EASIMIP™ PATULIN

Product Code: P250 / P250B

Molecularly imprinted polymer columns for use in conjunction with HPLC.
For in vitro use only.

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Test Principle

The procedure is based on molecularly imprinted polymer (MIP) technology, which makes the test specific, sensitive, rapid and simple to perform.

The columns contain a molecularly imprinted polymer specific to the toxin of interest. Following extraction of the toxin the sample extract is centrifuged, filtered, and passed through the MIP column. Any toxin which is present in the sample is retained by the MIP within the column. The column is washed to remove unbound material and the toxin is then released from the column following elution with solvent. The eluate is collected prior to analysis by HPLC.

The total extraction and clean-up time takes approximately 45 minutes to perform. The result is improved clean-up and concentration of the toxin from food samples giving a much cleaner chromatogram.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (Acetonitrile, Diethyl ether and Ethyl acetate)
- Perchloric acid (60 %)
- Acetic acid
- Sodium bicarbonate
- Pectinase (P129)*
- Patulin Standard (Please refer to Preparation of Standards section)

Accessory Products

- Immunoaffinity Column Rack (CR1)*
- Immunoaffinity Column Accessory Pack (AP01)*

* Available from R-Biopharm. Please contact your local R-Biopharm distributor for further information.

Hazards

Mycotoxins are very hazardous substances. Only laboratories equipped to handle toxic materials and solvents should perform analyses. Suitable protective clothing, including gloves, safety glasses and lab coats should be worn throughout the analysis.

Flammable solvents should be stored in an explosion-proof cabinet. Use a chemical hood and protective equipment as applicable.

Contact your local R-Biopharm distributor for a Material Safety Data Sheet for further information if required.

Decontamination

Prior to disposal, excess standard solutions should be treated with at least one-tenth their volume of 5 % sodium hypochlorite. Labware and contaminated waste should be immersed in 5 % sodium hypochlorite solution for 30 minutes followed by the addition of 5 % acetone for 30 minutes. Flush with copious amounts of water before disposal. After decontamination labware should be thoroughly washed. Recycle decontaminated plastic waste if local regulations permit.

Storage & Shelf Life

The columns have an expiry of 3 years from date of manufacture if stored at 21 - 25 °C. Do not freeze.

Sampling

A representative sample should be obtained by following one of the officially recognised sampling procedures. It is recommended that a minimum of 1 kg of representative sample is finely ground and a portion (10 - 50 g dependent on method used) of this is removed and extracted.

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A representative sample should be obtained by following one of the officially recognised sampling procedures. It is recommended that a minimum of 1 kg of representative sample is finely ground and a portion (5 - 50 g dependent on method used) of this is removed and extracted.

Recoveries

If an analyst wishes to account for losses during extraction it is recommended a spiked sample of the same commodity type as the material being tested is analysed following the complete procedure as a reference standard. The recoveries obtained with the spiked sample can be used to correct the results obtained with the test sample.

Column Preparation

Firmly attach the column to a glass syringe barrel using an adapter and place in an immunoaffinity column rack or clamp stand. MIP columns require a pre-conditioning before use, see sample preparation for further information.

Sample Preparation

• Clear Apple Juice

Note: A glass barrel and syringe pump will be required to push liquid through the MIP column as they do not run by gravity.

1. Measure 2.5 ml of sample into a centrifuge tube.

2. Add 2.5 ml of 2 % acetic acid. Vortex for 20 seconds.

   Note: Do not allow the column to dry out during the conditioning stage.

3. To condition the column, pass 2 ml of 100 % acetonitrile through the column at a flow rate of 1 ml per minute (1 drop per second). Leave a fine layer of acetonitrile above the white section of the column.

4. Immediately pass 1 ml of water through the column at a flow rate of 1 ml per minute (1 drop per second). Leave a fine layer of water above the white section of the column.

