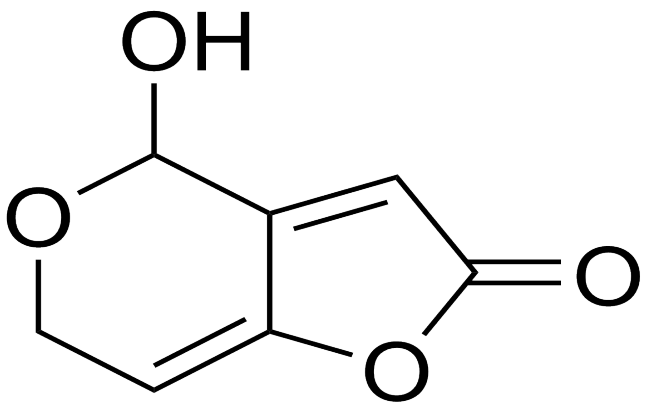
Determination of Patulin in Processed Foods Using QuEChERS Extraction and UHPLC-MS/MS Analysis



Chemical Structure of Patulin.

UCT Part Numbers

ECMSSC-MP
Mylar pouch containing 4g $MgSO_4$ and 1g NaCl

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)

CUMPSC1875CB2CT
2mL dSPE tube with 150mg $MgSO_4$, 50mg PSA, 50mg C18 and 7.5mg GCB

Or

ECQUEU615CT
15mL dSPE tube with 900mg $MgSO_4$, 150mg PSA and 45mg GCB

SLGRDHLDR-HPOPT
Guard cartridge holder High Pressure

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 food products.

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 QuEChERS procedure for the detection of patulin in processed food products. An apple-based baby food product was used as a representative sample matrix. The inclusion of graphitized carbon black (GCB) in the dispersive-SPE (dSPE) cleanup step produces a clean sample extract. 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.

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QuEChERS Procedure:

1. Sample Extraction

a) Weigh 10 g of sample into a 50 mL polypropylene centrifuge tube.
b) Add 10 mL of acetonitrile.
c) Add the contents of the ECMSSC-MP Mylar pouch and shake for a minimum of 1 minute (by hand or mechanically).
d) Centrifuge the samples at ≥ 3000 × g for 5 minutes.

2. Sample Clean-up of 1 mL Extract

a) Transfer 1 ml of supernatant to a 2 mL dSPE tube (**CUMPSC1875CB2CT**).
b) Vortex the sample for 30 seconds.
c) Centrifuge the samples at ≥ 3000 × g for 5 minutes
d) Transfer 500-600 µL of purified supernatant into an autosampler vial (filter if desired).

3. Sample Clean-up of 5 mL Extract (for increased sensitivity)

a) Transfer 6 ml of supernatant to a 15 mL dSPE tube (**ECQUEU615CT**).
b) Vortex the sample for 1 minute
c) Centrifuge the samples at ≥ 3000 × g for 5 minutes.
d) Transfer 5 mL of purified supernatant into a glass tube and evaporate the sample to ≈1 mL at 40°C under a gentle stream of nitrogen.
e) Transfer the sample into an autosampler vial (filter if desired).

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 × 2.1 mm, 1.8 µm **(p/n: SLDAGDC20-18UM)**
Guard column holder	p/n: **SLGRDHLDR-HP**
Column temperature	40°C
Injection volume	5 µL
Flow rate	400 µL/min

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

Note: No mobile phase additive was used as it was found to give better signal intensity in ESI^- for patulin.

