Extraction of Deoxynivalenol From Grain Using ISOLUTE® Myco prior to LC-MS/MS Analysis

This application note describes a solid phase extraction (SPE) protocol for the extraction of deoxynivalenol (DON) from wheat, maize and barley using ISOLUTE® Myco SPE columns with LC-MS/MS analysis.

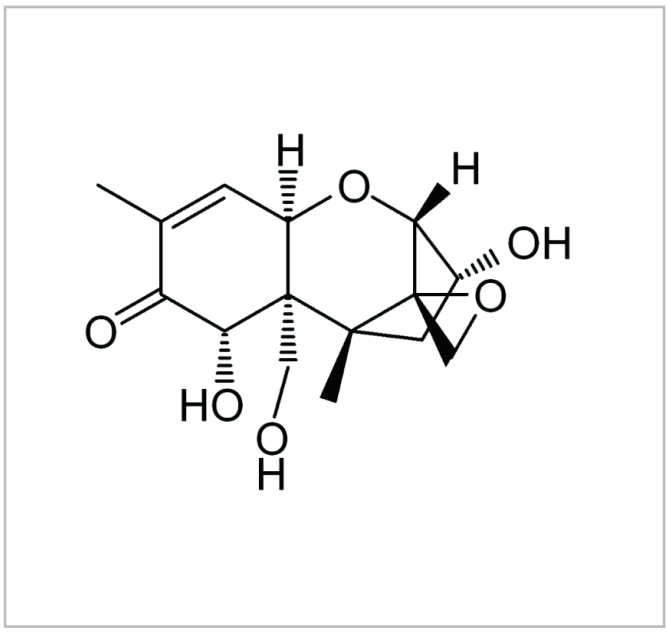

Figure 1. Structures of Deoxynivalenol

Introduction

Deoxynivalenol, also commonly known as vomitoxin, is a mycotoxin found predominantly in grain crops. Mycotoxins are toxic metabolites produced by fungal molds on food crops. Regulation and legislation for testing of mycotoxin contamination has established which mycotoxins are prevalent on a wide variety of food crops. This application note describes an SPE protocol appropriate for LC-MS/MS analysis of deoxynivalenol found on grain (maize, wheat, barley) crops.

The method described in this application note achieves high recoveries of deoxynivalenol from a range of different grain matrices with %RSDs and LOQs that all meet the requirements set in European regulations for measurement of these analytes in grains.

ISOLUTE Myco solid phase extraction columns provide robust, reliable sample preparation for multiple mycotoxin classes from a wide range of foodstuffs.

Using a single, easy to use sample preparation product, along with optimized matrix specific application notes, scientists can prepare diverse food/crop samples for analysis by LC-MS/MS.

Analyte

Deoxynivalenol

Sample Preparation Procedure

Column configuration ISOLUTE Myco 60 mg/3 mL column (Tabless) Part Number 150-0006-BG

Sample pre-treatment:

1. Sample processing: Grind the sample (wheat, maize, barley, 50 g) and store the ground sample in a sealed container at room temperature until required.
2. Extraction: Mix the ground whole grain (or flour) sample (5 g) with water (20 mL) and place on a shaking table for 30 minutes. Transfer the extract to a 50 mL centrifuge tube and centrifuge at 3000 g for 10 minutes.
3. Dilution: Take the supernatant (8 mL), transfer to a new 50 mL centrifuge tube and dilute with water (32 mL). Centrifuge diluted extract at 3000 g for a further 10 minutes.

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Solid Phase Extraction

Use flow rates of 1 mL min^{-1} throughout

Condition:	Condition the column with acetonitrile (2 mL)
Equilibration:	Equilibrate column with water (2 mL)
Sample loading:	Load pre-treated sample (3 mL) onto the column at a maximum flow rate of 1 mL min^{-1} (gravity load is recommended)
Interference wash 1	Wash the column with water (3 mL)
Elution	Elute deoxynivalenol with 10% (v/v) acetonitrile in water (3 mL)
Post elution:	Dry the eluate in a TurboVap® LV (1.5 bar at 40 °C for 100 minutes) or vacuum concentrator (minimum 2.5 hours at a 'high' heat setting) Reconstitute in 0.1 % acetic acid in 20 % acetonitrile : methanol (1 mL, 1:1, v/v). Syringe-filter using a 0.2 µm PTFE membrane prior to analysis.

