PuriTox Total Myco-MS

Product Code: TC-MT3000

Solid phase clean-up columns for use in conjunction with LC-MS/MS.
For in vitro use only.

TC-MT3000/V4/06.08.20

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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 LC-MS/MS 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)
- Standards (Please refer to Preparation of Standards section)
  - Aflatoxins
  - Deoxynivalenol
  - Fumonisins
  - Ochratoxin A
  - T-2 & HT-2
  - Zearalenone

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 you 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

• Cereal

This method has been tested on a number of cereals including wheat, maize and oats.

1. Weigh 25 g of ground sample to a 1 litre capacity, solvent resistent blender jar.
2. Add 100 ml of 80 % acetonitrile with 1 % acetic acid 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. Transfer 20 ml of sample filtrate to a glass beaker.
5. Add 200 µl of 100 % acetic acid and stir.
6. Pass 1.4 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.
7. Dilute 500 µl of eluate with 1.5 ml of 1 % acetic acid.
8. Add 900 µl of the diluted solution to 100 µl of 1 % acetic acid in 20 % acetonitrile
9. Inject 50 µl onto the LC-MS/MS system.

Preparation of Standards

Note: A wide range of crystalline or liquid standards and reference materials are available from Trilogy which can be used for the preparation of the Matrix Matched Calibration Series. Contact your local R-Biopharm distributor for further information.

• Aflatoxin Stock Solution

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. If required, dilute or reconstitute as per the instructions provided and leave 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.

• Deoxynivalenol Stock Solution

Preparation of 50,000 ng/ml deoxynivalenol stock solution:

1. Crystalline powder of deoxynivalenol can be purchased. Contact your local R-Biopharm distributor for further information. If required, dilute or reconstitute as per the instructions provided and leave overnight in the dark at room temperature to give a stock concentrate.

2. This is then used to prepare a 50,000 ng/ml deoxynivalenol stock solution.

• Fumonisin Stock Solution

Preparation of 150,000 ng/ml fumonisin stock solution:

1. Crystalline powder of fumonisin can be purchased. Contact your local R-Biopharm distributor for further information. If required, dilute or reconstitute as per the instructions provided and leave overnight in the dark at room temperature to give a stock concentrate.

2. This is then used to prepare a 150,000 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.

• Ochratoxin Stock Solution

Preparation of 1,000 ng/ml ochratoxin A Stock Solution:

1. Ready-to-use OCHRASTANDARD (P11 / P11A, 1,000 ng/ml) is available from R-Biopharm.

   or

1. Alternatively, crystalline powder of ochratoxin A can be purchased. Contact your local R-Biopharm distributor for further information. If required, dilute or reconstitute as per the instructions provided and leave overnight in the dark at room temperature to give a stock concentrate.

2. This is then used to prepare a 1,000 ng/ml ochratoxin A stock solution.

• T-2 & HT-2 Stock Solution

Preparation of 20,000 ng/ml T-2 and HT-2 toxin stock solutions:

1. Crystalline powder of T-2 and HT-2 can be purchased. Contact your local R-Biopharm distributor for further information. If required, dilute or reconstitute as per the instructions provided and leave overnight in the dark at room temperature to give a stock concentrate.

2. This is then used to prepare a 40,000 ng/ml T-2 and HT-2 (20,000 ng/ml each toxin) stock solution.

• Zearalenone Stock Solution

Preparation of 50,000 ng/ml zearalenone stock solution:

1. Crystalline powder of zearalenone can be purchased. Contact your local R-Biopharm distributor for further information. If required, dilute or reconstitute as per the instructions provided and leave overnight in the dark at room temperature to give a stock concentrate.

2. This is then used to prepare a 50,000 ng/ml zearalenone stock solution.

• Combined Working Solution

1. Take 5 ml of 1 % acetic acid in 20 % acetonitrile and remove 2.4 ml to waste.

2. Add the following volumes of stock solutions to prepare combined working solution.

Toxin	Stock Solution Concentration (ng/ml)	Volume of Stock Solution Added (µl)	Working Solution Concentration (ng/ml)
Aflatoxins	1,000 ng/ml (250 ng/ml each)	500 µl	25 ng/ml (each)
Ochratoxin A	1,000 ng/ml	500 µl	100 ng/ml
Zearalenone	50,000 ng/ml	500 µl	5,000 ng/ml
Fumonisins	150,000 ng/ml (total)	200 µl	6,000 ng/ml (total)
Deoxynivalenol	50,000 ng/ml	500 µl	5,000 ng/ml
T-2 & HT-2	20,000 ng/ml (each)	200 µl	800 ng/ml (each)

