



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

## UCT Part Numbers

### ECHLD126-P

ENVIRO-CLEAN<sup>®</sup> HLDVB  
200 mg/6mL SPE cartridge PE Frit

### SLDA50ID21-18UM

Selectra<sup>®</sup> DA UHPLC column  
(50 × 2.1 mm, 1.8 μm)

### SLDAGDC20-18UM

Selectra<sup>®</sup> DA guard cartridge  
(10 × 2.1 mm, 1.8 μm)

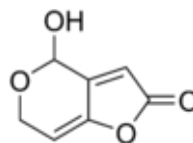
### SLGRDHLDLDR

Guard cartridge holder



## 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<sup>®</sup> DA column. The unique chemistry of the Selectra<sup>®</sup> 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.



**FOOD**

## 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 ( $\geq 5000$  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  $\leq 5$  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: SLDGRDHLDLDR
Column temperature	40°C
Flow rate	400 μL/min
Injection volume	5 μL



### 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 <sub>R</sub> (min)	Precursor ion	Product ion 1	Product ion 2
Patulin	2.32	153.0	109.0	81.0

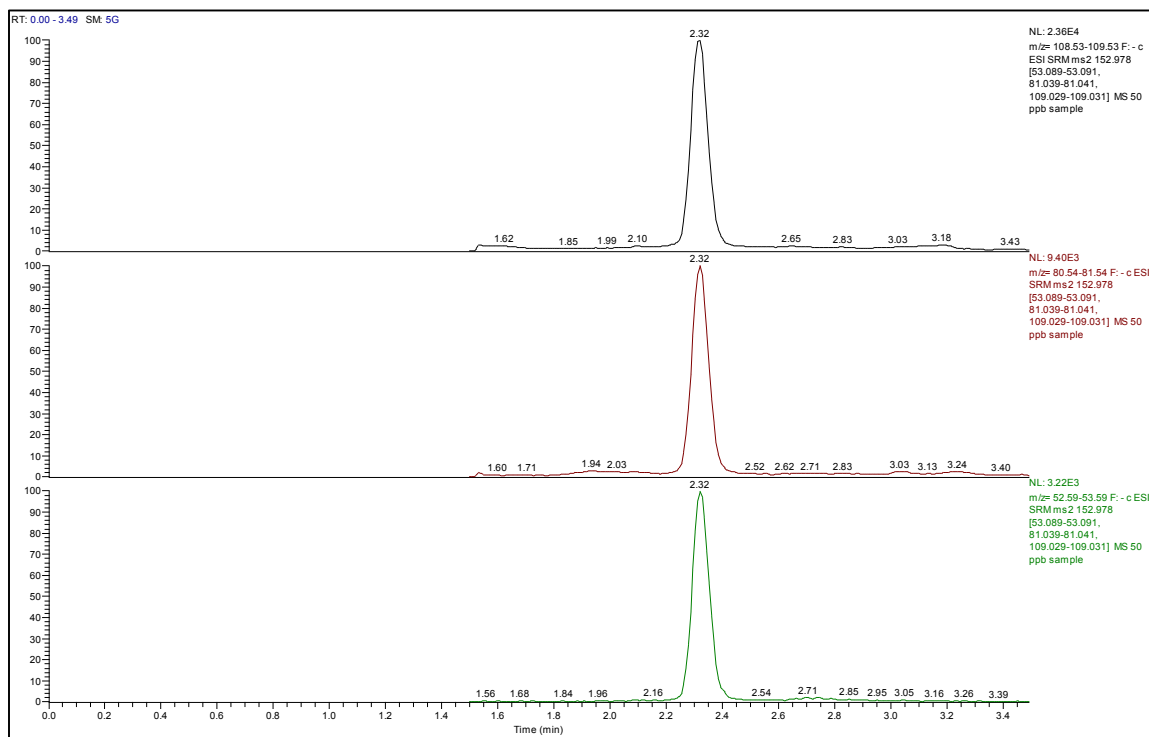
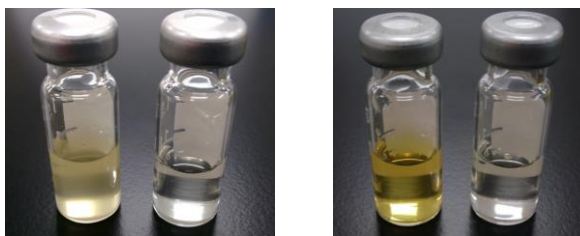


