TRICHOTHECENE P

Product Code: P51

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

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Contents

Page

Test Principle 4
Reagents Not Provided 4
Accessory Products 4
Recommended Methods 4
Hazards 4
Decontamination 4
Storage & Shelf Life 5
Sampling 5
Recoveries 5
Preparation of Diluent Solution (Mobile Phase A : Mobile Phase B (80 : 20 v/v)) 5
Sample Preparation 6
- Cereal and Animal Feed 6

Preparation of Standards 7
- Combined Working Solution 7

Calibration Curve Solvent Solutions 8
Matrix Matched Calibration Series 9
Recommended LC Conditions 10
Example LC-MS/MS Total Ion Count Chromatogram for Maize 11
Quality 12
Technical Support 12
Warranty 12

Test Principle

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

The solid phase clean-up columns contain material to help remove interfering components or pigments from cereal samples. The toxins are extracted from the sample, filtered and passed through the clean-up column prior to analysis by LC-MS/MS.

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

- Solvent (HPLC Grade Acetonitrile and Methanol)
- Mycotoxin Standards (Please refer to Preparation of Standards section)
- Ammonium Acetate
- Acetic Acid

Accessory Products

- Whatman No. 113 or No. 4 Filter Paper
- 0.2 µm Membrane Syringe Filter

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 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 (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.

Preparation of Diluent Solution (Mobile Phase A : Mobile Phase B (80 : 20 v/v))

The solution can be kept for 2 days if stored at room temperature.

1. Add 80 ml of mobile phase A (5 mM ammonimum acetate in water and 0.2 % acetic acid) into a flask.
2. Add 20 ml of mobile phase B (5 mM ammonimum acetate in methanol and 0.2 % acetic acid).

Sample Preparation

• Cereal and Animal Feed

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

1. Weigh 25 g of ground sample to a 1 litre capacity, solvent resistent blender jar.
2. Add 100 ml of 80 % 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. Measure 5 ml of the filtrate into the glass test tube.
5. Insert the solid phase clean-up column (orange side down) into the glass test tube. Apply pressure on the column pushing it down into the glass tube. The liquid should diffuse through the frit and up through the packing material of the column.
6. Transfer 2 ml of the cleaned-up solution from the top of the column packing into a glass test tube and evaporate to dryness under air at 60 - 70 °C.
7. Reconstitute with 1 ml of mobile phase A : mobile phase B (80:20 v/v). Vortex for 2 - 3 minutes.
8. Pass the reconstituted sample through a 0.2 µm syringe filter.
9. Take 675 µl of filtrate and add 75 µl of diluent solution. Vortex for 20 seconds.
10. Inject 40 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.

• Combined Working Solution

1. Crystalline powder containing a mix of trichothecenes can be purchased. Contact your local R-Biopharm distributor for further information. The powder is reconstituted as per the instructions provided and left overnight at room temperature to give a stock concentrate.

Type A and Type B Trichothecenes	
Type A	**Type B**
T-2	Fusarenon X
HT-2	3-Acetyl Deoxynivalenol
Diacetoxyscirpenol	15-Acetyl Deoxynivalenol
Neosolaniol	Deoxynivalenol
	Nivalenol

2. This is then used to prepare a 100,000 ng/ml of each toxin stock solution.

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 an eight point calibration curve (can be modified according to legislative requirements or contamination levels):

1. Add 1.5 ml of mobile phase A : mobile phase B (80:20 v/v) to a glass vial.
2. Remove 300 µl to waste.
3. Standard 8: Add 300 µl of 100,000 ng/ml stock solution (equivalent to 20,000 ng/ml each toxin). Vortex for 20 seconds.
4. Standard 7: Take 1 ml of standard 8 and add 1 ml of mobile phase A : mobile phase B (80:20 v/v) (equivalent to 10,000 ng/ml each toxin). Vortex for 20 seconds.
5. Standard 6: Take 1 ml of standard 7 and add 1 ml of mobile phase A : mobile phase B (80:20 v/v) (equivalent to 5,000 ng/ml each toxin). Vortex for 20 seconds.
6. Standard 5: Take 1 ml of standard 6 and add 1 ml of mobile phase A : mobile phase B (80:20 v/v) (equivalent to 2,500 ng/ml each toxin). Vortex for 20 seconds.
7. Standard 4: Take 1 ml of standard 5 and add 1 ml of mobile phase A : mobile phase B (80:20 v/v) (equivalent to 1,250 ng/ml each toxin). Vortex for 20 seconds.
8. Standard 3: Take 1 ml of standard 4 and add 1 ml of mobile phase A : mobile phase B (80:20 v/v) (equivalent to 625 ng/ml each toxin). Vortex for 20 seconds.
9. Standard 2: Take 1 ml of standard 3 and add 1 ml of mobile phase A : mobile phase B (80:20 v/v) (equivalent to 312.5 ng/ml each toxin). Vortex for 20 seconds.
10. Standard 1: Take 1 ml of standard 2 and add 1 ml of mobile phase A : mobile phase B (80:20 v/v) (equivalent to 156.25 ng/ml each toxin). Vortex for 20 seconds.

