PuriTox AflaZON
Product Code: TC-M160

Solid phase clean-up columns for use in conjunction with HPLC.
For in vitro use only.

TC-M160/V4/03.09.18

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R-BIOPHARM
RHÔNE LTD

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Test Principle

The toxins are extracted from the sample, filtered and passed through the solid phase clean-up column.

The clean-up columns can be used in conjunction with HPLC for the analysis of pigmented samples.

The total clean-up time takes approximately 20 minutes to perform. The result is reduced background interference therefore improving the accuracy of results.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (HPLC Grade Acetonitrile and Methanol)
- Mycotoxin (Please refer to Preparation of Standards section)

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper

Recommended Methods

Deviation from the methods described in our Instructions For Use may not result in optimum results. 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. Incinerate waste if regulations permit.

Storage & Shelf Life

The columns have an expiry of 3 years from date of manufacture if stored at room temperature. 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.

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

• Aflatoxin in Cereal

This method has been tested on a number of cereals including grains and cereal based animal feeds.

1. Weigh 25 g of ground sample to a 1 litre capacity, solvent resistent blender jar.
2. Add 100 ml of 84 % acetonitrile and blend at high speed for 3 minutes.
3. Filter the sample through Whatman No. 113 or No. 4 filter paper or centrifuge at 4,000 rpm for 10 minutes.
4. Pass 2 ml of the extract through the column by applying pressure with the plunger and collect in a glass tube. Pass air through the column to remove residual liquid.
5. Dilute 200 µl of the cleaned up filtrate with 880 µl of distilled water.
6. Inject 100 µl onto the HPLC system.

• Zearalenone in Cereal

This method has been tested on a number of cereals including grains and cereal based animal feeds.

1. Weigh 25 g of ground sample to a 1 litre capacity, solvent resistent blender jar.
2. Add 100 ml of 84 % acetonitrile and blend at high speed for 3 minutes.
3. Filter the sample through Whatman No. 113 or No. 4 filter paper or centrifuge at 4,000 rpm for 10 minutes.
4. Pass 4 ml of the extract through the column by applying pressure with the plunger and collect in a glass tube. Pass air through the column to remove residual liquid.
5. Evaporate the cleaned-up filtrate to dryness under air at 60 - 70 °C.
6. Reconstitute with 500 µl of mobile phase (water : methanol : acetonitrile : acetic acid (200 : 80 : 80 : 1.8 v/v/v)). Vortex for 20 seconds.
7. Inject 150 µl onto the HPLC system.

Preparation of Standards

• Aflatoxin Standard

Preparation of 1,000 ng/ml Aflatoxin Stock Solutions:

1. Ready-to-use AFLASTANDARD (P22 / P22A, 1,000 ng/ml) is available from R-Biopharm.

   or

1. Alternatively, crystalline powder of aflatoxins B1, B2, G1 and G2 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 1,000 ng/ml aflatoxin B1, B2, G1 and G2 stock solution.

   Note: The ratio of B1, B2, G1 and G2 may vary in each standard. Please note the correct ratio for the standard purchased.

• Zearalenone Standard

Preparation of 1,000 ng/ml zearalenone stock solutions:

1. Ready-to-use ZEASTANDARD (P44 / P44A, 1,000 ng/ml) is available from R-Biopharm.

   or

1. Alternatively, crystalline powder of zearalenone 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 1,000 ng/ml zearalenone stock solution.

Calibration Curve

• Aflatoxin

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.

Example of how to prepare a five point calibration curve (can be modified according to legislative requirements or contamination levels) :

1. Measure the appropriate volume of stock standard and 84 % acetonitrile into separate glass tubes, according to the table below, and make up to 10 ml with 84 % acetonitrile.

Total Aflatoxin Callibration Curve	
Volume of Stock Standard	Aflatoxin Concentration
5 µl	1 ng/ml B1 and G1 0.25 ng/ml B2 and G2
10 µl	2 ng/ml B1 and G1 0.5 ng/ml B2 and G2
15 µl	3 ng/ml B1 and G1 0.75 ng/ml B2 and G2
20 µl	4 ng/ml B1 and G1 1 ng/ml B2 and G2
25 µl	5 ng/ml B1 and G1 1.25 ng/ml B2 and G2

• Zearalenone

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.

