Analysis of Patulin in Fruit Juices and Extracts Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

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ABSTRACT

Purpose: Patulin is a mycotoxin produced by different types of fungi as their secondary metabolite. The regulatory authorities have imposed restrictions on maximum patulin levels in fruit products, which creates a need for sensitive analytical methods.

Methods: Two different LC-MS/MS methods were tested using Thermo Scientific™ TSQ Fortis™ Triple Quadrupole Mass Spectrometer and Thermo Scientific™ Vanquish™ UHPLC system. SPE was used to extract patulin from fruit juices.

Results: SPE LC-MS/MS method for quantification of patulin was developed and tested

INTRODUCTION

Patulin, 4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one (CAS#149-29-1), is a polyketide produced as a mycotoxin by several fungi, namely *Aspergillus* and *Penicillium*. Fruits that were damaged or improperly stored are susceptible to the growth of patulin-producing molds. If these fruits are used to make further consumer products, patulin can be present as a toxic contaminant. Because patulin is a heat-stable lactone that resists thermal denaturization, the normal pasteurization treatment is not sufficient to decompose it. The regulators in different jurisdictions around the world have imposed restrictions on maximum patulin levels in different products, especially in apple juice. These levels differ depending on the product and the country, but predominantly were set in the concentration range between 5-100ppb (50ug/kg in the US and EU).

MATERIALS AND METHODS

Chemicals and Sample Preparation

Patulin was obtained from Sigma Aldrich,; all solvents and reagents were obtained from Fisher Scientific. For method development the samples were prepared as neat in 0.1% acetic acid in the concentration range 0.5-500ppb. To prepare matrix samples, apple juice was purchased in a local market and spiked with patulin.

Mass Spectrometry

The TSQ Fortis Mass Spectrometer was used for all the examples described in this work.

Figure 1 Ion Source Properties

Figure 2 SRM Properties

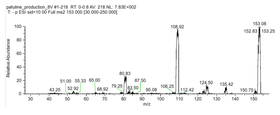


Figure 3 Product spectrum of patulin at 10V CID

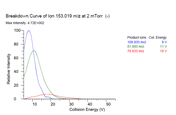
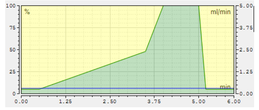


Figure 4 Patulin CID breakdown curves

Table 1. Patulin SRM parameters

Precursor m/z	Product m/z	CE (V)	Tube lens (V)	
153.0	108.9	8	76	Quan
153.0	81.2	11	76	Confirm

LC methodology



LC method #1

[A]	water
[B]	acetonitrile
Injection volume:	10uL
Temperature:	30°C
Column:	HSS T3 1.8µm; 2.1x100mm

LC method #2

[A]	0.2mM NH_4F in water
[B]	acetonitrile
Injection	volume: 10uL
Temperature:	30°C
Column:	HSS T3 1.8µm; 2.1x100mm

Solid-Phase Extraction

Format	Oasis Max 6cc
Sample pre-treatment	Spike apple juice at 1; 2.5; 5; 10; 50; 100 and 200 ppb (2 mL)
Condition	6 mL of methanol
Equilibration	6 mL water
Sample load	load 2 mL of spiked apple juice
Wash 1	3 mL 5mM ammonium acetate
Wash 2	3 mL water
Elution	Eluate patulin with 4 mL Methanol
Post elution and reconstitution	The extracts were evaporated to dryness under N_2 stream and reconstituted in 1 mL 0.1% acetic acid

Data Analysis

Thermo Scientific™ TraceFinder™ and Thermo Scientific™ FreeStyle™ software were used for data processing.

