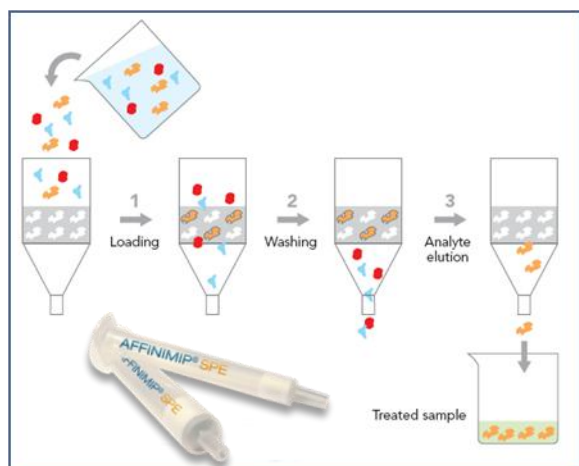


Selective Solid Phase Extraction of Patulin from fruit juice concentrates and thick juices using AFFINIMIP® SPE Patulin



Background

Patulin [4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one] is a mycotoxin produced by a variety of molds, particularly *Aspergillus* and *Penicillium species* (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.

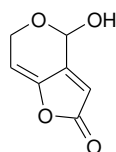


Figure 1. Chemical structure of Patulin, CAS N° 149-29-1

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, fruit juice concentrates as reconstituted and fruit nectars, 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 thick juices and fruit juice concentrates

The use of AFFINIMIP® SPE Patulin (6mL-200mg sorbent) removes most of HydroxyMethylFurfural (HMF), the main interference usually overlapping Patulin. Thanks to this removal, Patulin analysis becomes possible and reliable with recovery yields above 80%.

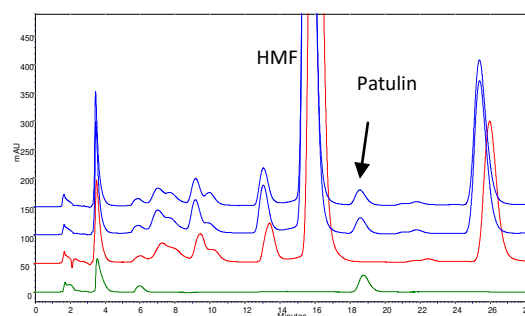


Figure 1. Chromatograms obtained after AFFINIMIP® SPE Patulin clean-up of apple mango juice spiked at 20µg/kg (blue) with Patulin or not spiked (red). In green, Patulin solution at 50ng/mL prepared by dilution of a 100µg/mL Patulin standard solution (REA-PAT-1mL) in mobile phase.

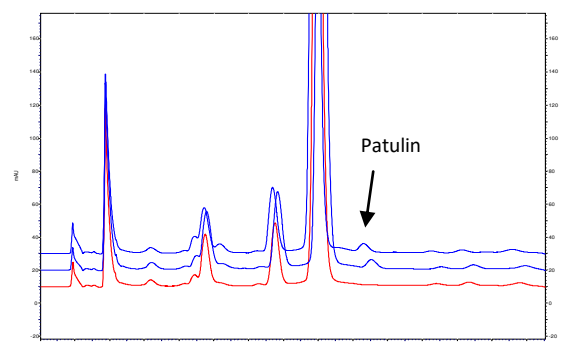


Figure 2. Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of grapefruit juice concentrate spiked at 10µg/kg (blue) with Patulin or not spiked (red).

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 fruit juice concentrate samples prior to SPE with AFFINIMIP® SPE Patulin Cartridge

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 prior to SPE with AFFINIMIP® SPE Patulin Cartridge

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.

Solid phase extraction (SPE) protocol

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 4mL of deionized water
- Load 4 to 6mL of the loading solution
- Wash the cartridge with 2mL of NaHCO₃ 1% in water
- Wash the cartridge with 4mL of deionized water
- Force the water down into the cartridge and out the bottom or apply vacuum 30 seconds
- Wash the cartridge with 1mL 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 20 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

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