

## Better Tools for Fast and Convenient Patulin Analysis

Analytical Standards and **NEW** SupelMIP® SPE Cartridges

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The mycotoxin patulin is often found in rotten apples. It is mostly produced by *Aspergillus*, *Penicillium* and *Byssoschlamys* and shows a variety of short-term and long-term effects in animal studies, ranging from gastrointestinal problems and neurotoxicity to genotoxicity and teratogenicity. Therefore, many countries set limits for patulin content in apple products. For a precise detection of patulin, Sigma-Aldrich offers **NEW** SPE cartridges for purification and a comprehensive range of analytical standards.

### Extraction of Patulin with Molecular Imprinted Polymer

Historically, analytical methods for patulin have employed liquid-liquid extraction (LLE) followed by HPLC separation with UV detection at 276 nm. Researchers have highlighted problems with these methodologies, including:

- Tedious sample preparation associated with liquid-liquid extraction
- Patulin instability in alkaline conditions resulting from sodium carbonate clean-up
- The requirement of extra clean-up or chromatographic method development to prevent the co-elution of patulin and the interfering matrix component 5-hydroxymethylfurfural (HMF).

Therefore, a quick, simple and robust sample preparation method for patulin analysis is needed. To propose an accurate solution, Supelco has developed a method based on the technology of molecularly imprinted polymers. These SupelMIP® SPE Patulin extraction cartridges selectively clean and concentrate patulin prior to analysis by HPLC.

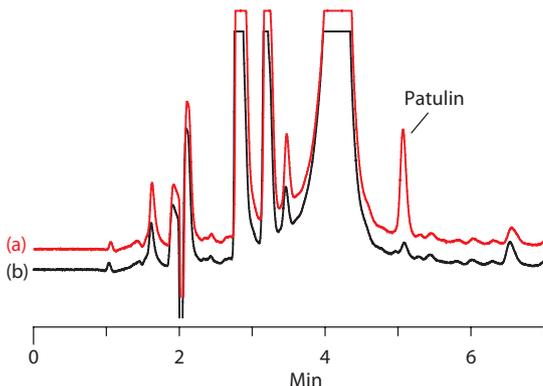
The process to extract patulin from apple juice is described below.

### SPE Procedure for Patulin Extraction and Cleanup

1. Dilute apple juice in a 1:1 ratio with 2% acetic acid.
2. At a drop rate of 1–2 drops per second, condition the cartridge with 2 mL of acetonitrile and 1 mL de-ionized water.
3. Load 4 mL of the pre-treated sample into the cartridge.
4. At a flow rate of 0.5–1 mL/minute, wash with 1 mL 1% sodium bicarbonate solution and 2 mL de-ionized water. Apply a strong vacuum (–0.8 bar or –20" of in Hg) for 10 seconds after the wash steps to dry the SPE tube.
5. Pulling a strong vacuum, elute the analyte with 0.5 mL diethyl ether and 2 mL ethyl acetate.
6. Evaporate the eluted samples to dryness, and reconstitute in 1 mL of 0.1% acetic acid in water

This MIP-phase SPE procedure yielded high analyte recovery with excellent reproducibility. The average recovery of patulin was calculated to be 84% with a relative standard deviation (RSD) of 2%. As seen in **Figure 1**, chromatographic analysis showed that no direct interferences with patulin detection were observed. Unlike LLE procedures, the clean-up procedure using SupelMIP SPE Patulin successfully removed HMF and other common interfering components from the final extract. As a result, patulin was easily detectable in apple juice at concentrations of 50 ng/mL.

