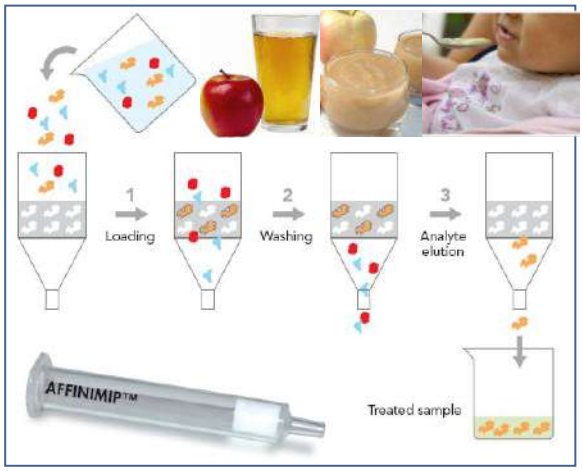
Selective Solid Phase Extraction of Patulin from Apple Products for Infants and Young Children Using Molecularly Imprinted Polymers



Introduction

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.

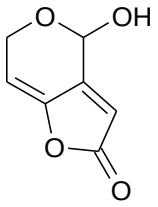


Figure 1. Chemical structure of Patulin, CAS N° 149-29-1

Studies have shown that it is genotoxic. Several countries have instituted Patulin restrictions in apple products. Member countries of the European Union have set maximum allowable levels of patulin at 10µg/kg in apple juice and solid apple products, including apple compote and apple puree, for infants and young children (European Commission Regulation (EC) 1881/2006).

Several analytical methods for the determination of Patulin have been developed in which a clean-up step is necessary and crucial. However by the use of the classical methods of clean-up, the main matrix interferent, 5-HydroxyMethylFurfural (HMF), is still present at a very high concentration, preventing a reliable quantitative Patulin determination. So there is an increasing need to improve the sensitivity and the specificity of this key step of clean-up.

To propose an accurate solution, we have developed a new class of intelligent polymers based on molecularly imprinted polymers specific to Patulin. Molecular imprinting polymer (MIP) is a synthetic material with artificially generated three-dimensional network able to specifically rebind a target molecule. MIP has the advantages to be not only highly selective and specific but also chemically and thermally stable, compatible with all solvents and cost-effective. So this polymer is used as a powerful technique for clean-up and pre concentration applications of Patulin. This study describes the solid phase extraction of Patulin from apple juice and apple puree utilizing a Molecular imprinting polymer SPE cartridge that is specific to Patulin (**AFFINIMIP® SPE Patulin**).

Experimental conditions for Apple Juice

Materials

All reagents and chemicals were ACS grade quality or better. Patulin was obtained from Sigma Aldrich (Fluka). Apple juice and apple puree were purchased in different supermarkets.

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

2.5mL of apple juice is diluted with 2.5mL of water-2% acetic acid and mixed.

Solid phase extraction (SPE) protocol for apple juice

The SPE procedure used 3mL **AFFINIMIP® SPE Patulin** Cartridges. The details of each step are as follows:

- Condition the SPE Cartridge with 2mL of acetonitrile (ACN), then with 1mL of deionized water
- Load 4mL of apple juice
- Wash the cartridge with 1mL of NaHCO3 1% in water
- Wash cartridge with 2mL of deionized water
- **Force the water down into the cartridge and out the bottom or apply vacuum 10 seconds**
- Wash the cartridge with 1mL of diethyl ether
- Elute Patulin with 2mL of ethyl acetate

The SPE procedure lasted approximately 20 minutes.
The elution fraction was then evaporated and dissolved in water containing 0.1% acetic acid.

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Analysis

HPLC was performed on a ThermoFinnigan Spectra System with an Atlantis T3 column (150mm x 2.1mm). Separation was accomplished using a mobile phase of deionized water/ACN (95/5, v/v) at a flow rate of 0.2mL/min. The detection system was a ThermoFinnigan Spectra System Model UV6000LP set to 276nm. The injection volume was 100µL.

