

Extraction of Patulin From Clear Apple Juice Using ISOLUTE® Myco prior to LC-MS/MS Analysis

This application note describes a Solid Phase Extraction (SPE) protocol for the extraction of patulin from clear apple juice using the mycotoxin specific ISOLUTE® Myco columns prior to quantitative LC-MS/MS analysis.

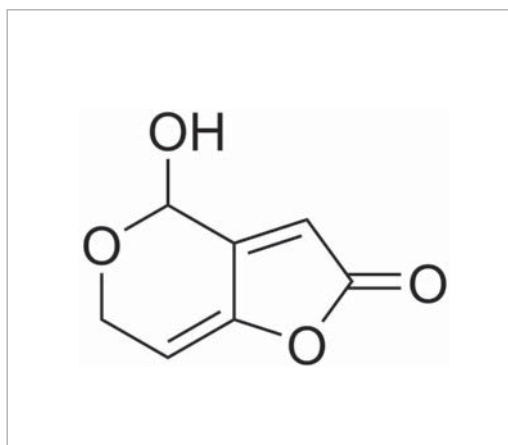


Figure 1. Structure of Patulin

Introduction

Patulin is a mycotoxin produced by *Aspergillum* and *Penicillium* mold species commonly found on rotting apples. Although not a particularly potent toxin, patulin has been shown to be genotoxic and potentially carcinogenic, requiring regulation and analysis in all apple based products. Recommended maximum limits for patulin are set globally at $10 \mu\text{g kg}^{-1}$ in apple juice, $25 \mu\text{g kg}^{-1}$ in solid apple foods and $10 \mu\text{g kg}^{-1}$ for apple based baby food. See Comments (page 5) for sample pre-treatment suggestions for solid apple samples and cloudy apple juice.

This application note achieves high recoveries of patulin from clear apple juice with RSDs below 10%. Limits of quantitation are $10 \mu\text{g kg}^{-1}$ (calculated from the signal to noise ratio).

ISOLUTE Myco SPE columns provide robust, reliable sample preparation for multiple mycotoxin classes from a wide range of foodstuffs. Using a single, easy to use sample preparation product, along with optimized matrix specific application notes, scientists can prepare diverse food/crop samples for analysis by LC-MS/MS.

Analytes

Patulin

Sample Preparation Procedure

Column configuration:	ISOLUTE Myco 60 mg/3 mL (Tablet) part number 150-0006-BG
Sample pre-treatment:	Dilute apple juice 1:1 (v/v) with 10 mM ammonium acetate pH 5. Adjust total volume to pH 5 using 25% ammonium hydroxide (conc) (62.5 μL per mL of apple juice).
Condition:	Condition the column with acetonitrile (2 x 1 mL).
Equilibration:	Equilibrate column with 10 mM ammonium acetate pH 5 (1 mL).
Sample loading:	Load pre-treated sample (1 mL) onto the column.
Interference wash 1:	Wash the column with 10 mM ammonium acetate pH 5 (3 x 1 mL).
Drying:	Dry the column for 5 minutes at maximum pressure, 2 bar/29 psi.
Interference wash 2:	Wash the column with toluene (1 mL).
Drying:	Dry the column for 5 minutes at maximum pressure, 2 bar/29 psi.
Elution	Elute with acetonitrile (1 mL).
Post elution:	The eluate is dried in a stream of air or nitrogen using a SPE Dry (20 to 40 L min^{-1}) or TurboVap LV (15 bar at 30 °C for 40 min). Reconstitute in water (500 μL , HPLC or deionized) prior to analysis.
Note:	This method was developed using the Biotage PRESSURE+ 48 positive pressure manifold, but columns can also be processed using VacMaster sample processing manifolds.

