

RAPID DETERMINATION OF PATULIN IN APPLE JUICE

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BACKGROUND

Patulin is a mycotoxin produced by several species of *Penicillium*, *Aspergillus* and *Byssoschlamys* molds that may grow on a variety of foods including grains, fruits and cheese. Of the fungi capable of producing patulin, *penicillium expansum* is probably the most commonly encountered specimen and is often isolated from decaying apples. The presence of residual patulin in apple juice serves as an indicator of the quality of fruit used in its production since an appreciable concentration of the mycotoxin remains in product after processing (Gökmen and Acar, 1996¹; Gökmen and Acar, 1999², Gökmen et al., 2005³).

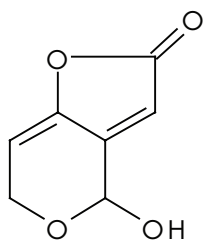


Figure 1. The chemical structure of patulin.

Owing to its toxicity, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) have established a provisional maximum tolerable daily intake for patulin of 0.4 mg/kg body weight/day. Due to concern for human health, especially amongst children (who consume higher amounts of apple juice relative to their body weight than other age groups), health authorities in many countries regard patulin contamination of foods as a problem. The World Health Organization recommends limiting its content in foods to 50 mg/l or mg/kg (WHO, 1995)⁴. Based upon an independent study, the US FDA “supports a 50 µg/kg action level for patulin in apple juice, apple juice concentrates, and apple juice products.”⁵ The European Commission also established this same action level in Commission Recommendation 2003/598/EC of 11

August 2003 concerning the prevention and reduction of patulin contamination in apple juice and apple juice ingredients in other beverages.

Several analytical methods based on high-performance liquid chromatography (HPLC) have been developed for detecting and quantifying patulin in apple juice. This report describes an improved analytical method for the identification of patulin in apple juice. It entails solid phase extraction/cleanup of patulin using Oasis® HLB and its chromatographic separation using XBridge™ C₁₈ column.

EXPERIMENTAL

Sample Preparation

A commercial HLB cartridge was conditioned by passing 1 mL of methanol and equilibrated by passing 1 mL of water at a flow rate of approximately two drops per second using a plastic syringe. 1.0 mL of single strength apple juice was eluted through the preconditioned cartridge at a flow rate of approximately one drop per second using a plastic syringe and the eluate was discarded. Then the cartridge was dried under the gentle stream of nitrogen. Patulin was eluted from the cartridge by passing 1 mL of diethyl ether at a flow rate of approximately one drop per second using a plastic syringe. The eluate was collected in a glass vial. The solvent was evaporated completely under the gentle stream of nitrogen. The residue was dissolved in 200 µL of 0.1% formic acid in water. 20 µL of the sample was injected onto the HPLC system.

| HPLC Conditions | |
|-----------------------|--|
| Column: | XBridge C ₁₈ , 4.6 x 100 mm, 3.5 µm |
| Mobile Phase: | 0.1% formic acid and acetonitrile (95:5, v/v) |
| Flow Rate: | 0.75 mL/min |
| Temperature: | 25 °C |
| Detection Wavelength: | 276 nm |
| Wavelength: | 240-340 nm |

**Sample Preparation**

- Condition an Oasis HLB cartridge (1 mL, 30 mg)
 - a. Elute 1 mL methanol
 - b. Elute 1 mL water
- Elute 1 mL apple juice at a rate one drop per second, discard eluate
- Dry cartridge under N₂ stream
- Elute 1 mL ethyl ether at a rate one drop per second, collect eluate in a glass vial
- Evaporate ethyl ether to complete dryness under N₂ stream
- Dissolve residue in 0.1% formic acid in water

HPLC Analysis

- Inject sample into XBridge C₁₈ column (4.6 x 100 mm, 3.5 µm)
 - a. Mobile Phase: the mixture of 0.1% formic acid:acetonitrile (95:5, v/v)
 - b. Flow Rate: 0.75 mL/min at 25 °C
 - c. Detection: 276 nm

Figure 2. Complete scheme for simple and rapid analysis of patulin in apple juice using Oasis HLB based sample preparation and XBridge based chromatographic separation.

RESULTS AND DISCUSSION

Hydroxymethylfurfural has been identified as one of the important interfering compounds during the chromatographic analysis of patulin in apple juice. Therefore, any chromatographic method used for this analysis must be capable of resolving patulin from hydroxymethylfurfural. In this method, patulin can be completely resolved from hydroxymethylfurfural using an XBridge C₁₈ column with an isocratic mixture of 0.1% formic acid in water and acetonitrile (95:5, v/v) at a flow rate of 0.75 mL/min (Figure 3).

