AOF MS-PREP®

Product Code: P115 / P115B

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

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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 antibodies 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 antibodies 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 LC-MS/MS.

The total extraction and clean-up time takes approximately 20 minutes to perform. The result is improved clean-up and concentration of the toxins from food and feed samples reducing ion suppression and removing the need to use matrix matched standards. This provides cleaner chromatography, improved sensitivity and greater accuracy. The columns also have the added advantage that they can be automated for large scale analysis of samples.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (HPLC Grade Methanol and Acetonitrile)
- Phosphate Buffered Saline (PBS) (RP202)*
- Mycotoxin Standards (Please refer to Preparation of Standards section)
- Ammonium Formate
- Formic Acid
- Acetic Acid
- Sodium Chloride
- Sodium Hydroxide (to pH filtrate if required)

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.

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.

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.

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 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.

Buffer Preparation

- ### Preparation of 20 mM Ammonium Acetate

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

1. Weigh 1.54 g of ammonium acetate into a flask.
2. Make up to 1 L with water.

Sample Preparation

- **Cereal**

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

2. Add 100 ml of 60 % 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. Dilute 10 ml of the filtrate with 15 ml of phosphate buffered saline (PBS).

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

6. Pass 5 ml of the filtrate (equivalent to 0.5 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 toxin by the antibody.

7. Wash the column by passing 20 ml of 20 mM of ammonium acetate through at a flow rate of approximately 5 ml per minute. Pass air though the column to remove residual liquid.

8. Elute the toxins from the column at a flow rate of 1 drop per second using 1 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 ml of water through the column and collect in the same vial to give a 2 ml total volume.

10. Inject 25 - 50 µl onto the LC-MS/MS system.

Sample Preparation

- **Animal Feed**

1. Weigh 25 g of ground sample into a 1 litre capacity, solvent resistant blender jar.
2. Add 100 ml of acetonitrile : acetic acid : water (74 : 1 : 25 v/v/v) 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. Dilute 5 ml of the filtrate with 75 ml of phosphate buffered saline (PBS).
5. Adjust to around pH 7.4 using 2 M sodium hydroxide.
6. Filter the diluted extract through glass microfibre filter paper.
7. Pass 32 ml of the filtrate (equivalent to 0.5 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.
8. Wash the column by passing 20 ml of 20 mM of ammonium acetate through at a flow rate of approximately 5 ml per minute. Pass air though the column to remove residual liquid.
9. Elute the toxins from the column at a flow rate of 1 drop per second using 1 ml of 100 % methanol and collect in an amber glass vial. Please refer to the Elution section for further information.
10. Following elution pass 1 ml of water through the column and collect in the same vial to give a 2 ml total volume.
11. Inject 25 - 50 µl onto the LC-MS/MS system.

Preparation of Standards

- ### Aflatoxin Stock Solution

It is advised to start with a 1,000 ng/ml total aflatoxin 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.

- ### Ochratoxin Stock Solution

It is advised to start with a 1,000 ng/ml ochratoxin A stock solution.

- ### Fumonisin Stock Solution

It is advised to start with a 150,000 ng/ml fumonisin 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 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. Standard 5:
- Take 5 ml of 100 % methanol and remove 200 µl to waste.
- Add 100 µl of 1,000 ng/ml total aflatoxin standard, 50 µl of 1,000 ng/ml ochratoxin A standard and 100 µl of 150,000 ng/ml fumonisin standard.
- Add 4.95 ml of water (equivalent to 10 ng/ml total aflatoxin, 5 ng/ml ochratoxin A and 1,500 ng/ml fumonisin).

2. Standard 4: Take 5 ml of standard 5 and add 5 ml of 50 % methanol (equivalent to 5 ng/ml total aflatoxin, 2.5 ng/ml ochratoxin A and 750 ng/ml fumonisin).

3. Standard 3: Take 4 ml of standard 4 and add 6 ml of 50 % methanol (equivalent to 2 ng/ml total aflatoxin, 1 ng/ml ochratoxin A and 300 ng/ml fumonisin).

