PuriTox Aflatoxin

Product Code: P25

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

P25/V11/30.06.21

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R-BIOPHARM
RHÔNE LTD

Contents

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 or for the clean-up of pigmented samples prior to analysis by ELISA.

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

For HPLC Method:
- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (HPLC Grade Methanol)
- Aflatoxin Standard (Please refer to Preparation of Standards section)

For HPLC Method Only:
- Nitric Acid (only required when derivatising with a KOBRA® CELL)
- Potassium Bromide (only required when derivatising with a KOBRA® CELL)

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper
- 0.45 µm Syringe Filter
- KOBRA® CELL (K01)*
- 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. Incinerate waste if regulations permit.

Storage & Shelf Life

The columns have an expiry of 2 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 (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.

HPLC Information

• Sample Preparation - Peanuts

1. Weigh 50 g of ground sample into a 1 litre capacity, solvent resistant blender jar.

2. Add 100 ml of 80 % methanol and blend at high speed for 2 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 6 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 2 ml of eluate with 1.2 ml of water.

6. Filter the diluted filtrate through a 0.45 µm syringe filter.

7. Inject 100 µl onto the HPLC system.

HPLC Information

• Preparation of Standards

Preparation of 1,000 ng/ml aflatoxin B1, B2, G1 and G2 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.

• 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.

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

1. Standard 4: Take 80 µl of 1,000 ng/ml total aflatoxin solution and make up to 2 ml with 50 % methanol (equivalent to 40 ng/ml).

2. Standard 3: Take 1 ml of 40 ng/ml and add 1 ml of 50 % methanol (equivalent to 20 ng/ml).

3. Standard 2: Take 1 ml of 20 ng/ml and add 1 ml of 50 % methanol (equivalent to 10 ng/ml).

4. Standard 1: Take 400 µl of 10 ng/ml and make up to 2 ml with 50 % methanol (equivalent to 2 ng/ml).

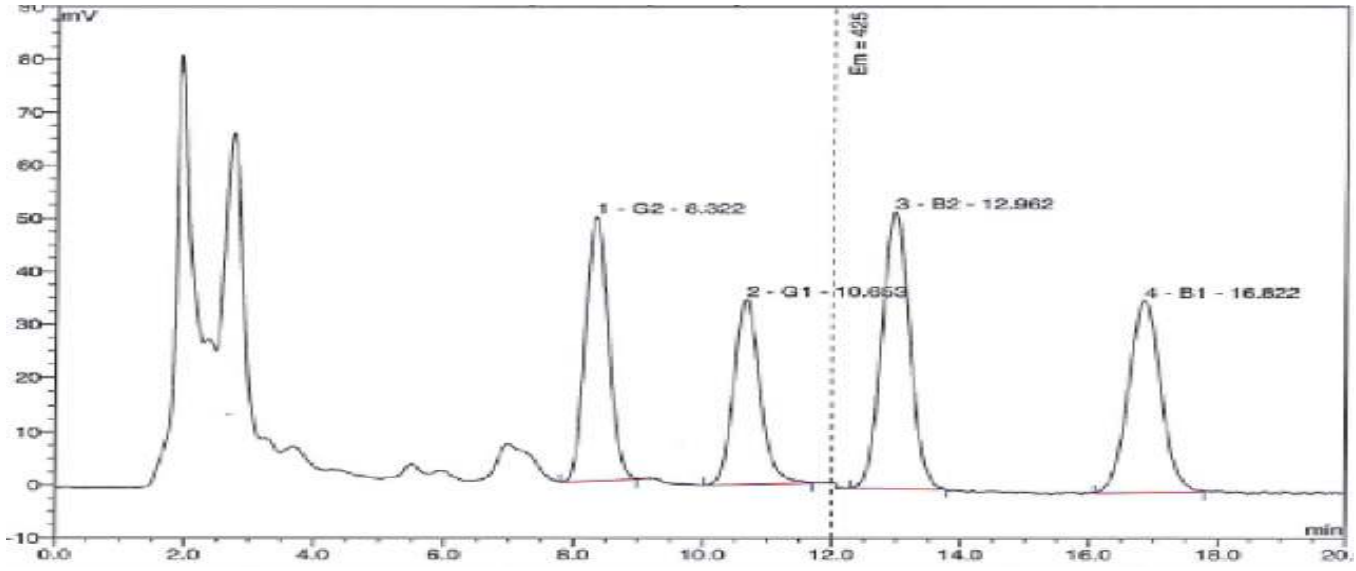
5. Inject 100 µl of each standard onto the HPLC system. The elution order is G2, G1, B2 and B1 when derivatising with a KOBRA® CELL.

HPLC Information

• Recommended HPLC Conditions

HPLC Conditions	
Derivatisation	KOBRA® CELL at 100 µA setting
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	Water : Methanol (60 : 40 v/v)
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. Prepare fresh on day of analysis.
Flow Rate	1.0 ml/minute
Fluorescence Detector	Excitation: 362 nm
	Emission: 425 nm (B1 and B2) 455 nm (G1 and G2)
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
Elution Order	G2, G1, B2, B1

• Example HPLC Chromatogram for Peanuts (Spiked at 8 ppb Total Aflatoxin)



ELISA Information

• Sample Preparation

1. Extract the sample in the appropriate solvent.

2. Filter the sample through Whatman No. 113 or No. 4 filter paper, or centrifuge at 4,000 rpm for 10 minutes.

3. Pass 5 - 10 ml of the filtrate through the solid phase clean-up column by applying pressure with the plunger and collect the cleaned-up filtrate.

4. If the filtrate is not clear the liquid can be passed through a second clean-up column.

   Note: The cleaned-up filtrate does not need to be colourless, but it must be clear.

5. Dilute and apply to ELISA as per instructions provided.

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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