HPLC Conditions

Instrument:	Waters Alliance 2795
Column	Kinetex phenyl-hexyl 50 mm x 2.1 mm 2.6 µm dp. (Phenomenex inc., Torrance CA)
Mobile phase:	A: Water (HPLC grade) B: Acetonitrile
Flow rate:	0.3 mLmin ⁻¹
Injection:	10 µL (partial loop) from 20 µL
Gradient:	Initial 15 % B, hold for 1 min Linear ramp to 100 % B in 1 min, hold for 1 min Linear ramp to initial conditions in 0.5 min, hold for 2 min Total run time 4.5 min
Column temp:	Room temperature
Sample temp:	15 °C

Mass Spectrometry Conditions

Instrument:	Waters Ultima Platinum QQQ
Source:	Electrospray, negative ion mode
Desolvation temp:	350 °C
Quadrupole:	100 °C
Capillary:	3000 kv
Mode:	MRM

Table 1. MRM Parameters

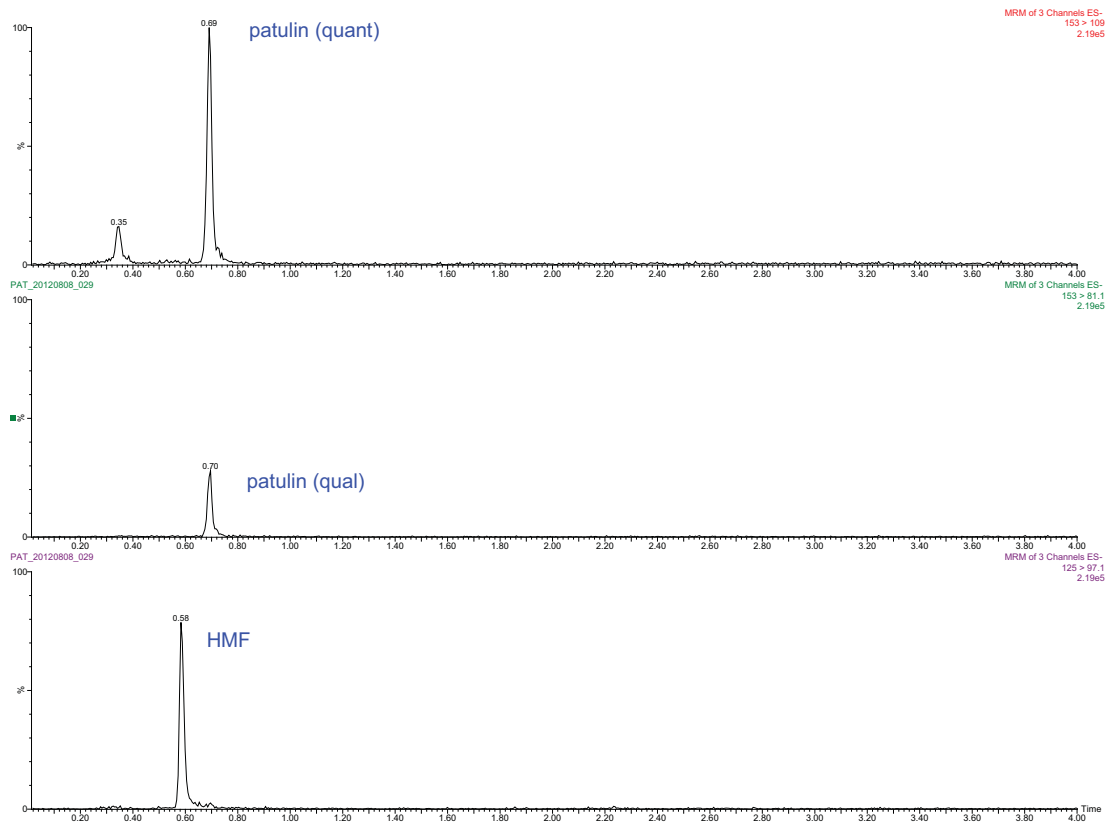
Analyte	MRM	Entrance potential	Collision energy (eV)
Patulin (quant ion)	153 > 109	40	8
Patulin (qual ion)	153 > 81	35	10
Hydroxymethylfurfural	125 > 97	40	8

Results

The extraction method demonstrates high recovery of patulin whilst effectively resolving hydroxymethylfurfural (HMF), which can be a source of chromatographic interference when analysed using less selective analytical methods. Mean analyte recoveries of 101% were achieved for patulin (n=7) with RSDs <10% as shown in table 2. Each chromatogram is shown separately in figure 2 and then overlaid to show actual resolution in figure 3. The limit of quantitation was calculated to be 10 µg kg⁻¹ based upon an observed signal to noise ratio of 16:1, easily meeting the regulatory limits set for patulin minimum residue limits.