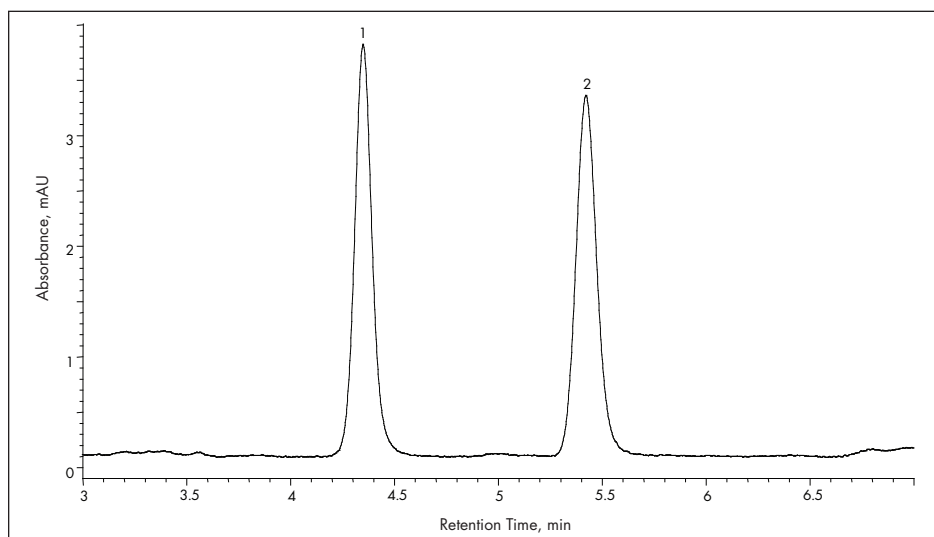


Figure 3. Separation of hydroxymethylfurfural (0.2 µg/mL, peak # 1) and patulin (0.2 µg/mL peak # 2) on XBridge C₁₈ column.

As illustrated in Figure 4, patulin could be completely resolved from the matrix co-extractives of apple juice under the chromatographic conditions applied here. Oasis HLB based extraction/cleanup of apple juice prior to chromatographic separation was found to be very useful as a sample preparation treatment. Apple juice was eluted through the preconditioned cartridge. Doing so, phenolic compounds and patulin were retained in the cartridge. Since the final extract was clean enough and the chromatograms with the least interfering peaks were obtained by using diethyl ether to elute patulin, consequent washing of the cartridge with basic and acidic solutions was not required. Limiting the volume of diethyl ether to 1 mL was sufficient to recover patulin from the cartridge completely, while much of the phenolic co-extractives were still retained in the cartridge. The recoveries of patulin from apple juice using the HLB cartridge ranged between $91 \pm 4\%$ and $94 \pm 2\%$ for spiking levels of 10–50 $\mu\text{g/L}$.

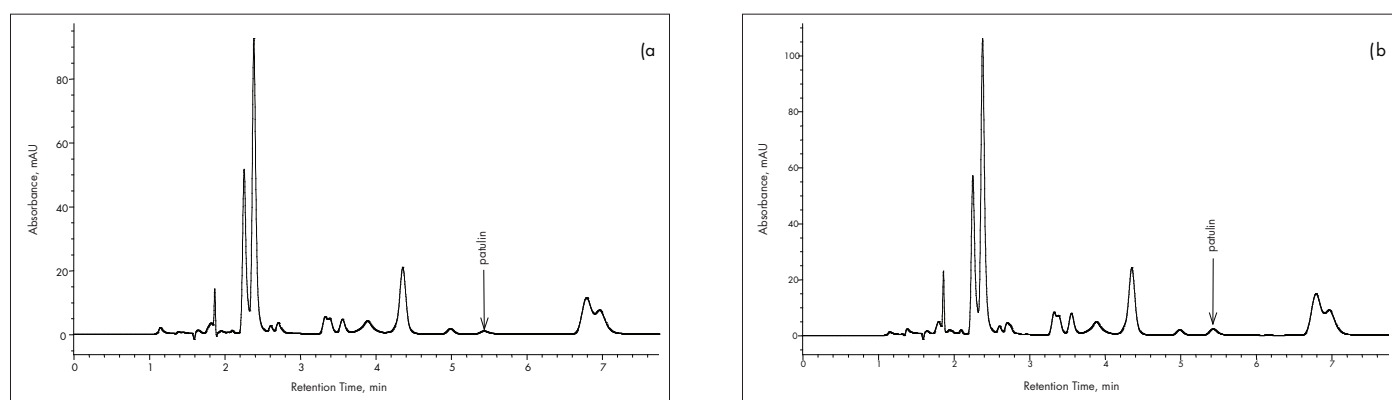


Figure 4. Patulin in (a) control apple juice (patulin level, 12 $\mu\text{g/L}$), (b) spiked apple juice (control + 20 $\mu\text{g/L}$ spiking)