Column: Ascentis Express C18, 15 cm x 2.1 mm,  
2.7 µm particles (53825-U)  
Mobile Phase: (A) 95:5 water:acetonitrile; (B) 100% acetonitrile  
Gradient: Hold at 100% A for 6 min; 0% to 80% B in 0.1 min;  
hold at 80% B for 3 min, 80% to 0% B in 0.1 min,  
hold at 100% A for 13 min  
Flow Rate: 0.2 mL/min.  
Column Temp.: 30 °C  
Detector: UV (276 nm)  
Injection: 10 µL

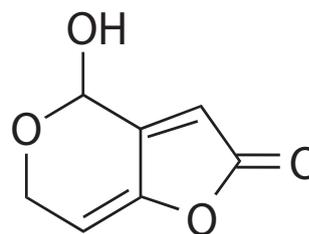


**Figure 1** Chromatogram of apple juice after SPE clean-up  
(a) spiked at 50 ng/mL with patulin and  
(b) with no patulin spike.

### Analytical Standards for Mycotoxin Analysis

For precise quality control of patulin in food and feed, we provide our customers with analytical neat standards and standard solutions. We also offer a stable, non-radioactive fully <sup>13</sup>C-isotopically labeled version of patulin, with the same physiochemical and chromatographic behaviors (**Figure 2**). This internal standard accounts for variations during sample preparation, clean-up and ionization.

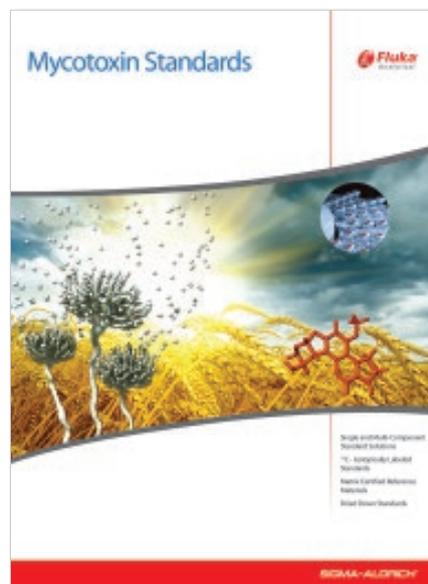
In addition to our patulin standards (**Table 1**), we offer a comprehensive range of standards for the precise detection of all regulated mycotoxins. Our portfolio includes mixture solutions, <sup>13</sup>C-isotopically labeled standards, certified reference materials (CRM) and dried down standards. For a complete product list, visit us at [sigma-aldrich.com/mycotoxins](http://sigma-aldrich.com/mycotoxins), or order our brochure (**Figure 3**) at [sigma-aldrich.com/analytical](http://sigma-aldrich.com/analytical).



**Figure 2** Molecular Structure of Patulin

### Did you know?

We offer valuable products for the entire mycotoxin analysis workflow. For more information, please order our mycotoxin analysis workflow brochure at [sigma-aldrich.com/analytical](http://sigma-aldrich.com/analytical)



**Figure 3** Mycotoxin Standards Brochure

Cat. No.	Brand	Description	Package Size
<b>SPE Column</b>			
52776-U	Supelco	SupelMIP® SPE Patulin	volume 3 mL, 50 EA
<b>HPLC Column</b>			
53825-U	Supelco	Ascentis® Express C18, 2.7 Micron HPLC Column	1 EA
<b>Analytical Solvents</b>			
34998	Sigma-Aldrich	Acetonitrile, CHROMASOLV® Plus, for HPLC, ≥99.9%	1 L, 6x1 L, 2.5 L, 4x2.5 L, 7 L, 18 L, 45 L
309966	Sigma-Aldrich	Diethyl Ether, CHROMASOLV®, for HPLC, ≥99.9%	100 mL, 1 L, 6x1 L, 25 L
650528	Sigma-Aldrich	Ethyl Acetate, CHROMASOLV® Plus, for HPLC, ≥99.9%	1 L
<b>Analytical Standards</b>			
32759	Fluka	Patulin	5 mg
34127	Fluka	Patulin, 100 µg/ml in acetonitrile	2 mL
46914-U	Supelco	Patulin, 100 µg/mL in chloroform	1 mL
35516	Fluka	Patulin- <sup>13</sup> C <sub>7</sub> , 25 µg/mL in acetonitrile	1.2 mL

**Table 1** Featured Products for Patulin Analysis