Table 2. Typical recoveries of patulin , quant and qual ions (n=7)

	Patulin Quant (153>109) % recovery	Patulin Qual (153>81) % recovery
1	99.60	104.30
2	95.40	98.40
3	97.30	100.00
4	103.60	100.10
5	92.40	91.90
6	104.50	98.20
7	113.60	107.50
Mean	100.91	100.90
% RSD	6.99	3.02

**Figure 2.** Typical chromatogram ($10 \mu\text{g kg}^{-1}$) using the ISOLUTE Myco method, showing extracted patulin and the resolved interference analyte HMF

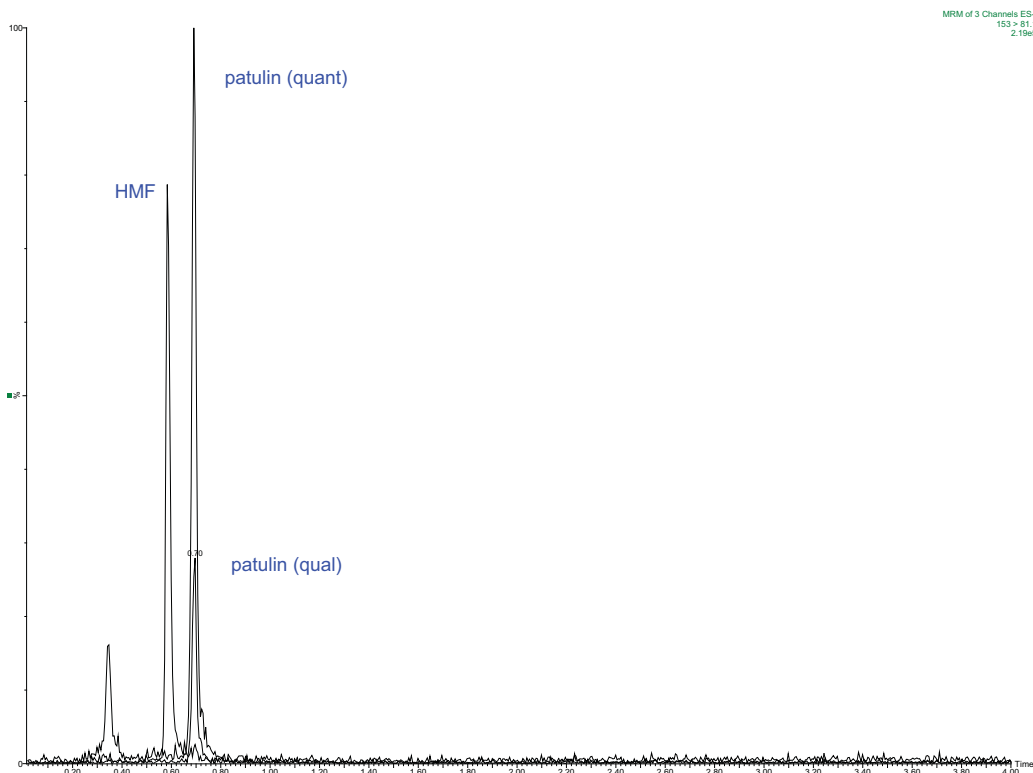


Figure 3. Typical overlaid chromatogram as 10 µg kg⁻¹ using the ISOLUTE Myco method, showing extracted patulin and the resolved interference analyte HMF

Calibration curves constructed using this method (from 2-200 ngmL⁻¹), demonstrated excellent linearity with coefficients of determination greater than 0.99 as shown in Figure 4.