Calibration Curve Solvent Solutions

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: Dilute 2 ml of combined working solution with 2 ml of 1 % acetic acid in 20 % acetonitrile.
2. Standard 4: Take 2 ml of combined standard 5 and add 2 ml of 1 % acetic acid in 20 % acetonitrile.
3. Standard 3: Take 2 ml of combined standard 4 and add 2 ml of 1 % acetic acid in 20 % acetonitrile.
4. Standard 2: Take 2 ml of combined standard 3 and add 2 ml of 1 % acetic acid in 20 % acetonitrile.
5. Standard 1: Take 2 ml of combined standard 2 and add 2 ml of 1 % acetic acid in 20 % acetonitrile.

Toxin	Standard 5 (ng/ml)	Standard 4 (ng/ml)	Standard 3 (ng/ml)	Standard 2 (ng/ml)	Standard 1 (ng/ml)
Aflatoxins	12.5 (each)	6.25 (each)	3.12 (each)	1.56 (each)	0.78 (each)
Ochratoxin A	50	25	12.5	6.25	3.12
Zearalenone	2,500	1,250	625	312	156
Fumonisins	3,000	1,500	750	375	188
Deoxynivalenol	2,500	1,250	625	312	156
T-2	400	200	100	50	25
HT-2	400	200	100	50	25

Matrix Matched Calibration Series

1. Prepare a matrix blank using Cereal Sample Preparation method.
2. Collect sufficient cleaned-up solution from step 6 of the Cereal Sample Preparation in order to prepare a matrix matched calibration series. For example, run the sample through 4 separate PuriTox Total Myco-MS and pool the solution.
3. Dilute 2.5 ml of the pooled collected solution with 7.5 ml 1 % acetic acid. This solution is then used to prepare the calibration standards.
4. Take 900 µl of matrix blank solution, as prepared in steps 1 to 3.
5. Add 100 µl of calibration curve solvent solution (Standard 5) as prepared above.
6. Vortex for 20 seconds to mix.
7. Inject 50 µl onto the LC-MS/MS system.
8. Repeat steps 5 to 7 for Standards 4 to 1.
9. Resulting concentrations are 1/10 of those outlined in table above (see Calibration Curve Solvent Solutions).