Table 2. Results overview for LC-MS/MS method #1 (left) and #2 (right); based on 3 replicate injections

Sample	Average diff. from theoretical value [%]	RSD [%]
0.5 ppb	6.1	11.93
1 ppb	13.8	3.65
2 ppb	6.5	7.29
5 ppb	13.8	11.8
10 ppb	6.5	2.7
20 ppb	9.7	1.1
50 ppb	5.9	4.0
100 ppb	0.8	1.5
200 ppb	2.6	0.7
500 ppb	4.0	0.9

Sample	Average diff. from theoretical value [%]	RSD [%]
0.5 ppb	9.3	14.8
1 ppb	6.0	3.9
2 ppb	3.1	1.7
5 ppb	3.7	4.8
10 ppb	3.4	2.3
20 ppb	3.3	1.2
50 ppb	3.5	3.9
100 ppb	4.8	1.1
200 ppb	1.5	2.0
500 ppb	1.4	2.0

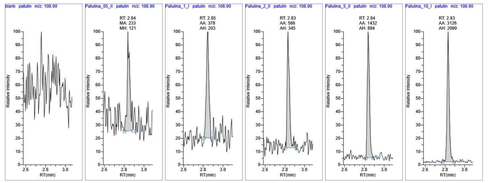


Figure 5 Extracted Ion chromatograms for lowest calibrators in neat solvent and solvent blank; LCMS method #1

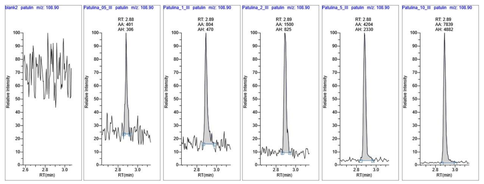
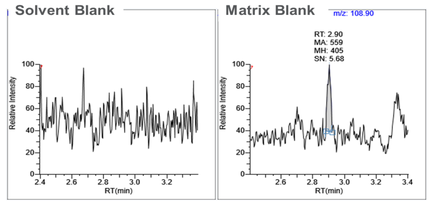


Figure 6 Extracted Ion chromatograms for lowest calibrators in neat solvent and solvent blank; LCMS method #2



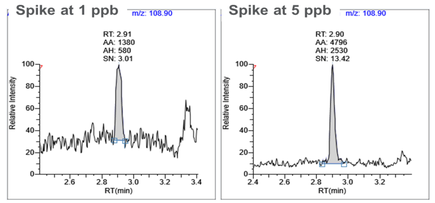


Figure 7 Patulin Extracted Ion Chromatograms in apple juice; LC-MS method #2 was used for apple juice matrix samples

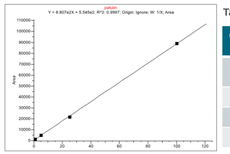


Figure 8 Patulin calibration curve in apple juice matrix.

Table 3. Overview of Patulin calibrators in apple juice

Calibrators in apple juice	Calculated Conc. (ppb)	CV %	Ion ratio
1 ppb	1.005	0.53%	36.38
5 ppb	5.11	2.17%	40.54
25 ppb	24.14	-3.45%	37.34
100 ppb	100.748	0.75%	37.49

Table 4. Sample preparation accuracy and precision *

Apple juice Samples N=4	Average concentration /ppb	Precision %	Accuracy %
Spiked at 1 ppb	1.01	10%	101%
Spiked at 5 ppb	4.90	9%	98%
Spiked at 25 ppb	28.37	11%	113%
Spiked at 100 ppb	97.02	12%	97%

*isotopically labeled internal standard is commercially available for patulin, but was not used in this work

CONCLUSIONS

- Method for LC-MS/MS quantitation of Patulin in negative ion mode was developed using TSQ Fortis mass spectrometer and Vanquish-Flex UHPLC system.
- Two different LC methods were evaluated, one with addition of NH_4F as modifier
- TSQ Fortis showed linearity with R^2 >0.999 in the 0.5-500 ppb concentration range in both neat and apple juice matrix
- Method #2 demonstrated better sensitivity, but required longer column equilibration than simpler method #1, which was based on the Chinese regulation GB5009.185-2016 (ref 6)
- Calibrators were analyzed in triplicates, all RSD were <15%
- Both methods showed very good precision and accuracy even without using any internal standard
- The SPE method provided a recovery of 80-90% with a signal suppression of ca.14%, affording lower limit of quantitation (LLOQ) of 1 ppb for patulin in apple juice (mean accuracy 101%; CV=10%) using LC-MS method #2 (with NH_4F in the mobile phase)

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6. GB5009.185 – 2016 National food safety standard Determination of patulin in in apple and hawthorn products, National Health and Family Planning of People's Republic of China (NFHPC), China 2016

TRADEMARKS/LICENSING

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