Determination of Patulin in Apple Juice Using SPE and UHPLC-MS/MS Analysis

UCT Part Numbers

ECHLD126-P
ENVIRO-CLEAN® HLDVB
200 mg/6mL SPE cartridge PE Frit

SLDA50ID21-18UM
Selectra® DA UHPLC column
(50 × 2.1 mm, 1.8 µm)

SLDAGDC20-18UM
Selectra® DA guard cartridge
(10 × 2.1 mm, 1.8 µm)

SLGRDHLDR
Guard cartridge holder

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Summary:

Patulin (Figure 1) is a naturally occurring mycotoxin that is produced by several species of fungi, such as Aspergillus, Penicillium and Byssochlamys. It typically grows on fruit, including apples, pears, peaches and grapes, but has also been reported in vegetables and cereal grains. Patulin has been implicated as a possible carcinogen and teratogen, although an official designation has not yet been made. The main risk arises when unsound fruit is used for the production of juices and other processed products. The amount of patulin in apple juice is also viewed as a measure of the quality of the apples used in production.

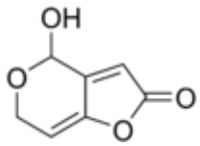

Figure 1. Structure of patulin.

The World Health Organization, U.S. Food and Drug Administration (FDA) and European Union (EU) have suggested a maximum limit of patulin in apple juice and apple juice ingredients at 50 µg/kg. Furthermore, the EU has set a limit of 25 µg/kg in solid apple products and 10 µg/kg in baby food (EC 1881/2006).

This application note outlines a simple solid-phase extraction (SPE) procedure for the low level detection of patulin in apple juice. Analysis is carried out by UHPLC-MS/MS using a Selectra® DA column. The unique chemistry of the Selectra® DA column, which contains a polyaromatic stationary phase, provides a high degree of retention and selectivity for aromatic compounds and improved retention of polar compounds.

Sample Pretreatment:

No additional preparation is necessary for filtered apple juice or clear unfiltered apple juice. Cloudy unfiltered apple juice should be centrifuged prior to application to the SPE cartridge (≥ 5000 rpm for 5-10 min).

SPE Procedure:

1. **SPE conditioning**
   a) 1 × 3 mL methanol.
   b) 1 × 3 mL deionized/ultrapure water.

2. **Sample extraction**
   Add 5 mL of apple juice to the SPE cartridge. If necessary, apply a low vacuum or positive pressure to force the sample through the SPE cartridge at ≤ 5 mL/min.

3. **Wash cartridge**
   a) 1 × 2 mL 1% sodium bicarbonate.
      - The high pH of the buffer aids in the removal of sugars that are co-extracted onto the SPE cartridge.
   b) 1 × 2 mL 1% acetic acid.
      - Reduces the pH of the sample (patulin is unstable at high PH).
   c) Dry SPE cartridge under full vacuum for 10 minutes to remove excess water. Do not overdry as this can result in reduced recovery of patulin.

4. **Elution**
   a) 1 × 3 mL n-hexane/acetone (7:3, v/v).
   b) Evaporate to dryness at 35-40°C under a gentle stream of nitrogen (the elution solvent is very volatile and is rapidly evaporated).
   c) Reconstitute in 1 ml methanol/water (1:1, v/v) containing 0.1% acetic acid or other suitable solvent solution.

LC-MS/MS Parameters:

Instrumentation	
HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000 UHPLC
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® DA, 50 × 2.1 mm, 1.8 µm (p/n: SLDA50ID21-18UM)
Guard column	UCT Selectra® DA, 10 × 2.0 mm, 1.8 µm (p/n: SLDAGDC20-18UM)
Guard column holder	p/n: SLDGRDHLDR
Column temperature	40°C
Flow rate	400 µL/min
Injection volume	5 µL

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Mobile Phase Gradient		
Time (min)	**% Mobile Phase A Water**	**% Mobile Phase B Methanol**
0.0	95	5
2.0	5	95
3.5	5	95
3.6	95	5
6.0	95	5

MRM transitions (ESI^-)				
Compound	**t_R (min)**	**Precursor ion**	**Product ion 1**	**Product ion 2**
Patulin	2.32	153.0	109.0	81.0

