.com
SilactSPE™ PRODUCT LIST (continued)

<table>
<thead>
<tr>
<th>Cartridges format, Sorbent amount</th>
<th>#/box</th>
<th>Polar sorbents</th>
<th>Others sorbents</th>
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<tr>
<td></td>
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<td>SilactSPE™ Alumina Acidic</td>
<td>SilactSPE™ Alumina Neutral</td>
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<td>1mL, 50mg</td>
<td>100</td>
<td>AluA-100.S.1.50</td>
<td>AluN-100.S.1.50</td>
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<tr>
<td>1mL, 100mg</td>
<td>100</td>
<td>AluA-100.S.1.100</td>
<td>AluN-100.S.1.100</td>
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<td>6mL, 1g</td>
<td>50</td>
<td>AluA-50.S.6.1g</td>
<td>AluN-50.S.6.1g</td>
</tr>
<tr>
<td>6mL, 2g</td>
<td>50</td>
<td>AluA-50.S.6.2g</td>
<td>AluN-50.S.6.2g</td>
</tr>
<tr>
<td>12mL, 2g</td>
<td>20</td>
<td>AluA-20.S.12.2g</td>
<td>AluN-20.S.12.2g</td>
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For other formats, please contact us
## SPE for Polycyclic Aromatic Hydrocarbons (PAHs) in soil

<table>
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<tr>
<th>Product</th>
<th>Vol</th>
<th>Sorbent</th>
<th>25 cartridges/box</th>
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<tbody>
<tr>
<td>SilactSPE™ CN/SiOH</td>
<td>3mL</td>
<td>500mg/1g</td>
<td>CNSiOH-25.S.3.500.1g</td>
<td>CNSiOH-50.S.3.500.1g</td>
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<tr>
<td></td>
<td>6mL</td>
<td>500mg/1g</td>
<td>CNSiOH-25.S.6.500.1g</td>
<td>CNSiOH-50.S.6.500.1g</td>
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<tr>
<td></td>
<td>6mL glass</td>
<td>500mg/1g</td>
<td>CNSiOH-25.G.6.500.1g</td>
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## SilactSPE™ SLE

<table>
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<th>Cartridge volume</th>
<th>Sorbent</th>
<th>25 cartridges/box</th>
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<tbody>
<tr>
<td>1mL</td>
<td>250mg</td>
<td>SLE-25.S.1.250</td>
<td>SLE-50.S.1.250</td>
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<tr>
<td>3mL</td>
<td>500mg</td>
<td>SLE-25.S.3.500</td>
<td>SLE-50.S.3.500</td>
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<tr>
<td>6mL</td>
<td>1g</td>
<td>SLE-25.S.6.1g</td>
<td>SLE-50.S.6.1g</td>
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<tr>
<td>15mL</td>
<td>3g</td>
<td>SLE-25.S.15.3g</td>
<td>SLE-50.S.15.3g</td>
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<tr>
<td>30mL</td>
<td>4.5g</td>
<td>SLE-25.S.30.4g</td>
<td>SLE-50.S.30.4g</td>
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<tr>
<td>70mL</td>
<td>14.5g</td>
<td>SLE-25.S.70.14g</td>
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## Fritted cartridges

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<th>SilactSPE™ Double fritted</th>
<th>SilactSPE™ Single fritted</th>
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<td>100 cartridges</td>
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<tr>
<td>1mL</td>
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<td>0-100.S.1.1F</td>
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<td>3mL</td>
<td>0-100.S.3.2F</td>
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<td>6mL</td>
<td>0-100.S.6.2F</td>
<td>0-100.S.6.1F</td>
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<tr>
<td>15mL</td>
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<td>25mL</td>
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<td>60mL</td>
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### Qcleanup™ EXTRACTION SALTS

