

HPLC Analysis of Patulin in Apple Juice

*Patulin, a mycotoxin produced by the apple rotting fungus *Penicillium expansum* and other molds, is of concern to producers of apple juice, apple cider, and other fruit juices. Juice samples were extracted according to a procedure described in Association of Official Analytical Chemists Method 974.18: Patulin in Apple Juice. When analyzed by HPLC, on a 25cm x 4.6mm SUPELCO SIL LC-18 column (5µm packing particles), in an acetonitrile:deionized water mobile phase (5:95) at a flow rate of 1.5mL/minute, patulin was eluted in approximately 6.5 minutes, well separated from other components of the extracts. Supelco can supply the chromatography column, the patulin reference standard, and associated apparatus needed for the sample preparation and analysis.*

Key Words:

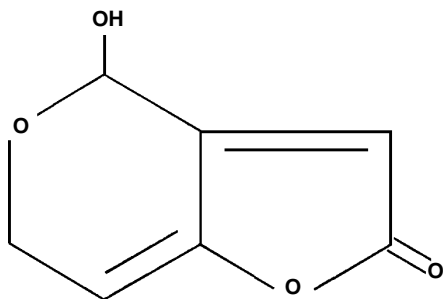
- mycotoxin • patulin • fruit juice • apples

Patulin (Figure A) is a mycotoxin produced by several molds, including *Penicillium*, *Aspergillus*, and *Bissochlamys* species. Patulin has been found in a number of foods, most often in apple products. The apple rotting fungus *Penicillium expansum* produces this toxin, making it a concern in apple products such as apple juice (1).

In tests with laboratory animals, patulin has shown teratogenic, carcinogenic, and mutagenic activity. No data are available concerning the toxicity of patulin in humans, but some governments (e.g., Switzerland, Belgium, Sweden, Norway) have established a maximum limit of 50µg/liter in apple juice (2).

Figure A. Chemical Structure of Patulin

CAS No. 149-29-1



796-0019

Although patulin is removed from juices during the fermentation process, common processes used to produce fruit juices decrease patulin levels by only about 20%. Consequently, a variety of methods have been studied to rapidly determine the presence of this mycotoxin in juices. The majority of these methods employ an extraction step, normally using ethyl acetate, followed by HPLC separation by reversed phase chromatography.

Supelco offers a complete selection of products needed for the analysis for patulin, including a quantitative standard, 100µg/mL patulin in chloroform. The standard is prepared and tested, using HPLC, according to guidelines established in *Method 974.18: Patulin in Apple Juice* in the *Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) (3)*.* As specified in the AOAC methodology, patulin standards are monitored at their maximum absorption wavelength, 275nm, and concentrations are calculated using the formula:

$$\mu\text{g patulin/mL} = \frac{(\text{absorbance}) (\text{mol. wt.}) (1000) (\text{dilution factor})}{\text{molar absorptivity in chloroform}}$$

where the molecular weight of patulin = 154 and molar absorptivity in chloroform = 14,600.

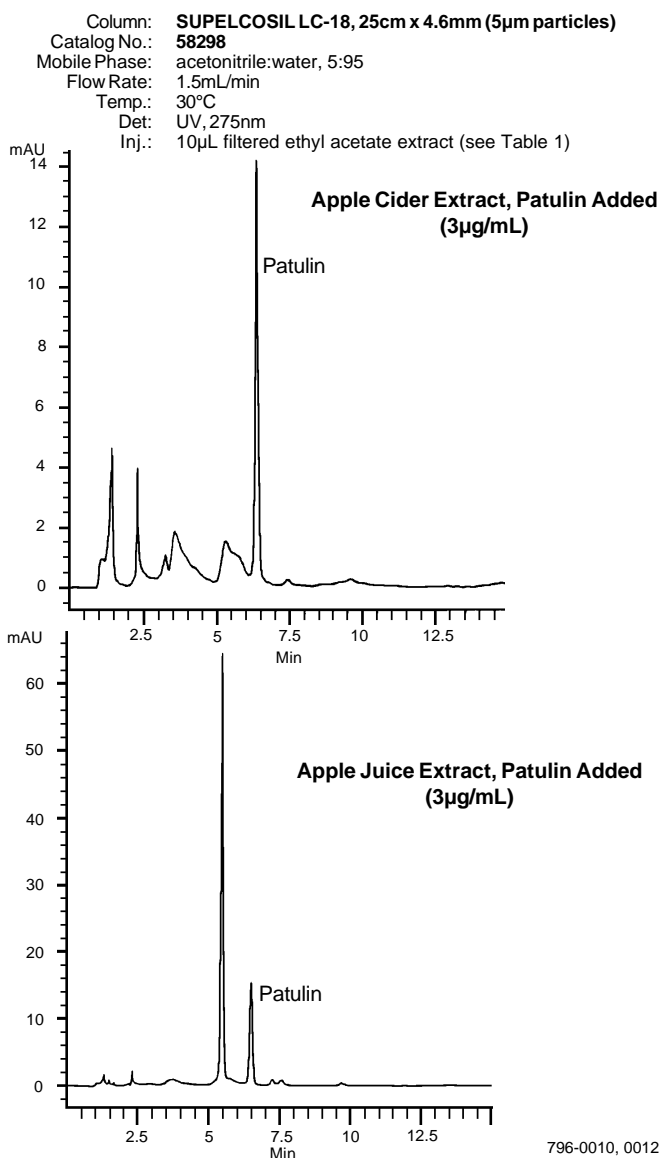
Extensive quality assurance and stability testing ensure that the new standard is of the highest quality. A certificate of analysis accompanies each ampul, indicating the purity and analytical concentration of the lot of material from which the ampul was prepared. Using this standard and a SUPELCO SIL™ LC-18 HPLC column, apple juice and a variety of other juices can be rapidly screened for patulin.

