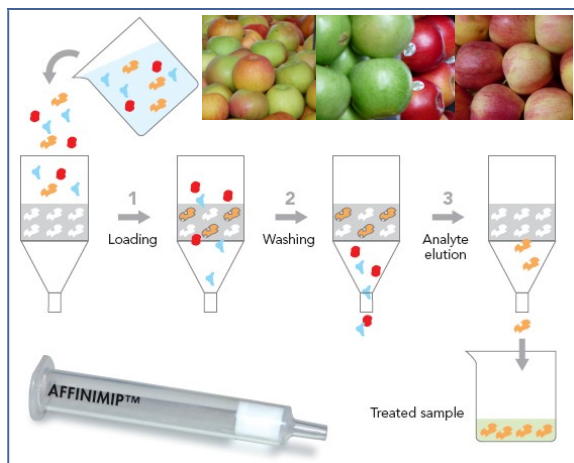


Selective Solid Phase Extraction of Patulin from Cider Using Molecularly Imprinted Polymers



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.

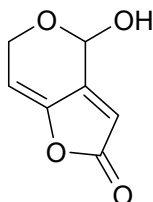


Figure 1. Chemical structure of Patulin, CAS N° 149-29-1

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 50µg/kg in fruit juices, spirit drink and cider, 25µg/kg in solid apple products, including apple compote, apple puree intended for direct consumption and 10µg/kg in apple juice and solid apple products, including apple compote and apple puree, for infants and young children and in baby foods (European Commission Regulation (EC) 1881/2006 [1]).

Several analytical methods for the determination of Patulin have been developed in which a clean-up step is necessary and crucial.

To propose an accurate solution, we have developed a new class of intelligent polymers based on molecularly imprinted polymers specific to Patulin. Molecularly Imprinted 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. 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 cider using a Molecularly Imprinted Polymer (MIP) SPE cartridge that is specific for Patulin (AFFINIMIP® SPE Patulin).

Experimental conditions for Cider

Materials

All reagents and chemicals were ACS grade quality or better. Patulin was obtained from Sigma Aldrich (Fluka). Cider was purchased at a supermarket.

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

The cider is degassed by sonicating sample for 1 hour. Then the degas cider is diluted by 2 with water containing 2% of acetic acid. This solution is mixed and used as the loading solution.

Solid phase extraction (SPE) protocol for Cider

The SPE procedure used a 3mL AFFINIMIP® SPE Patulin Cartridge. 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 the loading solution
- 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 0.5mL 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. 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 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

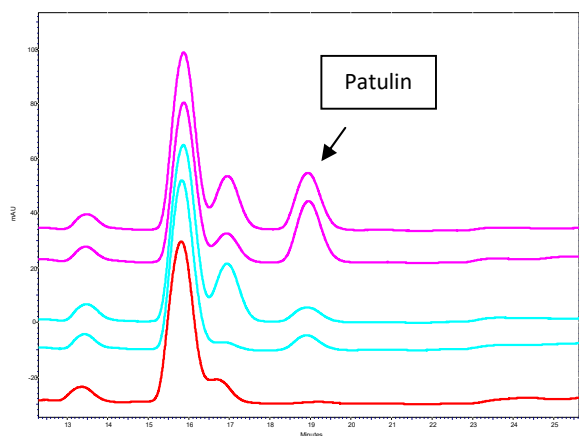


Figure 2. Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of a cider spiked at 40µg/kg (tested twice, pink) or at 10µg/kg (tested twice, blue) with Patulin or not spiked (red)

Table 1. 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.

Concentration of Patulin (ng/mL)	Recoveries %	% RSD _R
10	87.5 (n=2)	-
40	80.5 (n=5)	7.5

Conclusion

The use of an 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 [2]. This 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.

The use of AFFINIMIP® SPE Patulin enables to obtain recoveries above 80%. This method is well-suited for the analysis of Patulin in apple products.

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.

Related products

- AFFINIMIP® SPE Patulin

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