# Selective Automated Solid Phase Extraction of Patulin from Apple products Using Molecularly Imprinted Polymers

Application Note FBO311


## Keywords

Automation, Patulin, GX-271 ASPEC™, Apple, Apple Juice, Apple Puree, HPLC, Sample Preparation, Solid Phase Extraction, SPE, MIP, Molecularly Imprinted Polymers


## Introduction

*This study was performed by Polyintell - located in Val de Reuil, France*

***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* (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.

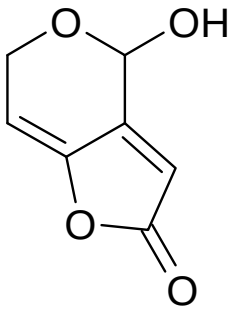


**Figure 1.** Chemical structure of Patulin, CAS N° 149-29-1.

Studies have shown that patulin is genotoxic, resulting in several countries instituting patulin restrictions in apple products. Member countries of the European Union have set maximum allowable levels of patulin at 50µg/kg in fruit juices, 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]).


**Gilson, Inc. World Headquarters**
Middleton, WI 53562 USA
Telephone: 800-445-7661

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 both sensitivity and specificity of this key step of clean-up, as well as provide an automated solution for this extraction process. This study describes the solid phase extraction of Patulin from apple juice using a Molecularly Imprinted Polymer (MIP) SPE cartridge that is specific for Patulin (AFFINIMIP® Patulin), and further automates the SPE process using the Gilson GX-271 ASPEC™.

To propose an accurate solution, a new class of intelligent polymers based on molecularly imprinted polymers specific to Patulin was used. 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.

**Figure 2.** Gilson GX-271 ASPEC™ System with 406 Syringe Pump (part no. 2614007).

## Automated Experimental conditions for Apple Juice on a Gilson GX-271 ASPEC

### *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® 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 a 3mL POLYINTELL AFFINIMIP® Patulin Cartridge that were sealed with Gilson polypropylene caps prior to automation. The details of each step are as follows:

- Condition the SPE Cartridge with 2mL of Acetonitrile (ACN), then with 1mL of deionized water with a flow of 1mL/min
- Load 4mL of the loading solution at a flow rate of 0.5mL/min
- Wash the cartridge with 1mL of $NaHCO_3$ 1% in water at a flow rate of 1mL/min
- Wash cartridge with 2mL of deionized water at a flow rate of 1mL/min, using an air push of 1000 uL to force the water out the bottom of the cartridge
- Wash the cartridge with 500µL of diethyl ether at a flow rate of 1mL/min
- Elute patulin with 2mL of ethyl acetate at a flow rate of 0.8mL/min

The SPE procedure lasted approximately 35 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.