Compound name: patulin 153>109
 Correlation coefficient: $r = 0.998134$, $r^2 = 0.996271$
 Calibration curve: $115.582 * x + -141.87$
 Response type: External Std, Area
 Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None

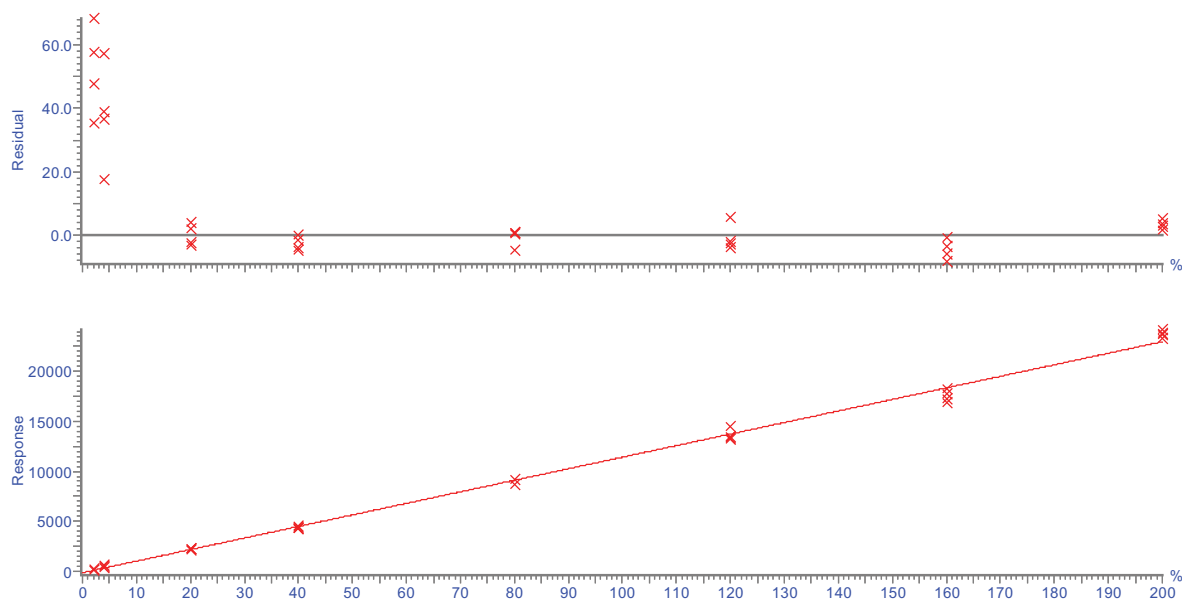


Figure 4. Calibration curves constructed using this method from 2-200 ngmL⁻¹

Comments

Unlike clear apple juice, solid apple samples and cloudy apple juices require digestion with pectinase to ensure high analyte recoveries. A typical procedure is suggested below:

Homogenize apples. Weigh 10 g of sample and add pectinase enzyme followed by 10 mL water. Mix. Leave at room temperature overnight OR for 2 hr at 40°C. Centrifuge at 4500 g for 5 min and filter the supernatant with 0.2 µm filter. Use 150 µL of pectinase aqueous solution at 3800 units/mL (Sigma-Aldrich Cat. No. P2611). Use a similar process for cloudy apple juice.

Ordering information

Part Number	Description	Quantity
150-0006-BG	ISOLUTE Myco 60 mg/3 mL column (Tabletless)	50
PPM-48	Biotage PRESSURE+ 48 Positive Pressure Manifold 48 Well	1
121-1016	VacMaster-10 Sample Processing Manifold complete with 16 mm collection rack	1
121-2016	VacMaster-20 Sample Processing Manifold complete with 16 mm collection rack	1
C103198	TurboVap LV, 110V	1
C103199	TurboVap LV, 220V	1

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