FUMONIPREP®

Product Code: DP31 / P31B

Immunoaffinity columns for use in conjunction with HPLC or LC-MS/MS.
For in vitro use only.

P31/V19/27.05.22

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R-BIOPHARM
RHÔNE LTD

Contents

Test Principle

The procedure is based on monoclonal antibody technology, which makes the test highly specific, sensitive, rapid and simple to perform.

The columns contain a gel suspension of monoclonal antibody specific to the toxins of interest. Following extraction of the toxins the sample extract is filtered, diluted and passed slowly through the immunoaffinity column. Any toxins which are present in the sample are retained by the antibody within the gel suspension. The column is washed to remove unbound material and the toxins are then released from the column following elution with solvent. The eluate is collected prior to analysis by HPLC or LC-MS/MS. Fumonisins are required to be derivatised when analysed by HPLC.

The total extraction and clean-up time takes approximately 30 minutes to perform. The result is improved clean-up and concentration of the toxins from food and feed samples giving a much cleaner chromatogram and therefore providing more accurate and sensitive detection. The columns also have the added advantage that they can be automated for large scale analysis of samples.

Reagents Not Provided

For HPLC and LC-MS/MS Methods:

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (HPLC Grade Methanol and Acetonitrile)
- Phosphate Buffered Saline (PBS) (RP202)*
- Fumonisin B1 and B2 Standard (Please refer to Preparation of Standards section)
- Sodium Chloride
- Sodium Dihydrogen Phosphate

For HPLC Methods Only:

- Disodium Tetraborate
- O-phosphoric acid (H_3PO_4) >85 %
- O-phthaldialdehyde (OPA)
- 1-Thioglycerol

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper
- Glass Microfiber Filter Paper
- Immunoaffinity Column Rack (CR1)*
- Immunoaffinity Column Accessory Pack (AP01)*

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

Recommended Methods and Application Notes

Methods are available for all matrices covered by legislation as well as additional commodities. Deviation from the methods described in our Instructions For Use and Application Notes may not achieve 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 expire 18 months from date of manufacture if stored at 2 - 8 °C or 12 months from date of manufacture if stored at 21 - 25 °C. Do not freeze.

Ensure the column has not dried out and contains buffer above the gel. It is important to note the antibody included in the immunoaffinity column can be denatured by extreme temperature or pH change.

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.

Sensitivity

The sensitivity is dependent on the final detection system employed by the analyst. However the test sensitivity may be improved if required by increasing the volume of sample passed through the immunoaffinity column. Please note the ratio of solvent to phosphate buffered saline (PBS) should be maintained.

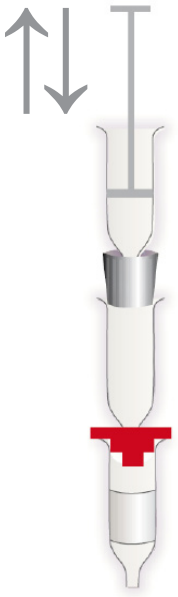
Recoveries

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

Immunoaffinity columns should be at ambient temperature before use. Remove the cap from the top of the column and discard. Firmly attach the column to a glass syringe barrel using an adapter and place in an immunoaffinity column rack or clamp stand.

Elution



In order to fully elute the toxin/s from the immunoaffinity column it is vital that the solvent is in contact with the antibody within the gel suspension for a sufficient period of time. This ensures that all of the bonds between the antibody and the toxin are broken, ultimately releasing all of the toxin from the column for analysis with the detection system of choice

To ensure that the solvent is in contact with the antibody gel for a sufficient period of time any of the following elution methods can be used: -

Backflushing (this is the preferred method of choice at R-Biopharm): backflush by gently raising and lowering the syringe plunger during passage of the solvent through the column. This process will reverse the direction of flow of the eluate through the gel. This should be repeated 3 times before collecting the eluate. Proceed to the next step in the method.

Application of small volumes of solvent: apply the volume of solvent required for elution in two or three smaller aliquots. Allow each aliquot to remain in contact with the gel suspension for a minimum of 30 seconds before allowing each to pass fully through the gel suspension for collection. Proceed to the next step in the method.

Incubation with solvent: apply the full volume of solvent required for elution and allow 2-3 drops of the solvent to pass through the column for collection. Allow the remainder of the solvent to remain in contact with the gel suspension for a minimum of 60 seconds before allowing it to pass through the gel suspension for collection. Proceed to the next step in the method.

Derivitisation Prior to HPLC Detection

• Preparation of 0.1 M Borate Buffer

The buffer should be prepared fresh on the day of analysis.

1. Weigh 3.8 g of sodium teraborate decahydrate into a glass jar.
2. Make up to 100 ml with water.

• Preparation of OPA Reagent (with Mercaptoethanol or 1-Thioglycerol)

The reagent can be kept for up to 5 days if stored at 2 - 8 °C. The same reagent should be used throughout the procedure.

