GC/MS of Native Patulin in Apple Juice and Cider

Instrument: LECO Pegasus III TOF MS with Agilent 6890 Plus GC

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.

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

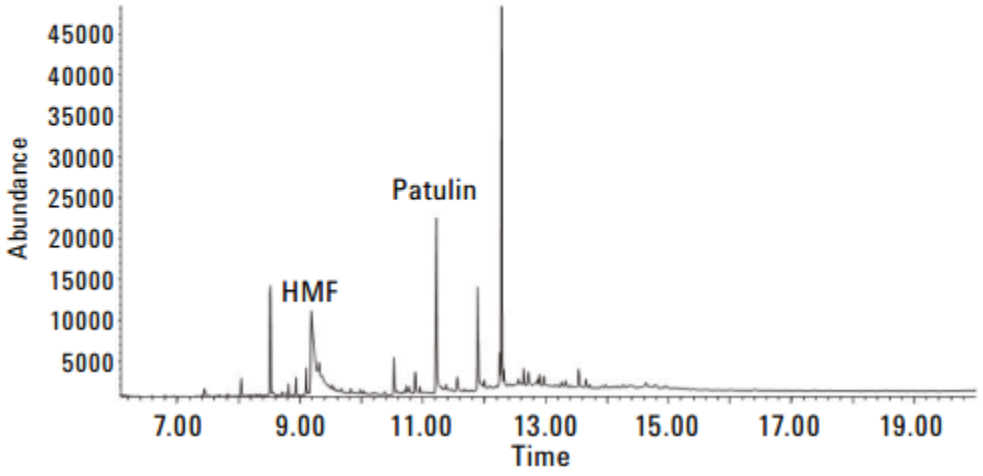
MS

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)

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.

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.



Reference: http://hpst.cz/sites/default/files/uploaded_files/5991-2799en_-_gcms_of_native_patulin_in_apple_juice_and_cider.pdf