HPLC Conditions*

Instrument:	Shimadzu Nexera UHPLC (Shimadzu Europe Gmbh)
Column	Kinetex XB-C18 50 x 2.1 mm 2.6 µm dp (Phenomenex, Macclesfield UK)
Mobile Phase:	A: 1 mM ammonium acetate, 0.5% acetic acid B: 1 mM ammonium acetate, 0.5% acetic acid in 95% methanol (aq)
Flow rate:	0.45 mL min^{-1}
Injection:	20 µL
Gradient:	Initial 20% B, hold 1.0 min linear ramp to 73% B in 6 min linear ramp to 100% B in 0.2 min, hold 2.3 min linear ramp to initial conditions in 0.2 min hold 2.3 min, total run time 10.0 min
Column temperature	40 °C
Sample temperature:	15 °C

*Used to generate the data in this application note. Other HPLC conditions may be appropriate.

Table 1: Typical retention time for deoxynivalenol using the LC-MS/MS method described.

Compound	Retention Time (min)
deoxynivalenol	0.7

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MS Conditions

Ions were selected in order to achieve maximum sensitivity, and the MS was operated in negative ion mode, using multiple reaction monitoring.

Instrument: AB Sciex Triple Quad 5500 (Warrington, UK)

Source: Turbo-V ESI

Desolvation temperature: 500 °C

Curtain gas: 30 psi

Spray voltage: -4.5 kV

Gas 1: 60 psi

Gas 2: 60 psi

Collision gas: 7 psi

Table 2. Negative Ion Mode - MRM Parameters

MRM transition	RT	Compound ID	DP, V	EP, V	CE, V	CXP, V
355.1>59.0	0.7	deoxynivalenol 1	-50	-10	-45	-15
335.1>295.1	0.7	deoxynivalenol 2	-50	-10	-13	-15
335.1>265.1	0.7	deoxynivalenol 3	-50	-10	-20	-15

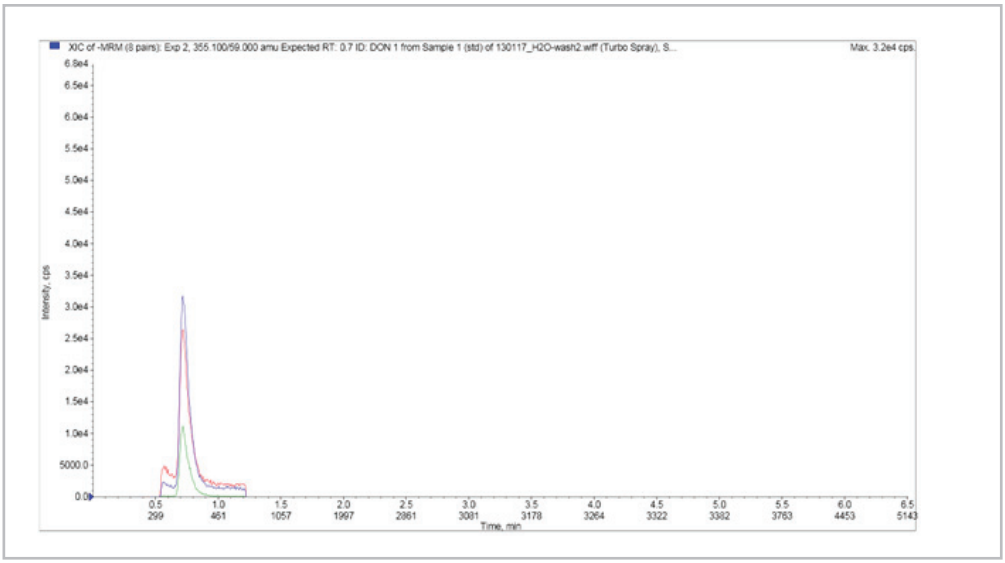

Figure 2. Extracted ion chromatogram for deoxynivalenol in negative ion mode using ISOLUTE® protocol at 100 µg kg^{-1} from wheat

Validation Criteria

Method linearity was determined using matrix-matched calibration standards in six replicates over seven levels; the range is shown below.