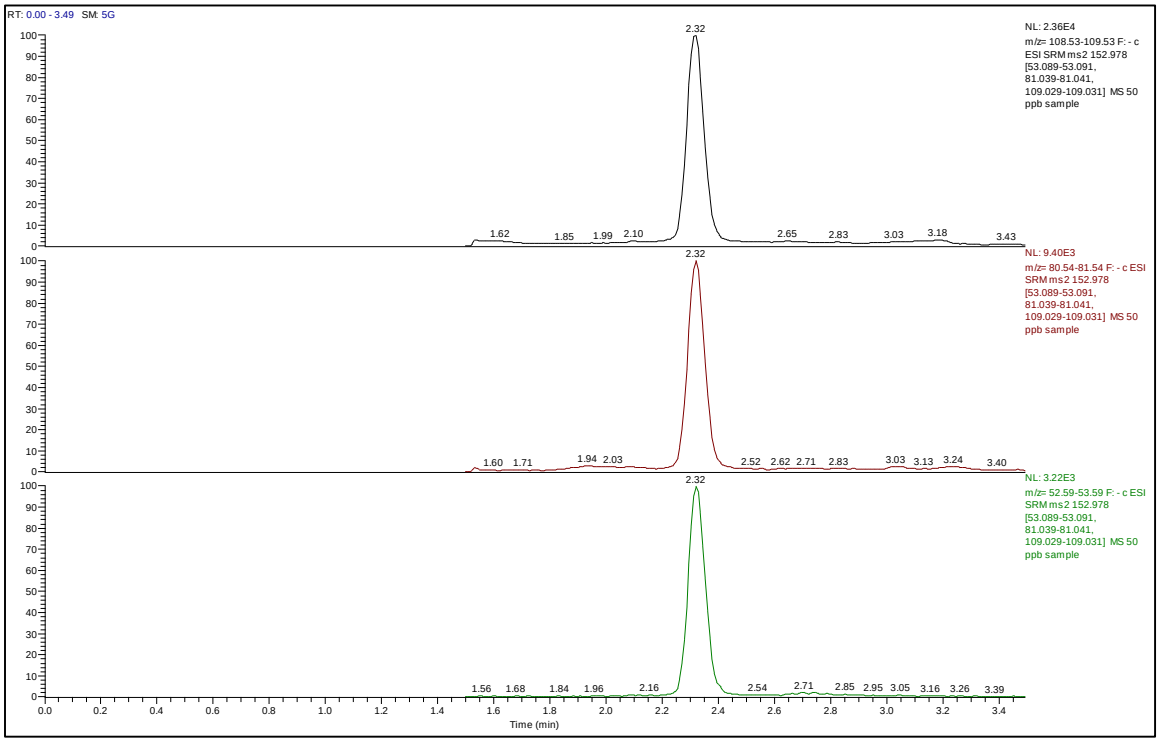

Figure 2. Chromatogram of Patulin at 50 µg/L.

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Results:

Recovery and Reproducibility		
	5 µg/kg	**50 µg/kg**
Sample 1	79.0	100.3
Sample 2	87.0	94.7
Sample 3	82.2	83.1
Sample 4	81.1	86.8
Sample 5	86.4	85.7
Sample 6	78.1	99.9
Mean Recovery (%)	**82.3**	**91.7**
RSD (%)	**4.54**	**8.2**

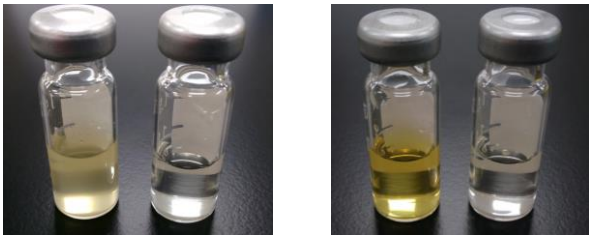

Figure 3. Effect of wash and elution solvent on cleanliness of final extract. Left picture - Rinsing of SPE sorbent with 100% water (L) vs. 1% sodium bicarbonate (R). Right picture: Eluting with 100% methanol (L) vs. n-hexane/acetone (7:3, v/v) (R).

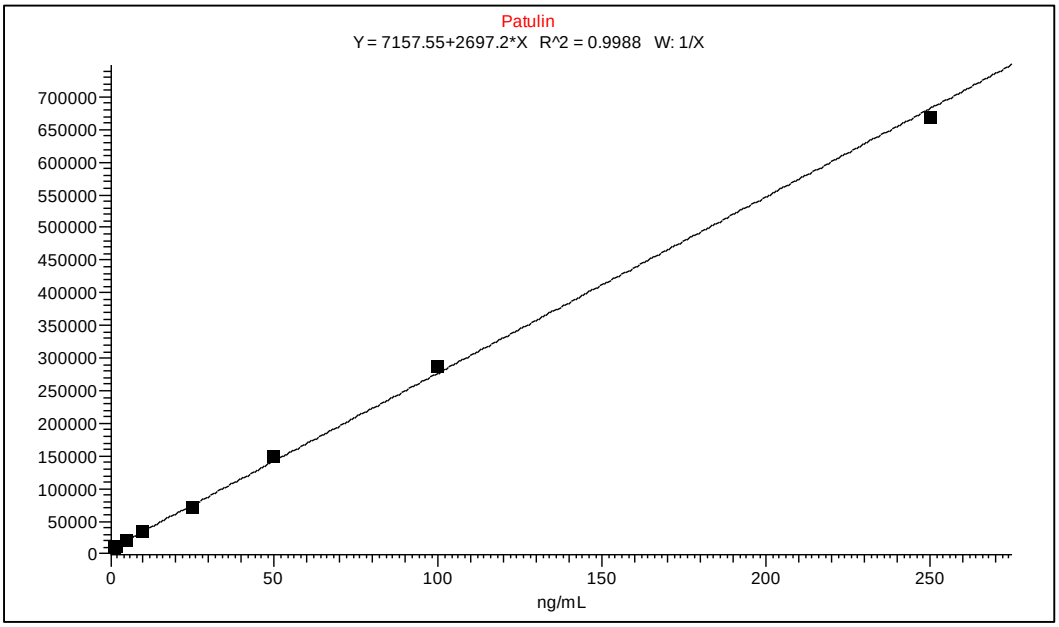

Figure 4. Example of an eight-point matrix-matched calibration curve (1-250 ng/mL).

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