<table>
<thead>
<tr>
<th>QuEChERS methods</th>
<th>Description</th>
<th>Pouches / box</th>
<th>Product reference</th>
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</thead>
<tbody>
<tr>
<td>Original method</td>
<td>4g MgSO₄, 1g NaCl</td>
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<td>EXT.ORL.50</td>
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<tr>
<td>EN 15662</td>
<td>1g Trisodium citrate Dihydrate, 0.5g Disodium hydrogen citrate sesquihydrate, 1g NaCl and 4g MgSO₄</td>
<td>50</td>
<td>EXT.EN.50</td>
</tr>
<tr>
<td>AOAC 2007.01</td>
<td>1.5g Sodium Acetate and 6g MgSO₄</td>
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<td>EXT.AOAC.50</td>
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### Qcleanup™ DISPERSIVE SPE PRODUCTS

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Nber / box</th>
<th>Product reference</th>
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<tbody>
<tr>
<td>EN 15662</td>
<td>For General Fruits &amp; Vegetables</td>
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<tr>
<td></td>
<td>150mg MgSO₄ + 25mg PSA</td>
<td>100 tubes of 2mL</td>
<td>dSPE.EN.GFV.100.2</td>
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<tr>
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<td>900mg MgSO₄ + 150mg PSA</td>
<td>50 tubes of 15mL</td>
<td>dSPE.EN.GFV.50.15</td>
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<tr>
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<td>150mg MgSO₄ + 50mg PSA</td>
<td>100 tubes of 2mL</td>
<td>dSPE.AOAC.GFV.100.2</td>
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<tr>
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<td>1200mg MgSO₄ + 400mg PSA</td>
<td>50 tubes of 15mL</td>
<td>dSPE.AOAC.GFV.50.15</td>
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<tr>
<td>EN 15662</td>
<td>For Pigmented Fruits &amp; Vegetables</td>
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<tr>
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<td>150mg MgSO₄ + 25mg PSA + 2.5mg CB</td>
<td>100 tubes of 2mL</td>
<td>dSPE.EN.PFV.100.2</td>
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<td>900mg MgSO₄ + 150mg PSA + 15mg CB</td>
<td>50 tubes of 15mL</td>
<td>dSPE.EN.PFV.50.15</td>
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<tr>
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<td>150mg MgSO₄ + 50mg PSA + 50mg CB</td>
<td>100 tubes of 2mL</td>
<td>dSPE.AOAC.PFV.100.2</td>
</tr>
<tr>
<td></td>
<td>1200mg MgSO₄ + 400mg PSA + 400mg CB</td>
<td>50 tubes of 15mL</td>
<td>dSPE.AOAC.PFV.50.15</td>
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<tr>
<td>EN 15662</td>
<td>For Highly Pigmented and Fatty Fruits &amp; Vegetables</td>
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<td>150mg MgSO₄ + 25mg PSA + 7.5mg CB</td>
<td>100 tubes of 2mL</td>
<td>dSPE.EN.HPFV.100.2</td>
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<td>50 tubes of 15mL</td>
<td>dSPE.EN.HPFV.50.15</td>
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<tr>
<td>AOAC 2007.01</td>
<td>150mg MgSO₄ + 50mg PSA + 50mg CB + 50mg C18</td>
<td>100 tubes of 2mL</td>
<td>dSPE.AOAC.HPFV.100.2</td>
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<tr>
<td></td>
<td>1200mg MgSO₄ + 400mg PSA + 400mg CB + 400mg C18</td>
<td>50 tubes of 15mL</td>
<td>dSPE.AOAC.HPFV.50.15</td>
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<tr>
<td>EN 15662</td>
<td>For Fatty and waxed Fruits &amp; Vegetables</td>
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</tr>
<tr>
<td></td>
<td>150mg MgSO₄ + 25mg PSA + 25mg C18</td>
<td>100 tubes of 2mL</td>
<td>dSPE.EN.FWFV.100.2</td>
</tr>
<tr>
<td></td>
<td>900mg MgSO₄ + 150mg PSA + 150mg C18</td>
<td>50 tubes of 15mL</td>
<td>dSPE.EN.FWFV.50.15</td>
</tr>
<tr>
<td>AOAC 2007.01</td>
<td>150mg MgSO₄ + 50mg PSA + 50mg C18</td>
<td>100 tubes of 2mL</td>
<td>dSPE.AOAC.FWFV.100.2</td>
</tr>
<tr>
<td></td>
<td>1200mg MgSO₄ + 400mg PSA + 400mg C18</td>
<td>50 tubes of 15mL</td>
<td>dSPE.AOAC.FWFV.50.15</td>
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</tbody>
</table>
## POCIS PRODUCT LIST