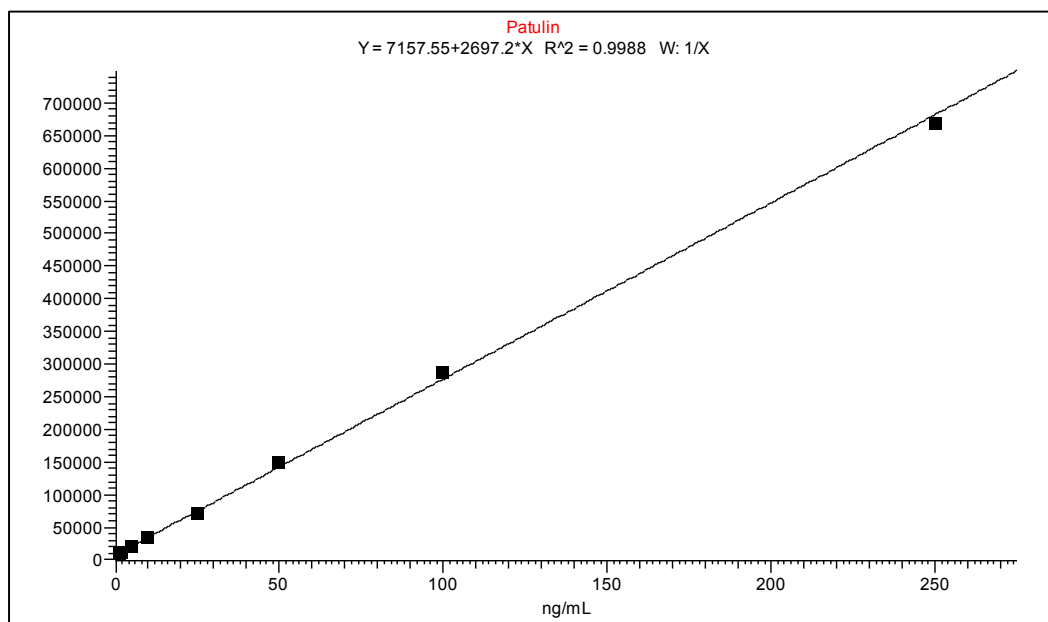
Figure 2. Chromatogram of Patulin at 50 µg/L.

## 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
<b>Mean Recovery (%)</b>	<b>82.3</b>	<b>91.7</b>
<b>RSD (%)</b>	<b>4.54</b>	<b>8.2</b>



**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).

6103-01-01



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

## UCT Part Numbers

### ECMSSC-MP

Mylar pouch containing 4g MgSO<sub>4</sub>  
and 1g NaCl

### CUMPSC1875CB2CT

2mL dSPE tube with 150mg  
MgSO<sub>4</sub>, 50mg PSA, 50mg C18 and  
7.5mg GCB

or

### ECQUEU615CT

15mL dSPE tube with 900mg  
MgSO<sub>4</sub>, 150mg PSA and 45mg GCB

### 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



## Summary:

Patulin (Figure 1) is a naturally occurring mycotoxin that is produced by several species of fungi, such as *Aspergillus*, *Penicillium* and *Byssoschlamys*. 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.

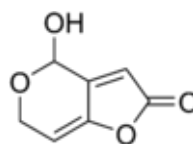


Figure 1. Structure of patulin.

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 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.



FOOD



## QuEChERS Procedure:

### Sample Extraction:

1. Weigh 10 g of sample into a 50 mL polypropylene centrifuge tube.
2. Add 10 mL of acetonitrile.
3. Add the contents of the **ECMSSC-MP** Mylar pouch and shake for a minimum of 1 minute (by hand or mechanically).
4. Centrifuge the samples at  $\geq 3000 \times g$  for 5 minutes.

### Sample Clean-up of 1 mL Extract:

1. Transfer 1 ml of supernatant to a 2 mL dSPE tube (**CUMPSC1875CB2CT**).
2. Vortex the sample for 30 seconds.
3. Centrifuge the samples at  $\geq 3000 \times g$  for 5 minutes.
4. Transfer 500-600  $\mu\text{L}$  of purified supernatant into an autosampler vial (filter if desired).

