GC/MS of Native Patulin in Apple Juice and Cider

Application Note

Food Testing & Agriculture

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Abstract

As growing seasons for apple production and the ability to export apples and apple juice concentrates become more extensive, the potential to consume contaminated produce rises. Patulin, a mycotoxin produced as apples decay, is monitored using an Agilent Ultra Inert column without derivatization. The assay chromatographically resolves 5-hydroxymethylfurfural (HMF), which can be produced from sugars in apple products that have been excessively heat treated. Sample preparation involves solid phase extraction using a polystyrene-divinyl benzene SPE cartridge followed by liquid/liquid extraction in ethyl acetate prior to injection into the GC/MS system.

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Introduction

Apple cider and juice are among the top 10 most consumed fruit beverages globally. The top three producers of apples and apple-based products are China, the United States, and Poland. All of these countries have regions that offer excellent climate conditions for a multitude of apple varieties. Apple juice for consumers is processed through filtration, pasteurization, and vacuum packing to provide superior qualities of flavor, aroma, and shelf life. The mycotoxin patulin, formed by penicillin mold, is almost completely avoided and has been regulated at less than 10 ng/g in packaged products [1]. Numerous techniques are used to measure patulin during juice production, including HPLC/UV, GC, and GC/MS, with and without derivatization [2,3]. Although each of these techniques can be done reliably and inexpensively, separation of patulin from HMF can be a challenge for reversed-phase HPLC. However, confirmation can be performed using LC/MS/MS if there are concerns [4]. In the production of fresh cider, the processing steps allow this beverage to include the fruit pectin, which offers added health benefits by possibly lowering serum cholesterol. However, this reduced processing comes at added risk of much shorter shelf life and significant likelihood of patulin formation [5].

Materials and Methods

An Agilent 7890A GC was coupled to an Agilent 5975C mass spectrometer with the inert EI 350 °C noncoated source. An Agilent J&W DB-35ms UI column was fitted to the multi-mode inlet (MMI) operated in cold-splitless mode to allow optimum sample transfer. Patulin and HMF were obtained from Sigma-Aldrich Corp as 100 µg/mL (patulin) or in pure form (HMF).

Conditions

GC

Column:	Agilent J&W DB-35ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 122-3832UI)
Sample prep:	Agilent Bond Elut LMS, 1 g, 6 mL, 30/pk (p/n 12255022)
Sample:	10 g juice or cider
Carrier:	MSD helium, 1 mL/min constant flow
Oven:	50 °C (hold 5 minutes), then to 300 °C at 40 °C/min (hold 8.75 minutes)
Injection:	Cold-splitless, 67 °C (hold 0.1 minutes), then to 160 °C at 720 °C/min, split vent on at 1 minute (30 mL/min), gas saver on at 3 minutes (20 mL/min)
MSD transfer aux temperature:	300 °C
GC:	Agilent 7890A GC
Sampler:	Agilent 7693 Automatic Liquid Sampler, 1 µL volume injection

MS

MS:	Agilent 5975C Series MSD with inert EI 350 source, tandem axis detector
Solvent delay:	6 minutes
MS temperature:	300 °C (source), 150 °C (quad)
SIM mode:	Mass 55.00, 97.00*, 110.00*, 126.00 dwell 100 ms for each (*quant ions)

Agilent supplies (unless otherwise stated)

Drying tubes:	Pyrex centrifuge tubes, conical with screw cap, 15 mL, with graduations (Sigma-Aldrich Corp., catalog no. CLS808215-12EA)
Vials:	Amber screw cap (p/n 5182-0716)
Caps:	Blue screw cap (p/n 5282-0723)
Inlet liner:	Ultra Inert splitless single taper liner (p/n 5190-3162)

Standards

Patulin and HMF mixed standard solutions were prepared in ethyl acetate in the range 10 to 1,000 ng/g, to demonstrate linearity and detection limit. Apple juices and ciders were purchased and stored refrigerated at all times. After initial screening, it would also be possible to prepare a matrix-matched calibration set using blank extracts as dilution solvent.

Sample preparation

1. Weigh 10 g (10 mL) of juice or cider into a clean container.
2. Spike samples at 10 ng/g (10 ppb) to demonstrate adequate recovery from the juice.
3. Condition SPE tubes with 4 mL MeOH, followed by 4 mL deionized HPLC-grade water.
4. Load 10 mL sample to the preconditioned SPE cartridge under gravity (slight vacuum required for cider, depending on clarity).
5. Wash with 8 mL 1% aqueous sodium bicarbonate under gravity.
6. Wash with 8 mL 1% aqueous acetic acid under gravity.
7. Replace waste tubes with collection tube.
8. Elute with 8 mL HPLC-grade methanol under gravity.
9. Dry eluate to remove methanol in a warm water bath, approximately 4 mL aqueous should remain.
10. Liquid/liquid extract the eluate with 3 × 3 mL portions of ethyl acetate, combining them into a conical tube for drying.
11. Dry the ethyl acetate extract to 1 mL, transfer to autosampler vial for injection.

Results and Discussion

Figure 1 is a scan mode representation of a 10 ng/g mix of HMF and patulin. Peak shape and resolution from the matrix were satisfactory.

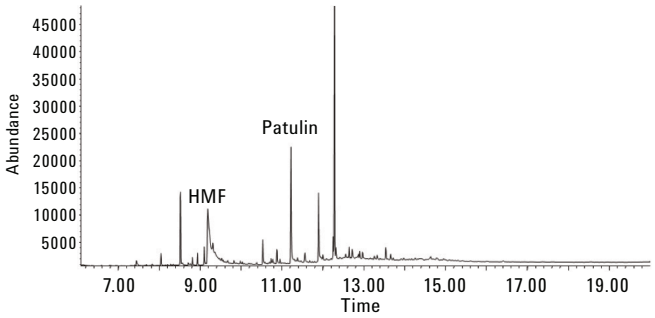

Figure 1. Scan mode of a 10 ng/g mix of HMF and patulin with acceptable peak shape and satisfactory resolution from the matrix components.

Recovery of spiked samples was greater than 90%, making the use of Bond Elut LMS SPE a viable tool for low level isolation. The extraction procedure removed the sugars which were present at 10% in juice and would cause injector fouling in a short time frame and make it necessary to perform inlet maintenance much more often. Also removed was a fairly high vitamin C content which would closely coelute with HMF, introducing peak integration challenges. A number of higher boiling solutes such as polyphenols were co-extracted. These tend to accumulate in the inlet but can be removed with a periodic bakeout at the maximum allowable temperature. Benzaldehyde can be isolated by this technique. It is a marker compound from apple seed degradation. While there was a fair amount of tailing, the MMI produced a peak symmetry that was comparable to cool on-column.

Conclusions

Patulin and 5-hydroxymethylfurfural were well resolved on an Agilent J&W DB-35ms Ultra Inert column after sample preparation with a Bond Elut LMS cartridge and liquid/liquid extraction. It would be time efficient to further refine and automate the extraction procedure if a large sample volume is anticipated.

References

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