# ASPEC® 271 System: Automated Extraction of Patulin from Apple Products

## APPLICATION NOTE AN0988

### APPLICATION BENEFITS

Mycotoxins are toxic compounds produced by fungi that are harmful to humans and animals. Patulin, found in rotting apples, is a compound that is regulated in many countries. Food safety testing for patulin is crucial to limit exposure; however, many sample cleanup methods do not remove closely related compounds, which can lead to false positive results.

### SOLUTIONS

We automated a solid phase extraction (SPE) sample cleanup method using AFFINIMIP® cartridges packed with molecularly imprinted polymers (MIPs). This cleanup method isolates patulins from apple juice while removing interfering compounds. The automated method using a Gilson ASPEC® 271 System is reproducible, can run unattended, and can be applied to many types of apple products.

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## INTRODUCTION

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, that is commonly found in rotting apples. The patulin content is routinely used as a measure of the quality of the apples used in production. 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 from 50 µg/kg to 25 µg/kg depending on the food item, with a lower limit of 10 µg/kg for products intended for consumption by infants [European Commission (EC) regulation 1881/2006].[1]

Several analytical methods have been developed for the determination of patulin in which a sample cleanup step is necessary and crucial. However, after classical methods of sample clean-up the main matrix interferent, 5-Hydroxymethylfurfural (HMF), is still present at a very high concentration, preventing a reliable quantitative Patulin determination.

As food safety concerns continue to rise, there is an increasing need to improve both sensitivity and specificity of this key step of cleanup, as well as provide an automated solution for this extraction process. To accomplish these goals, we carried out solid phase extraction of patulin from apple products using a molecularly imprinted polymer (MIP) SPE cartridge that is specific for patulin (AFFINIMIP Patulin), and automated the SPE process using the Gilson ASPEC 271 System (Figure 1).[2]

**Figure 1**
ASPEC® 271 System

AFFINISEP

GILSON®

## MATERIALS AND METHODS

### Materials

All reagents and chemicals were ACS grade-quality or better. Patulin was obtained from Sigma Aldrich (Fluka). Apple juice was purchased in local supermarkets. Some samples were spiked with patulin to achieve a final concentration of 25 µg/kg or 40 µg/kg to allow determination of %recovery.

### Solid Phase Extraction (SPE) Protocol for Apple Juice

Prior to the procedure, 2.5 mL of apple juice was diluted with 2.5 mL of 2% (v/v) acetic acid in water and mixed. The automated SPE procedure used 3 mL Affinisep AFFINIMIP® Patulin cartridges sealed with Gilson polypropylene caps. The details of the automated method, carried out on a ASPEC 271 System controlled by TRILUTION® LH Software are as follows:

1. Condition the SPE Cartridge with 2 mL of acetonitrile (ACN), then with 1 mL of deionized water with a flow of 1 mL/min
2. Load 4 mL of the loading solution at a flow rate of 0.5 mL/min
3. Wash the cartridge with 1 mL of Na-$HCO_3$ 1% in water at a flow rate of 1 mL/min
4. Wash the cartridge with 2 mL of deionized water at a flow rate of 1 mL/min, using an air push of 1000 µL to force the water out the bottom of the cartridge
5. Wash the cartridge with 500 µL of di-ethyl ether at a flow rate of 1 mL/min
6. Elute patulin with 2 mL of ethyl acetate at a flow rate of 0.8 mL/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 was approximately 10 minutes.

### Analysis

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

## RESULTS AND DISCUSSION

In this application note we demonstrate automated solid phase extraction sample cleanup using cartridges packed with 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, shown in Figure 2.

The sample cleanup method was automated on an ASPEC 271 System controlled by TRILUTION LH. The ASPEC system enables unattended sample cleanup and employs positive pressure SPE to generate reproducible results. The automated method is outlined in Figure 3.

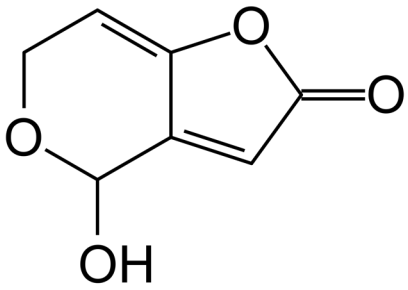


**Figure 2**
Chemical structure of Patulin, CAS N° 149-29-1


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

A schematic showing the patulin cleanup process using AFFINIMIP cartridges is shown in Figure 4.

HPLC analysis was used to measure patulin levels in samples. Figure 5 compares chromatograms of samples of apple juice (spiked with 25 µg/kg patulin) before and after AFFINIMIP Patulin cleanup. Figure 6 shows two post-cleanup chromatograms of apple juice samples that had been spiked with 40 µg/kg patulin; the X axes of the two chromatograms have been offset to accentuate the the reproducibility of the results obtained using this method.

This method complies with the performance criteria for patulin established by the EC regulation 401/2006.[3] This regulation requires recovery values for patulin higher than 70% for analysis done between 20 to 50 µg/kg and higher than 50% for analysis done below 20 µg/kg. As shown in Table 1, this automated method of AFFINIMIP Patulin cleanup of apple juice spiked at 40 µg/kg achieved 80% recovery, which is above the required level set by the EC.

In conclusion, this method is well suited for the analysis of patulin in apple products. In addition, this protocol is easily automated with the Gilson ASPEC 271. The Affinimip SPE system has been adopted by more than 50 customers, mainly in industrial QC labs, in 20 different countries.[4] The performance of the AFFINIMIP Patulin cartridges has been confirmed and expanded using a variety of apple products and concentrations of spiked patulin.

