Extraction of Multiple Mycotoxins From Nuts Using ISOLUTE® Myco prior to LC-MS/MS Analysis

This application note describes a Solid Phase Extraction (SPE) protocol for the extraction of a range of mycotoxins from brazil nut and peanut (groundnut) using ISOLUTE® Myco SPE columns with LC-MS/MS analysis.

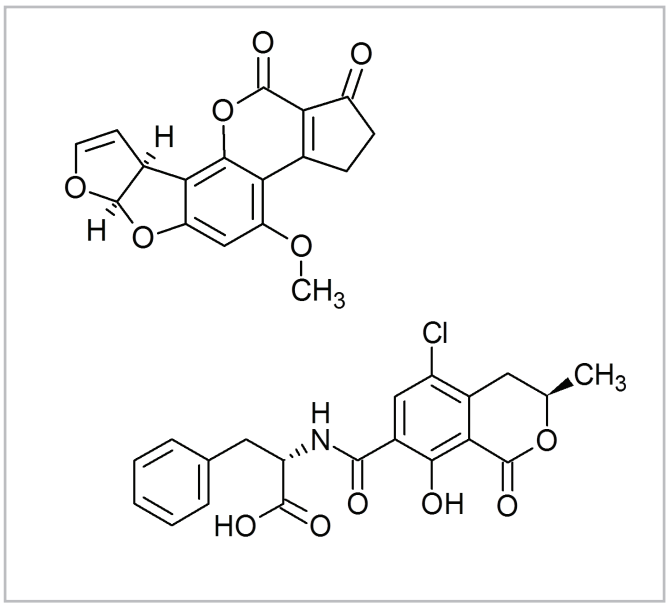

Figure 1. Structures of Aflatoxin B1 Ochratoxin A

Introduction

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 a range of mycotoxins found on nuts.

The method described in this application note achieves high recoveries of all relevant mycotoxins from brazil nut and peanut (groundnut) matrices with %RSDs and LOQs that meet the requirements set in European regulations for measurement of these analytes in nuts.

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.

Analytes

Aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ochratoxin A

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 (brazil nut, peanut, 50 g). Store ground sample in a sealed container at 2 to 8 °C until required.
2. Extraction: Mix the ground sample (5 g) with acetonitrile:water (80:20, v/v, 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 (4 mL), transfer to a new 50 mL centrifuge tube and dilute with water (28 mL) to a total volume of 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 10 mM ammonium acetate (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 10 mM ammonium acetate (3 mL)
Interference wash 2:	Wash the column with 10 mM ammonium acetate:acetonitrile (90:10, v/v, 3 mL)
Drying:	Dry the column for 30 seconds at maximum vacuum, -0.5 bar/7 psi
Elution 1:	Elute with 0.1% formic acid in acetonitrile (2 mL)
Elution 2:	Elute with 0.1% formic acid in methanol (2 mL)
Post elution:	The combined eluates are dried in a stream of air or nitrogen using a SPE Dry (35 °C, 20 to 40 L min^{-1}) or TurboVap® LV (1.5 bar at 35 °C for 40 min). 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 times for a range of mycotoxins using the LC-MS/MS method described

Compound	Retention Time (min)
aflatoxin G2	3.3
aflatoxin G1	3.6
aflatoxin B2	3.9
aflatoxin B1	4.1
ochratoxin A	6.1

MS Conditions

Ions were selected in order to achieve maximum sensitivity, and the MS was operated in positive ion polarity 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: +5.0 kV

Gas 1: 60 psi

Gas 2: 60 psi

Collision gas: 7 psi

Table 2. Positive Ion Mode - MRM Parameters

MRM transition	RT	Compound ID	DP, V	EP, V	CE, V	CXP, V
331.1>313.1	3.3	aflatoxin G2 1	100	10	33	12
331.1>245.1	3.3	aflatoxin G2 2	100	10	41	12
331.1>257.1	3.3	aflatoxin G2 3	100	10	41	12
329>243.1	3.6	aflatoxin G1 1	80	10	37	12
329>200	3.6	aflatoxin G1 2	80	10	53	12
315.1>287	3.6	aflatoxin B2 1	100	10	35	12
315.1>259.1	3.9	aflatoxin B2 2	100	10	40	12
315.1>243.1	3.9	aflatoxin B2 3	100	10	51	12
313.1>285	4.1	aflatoxin B1 1	100	10	31	18
313.1>241.1	4.1	aflatoxin B1 2	100	10	49	18
313.1>185	4.1	aflatoxin B1 3	100	10	65	18
404.1>239	6.1	ochratoxin A 1	165	10	32	12
404.1>221	6.1	ochratoxin A 2	165	10	47	12
404.1>102	6.1	ochratoxin A 3	165	10	84	12