5. Pass 4 ml of the diluted sample (equivalent to 2 ml of sample) through the column at a flow rate of 0.5 ml per minute (1 drop every 2 seconds). A slow, steady flow rate is essential for the capture of the toxin.

6. Wash the column by passing 1 ml of 1 % sodium bicarbonate through at a flow rate of 1 ml per minute (1 drop per second) to remove any salt and polar matrix components.

7. Immediately pass 2 ml of water through the column at a flow rate of 1 ml per minute (1 drop per second). Dry the column by passing air through to remove residual liquid.

8. Under a fume hood, immediately pass 500 µl of 100 % diethyl ether through the column at a flow rate of 1 ml per minute (1 drop per second). Dry the column by passing air through to remove residual liquid.

9. Under a fume hood, elute the toxin from the column at a flow rate of 1 drop per second using 2 ml of 100 % ethyl acetate and collect in a glass tube.

10. Add 1 drop (approximately 10 µl) of 100 % acetic acid to the eluate. Vortex for 20 seconds.

11. Evaporate the eluate to dryness under nitrogen at 35 - 45 °C.

    Note: Ensure that the eluate is not over dried as this will degrade the toxin.

12. Immediately reconstitute with 1 ml of 0.1 % acetic acid. Vortex for 20 seconds.

13. Inject 100 µl of reconstituted eluate onto the HPLC system.

Sample Preparation

• Cloudy Apple Juice

Note: A glass barrel and syringe pump will be required to push liquid through the MIP column as they do not run by gravity.

1. Measure 20 ml of sample into a centrifuge tube.
2. Add 130 µl of pectinase enzyme. Vortex for 20 seconds.
3. Incubate for 2 hours at 40 °C or overnight at 21 - 25 °C.
4. Centrifuge the sample at 4,000 rpm for 10 minutes.
5. Filter the centrifuged sample through a 0.2 µm syringe filter (regenerated cellulose) if required.

   Note: Do not allow the column to dry out during the conditioning stage.
6. Measure 2.5 ml of sample into a centrifuge tube.
7. Add 2.5 ml of 2 % acetic acid. Vortex for 20 seconds.

   Note: Do not allow the column to dry out during the conditioning stage.
8. To condition the column, pass 2 ml of 100 % acetonitrile through the column at a flow rate of 1 ml per minute (1 drop per second). Leave a fine layer of acetonitrile above the white section of the column.
9. Immediately pass 1 ml of water through the column at a flow rate of 1 ml per minute (1 drop per second). Leave a fine layer of water above the white section of the column.
10. Pass 4 ml of the diluted sample (equivalent to 2 ml of sample) through the column at a flow rate of 0.5 ml per minute (1 drop every 2 seconds). A slow, steady flow rate is essential for the capture of the toxin.
11. Wash the column by passing 1 ml of 1 % sodium bicarbonate through at a flow rate of 1 ml per minute (1 drop per second) to remove any salt and polar matrix components.
12. Immediately pass 2 ml of water through the column at a flow rate of 1 ml per minute (1 drop per second). Dry the column by passing air through to remove residual liquid.
13. Under a fume hood, immediately pass 500 µl of 100 % diethyl ether through the column at a flow rate of 1 ml per minute (1 drop per second). Dry the column by passing air through to remove residual liquid.
14. Under a fume hood, elute the toxin from the column at a flow rate of 1 drop per second using 2 ml of 100 % ethyl acetate and collect in a glass tube.
15. Add 1 drop (approximately 10 µl) of 100 % acetic acid to the eluate. Vortex for 20 seconds.
16. Evaporate the eluate to dryness under nitrogen at 35 - 45 °C.

    Note: Ensure that the eluate is not over dried as this will degrade the toxin.
17. Immediately reconstitute with 1 ml of 0.1 % acetic acid. Vortex for 20 seconds.
18. Inject 100 µl of reconstituted eluate onto the HPLC system.

Sample Preparation

• Apple Purée

Note: A glass barrel and syringe pump unit will be required to push liquid through the MIP column as they do not run by gravity.