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

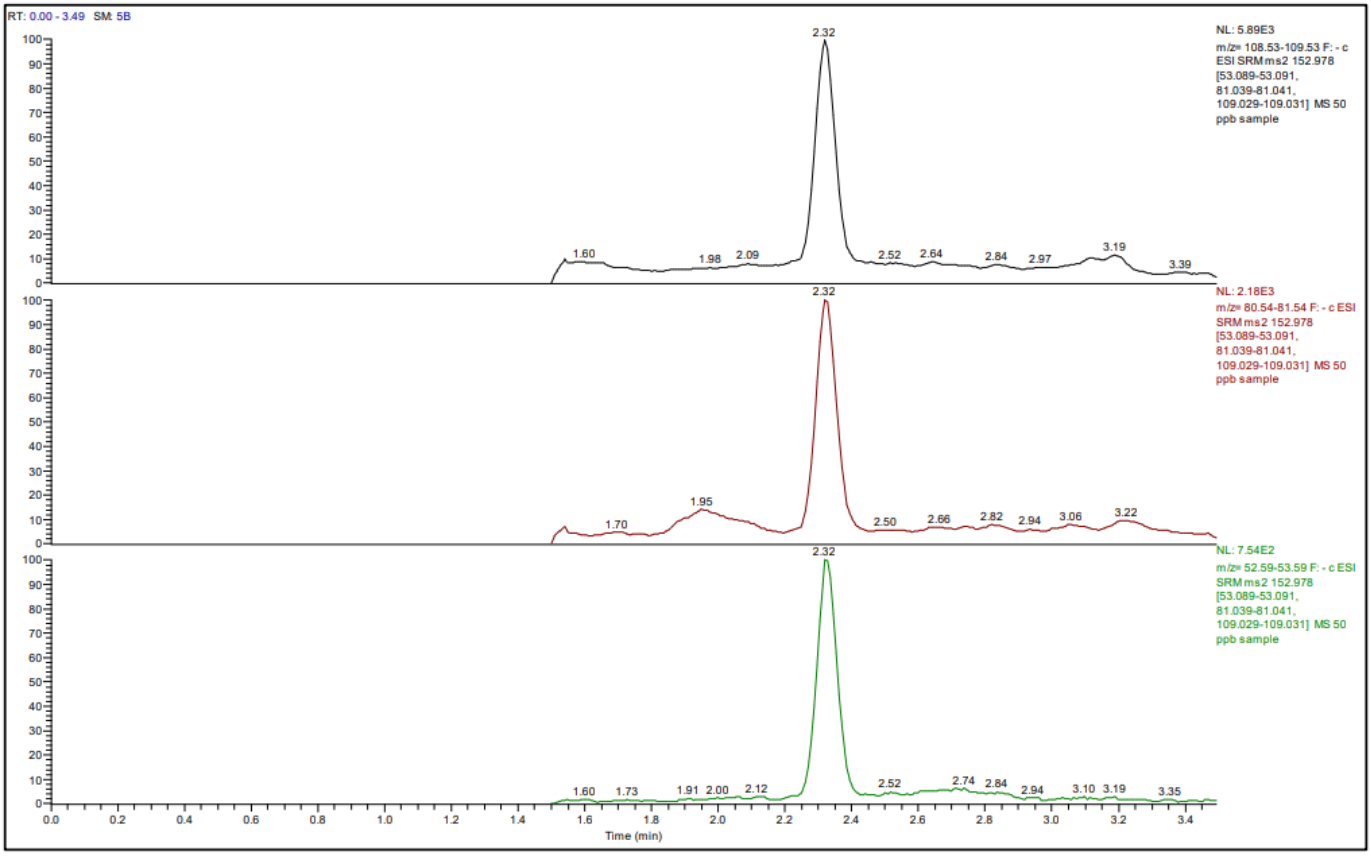

Figure 1. Chromatogram of an extracted baby food sample fortified with patulin at 50 µg/kg.

Results:

Recovery and Reproducibility		
	10 µg/kg [a]	**50 µg/kg [b]**
Sample 1	102.3	90.1
Sample 2	101.8	83.4
Sample 3	99.2	86.0
Sample 4	103.8	88.3
Sample 5	104.8	87.9
Sample 6	106.1	83.9
Mean Recovery (%)	**103.0**	**86.6**
RSD (%)	**2.35**	**0.30**

[a] used 5 mL of sample extract and included a concentration step.
[b] used 1 mL of sample extract and no concentration step.

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