Analytes	Working Range, µg kg^{-1} (pg μL^{-1} on column)
deoxynivalenol	13.3 to 1333 (2 to 200)

LOQ was determined from the lowest matrix-matched standard meeting EU repeatability and recovery criteria. Repeatability (%RSD_r) was determined from single acquisitions of 5 SPE replicates of a single sample extraction. The RSDs generated gave close agreement when a single sample was extracted and processed using ISOLUTE Myco from three separate sorbent batches.

Recovery was determined as a % of ISOLUTE® Myco extract spike before sample prep to spike after at the EU MRL.

Results

The extracted ion chromatogram in figure 2 demonstrates chromatography at 100 µg kg^{-1} from a spiked extraction of 10 g ground wheat. Good linearity was achieved for deoxynivalenol in the different matrices as demonstrated in the example chart shown in figure 3.

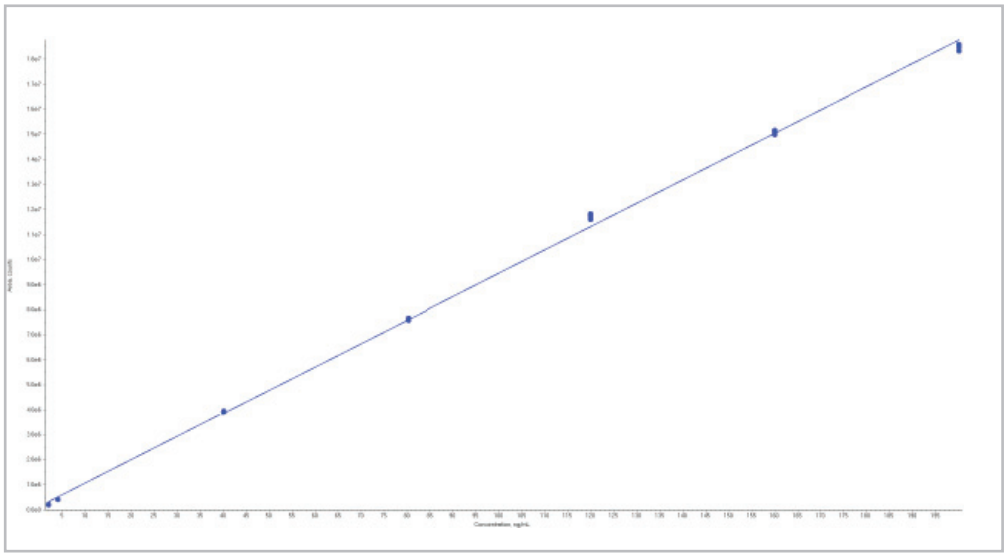

Figure 3. Calibration curve for deoxynivalenol from ground wheat using the ISOLUTE® Myco protocol from 2–200 ng mL^{-1}

Using the ISOLUTE Myco protocol the limits of quantitation and recovery required by the current European standards for deoxynivalenol analysis were achieved as shown in table 4.

Table 4. Analyte recovery and limit of quantitation data for for deoxynivalenol from wheat, maize and barley using the ISOLUTE® Myco protocol

Analyte	r^2	LOQ / µg kg^{-1}		%RSD_r		Recovery %	
Deoxynivalenol		**Target**	**Actual**	**Target**	**Actual**	**Target**	**Actual**
Wheat	0.9997	75	26.7	20	2.5	60 to 110	78
Maize	0.9990	75	26.7	20	5.6	60 to 110	85
Barley	0.9998	75	26.7	20	10.7	60 to 110	67

Ordering Information

Part Number	Description	Quantity
150-0006-BG	ISOLUTE Myco 60 mg/3 mL column (Tabless)	50
121-1016	VacMaster-10 Sample Processing Manifold complete with 16 mm collection rack	1
121-2016	VacMaster-20 Sample Processing Manifold complete with 16 mm collection rack	1
C103198	TurboVap LV, 110V	1
C103199	TurboVap LV, 220V	1

For the latest application notes and more information about ISOLUTE® Myco, please visit www.biotage.com/isolutemyco, or scan the QR code with your smartphone to go direct.

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