1. Weigh 10 g of sample into a centrifuge tube.

2. Add 130 µl of pectinase and 10 ml of water. Vortex for 20 seconds.

3. Incubate for 2 hours at 40 °C or overnight at 21 - 25 °C.

4. Centrifuge the diluted sample at 4, 000 rpm for 10 minutes.

5. Filter the centrifuged sample through a 0.2 µm syringe filter (regenerated cellulose).

   Note: Do not allow the column to dry out during the conditioning stage.

6. To condition the column, pass 2 ml of 100 % acetonitrile through the column at a flow rate of 1 ml per minute (1 drop per second). Leave a fine layer of acetonitrile above the white section of the column.

7. Immediately pass 1 ml of water through the column at a flow rate of 1 ml per minute (1 drop per second). Leave a fine layer of water above the white section of the column.

8. Pass 5 ml of the filtrate (equivalent to 2.5 g of sample) through the column at a flow rate of 0.5 ml per minute (1 drop every 2 seconds). A slow, steady flow rate is essential for the capture of the toxin.

9. Wash the column by passing 4 ml of 1 % acetic acid through at a flow rate of 1 ml per minute (1 drop per second). Dry the column by passing air through to remove residual liquid.

10. Immediately pass 4 ml of water through at a flow rate of 1 ml per minute (1 drop per second). Dry the column by passing air through to remove residual liquid.

11. Under a fume hood, immediately pass 500 µl of 100 % diethyl ether through the column at a flow rate of 1 ml per minute (1 drop per second). Dry the column by passing air through to remove residual liquid.

12. Under a fume hood, elute the toxin from the column at a flow rate of 1 drop per second using 2 ml of 100 % ethyl acetate and collect in a glass tube.

13. Add 1 drop (approximately 10 µl) of 100 % acetic acid to the eluate. Vortex for 20 seconds.

14. Evaporate the eluate to dryness under nitrogen at 35 - 45 °C.

    Note: Ensure that the eluate is not over dried as this will degrade the toxin.

15. Immediately reconstitute with 1 ml of 0.1 % acetic acid. Vortex for 20 seconds.

16. Inject 100 µl of reconstituted eluate onto the HPLC system.

Preparation of Standards

Preparation of 10,000 ng/ml patulin stock solutions:

1. Crystalline powder of Patulin can be purchased. Contact your local R-Biopharm distributor for further information. The powder is reconstituted as per the instructions provided and left overnight in the dark at room temperature to give a stock concentrate.

2. This is then used to prepare a 10,000 ng/ml patulin stock solution.

Calibration Curve

It is recommended to run at least a 3 - 6 point calibration curve. In constructing a suitable curve the levels of the calibration standards should bracket or include the range of expected results. The diluted standard solutions should be prepared fresh on the day of analysis and used within a 24 hour period.

To prepare a four point calibration curve:

1. Standard 5: Take 200 µl of 10,000 ng/ml and make up to 8 ml with 0.1 % acetic acid (equivalent to 250 ng/ml).

2. Standard 4: Take 5 ml of 250 ng/ml and add 5 ml of 0.1 % acetic acid (equivalent to 125 ng/ml).

3. Standard 3: Take 2 ml of 125 ng/ml and add 3 ml of 0.1 % acetic acid (equivalent to 50 ng/ml).

4. Standard 2: Take 1 ml of 50 ng/ml and add 1 ml of 0.1 % acetic acid (equivalent to 25 ng/ml).

5. Standard 1: Take 1 ml of 25 ng/ml and add 1 ml of 0.1 % acetic acid (equivalent to 12.5 ng/ml).

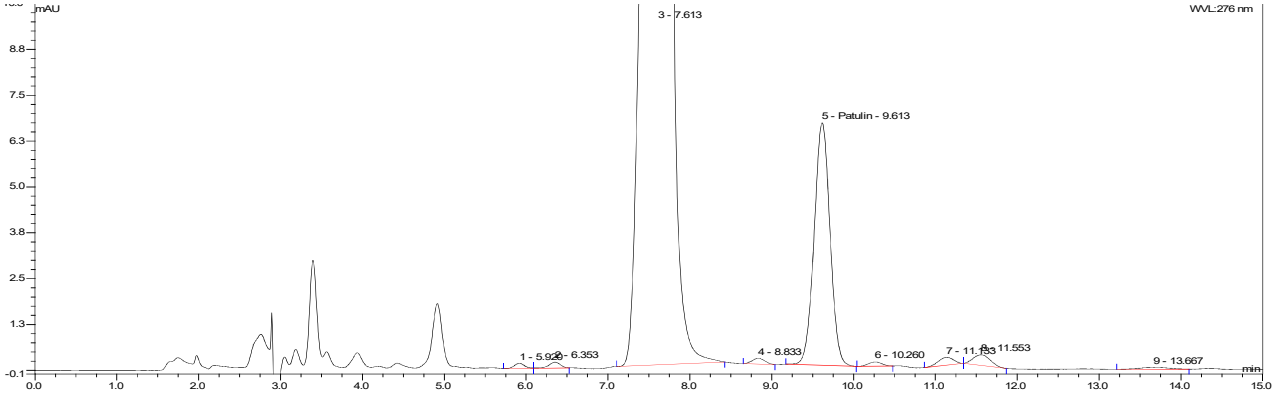
6. Inject 100 µl of each standard onto the HPLC system.

Recommended HPLC Conditions

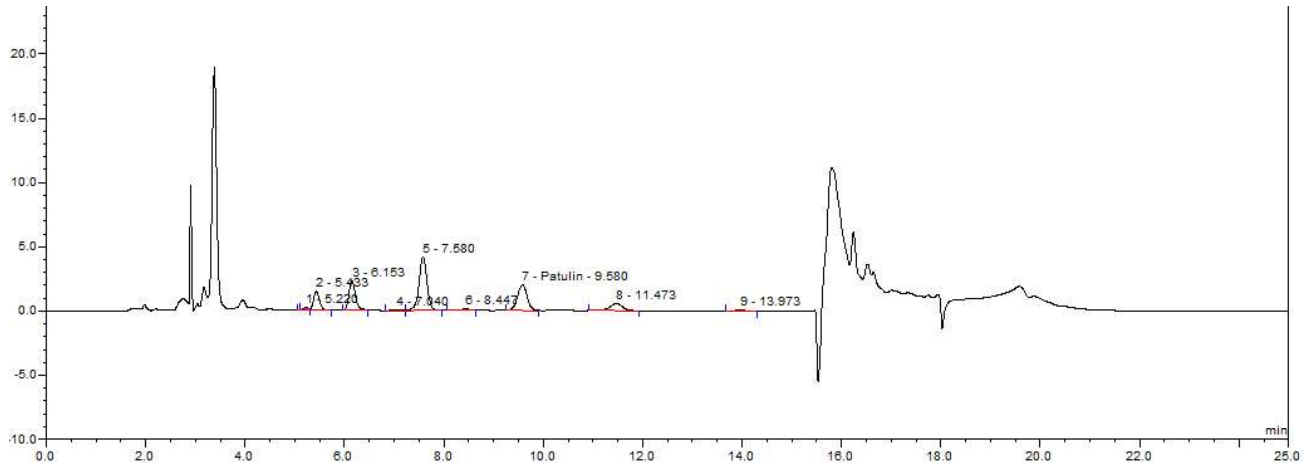
HPLC Conditions			
Guard Cartridge	Inertsil ODS-3 5 µm, 4 mm x 10 mm (Hichrom) or equivalent		
Analytical Column	Inertsil ODS-3V 5 µm, 4.6 mm x 150 mm (Hichrom) or equivalent		
Mobile Phase	Solution A: Water : Acetonitrile : 60 % Perchloric Acid (95 : 5 : 0.1 v/v/v) Solution B: Acetonitrile Prepare fresh on day of analysis.		
Gradient Conditions	Time (min)	% Solution A	% Solution B
	0	100	0
	13	100	0
	13.1	20	80
	16	20	80
	16.1	100	0
	25	100	0
HPLC Pump	To deliver mobile phase		
Flow Rate	1.0 ml per minute		
UV Detector	276 nm		
Column Heater	Maintain guard and analytical columns at 30 °C		
Integrator / Data Control System	From preferred supplier		
Injector	Autosampler / Rheodyne valve		
Injection Volume	100 µl		