Matrix Matched Calibration Series

1. Prepare a matrix blank using Cereal Sample Preparation method.
2. Collect sufficient cleaned-up solution from step 8 of the Cereal Sample Preparation in order to prepare a matrix matched calibration series. For example, run the sample through 12 separate TRICHOTHECENE P columns and pool the solution.
3. Standard 8: Take 100 µl of 20,000 ng/ml and add 900 µl of pooled matrix blank (equivalent to 2,000 ng/ml each toxin). Vortex for 20 seconds.
4. Standard 7: Take 100 µl of calibration curve solvent (standard 7) as previously prepared. Add 900 µl of matrix blank (equivalent to 1,000 ng/ml each toxin). Vortex for 20 seconds.
5. Standard 6: Take 100 µl of calibration curve solvent (standard 6) as previously prepared. Add 900 µl of matrix blank (equivalent to 500 ng/ml each toxin). Vortex for 20 seconds.
6. Standard 5: Take 100 µl of calibration curve solvent (standard 5) as previously prepared. Add 900 µl of matrix blank (equivalent to 250 ng/ml each toxin). Vortex for 20 seconds.
7. Standard 4: Take 100 µl of calibration curve solvent (standard 4) as previously prepared. Add 900 µl of matrix blank (equivalent to 125 ng/ml each toxin). Vortex for 20 seconds.
8. Standard 3: Take 100 µl of calibration curve solvent (standard 3) as previously prepared. Add 900 µl of matrix blank (equivalent to 62.5 ng/ml each toxin). Vortex for 20 seconds.
9. Standard 2: Take 100 µl of calibration curve solvent (standard 2) as previously prepared. Add 900 µl of matrix blank (equivalent to 31.25 ng/ml each toxin). Vortex for 20 seconds.
10. Standard 1: Take 100 µl of calibration curve solvent (standard 1) as previously prepared. Add 900 µl of matrix blank (equivalent to 15.625 ng/ml each toxin). Vortex for 20 seconds.