Example of how to prepare a five point calibration curve (can be modified according to legislative requirements or contamination levels) :

1. Measure the appropriate volume of stock standard and mobile phase into separate glass tubes, according to the table below, and make up to the required volume with zearalenone mobile phase.

Zearalenone Calibration Curve		
Volume of Stock Standard	Total Volume of Mobile Phase	Zearalenone Concentration
10 µl	5 ml	50 ng/ml
20 µl	5 ml	100 ng/ml
100 µl	4.9 ml	500 ng/ml
200 µl	4.8 ml	1,000 ng/ml
600 µl	4.4 ml	3,000 ng/ml

Recommended HPLC Conditions

• Aflatoxin

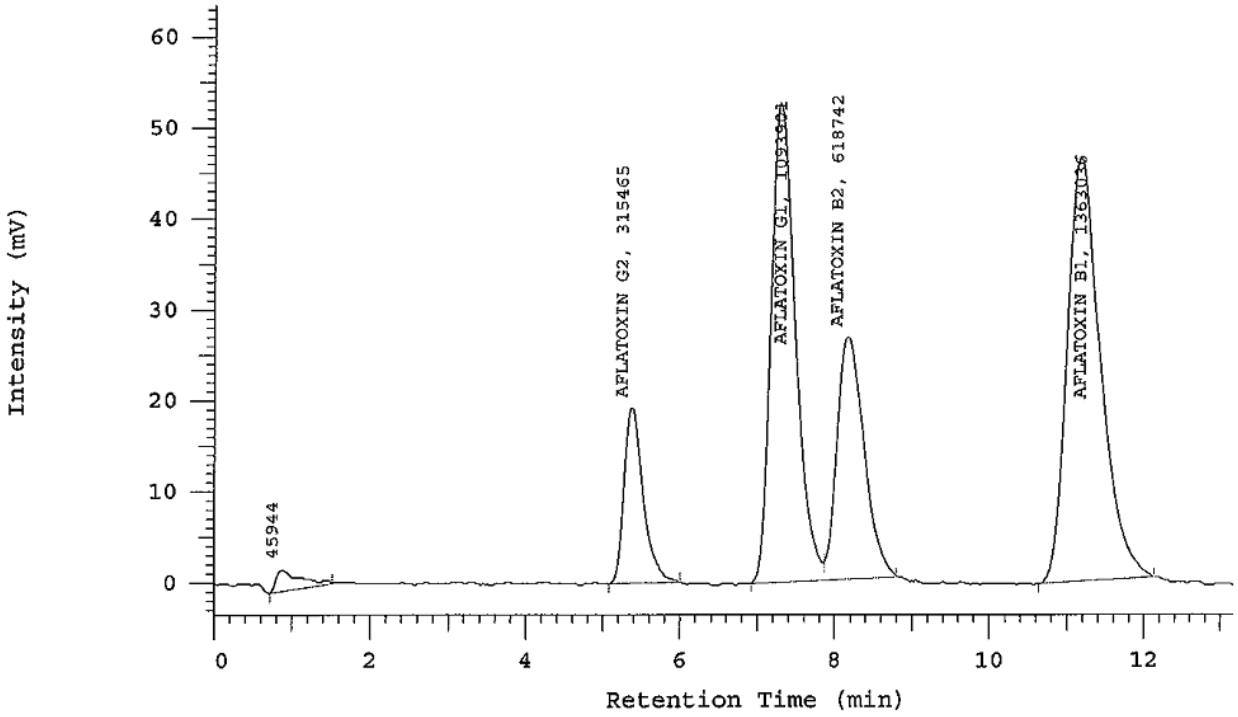
HPLC Conditions	
Derivatisation	KOBRA® CELL at 100 µA setting
Guard Cartridge	Inertsil ODS-3 5 µm, 4 mm x 10 mm or (Hichrom) equivalent
Analytical Column	Inertsil ODS-3V 5 µm, 4.6 mm x 150 mm (Hichrom) or equivalent
Mobile Phase	Water : Acetonitrile : Methanol (70 : 15 : 15 (v/v/v)) Prepare fresh on day of analysis.
HPLC Pump	To deliver mobile phase Add 119 mg of potassium bromide and 350 µl 4 M Nitric Acid to 1 litre of mobile phase
Flow Rate	1.2 ml/minute
Fluorescence Detector	Excitation: 360 nm
	Emission: 440 nm
Column Heater	Maintain guard and analytical columns at 40 °C
Integrator / Data Control System	From preferred supplier
Injector	Autosampler / Rheodyne valve
Injection Volume	100 µl

