

## DETERMINATION OF PATULIN IN WHOLE APPLE

**Regulations for solid apple products:**  
Europe (EC 1881/2006) : 25µg/Kg

### PROTOCOL OF PURIFICATION

#### Sample preparation

##### Preparation with microwave

Whole apple is cut into pieces and put in a microwave for 90s before crushing the pieces. 15g sample and 7.5mL water are mixed with 150µL pectinase solution and put during 15min at 60°C before a filtration with filter 4-7µm to obtain the loading solution.

##### Preparation with a blender

Whole apple is cut into pieces, put in a blender with Water (2:1 Apple: Water) and mix for 1min. 15g sample and 300µL pectinase solution are put during 15min at 60°C before a filtration with filter 4-7µm to obtain the loading solution.

#### Purification with a 3mL/100mg AFFINIMIP® SPE Patulin cartridge

##### Equilibration

- 2mL Acetonitrile
- 1mL Water

##### Loading

- 3mL of loading solution

##### Washing of interferents (W1)

- 3mL Water-2% Acetic Acid

##### Drying by applying vacuum 10 seconds

##### Washing of interferents (W2)

- 250µL Diethyl Ether

##### Drying by applying vacuum 10 seconds

##### Elution (E)

- 1mL Ethyl Acetate

The elution fraction was then evaporated and dissolved in water containing 0.1% acetic acid before HPLC analysis.

#### HPLC Method

Column: Atlantis T3 column, 150mm x 2.1mm  
Mobile phase: gradient profile

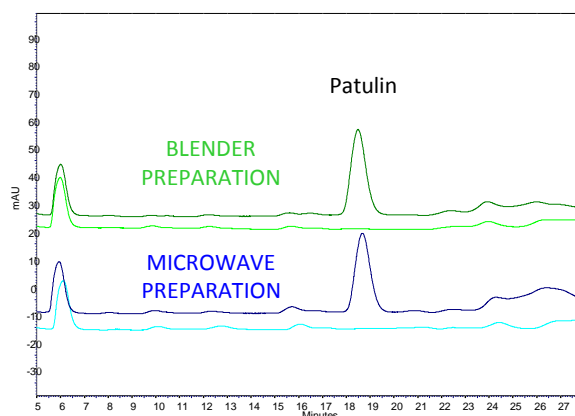
| Time (min) | % water | % ACN |
|------------|---------|-------|
| 0          | 98      | 2     |
| 20         | 98      | 2     |
| 21         | 50      | 50    |
| 25         | 50      | 50    |
| 26         | 98      | 2     |

Flow rate: 0.2mL/min

Detection: UV - 276nm

Injection volume: 100µL.

### RESULTS



Chromatograms obtained after AFFINIMIP® SPE Patulin Clean-up of whole apple spiked at 40µg/kg with Patulin (dark colors) or not spiked (light colors).

Recovery yields obtained after AFFINIMIP® SPE Patulin Clean-up of spiked whole apple with 40µg/kg of Patulin. Whole apples are prepared according to 2 different methods

| Whole apple prepared with blender |    | Whole apple prepared with microwave |    |
|-----------------------------------|----|-------------------------------------|----|
| 96                                | 96 | 95                                  | 88 |

#### Catalog number:

##### 3mL-100mg sorbent

FS102-02 for 25 cartridges

FS102-03 for 50 cartridges

FS102-02K for a kit of 25 cartridges + 50mL Pectinase

FS102-03K for a kit of 50 cartridges + 50mL Pectinase

REA-001-50mL for 50mL Pectinase solution