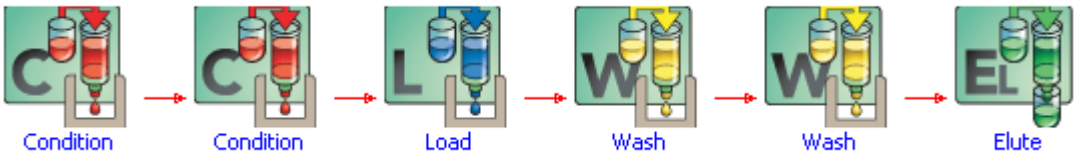


**Figure 3.** TRILUTION® LH Basic SPE Tasks for Solid Phase Extraction of Patulin from Apple Juice

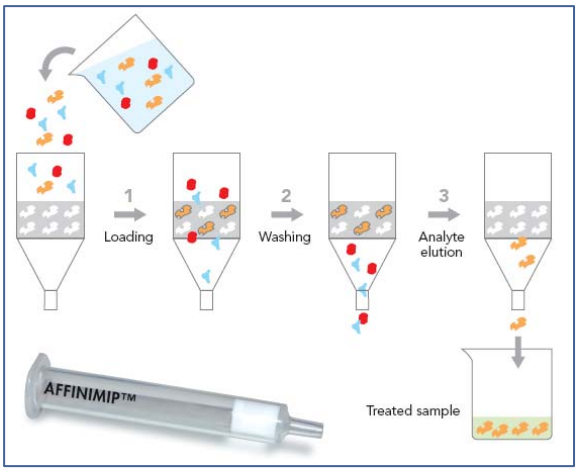


**Figure 4.** AFFINIMIP® Patulin Clean-up Process.

## 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

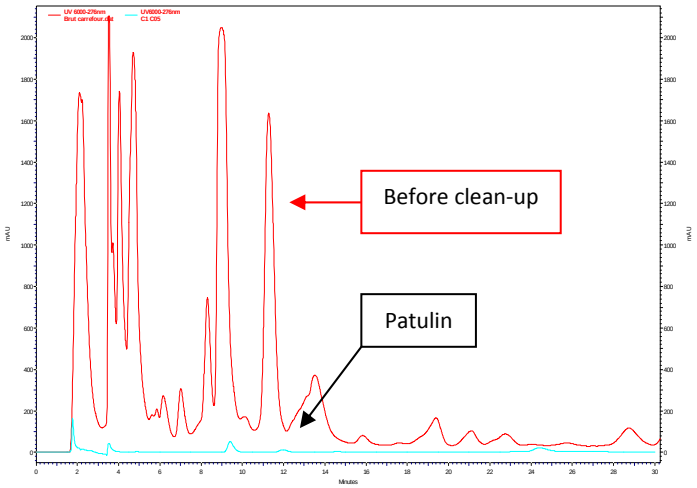


**Figure 5.** Chromatograms of apple juice containing 25 µg/kg of Patulin before (Red) and after (Blue) AFFINIMIP® Patulin Clean-up.

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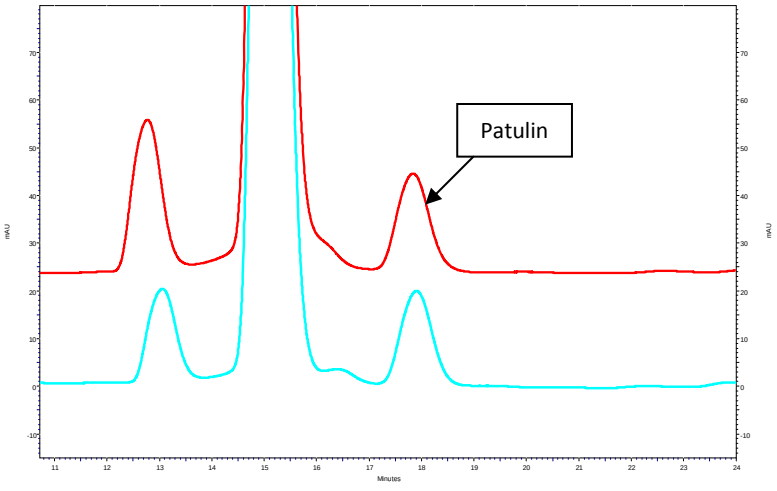


**Figure 6.** Chromatograms obtained after AFFINIMIP® Patulin Clean-up of an apple juice spiked at 40µg/kg (tested twice, red and blue) using a Gilson GX-271 ASPEC™.

**Table 1.** Recovery of Patulin at a contamination level of 40µg/kg in apple juice after AFFINIMIP® Patulin Clean-up using the GX-271 ASPEC.

| Concentration of Patulin (ng/mL) | Recoveries % | % $RSD_R$ |
|---|---|---|
| **40** **(n=2)** | 80 | NA |

## Conclusion

The use of an AFFINIMIP® Patulin SPE 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® Patulin enables to obtain recoveries at 80%, which is above the required level set by the European commission. This method is well-suited for the analysis of patulin in apple products. In addition, this protocol is easily automated with the Gilson GX-271 ASPEC™.

## 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.