<table>
<thead>
<tr>
<th>Designation</th>
<th>Definition</th>
<th>Composition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFFINIMIP® POCIS GLYPHOSATE</td>
<td>POCIS containing AFFINIMIP® GLYPHOSATE - AMPA for the retention of glyphosate and AMPA</td>
<td>1 POCIS Kit of 10 POCIS + empty fritted cartridges</td>
<td>POCIS.GLY.90.55.A.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kit of 50 POCIS + empty fritted cartridges</td>
<td>POCIS.GLY.90.55.kit.50</td>
</tr>
<tr>
<td>AFFINIMIP® POCIS EDC</td>
<td>POCIS containing AFFINIMIP® Estrogens and AFFINIMIP® Bisphenols for the retention of endocrine disrupters such as natural/synthetic estrogens, Bisphenols...</td>
<td>1 POCIS Kit of 10 POCIS + empty fritted cartridges</td>
<td>POCIS.EDC.90.55.A.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kit of 50 POCIS + empty fritted cartridges</td>
<td>POCIS.EDC.90.55.kit.50</td>
</tr>
<tr>
<td>Attract POCIS Pesticides</td>
<td>POCIS containing mixture of sorbent for the retention of several pesticides</td>
<td>1 POCIS Kit of 10 POCIS + empty fritted cartridges</td>
<td>POCIS.PEST.90.55.A.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kit of 50 POCIS + empty fritted cartridges</td>
<td>POCIS.PEST.90.55.kit.50</td>
</tr>
<tr>
<td>Attract POCIS HLB</td>
<td>POCIS containing Attract HLB for the retention of pharmaceutical drug residues</td>
<td>1 POCIS Kit of 10 POCIS + empty fritted cartridges</td>
<td>POCIS.HLB.90.55.A.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kit of 50 POCIS + empty fritted cartridges</td>
<td>POCIS.HLB.90.55.kit.50</td>
</tr>
<tr>
<td>CANISTER – 3 POCIS</td>
<td>Canister for 3 POCIS Requires a holder</td>
<td>1 canister</td>
<td>CAN-3P.A.1</td>
</tr>
<tr>
<td>HOLDER – 3 POCIS</td>
<td>Holder for 3 POCIS</td>
<td>1 holder</td>
<td>HOLD-3P.A.1</td>
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### On-line SPE columns – Product list

<table>
<thead>
<tr>
<th>Product</th>
<th>Product reference</th>
<th>Nber column</th>
<th>I.D. (mm)</th>
<th>Lenght (mm)</th>
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<tbody>
<tr>
<td>On-line AttractSPE™ HLB columns</td>
<td>OnlineSPE-HLB-1.2.20</td>
<td>1</td>
<td>2.1</td>
<td>20</td>
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<tr>
<td></td>
<td>OnlineSPE-HLB-1.5.20</td>
<td>1</td>
<td>4.6</td>
<td>20</td>
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<tr>
<td>On-line AFFINIMIP® PHENOLICS columns</td>
<td>OnlineSPE-PHE-1.2.20</td>
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<td>2.1</td>
<td>20</td>
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<tr>
<td></td>
<td>OnlineSPE-PHE-1.5.20</td>
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<td>4.6</td>
<td>20</td>
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<tr>
<td>On-line AFFINIMIP® ESTROGENS columns</td>
<td>OnlineSPE-EST-1.2.20</td>
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<td>2.1</td>
<td>20</td>
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<td></td>
<td>OnlineSPE-EST-1.5.20</td>
<td>1</td>
<td>4.6</td>
<td>20</td>
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</tbody>
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### SPE ACCESSORIES – Product list