4. Standard 2: Take 5 ml of standard 3 and add 5 ml of 50 % methanol (equivalent to 1 ng/ml total aflatoxin, 0.5 ng/ml ochratoxin A and 150 ng/ml fumonisin).

5. Standard 1: Take 5 ml of standard 2 and add 5 ml of 50 % methanol (equivalent to 0.5 ng/ml total aflatoxin, 0.25 ng/ml ochratoxin A and 75 ng/ml fumonisin).

6. Inject 25 - 50 µl of each standard onto the LC-MS/MS system.

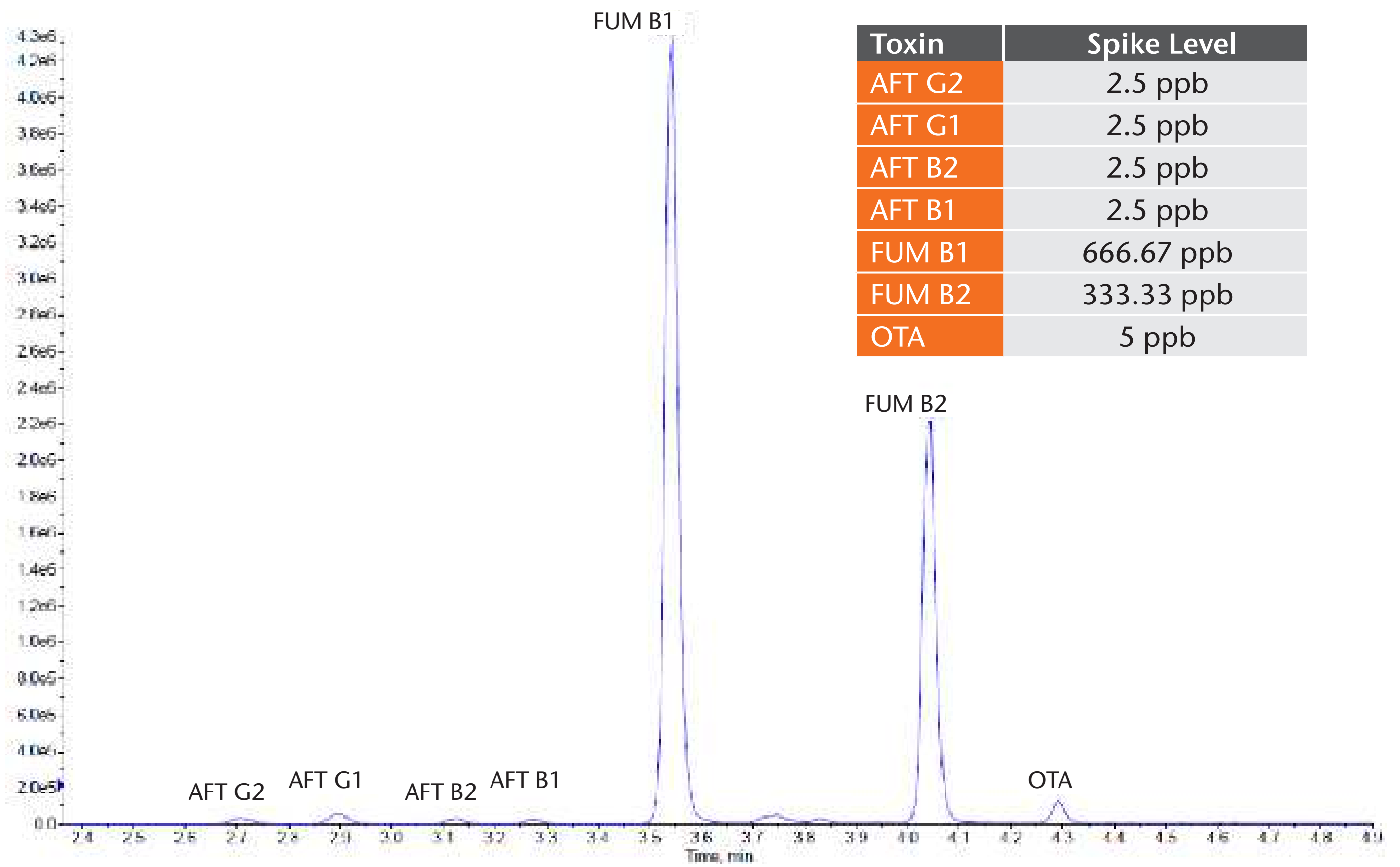
Recommended LC Conditions

LC Conditions			
Guard Cartridge	Phenomenex Luna Omega 3 4 mm x 2 mm or equivalent		
Analytical Column	Phenomenex Luna Omega 3 µm Polar C18, 100 x 3 mm		
Mobile Phase	Solution A: Water : Methanol : Formic Acid (95 : 5 : 0.1 v/v/v) containing 1 mM Ammonium Formate Solution B: Water : Methanol : Formic Acid (2 : 98 : 0.1 v/v/v) containing 1 mM Ammonium Formate Prepare fresh on day of analysis.		
Gradient Conditions	Time (min)	% Solution A	% Solution B
	0	60	40
	0.5	60	40
	4.0	0	100
	6.0	0	100
	6.1	60	40
	8.0	60	40
HPLC Pump	To deliver mobile phase		
Flow Rate	0.6 ml per minute		
Column Heater	Maintain guard and analytical columns at 40 °C		
Integrator / Data Control System	From preferred supplier		
Injector	Autosampler / Rheodyne valve		
Injection Volume	25 or 50 µl		

MS/MS Conditions	
Instrument	SCIEX QTRAP 3500 - 5500 with Electrospray Ionisation mode Scheduled Multiple Reaction Monitoring (MRM) in positive polarity
Scheduled MRM Parameters	
MRM Window	60 s
Target Cycle Time	0.35 s
Minimum Dwell Time	10 ms
Maximum Dwell Time	175 ms
Ion Source / Gas Settings	
Curtain gas	30
IonSpray Voltage	+ 4,500 V
Turbo Gas Temperature	500 °C
Ion Source Gas 1 (Nebuliser gas)	40
Ion Source Gas 2 (Heater gas)	50
Collision gas	7

