PECTINASE

Product Code: P129

Enzyme for sample clarification prior to patulin analysis.
For *in vitro* use only.

P129/V2/03.09.18

www.r-biopharm.com

R-BIOPHARM
RHÔNE LTD

Contents

Page

Test Principle 3
Kit Components 3
Reagents Not Provided 3
Hazards 3
Decontamination 3
Storage & Shelf Life 3
Sampling 4
Recoveries 4
Sample Preparation 4
- Cloudy Apple Juices 5
- Apple Purée 6

Recommended LC Conditions 7
Quality 7
Technical Support 7
Warranty 7

Test Principle

Sample is extracted with ethyl acetate and then cleaned-up by extraction with sodium carbonate solution. Samples are pre-treated with pectinase enzyme. The ethyl acetate extract is dried with anhydrous sodium sulphate. After evaporation of the solvent, the toxin is quantitatively determined by LC with UV detection.

The total sample extraction and clean-up takes approximately 2 hours to perform. The result is improved clean-up of the toxin from the sample giving a much cleaner chromatogram.

Kit Components

- 1 bottle containing 16 ml of pectinase

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (Ethyl Acetate)
- Anhydrous sodium carbonate
- Anhydrous sodium sulfate
- 1.5 % sodium carbonate solution

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. Incinerate waste if regulations permit.

Storage & Shelf Life

The enzyme has an expiry of 3 years from date of manufacture if stored at 2 - 8 °C.

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 (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 that a spiked sample of the same commodity type as the material being tested be analysed following the complete procedure as a reference standard. The recoveries obtained with the spiked sample can then be used to correct the results obtained with the test sample.

Sample Preparation

Pectinase is an enzyme used to clarify cloudy apple juice and apple purée samples to aid in extraction of patulin. The enzyme is used in AOAC Official Method 2000.02 as summarised in the following method.

Pectinase can also be used in conjunction with EASIMIP™ PATULIN (P250 / P250B), see kit instructions for use. Please contact your local R-Biopharm distributor for further information.

• Cloudy Apple Juice (AOAC Official Method 2000.02)

1. Measure 20 ml of sample into a centrifuge tube.
2. Add 150 µl of pectinase enzyme and leave overnight at room temperature, or at 40 °C for 2 hours.
3. Centrifuge at 4,000 rpm for 5 minutes.
4. Measure 10 ml of sample into a 100 ml separating funnel.
5. Add 20 ml of ethyl acetate and shake for 1 minute.
6. Allow the layers to separate and drain each into different conical flasks.
7. Transfer the aqueous layer back into the separating funnel and re-extract by adding 20 ml of ethyl acetate.
8. Allow the layers to separate and drain the lower aqueous layer into a conical flask containing ethyl acetate from the first extraction.
9. Repeat the extraction for a third time. Allow the layers to separate and drain the lower aqueous layer to waste.
10. Combine the three ethyl acetate layer phases in a separating funnel. Rinse the conical flask used to collect the ethyl acetate phases with 5 ml of ethyl acetate and add this to the separating funnel.
11. Add 4 ml of 1.5 % sodium carbonate solution into the separating funnel and shake for 30 seconds.
12. Allow the layers to separate and drain the lower aqueous layer into a conical flask.
13. Filter the top layer through Whatman No. 113 or No. 4 filter paper containing 15 g of anhydrous sodium sulphate.
14. Transfer the aqueous layer back into the separating funnel. Rinse the flask with 10 ml of ethyl acetate and add this to the separating funnel. Shake for 30 seconds.
15. Allow the layers to separate and drain the lower layer to waste.
16. Filter the top layer through the same Whatman No. 113 or No. 4 filter paper containing 15 g of anhydrous sodium sulphate.
17. Wash the separating funnel with 20 ml of ethyl acetate and collect in the same flask.

    Note: Patulin is not stable in alkaline conditions therefore perform this stage as quickly as possible to avoid losses.
18. Evaporate the extract to dryness under air at 60 - 70 °C.
19. For juices, reconstitute with 1 ml of water (pH 4), and for purée samples use 500 µl of water (pH 4). Vortex for 20 seconds.
20. If required filter the reconstituted eluate through a syringe filter (0.45 µm pore size).
21. Transfer to a vial and inject onto the LC system.

Sample Preparation

• Apple Purée (AOAC Official Method 2000.02)

1. Weigh 10 g of sample into a centrifuge tube.
2. Add 150 µl of pectinase enzyme and leave overnight at room temperature, or at 40 °C for 2 hours.
3. Centrifuge at 4,000 rpm for 5 minutes.
4. Measure 10 ml of sample into a 100 ml separating funnel.
5. Add 20 ml of ethyl acetate and shake for 1 minute.
6. Allow the layers to separate and drain each into different conical flasks.
7. Transfer the aqueous layer back into the separating funnel and re-extract by adding 20 ml of ethyl acetate.
8. Allow the layers to separate and drain the lower aqueous layer into a conical flask containing ethyl acetate from the first extraction.
9. Repeat the extraction for a third time. Allow the layers to separate and drain the lower aqueous layer to waste.
10. Combine the three ethyl acetate layer phases in a separating funnel. Rinse the conical flask used to collect the ethyl acetate phases with 5 ml of ethyl acetate and add this to the separating funnel.
11. Add 4 ml of 1.5 % sodium carbonate solution into the separating funnel and shake for 30 seconds.
12. Allow the layers to separate and drain the lower aqueous layer into a conical flask.
13. Filter the top layer through Whatman No. 113 or No. 4 filter paper containing 15 g of anhydrous sodium sulphate.
14. Transfer the aqueous layer back into the separating funnel. Rinse the flask with 10 ml of ethyl acetate and add this to the separating funnel. Shake for 30 seconds.
15. Allow the layers to separate and drain the lower layer to waste.
16. Filter the top layer through the same Whatman No. 113 or No. 4 filter paper containing 15 g of anhydrous sodium sulphate.
17. Wash the separating funnel with 20 ml of ethyl acetate and collect in the same flask.

    Note: Patulin is not stable in alkaline conditions therefore perform this stage as quickly as possible to avoid losses.
18. Evaporate the extract to dryness under air at 60 - 70 °C.
19. For juices, reconstitute with 1 ml of water (pH 4), and for purée samples use 500 µl of water (pH 4). Vortex for 20 seconds.
20. If required filter the reconstituted eluate through a syringe filter (0.45 µm pore size).
21. Transfer to a vial and inject onto the LC system.

Recommended LC Conditions

LC Conditions	
Analytical Column	A 4.3 mm id octadecylsilane (ODS) pre column with 5 µm particle stationary phase. An analytical reversed-phase LC column such as ODS, fully end capped with 5 µm particle stationary phase, 12 nm pore size, and carbon loading of 17 %.
UV Detector	276 nm
Integrator / Data Control System	From preferred supplier

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 users of our products:

- 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.

R-Biopharm Rhône Ltd
Block 10 Todd Campus
West of Scotland Science Park
Acre Road, Glasgow G20 0XA
www.r-biopharm.com