As illustrated in the chromatograms shown in Figure 4, the peak eluted at 5.416 min was assigned as patulin comparing its retention time to the retention time of pure patulin standard. Absorbance spectrum of peak recorded in a wavelength range between 240 and 340 nm was also monitored to confirm the patulin peak. As shown in Figure 5, the peak eluted at 5.416 min in the chromatogram of apple juice exhibited a maximum absorbance at 276 nm as in the case for pure patulin standard.

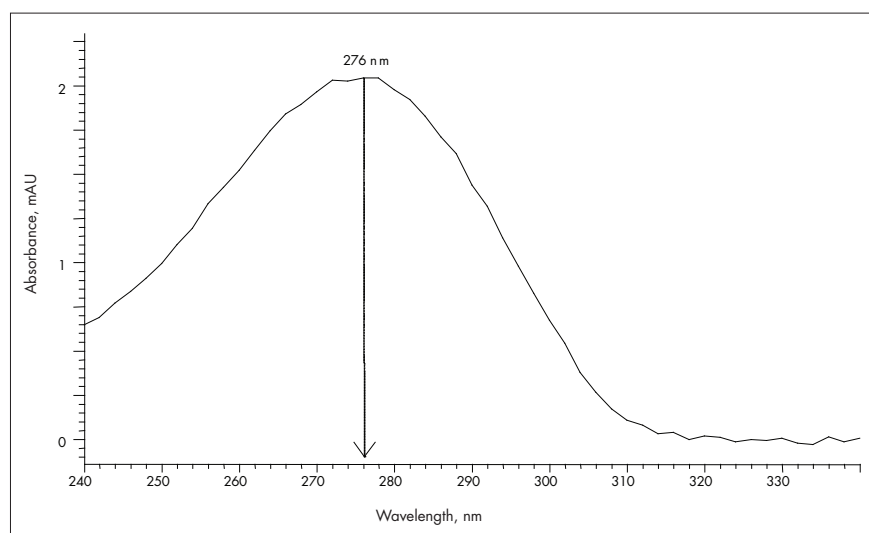


Figure 6. Spectrum of the peak assigned as patulin (ret time 5.416 min).

CONCLUSION

Analysis of patulin has become one of the most important applications in the field of food safety. Significant progresses have been made over the years on the chromatographic analysis of patulin. Combining the Oasis HLB based cleanup/extraction with the XBridge C₁₈ column based chromatographic separation step was found very useful allowing a rapid determination of patulin in apple juice.

REFERENCES

1. V. Gökmen, J. Acar, J. Chromatogr. A 730 (1996) 53.
2. V. Gökmen, J. Acar, J. Chromatogr. A 847 (1999) 69.
3. V. Gökmen, J. Acar, K. Sarıoğlu, Anal. Chim. Acta (2005) 64.
4. World Health Organization (WHO), 44th Report of the Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 859, Geneva, Switzerland, 1995, p. 36.
5. Guidance for FDA Components and Industry: Apple Juice, Apple Juice Concentrates, and Apple Juice Products – Adulteration with Patulin. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant and Dairy Foods and Beverages. October 22, 2001.

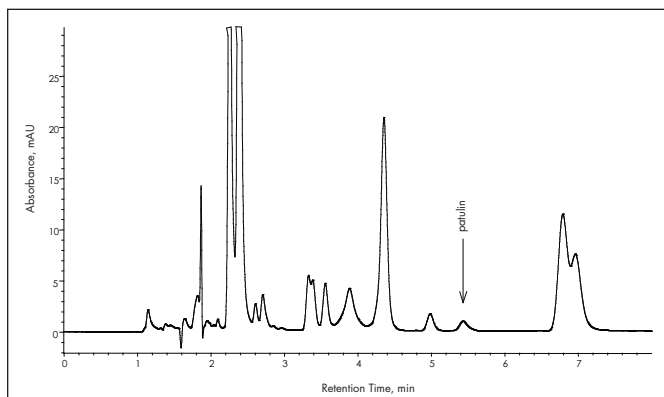


Figure 7. Overlaid chromatograms for control and spiked apple juice samples shown in Figure 4.

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