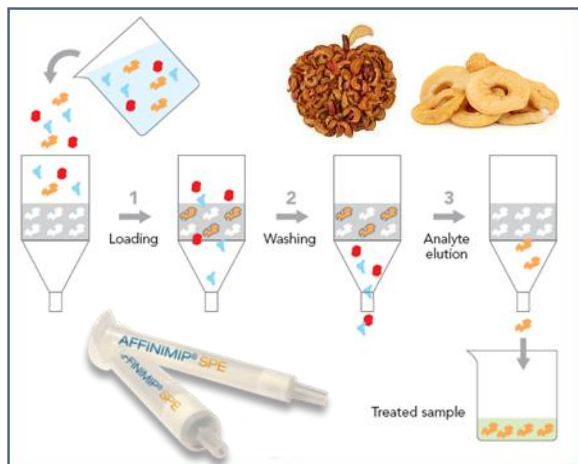


Selective Solid Phase Extraction of Patulin from Dried Apples using AFFINIMIP® SPE Patulin



Background

Patulin [4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one] is a mycotoxin produced by a variety of molds, particularly *Aspergillus* and *Penicillium* species (see figure 1). It is commonly found in rotting apples, and the amount of patulin in apple products is generally viewed as a measure of the quality of the apples used in production.

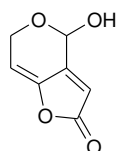


Figure 1. Chemical structure of Patulin, CAS N° 149-29-1

Studies have shown that it is genotoxic and several countries have instituted patulin restrictions in apple products. Member countries of the European Union have set maximum allowable levels of patulin European Commission Regulation (EC) 1881/2006 [1]:

- 50µg/kg in fruit juices, spirit drink and cider
- 25µg/kg in solid apple products, including apple compote, apple puree intended for direct consumption
- 10µg/kg in apple juice and solid apple products for infants and young children and in baby foods.

Due to these low levels and the presence of matrix interferences, a cleanup step is crucial to ensure an accurate quantification.

AFFINIMIP® SPE Patulin: highly selective cleanup of Patulin from complex matrices

AFFINIMIP® SPE Patulin uses a new class of intelligent polymers based on molecularly imprinted polymers

specific to Patulin ensuring extremely clean extracts for an easy quantification by all chromatography techniques.

AFFINIMIP® SPE products remove matrix components and are chemically and thermally stable, compatible with all solvents and cost-effective. For the tested matrices, the provided protocols require no further development.

High analyte recoveries in dried apple dices

The use of AFFINIMIP® SPE Patulin (6mL-200mg sorbent) enables to obtain recoveries above 90% at 10µg/kg in dried apple.

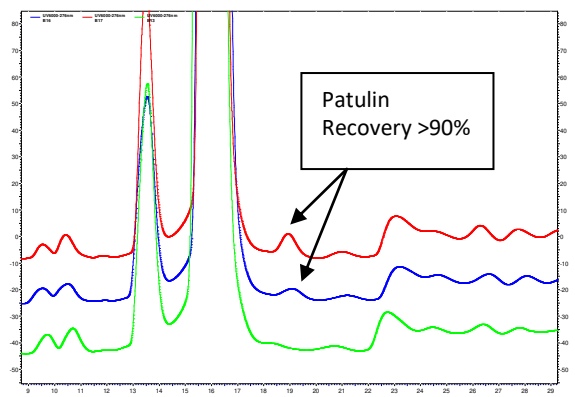


Figure 2. Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of apple dices spiked at 20µg/kg (red) or at 10µg/kg (blue) with Patulin or not spiked (green).

This method complies with the performance criteria for Patulin established by the European Commission Regulation (EC) 401/2006 [2]. The regulation requires recovery values for Patulin higher than 70% for analysis done between 20 to 50µg/kg and higher than 50% for analysis done below 20µg/kg.

Experimental conditions

Preparation of samples prior to SPE with AFFINIMIP® SPE Patulin Cartridge

Weigh 3g of dried apple dices; add 150µL of a pectinase enzyme solution followed by 30mL water and mix. Leave the 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.

Solid phase extraction (SPE) protocol for dried apple dices

The SPE procedure uses a 6mL AFFINIMIP® SPE Patulin Cartridge – 200mg (FS102-03B-200mg):

- Condition the SPE Cartridge with 4mL of Acetonitrile (ACN), then with 2mL of deionized water
- Load 10mL of the loading solution
- Wash the cartridge with 5mL of deionized water containing 2% of acetic acid
- Wash the cartridge with 5mL of deionized water
- Force the water down into the cartridge and out the bottom or apply vacuum 30 seconds
- Wash the cartridge with 500µL of diethyl ether
- Elute Patulin with 2mL of Ethyl acetate

The SPE procedure lasts approximately 30 minutes. Then the elution fraction is evaporated and dissolved in water containing 0.1% acetic acid. The evaporation time of the elution fraction is approximately 10 minutes.

Analysis

HPLC was performed on a ThermoFinnigan Spectra System with an Atlantis T3 column 150mm x 2.1mm (Waters). The separation was carried out using a gradient (see table 1) at a flow rate of 0.2mL/min:

Table 1. Gradient for the analysis of Patulin after an extraction from apple dices.

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

The detection system was a ThermoFinnigan Spectra System Model UV6000LP set to 276nm. The injection volume was 100µL.

References

[1] Commission Regulation (EC) No. 1881/2006 of 19 December 2006, Official Journal of the European Union.

[2] Commission Regulation (EC) No. 401/2006 of 23 February 2006, Official Journal of the European Union.

Ordering information

AFFINIMIP® SPE Patulin 3mL

| Catalog number | Description |
|----------------|---|
| FS102-02 | 25 cartridges 3mL/100mg |
| FS102-03 | 50 cartridges 3mL/100mg |
| FS102-02K | kit of 25 cartridges 3mL/100mg + 50mL pectinase enzyme solution |
| FS102-03K | kit of 50 cartridges 3mL/100mg + 50mL pectinase enzyme solution |

AFFINIMIP® SPE Patulin 6mL/200mg

| Catalog number | Description |
|------------------|---|
| FS102-02B-200mg | 25 cartridges 6mL/200mg |
| FS102-03B-200mg | 50 cartridges 6mL/200mg |
| FS102-02BK-200mg | kit of 25 cartridges 6mL/200mg + 50mL pectinase enzyme solution |
| FS102-03BK-200mg | kit of 50 cartridges 6mL/200mg + 50mL pectinase enzyme solution |

Reagents

| Catalog number | Description |
|----------------|---|
| REA-001-50mL | 50mL Pectinase enzyme solution |
| REA-PAT-1mL | 1mL of Patulin standard solution at 100 µg/mL in acetonitrile |