1. Weigh 120 mg of OPA into a glass jar.
2. Add 3 ml of 100 % methanol, 15 ml of borate buffer and either 150 µl of Mercaptoethanol or 179 µl of 1-Thioglycerol.
3. Leave overnight in the dark at room temperature.

• Programming the Autosampler

Note: It is important that the standards and the sample eluates are injected onto the HPLC within 3 minutes if they contain OPA reagent with Mercaptoethanol or 10 minutes if they contain OPA reagent with 1-Thioglycerol. It is also recommended that the time period following addition of the OPA and injection onto the HPLC is kept constant for both samples and standards in order to reduce variability.

Program is set up such that an empty vial is placed in an autosampler position preceding that of the sample solution, e.g. for the first injection of a run, the mix vial is placed in position 1 while the sample solution to be injected into the mix vial is in position 2 on the autosampler.

1. 200 µl of OPA reagent is injected into the mixing vial.
2. 200 µl of eluate is injected into the mixing vial.
3. 200 µl of mix is drawn up and dispensed 3 times.
4. 100 µl of derivatised sample is injected onto the system.

Sample Preparation

- **Cereal**

This method has been tested on a number of cereals including wheat, barley, maize, cornflour, popcorn, cornflakes and other cereal based products.

1. Weigh 25 g of sample and 5 g of sodium chloride into a 1 litre capacity, solvent resistent blender jar.

2. Add 125 ml of acetonitrile : methanol : water (25 : 25 : 50 v/v/v) and blend at high speed for 2 minutes.

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

4. Dilute 10 ml of the filtrate with 40 ml of phosphate buffered saline (PBS).

5. Filter the diluted extract through glass microfibre filter paper.

6. Pass 10 ml of the diluted filtrate (equivalent to 0.4 g of sample) through the column at a flow rate of 2 ml per minute (or the sample can be allowed to pass through the column by gravity if preferred). A slow, steady flow rate is essential for the capture of the toxins by the antibody.

7. **HPLC:** Wash the column with 10 ml of PBS.
   LC-MS/MS: Wash the column with 20 ml of water.
   The column should be washed at a flow rate of approximately 5 ml per minute. Pass air through the column to remove residual liquid.

8. Elute the toxins from the column at a flow rate of 1 drop per second using 1.5 ml of 100 % methanol and collect in an amber glass vial. Please refer to the Elution section for further information.

9. Following elution pass 1.5 ml water through the column and collect in the same vial to give a 3 ml total volume.

HPLC:

10. Add 200 µl of eluate to 200 µl of OPA reagent. Program the autosampler to perform derivatisation of the sample. Please refer to the Programming the Autosampler section for further information.

11. Inject 100 µl onto the HPLC system.

LC-MS/MS:

10. Inject 50 µl onto the LC-MS/MS system.

Preparation of Standards

- ## Fumonisin Stock Solution

It is advised to start with a 100,000 ng/ml fumonisin (FB1 and FB2) stock solution.

Note: The ratio of FB1 to FB2 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 solution should be prepared fresh on the day of analysis and used within a 24 hour period.

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

1. Measure 7.5 ml of 50 % methanol into an amber vial.
2. Remove 300 µl to waste.
3. Add 300 µl of 100,000 ng/ml total fumonisin standard to give a 4,000 ng/ml total fumonisin solution.

HPLC:

4. Standard 3: Take 500 µl of 4,000 ng/ml of total fumonisin solution and add 1.5 ml of 50 % methanol (equivalent to 1,000 ng/ml).
5. Standard 2: Take 1 ml of Standard 3 and add 1 ml of 50 % methanol (equivalent to 500 ng/ml).
6. Standard 1: Take 1 ml of Standard 2 and add 1 ml of 50 % methanol (equivalent to 250 ng/ml).
7. Add 200 µl of each standard to 200 µl of OPA reagent. Program the autosampler to perform derivatisation of each solution. Please refer to the Programming the Autosampler section for further information.
8. Inject 100 µl of each solution onto the HPLC system.

LC-MS/MS:

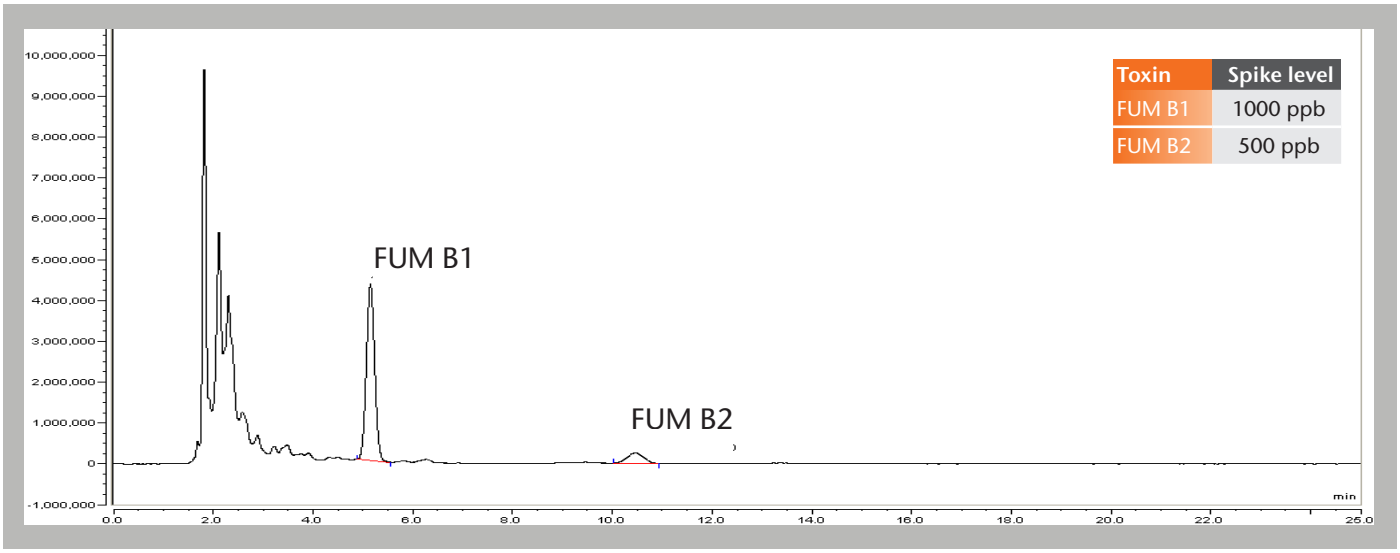
4. Standard 5: Take 500 µl of 4,000 ng/ml of total fumonisin solution and add 1.5 ml of 50 % methanol (equivalent to 1,000 ng/ml).
5. Standard 4: Take 1 ml of Standard 5 and add 1 ml of 50 % methanol (equivalent to 500 ng/ml).
6. Standard 3: Take 1 ml of Standard 4 and add 1 ml of 50 % methanol (equivalent to 250 ng/ml).
7. Standard 2: Take 1 ml of Standard 3 and add 1 ml of 50 % methanol (equivalent to 125 ng/ml).
8. Standard 1: Take 1 ml of Standard 2 and add 1 ml of 50 % methanol (equivalent to 62.5 ng/ml).
9. Inject 50 µl of each solution onto the LC-MS/MS system.

Recommended HPLC Conditions

HPLC Conditions	
Derivatisation	OPA Reagent
Guard Cartridge	C18 deactivated reverse phase cartridge or equivalent 10 mm x 4.6 mm i.d.
Analytical Column	Inertsil ODS-3V 5 µm, 4.6 mm x 150 mm (Hichrom) or equivalent
Mobile Phase	Methanol : 0.1 M Sodium Dihydrogen Phosphate (77 : 23 v/v) Add 11.998 g of sodium phosphate to 1 l of water to give a 0.1 M solution. Adjust to pH 3.3 with o-phosphoric acid. Prepare fresh on day of analysis.
HPLC Pump	To deliver mobile phase
Flow Rate	1.0 ml per minute
Fluorescence Detector	Excitation: 335 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

Example HPLC Chromatogram

- **Maize**



Recommended LC-MS/MS Conditions

LC Conditions			
Analytical Column	Phenomenex Gemini 5 µm C18 110 A, 150 mm x 3 mm or equivalent		
Mobile Phase	**Mobile Phase A:** 1 mM Ammonium Formate and 0.1 % Formic Acid in 5 % Methanol **Mobile Phase B:** 1 mM Ammonium Formate and 0.1 % Formic Acid in 98 % Methanol Prepare fresh on day of analysis.		
Gradient Conditions	Time (min)	% Solution A	% Solution B
	0	80	20
	0.1	80	20
	10	10	90
	15	10	90
	15.1	80	20
	20	80	20
HPLC Pump	To deliver mobile phase		
Flow Rate	0.3 ml per minute		
Column Heater	Maintain analytical column at 40 °C		
Integrator / Data Control System	From preferred supplier		
Injector	Autosampler / Rheodyne valve		
Injection Volume	50 µl		

Mass Spectrometry Conditions	
Instrument	Waters® ACQUITY TQ Detector with Electrospray Ionisation
Mode	Multiple Reaction Monitoring (MRM) Mode with positive polarity
Capillary Voltage	+1,500 Volts
Source Temperature	150 °C
Desolvation Gas Temperature	350 °C
Desolvation Gas Flow	600 L/hr (N)
Cone Gas Flow	50 L/hr (N)

Instrument Setting

Toxin	Time Segment (min)	Precursor Ion (m/z)	Product Ions (m/z)	Dwell Time (s)	Cone Voltage (V)	Collision Voltage (eV)
Fumonisin B1	6.5 - 9.5	722.39 [M+H]+	334.39 (Quantifier) 352.40 (Qualifier)	0.105	52 52	40 38
Fumonisin B2	8.5 - 10.5	706.39 [M+H]+	336.40 (Quantifier) 318.39 (Qualifier)	0.105	56 56	40 42

Example LC-MS/MS Chromatogram

- **Maize**

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

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