Recommended LC Conditions

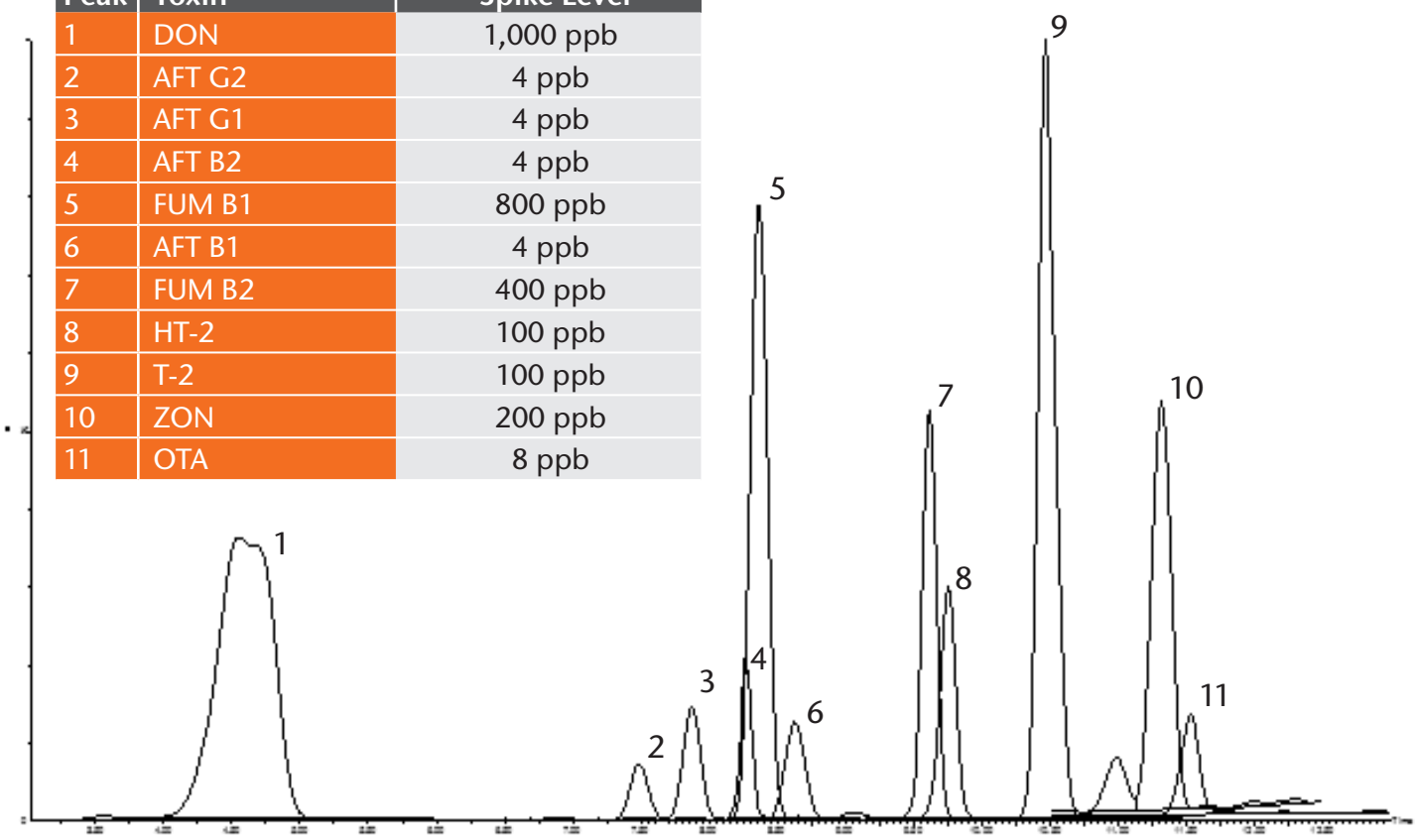
LC Conditions			
Guard Cartridge	Phenomenex Gemini C18 4 mm x 2 mm or equivalent		
Analytical Column	Phenomenex Gemini 5 µm C18 110 A, 150 mm x 3 mm or equivalent		
Mobile Phase	Solution A: 1 mM Ammonium Formate and 0.1 % Formic Acid in 5 % Methanol Solution B: 1 mM Ammonium Formate and 0.1 % Formic 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 guard and analytical columns 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 Settings						
Time (min)	**Toxin**	**Precursor Ion (m/z)**	**Product Ions (m/z)**	**Dwell Time (s)**	**Cone Voltage (V)**	**Collision Voltage (eV)**
3.0 - 6.0	DON	297.01 [M+H]+	249.10 (Quantifier) 231.08 (Qualifier)	0.661	24 24	10 12
6.5 - 9.5	AFT G2	331.01 [M+H]+	245.13 (Quantifier) 189.07 (Qualifier)	0.105	48 48	32 40
6.5 - 9.5	AFT G1	329.01 [M+H]+	243.06 (Quantifier) 199.88 (Qualifier)	0.105	50 50	28 44
6.5 - 9.5	AFT B2	315.07 [M+H]+	287.12 (Quantifier) 259.15 (Qualifier)	0.105	56 56	26 30
6.5 - 9.5	AFT B1	313.00 [M+H]+	284.93 (Quantifier) 241.10 (Qualifier)	0.105	52 52	22 40
6.5 - 9.5	FUM B1	722.39 [M+H]+	334.39 (Quantifier) 352.40 (Qualifier)	0.105	52 52	40 38
8.5 - 10.5	FUM B2	706.39 [M+H]+	336.40 (Quantifier) 318.39 (Qualifier)	0.105	56 56	40 42
9.5 - 11	HT-2	442.21 $[M+NH_4]+$	263.16 (Quantifier) 215.10 (Qualifier)	0.272	18 18	12 14
9.5 - 11.5	T-2	484.21 $[M+NH_4]+$	305.14 (Quantifier) 245.12 (Qualifier)	0.272	26 26	14 14
10.5 - 13.0	OTA	403.9 [M+H]+	239.0 (Quantifier) 358.1 (Qualifier)	0.428	32 32	22 14
10.5 - 12.5	ZON	319.11 [M+H]+	283.17 (Quantifier) 187.1 (Qualifier)	0.256	22 22	12 20

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

Peak	Toxin	Spike Level
1	DON	1,000 ppb
2	AFT G2	4 ppb
3	AFT G1	4 ppb
4	AFT B2	4 ppb
5	FUM B1	800 ppb
6	AFT B1	4 ppb
7	FUM B2	400 ppb
8	HT-2	100 ppb
9	T-2	100 ppb
10	ZON	200 ppb
11	OTA	8 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

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