### Sample Clean-up of 5 mL Extract (for increased sensitivity):

1. Transfer 6 ml of supernatant to a 15 mL dSPE tube (**ECQUEU615CT**).
2. Vortex the sample for 1 minute.
3. Centrifuge the samples at  $\geq 3000 \times g$  for 5 minutes.
4. Transfer 5 mL of purified supernatant into a glass tube and evaporate the sample to  $\approx 1$  mL at  $40^\circ\text{C}$  under a gentle stream of nitrogen.
5. Transfer the sample into an autosampler vial (filter if desired).

## LC-MS/MS Parameters:

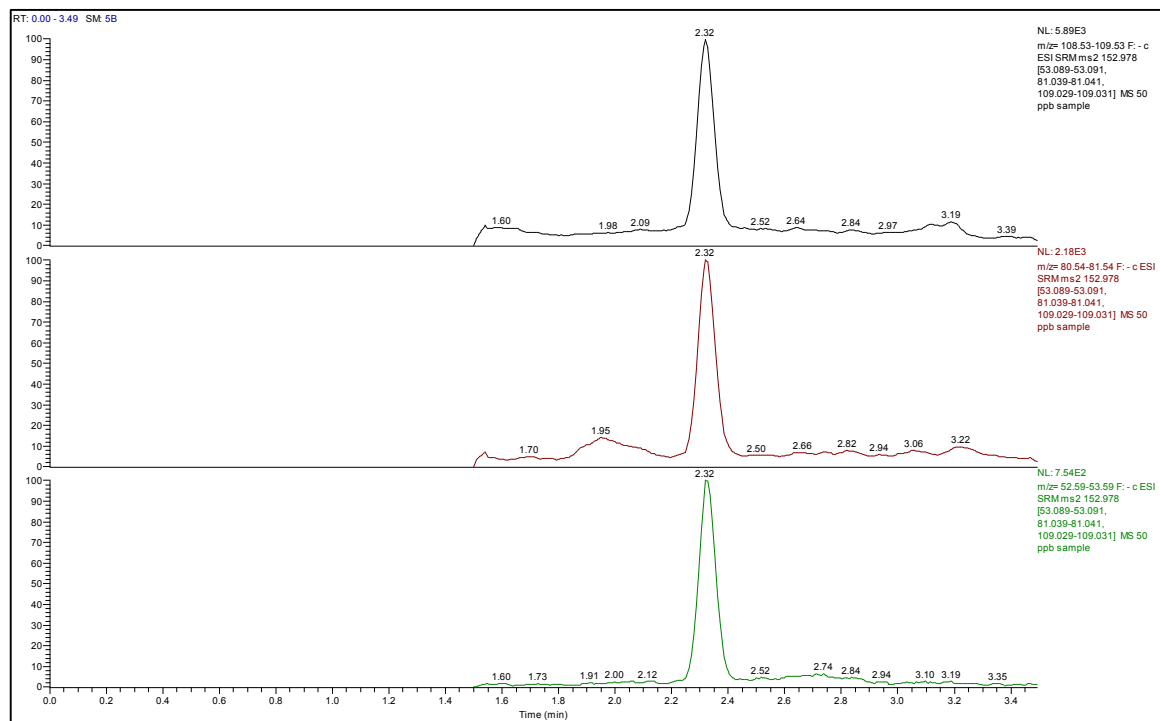
Instrumentation	
HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000 UHPLC
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® DA, 50 × 2.1 mm, 1.8 $\mu\text{m}$ (p/n: SLDA50ID21-18UM)
Guard column	UCT Selectra® DA, 10 × 2.1 mm, 1.8 $\mu\text{m}$ (p/n: SLDAGDC20-18UM)
Guard column holder	p/n: SLGRDHLDLDR
Column temperature	$40^\circ\text{C}$
Flow rate	400 $\mu\text{L}/\text{min}$
Injection volume	5 $\mu\text{L}$

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<sup>-</sup> for patulin.



MRM transitions (ESI <sup>-</sup> )				
Compound	t <sub>R</sub> (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 <sup>a</sup>	50 µg/kg <sup>b</sup>
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
<b>Mean Recovery (%)</b>	<b>103.0</b>	<b>86.6</b>
<b>RSD (%)</b>	<b>2.35</b>	<b>0.30</b>

<sup>a</sup> used 5 mL of sample extract and included a concentration step.

<sup>b</sup> used 1 mL of sample extract and no concentration step.

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