Supelco chemists obtained samples of apple juice and apple cider from local supermarkets, and extracted aliquots of each according to the AOAC extraction procedure, as summarized in Table 1. Extracted juice samples and patulin standards were analyzed by HPLC, using a 25cm x 4.6mm SUPELCO SIL LC-18 column (5µm packing particles) and an acetonitrile:deionized water mobile phase (5:95) at a flow rate of 1.5mL/minute. Under these conditions, patulin, if present, was eluted in approximately 6.5 minutes (Figure B). For the purposes of this project, patulin was added to the final extracts of apple juice and apple cider, at a concentration of 3µg/mL. Extraction efficiencies for the AOAC extraction procedure, and identification of other peaks in the extracts, will vary among samples and laboratories, and must be evaluated by each analyst.

In addition to the patulin standard, we offer other mycotoxin standards, including domoic acid, ochratoxins A and B, and several aflatoxins. For information about these standards, refer to the current Supelco catalog, or contact your Supelco distributor.

Table 1. Extraction of Patulin from Fruit Juice

1. Transfer 50g juice or cider to a 250mL separatory funnel.
2. Add 50mL ethyl acetate. Shake for 1 minute. Allow layers to separate.
3. Remove bottom (juice) layer. Re-extract 2 additional times (step 2). Combine ethyl acetate (top) layers (150mL total) in a 250mL beaker.
4. Add 20g anhydrous sodium sulfate (Na_2SO_4) to the ethyl acetate extracts. Allow to stand for 30 minutes.
5. Decant dried ethyl acetate into a new container and evaporate to <25mL on a steam bath under a gentle stream of clean nitrogen.
6. Cool to room temperature and adjust volume to 25.0mL with fresh ethyl acetate.
7. Prior to injection, filter aliquots through a syringe-tip filter (0.45 μm pores).

Figure B. Patulin in Fruit Juice and Cider**Ordering Information:**

Description	Cat. No.
SUPELCO SIL LC-18 HPLC Column 25cm x 4.6mm ID, 5 μm particles	58298
Patulin Standard 100 $\mu\text{g}/\text{mL}$ in chloroform, 1mL	46914-U
Syringe-Tip Filters nylon, 25mm, 0.45 μm pores, pk of 50	59230-U

References

1. Bartolome, B., M.L. Bengoechea, F.J. Perez-Illzarbe, T. Hernandez, I. Estrella, and C. Gomez-Cordoves, *J. Chromatogr. A* **644**: 39-43 (1994).
2. Brackett, R.E. and E.H. Marth in *Liquid Chromatographic Analysis of Food and Beverages* (G. Charalambous, ed.), Academic Press, New York 1979, p.499.
3. Method 974.18 in *Official Methods of Analysis of the Association of Official Analytical Chemists* (16th edition). Order from AOAC International, 481 North Frederick Avenue, Suite 500, Gaithersburg, Maryland 20877-2504 USA. Tel.: +1-301-924-7077; FAX: +1-301-924-7089.

*AOAC Method 974.18 describes analysis by thin-layer chromatography. We evaluate the patulin standard using some of the same criteria (e.g., absorbance wavelength), but by a more demanding HPLC analysis.

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