<table>
<thead>
<tr>
<th>SPE Accessories</th>
<th>Designation</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manifold</td>
<td>SPE Vacuum Manifold</td>
<td>12-port model</td>
<td>ACC-MAN1</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>
NONE EXHAUSTIVE LIST OF PUBLICATIONS AND POSTERS

Be selective

- Food / Feed Safety
- Environment
- Cosmetics
- Pharmaceutical R&D
Analysis of Mycotoxins

Analysis of Endocrine Disrupting Compounds


The survey results are published by ANSES, the French Health Agency: Assessment of dietary exposure to bisphenol A in the French population with a special focus on risk characterisation for pregnant French women, Nawel Bemrah, Julien Jean, Gilles Rivière, Moez Sanaa, Stéphane Leconte, Morgane Bachelot, Yoann Deceuninck, Bruno Le Bizec, Xavier Dauchy, Alain-Claude Roudot, Valérie Camel, Konrad Grob, Cyril Feidt, Nicole Picard-Hagen, Pierre-Marie Badot, Franck Foure, Jean-Charles Leblanc, Food and Chemical Toxicology 72, 90-97 (2014).

**French Health Agency reports on Health risks assessment of BPA**

A new report of the French Health Agency (ANSES) on assessment of the health risks associated with bisphenol A (BPA) was published on 9 April 2013. Quantitative analysis of Bisphenol A in all liquid or solid food matrices were carried out by using AFFINIMIP® SPE Bisphenols (Analyses carried out by LABERCA and described in Annex 12 of Annexes of the report p132 (in French) and summary of the study).

Quantitative analysis of Bisphenol A in all liquid or solid food matrices were carried out by using AFFINIMIP® SPE Bisphenols (p132, Annex 12 of Annexes to the report on the assessment of the risks associated with bisphenol A (BPA) for human health, and on toxicological data and data on the use of bisphenols S, F, M, B, AP, AF, and BADGE (In French)), ANSES April 2013.


**Determination of Nonylphenol (NP), 4-tert-Octylphenol (t-OP) and Bisphenol A (BPA)**


Determination of 7 Bisphenol analogues (BPS, BPF, BPA, BBP, bisphenol AF (BPAF), tetrachlorobisphenol A (TCBPA) and TBBPA)


Determination of 18 Bisphenol analogues: Bisphenol B (BPP), bisphenol AP (BPAP), bisphenol AF (BPAF), bisphenol BP (BPPB), bisphenol C (BPC), bisphenol Cl2 (BPCI2), bisphenol E (BPE), bisphenol PH (BPPH), bisphenol S (BPS), bisphenol F (BPF), [4,4’-dihydroxydiphenyl ether (DHDPE), bisphenol FL (BPLF), bisphenol Z (BPZ), biphenyl-4,4’-diol (BP4,4’), bisphenol M (BPM), bisphenol P (BPP), bis-2(hydroxyphenyl)methane (BIS2) and biphenyl-2,2’-diol (BP2,2’).

Analysis of Antibiotics and Drug residues


Analysis of other residues and miscellaneous

- Preliminary study on brominated dioxins/furans and hydroxylated/methoxylated PBDEs in Baltic cod (Gadus morhua) liver. Comparison to the levels of analogue chlorinated co-occurring pollutant, M. Roszko, K. Szymczyk, M. Rzepkowska, R. Jedrzejczak, *Marine Pollution Bulletin*, 96, 165-175, 2015.
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 and for passive sampling:

<table>
<thead>
<tr>
<th>Products</th>
<th>Applications</th>
<th>Matrices</th>
<th>Technologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• SPE</td>
<td>• Sample preparation</td>
<td>• Water</td>
<td>• Molecularly imprinted polymers (MIP)</td>
</tr>
<tr>
<td>• POCIS</td>
<td>• Passive sampling</td>
<td>• Biological fluids</td>
<td>• Other modified polymers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Food and feed</td>
<td>• Modified silica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Soil</td>
<td></td>
</tr>
</tbody>
</table>

By offering you a most comprehensive portfolio of solid phase extraction products and POCIS in a 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.

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