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 Byssochlamys 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

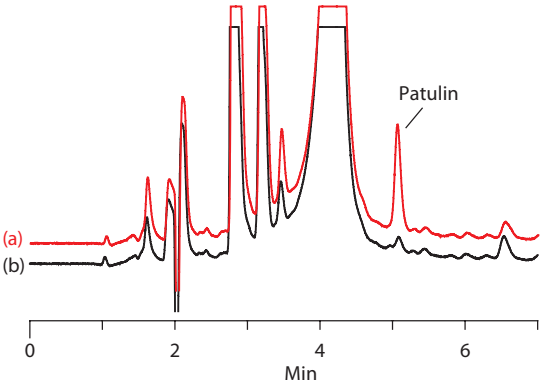

Figure 1 Chromatogram of apple juice after SPE clean-up (a) spiked at 50 ng/mL with patulin and (b) with no patulin spike.

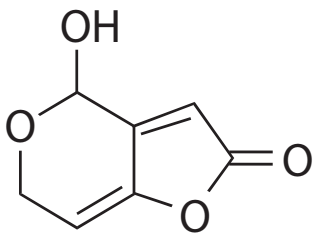

Figure 2 Molecular Structure of Patulin

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**

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 ^{13}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, ^{13}C-isotopically labeled standards, certified reference materials (CRM) and dried down standards. For a complete product list, visit us at **sigma-aldrich.com/mycotoxins**, or order our brochure (**Figure 3**) at **sigma-aldrich.com/analytical**.

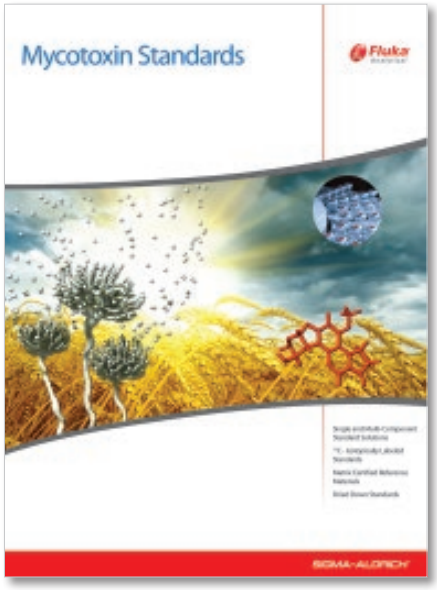

Figure 3 Mycotoxin Standards Brochure

Cat. No.	Brand	Description	Package Size
SPE Column			
52776-U	Supelco	SupelMIP® SPE Patulin	volume 3 mL, 50 EA
HPLC Column			
53825-U	Supelco	Ascentis® Express C18, 2.7 Micron HPLC Column	1 EA
Analytical Solvents			
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
Analytical Standards			
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-$^{13}C_7$, 25 µg/mL in acetonitrile	1.2 mL

Table 1 Featured Products for Patulin Analysis