Recommended LC Conditions

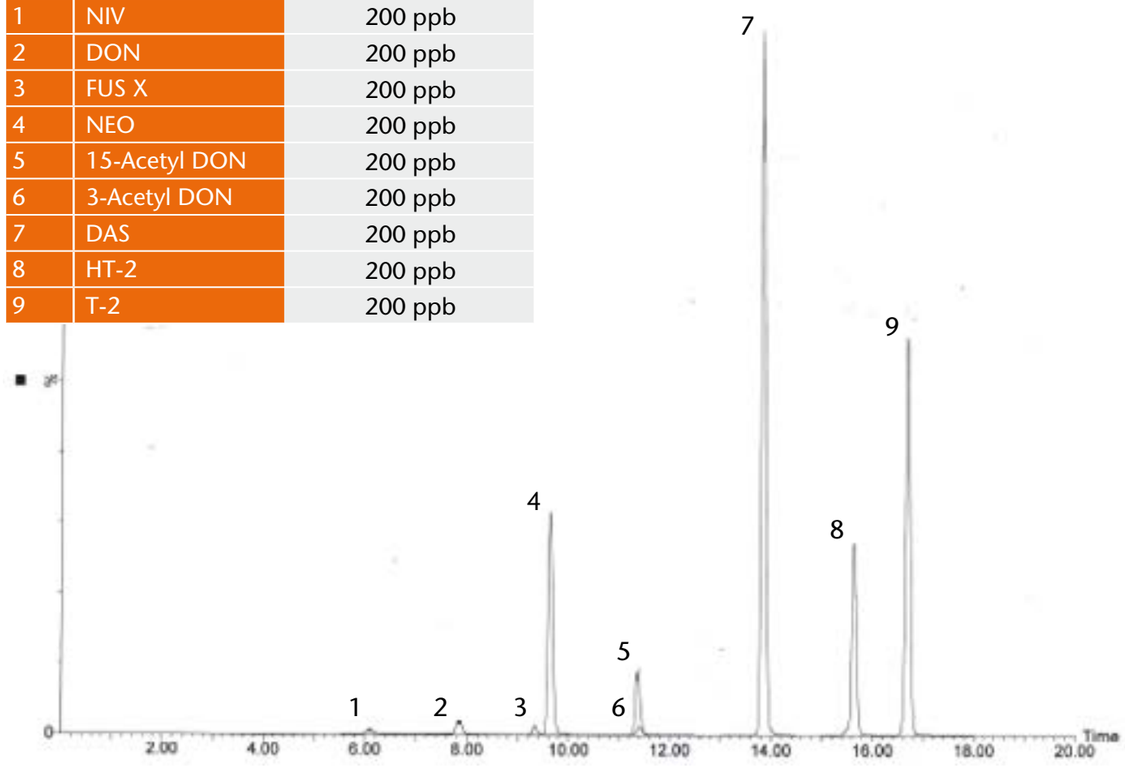
LC Conditions			
Guard Cartridge	Phenomenex Gemini C18 4 mm x 2 mm or equivalent		
Analytical Column	Phenomenex Gemini 5 µm C18 110 A, 150 mm x 3 mm or equivalent		
Mobile Phase	Solution A: 5 mM Ammonium Acetate in Water and 0.2 % Acetic Acid Solution B: 5 mM Ammonium Acetate in Methanol and 0.2 % Acetic Acid Prepare fresh on day of analysis.		
Gradient Conditions	Time (min)	% Solution A	% Solution B
	0	90	10
	15	20	80
	16	5	95
	17	5	95
	18	90	10
	20	90	10
HPLC Pump	To deliver mobile phase		
Flow Rate	0.275 ml per minute		
Column Heater	Maintain guard and analytical columns at 45 °C		
Integrator / Data Control System	From preferred supplier		
Injector	Autosampler / Rheodyne valve		
Injection Volume	40 µl		

Mass Spectrometry Conditions	
Instrument	Waters® ACQUITY TQ Detector with Electrospray Ionisation
Mode	Multiple Reaction Monitoring (MRM) Mode
Capillary Voltage	+1,700 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)
4.8 - 6.8	NIV	313.15 [M+H]+	205.14 (Quantifier) 159.07 (Qualifier)	0.161	23 23	11 21
6.8 - 8.8	DON	297.16 [M+H]+	249.18 (Quantifier) 203.12 (Qualifier)	0.050	25 25	11 15
8.0 - 10.0	FUS X	355.13 [M+H]+	247.00 (Quantifier) 229.00 (Qualifier)	0.050	30 30	12 12
8.5 - 10.5	NEO	400.19 [M+NH_4]+	305.00 (Quantifier) 215.00 (Qualifier)	0.045	30 30	12 12
10.0 - 12.0	15-Ac DON	339.17 [M+H]+	137.06 (Quantifier) 321.18 (Qualifier)	0.045	25 25	11 11
10.0 - 12.0	3-Ac DON	337.23 [M-H]-	317.18 (Quantifier) 173.05 (Qualifier)	0.045	29 29	11 9
12.5 - 14.5	DAS	384.18 [M+NH_4]+	307.19 (Quantifier) 105.06 (Qualifier)	0.161	18 18	12 26
14.5 - 16.5	HT-2	442.20 [M+NH_4]+	215.10 (Quantifier) 263.10 (Qualifier)	0.078	22 22	14 16
15.5 - 17.5	T-2	484.25 [M+NH_4]+	185.00 (Quantifier) 215.00 (Qualifier)	0.078	28 28	14 14

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

Peak	Toxin	Spike Level
1	NIV	200 ppb
2	DON	200 ppb
3	FUS X	200 ppb
4	NEO	200 ppb
5	15-Acetyl DON	200 ppb
6	3-Acetyl DON	200 ppb
7	DAS	200 ppb
8	HT-2	200 ppb
9	T-2	200 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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