## CONCLUSIONS AND BENEFITS

- The use of an AFFINIMIP Patulin SPE cartridge is a simple, fast, sensitive and selective tool for the extraction of patulin from apple products.
- The SPE cleanup method employing AFFINIMIP Patulin cartridges was automated using ASPEC 271 providing reproducible results.
- Recovery of patulin from apple juice was 80%, well above the required level set by the EC.

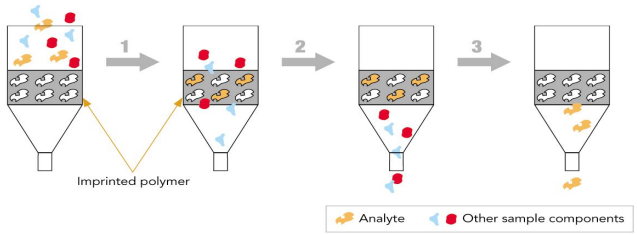


**Figure 4**
AFFINIMIP® Patulin Cleanup Process

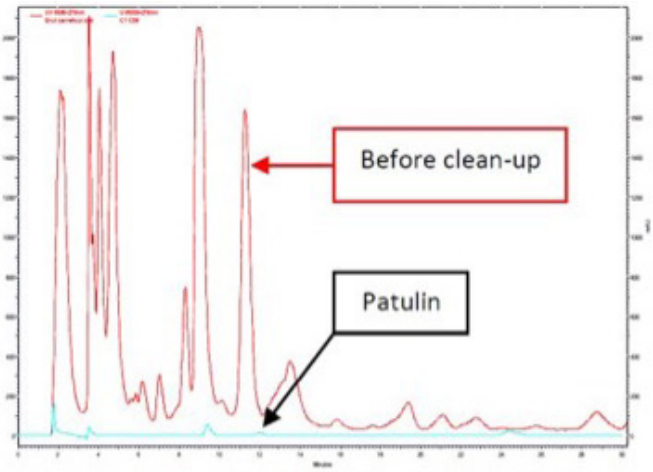


**Figure 5**
Chromatograms of apple juice containing 25 µg/kg of patulin before (Red) and after (Blue) AFFINIMIP® Patulin Clean-up

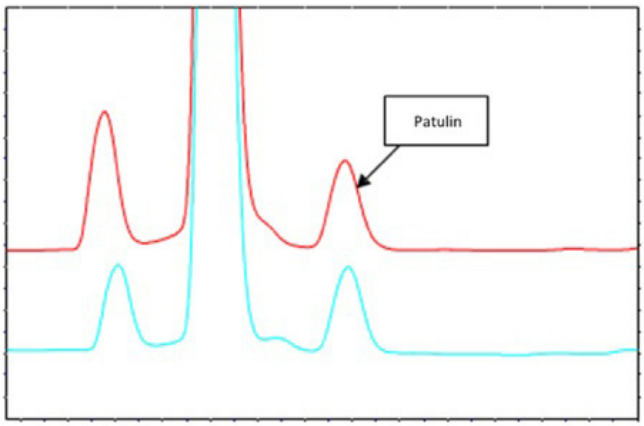


**Figure 6**
Chromatograms obtained after AFFINIMIP® Patulin cleanup of an apple juice spiked at 40 µg/kg (tested twice, red and blue) using an ASPEC® 271 System

**Table 1**
Recovery of Patulin at a contamination level of 40 µg/kg in apple juice after AFFINIMIP® Patulin Cleanup using the ASPEC® 271 System

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

## REFERENCES


1. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Official Journal of the European Union, http://data.europa.eu/eli/reg/2006/1881/oj
2. Gilson Guide to SPE Automation. https://www.gilson.com/pub/static/frontend/Gilson/customtheme/en_US/images/docs/SPE_BKLT_LT303139-05.pdf
3. Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs, Official Journal of the European Union, http://data.europa.eu/eli/reg/2006/401/oj.
4. https://www.affinisep.com/media/affinimip_spe_patulin__093470500_1233_24012013.pdf




## ORDERING INFORMATION

| Description | PN | QTY |
|---|---|---|
| GX-271, ASPEC, WITH VERITY 4060 SINGLE | 2614007 | 1 |
| SYRINGE, 10 mL | 25025345 | 1 |
| PLUMB PKG,GX241/GX271 ASPEC 10 mL | 2644701 | 2 |
| PROBE, NON-SP 221X1.5X1.1, BVTAPER | 27067374 | 1 |
| RINSE STATION,GX 175 mm | 26034551 | 1 |
| RINSE STATION,GX 175 mm FC | 26034555 | 1 |
| GDE ASSY,1.5MM ASPEC PROBE GX-271 | 26046228 | 1 |
| PLUMB PKG,GX241/GX271 ASPEC AIR/GAS | 2644703 | 1 |
| SPE PRESSURE REGULATOR ASSY 0-30 psi | 25051376 | 1 |
| SHIELD ASSEMBLY, GX27X | 2604706 | 1 |
| LOCATOR 5 20 SERIES, GX-271 | 26041033 | 1 |
| RACK,CODE 343 80 13x100 mm TUBES | 260440025 | 1 |
| DEC ACCY SET, 3 mL GX ASPEC | 2604702 | 1 |
| TRILUTION LH 4.0 LICENSE, LIFETIME | 21063024 | 1 |
| GILSON SOFTWARE, USB | 21060005 | 1 |