Typical HPLC Trace for Analysis of Patulin Using EASMIP™ PATULIN Molecularly Imprinted Columns

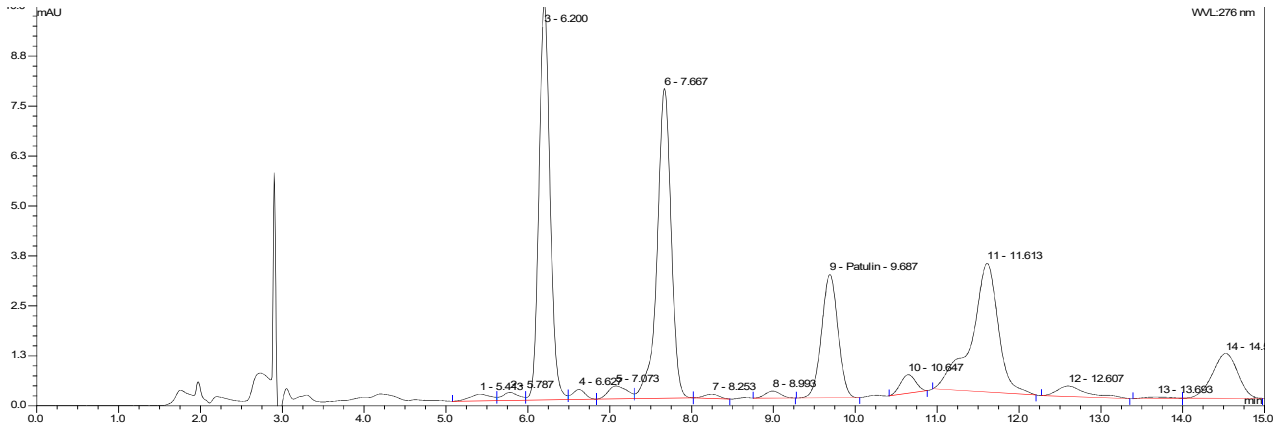
- **Clear Apple Juice**



- **Cloudy Apple Juice**



- **Apple Purée**



Quality

RBR products are developed, manufactured, tested and dispatched under an ISO 9001 registered Quality Management System, guaranteeing a consistent product, which always meets our performance specifications. Our products have been used in many collaborative studies to develop standard European and International Methods and are widely used by key institutions, food companies and government laboratories. Customer references for RBR products are available on request.

Technical Support

RBR understand that from time to time users of our products may need assistance or advice. Therefore, we are pleased to offer the following services to our customers:

- Analysis of problem samples.
- Application notes for difficult samples.
- References from the RBR library.
- Installation and support of the KOBRA® CELL.
- Advice on detection parameters.
- Advice on preparation and handling of standards.
- Updates on legislation, sampling and other news by e-mail.
- Provision of spiked samples.

Please contact your local R-Biopharm distributor for further information.

Warranty

R-Biopharm Rhône Ltd makes no warranty of any kind, express or implied, except that all products made by R-Biopharm Rhône Ltd are made with materials of suitable quality. If any materials are defective, R-Biopharm Rhône Ltd will provide a replacement product. The user assumes all risk and liability resulting from the use of R-Biopharm Rhône Ltd products and procedures. R-Biopharm Rhône Ltd shall not be liable for any damages, including special or consequential damages, loss or expense arising directly or indirectly from the use of R-Biopharm Rhône Ltd products or procedures.

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