Results

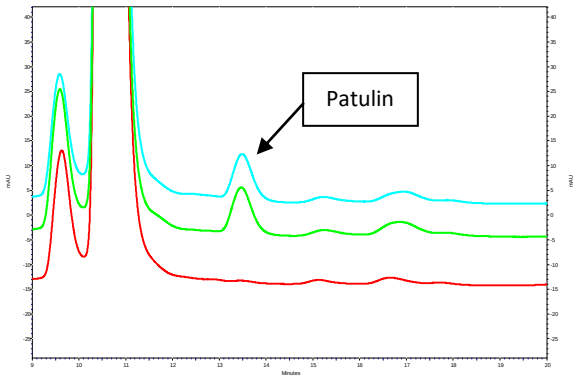

Figure 2. Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of an apple juice spiked at 10µk/kg with Patulin (Green and blue) or not spiked (Red)

Table 1. Recovery of Patulin (n=9) at a contamination level of 10µg/kg in apple Juice after **AFFINIMIP® SPE Patulin** Clean-up.

Recoveries % (n=9)	% RSD_R
97.9	11

Experimental conditions for Apple puree

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

Weigh 10g of apple puree; add 150µL of a pectinase enzyme solution followed by 10mL water and mix. 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.

Solid phase extraction (SPE) protocol for apple puree

The SPE procedure used 3mL POLYINTELL **AFFINIMIP® SPE Patulin** Cartridges. The details of each step are as follows:

- Condition the SPE Cartridge with 2mL of acetonitrile (ACN), then with 1mL of deionized water
- Load 5mL of the loading solution
- Wash the cartridge with 4mL of deionized water containing 1% of acetic acid
- Wash the cartridge with 4mL of deionized water
- **Force the water down into the cartridge and out the bottom or apply vacuum 10 seconds**
- Wash the cartridge with 1mL of chloroform (or 500µL diethyl ether)
- Elute Patulin with 2mL of ACN containing 1% acetic acid (Elution solvent may be changed by 2mL of Ethyl acetate)

The SPE procedure lasted approximately 30 minutes.
The elution fraction was then evaporated and dissolved in water containing 0.1% acetic acid.

Analysis

HPLC was performed on a ThermoFinnigan Spectra System with an Atlantis T3 column (150mm x 2.1mm). Separation was accomplished using a gradient (see table 2) at a flow rate of 0.2mL/min:

Table 2. Gradient for the analysis of Patulin after an extraction from an apple puree.

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.

Results

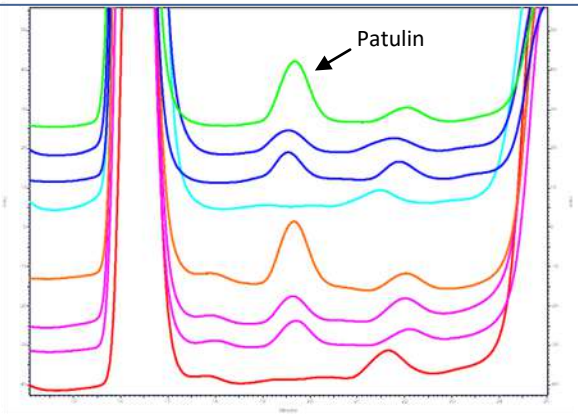

Figure 3. Chromatograms obtained after **AFFINIMIP® SPE Patulin** Clean-up of different apple puree.
In the lower part, clean-up of an apple puree from a well-known brand spiked at 25µg/kg (orange), 10µk/kg with Patulin (pink, tested twice) or not spiked (red).

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

Table 3. Recovery and repeatability of Patulin (n=4) at a contamination level of 10µg/kg in apple puree after **AFFINIMIP® SPE Patulin** Clean-up.

Recoveries % (n=4)	% RSD_R
81.2	2.1

Conclusion

The use of **AFFINIMIP® SPE Patulin** cartridge is a simple, fast, sensitive and selective tool for the extraction of Patulin from apple products.

This method complies with the performance criteria for Patulin established by the European Commission Regulation (EC) 401/2006. This regulation requires recovery values for Patulin between 50 to 120% for analysis done below 20µg/kg with reproducibility relative standard deviation lower than 40%.

The use of **AFFINIMIP® SPE Patulin** permits to obtain recoveries above 75% with reproducibility relative standard deviation lower than 20%.

This method is well-suited for the analysis of Patulin in apple products for infants and young children.

References

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

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

Related products

- **AFFINIMIP® SPE Patulin**

Catalog number: FS102-02 for 25 columns
FS102-03 for 50 columns

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