• Zearalenone

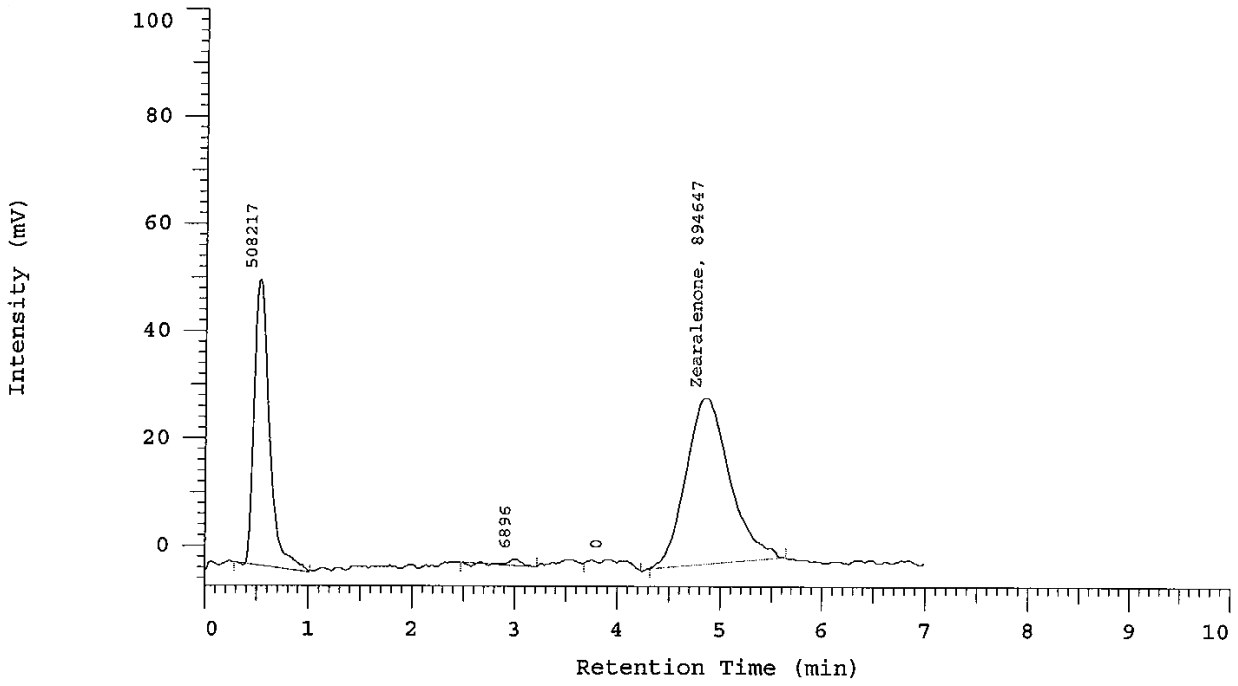
HPLC Conditions	
Guard Cartridge	Inertsil ODS-3 5 µm, 4 mm x 10 mm or (Hichrom) equivalent
Analytical Column	Inertsil ODS-3V 5 µm, 4.6 mm x 150 mm (Hichrom) or equivalent
Mobile Phase	Water : Methanol : Acetonitrile : Acetic Acid (200 : 80 : 80 : 1.8 (v/v/v/v)) Prepare fresh on day of analysis.
HPLC Pump	To deliver mobile phase
Flow Rate	2.0 ml/minute
Fluorescence Detector	Excitation: 285 nm
	Emission: 460 nm
Column Heater	Maintain guard and analytical columns at 40 °C
Integrator / Data Control System	From preferred supplier
Injector	Autosampler / Rheodyne valve
Injection Volume	150 µl

Typical HPLC Trace Using PuriTox AflaZON Columns

- **Total Aflatoxins**



- **Zearalenone**



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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