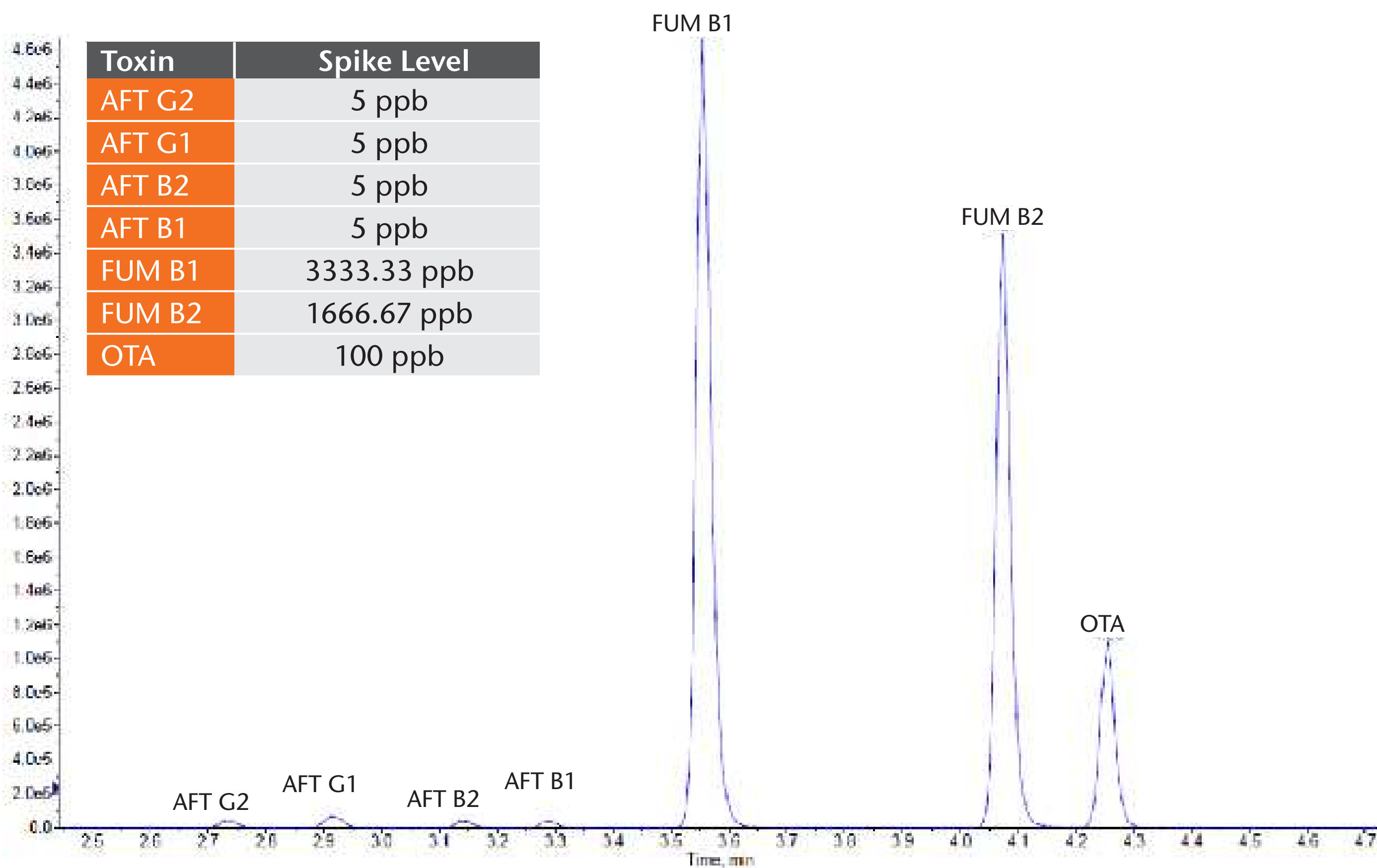
Instrument Setting						
Toxin	**Q1 Precursor Ion (m/z)**	**Q3 Product Ion (m/z)**	**Dwell Time (ms)**	**Declustering Potential (V)**	**Collision Energy (V)**	**Collision Exit Potential (V)**
Aflatoxin G2	331.1 [M+H]+	189.0 (Quantifier) 256.9 (Qualifier)	20 20	140	54.4 40.9	14.0 20.0
Aflatoxin G1	329.1 [M+H]+	243.0 (Quantifier) 199.9 (Qualifier)	20 20	140	38.6 53.7	15.0 15.0
Aflatoxin B2	315.2 [M+H]+	287.1 (Quantifier) 259.0 (Qualifier)	20 20	170	34.9 38.6	17.0 17.0
Aflatoxin B1	313.0 [M+H]+	285.1 (Quantifier) 189.0 (Qualifier)	20 20	170	30.7 48.2	17.0 17.0
Fumonisin B1	722.4 [M+H]+	336.4 (Quantifier) 318.4 (Qualifier)	20 20	100	55.1 48.3	16.0 20.0
Fumonisin B2	706.4 [M+H]+	336.2 (Quantifier) 318.2 (Qualifier)	20 20	120	49.3 50.8	20.0 20.0
Ochratoxin A	404.1 [M+H]+	239.0 (Quantifier) 358.0 (Qualifier)	20 20	78	31.2 19.1	15.0 18.0

Example LC-MS/MS Total Ion Count Chromatogram for Maize



Toxin	Spike Level
AFT G2	2.5 ppb
AFT G1	2.5 ppb
AFT B2	2.5 ppb
AFT B1	2.5 ppb
FUM B1	666.67 ppb
FUM B2	333.33 ppb
OTA	5 ppb

Example LC-MS/MS Total Ion Count Chromatogram for Animal Feed



Toxin	Spike Level
AFT G2	5 ppb
AFT G1	5 ppb
AFT B2	5 ppb
AFT B1	5 ppb
FUM B1	3333.33 ppb
FUM B2	1666.67 ppb
OTA	100 ppb

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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