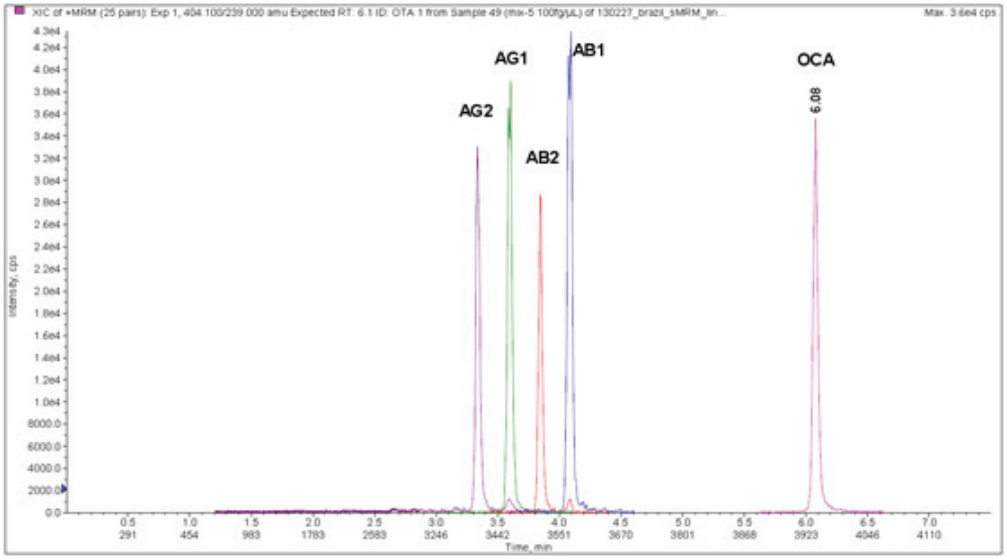

Figure 2. Extracted ion chromatogram (positive ion mode) using ISOLUTE Myco protocol at 5 µg kg-1 (aflatoxins and ochratoxin A) from brazil nut

Validation Criteria

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

Analytes	Working Range, µg kg^{-1} (pg µL^{-1} on column)
aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ochratoxin A	1.07 to 107 (0.1 to 10)

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 5 µg kg^{-1} (aflatoxins and ochratoxin A) from a spiked extraction of 10 g ground brazil nut. Good linearity was achieved for all analytes in all both matrices as demonstrated in the example charts shown in figures 3 and 4.

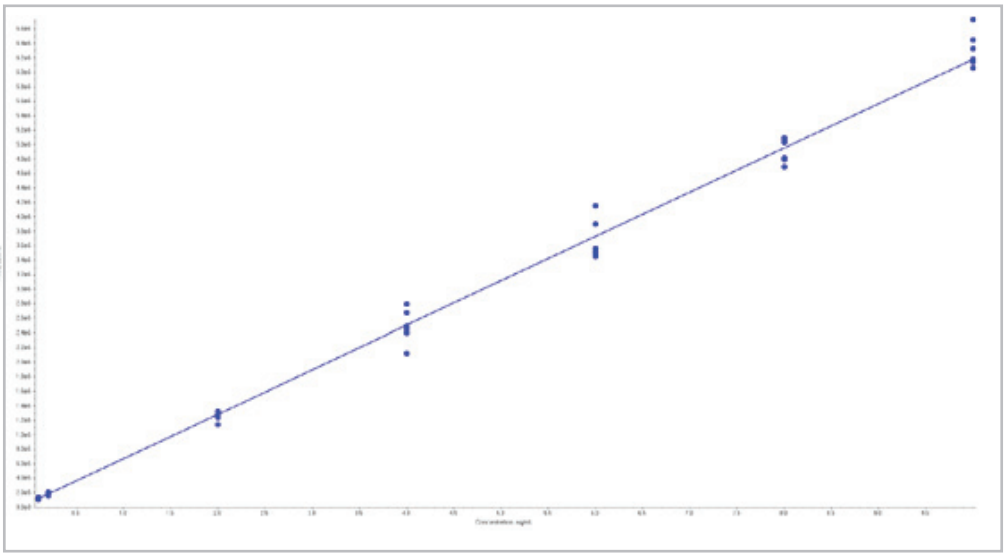

Figure 3 Calibration curve for aflatoxin B1 from ground brazil nut using the ISOLUTE® Myco protocol from 0.1 – 10 ngmL^{-1}

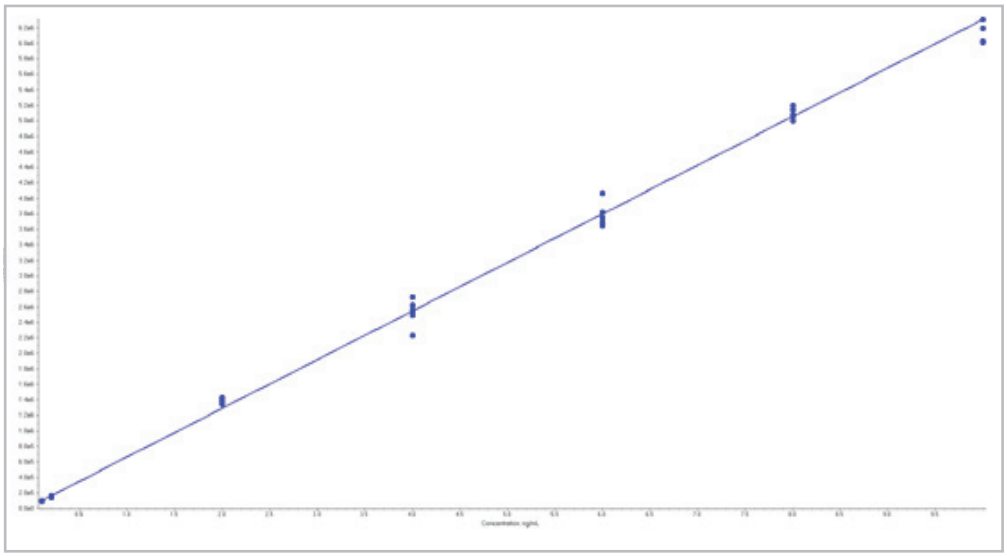

Figure 4 Calibration curve for ochratoxin A from ground brazil nut using the ISOLUTE® Myco protocol from 0.1 – 10 ngmL^{-1}

Table 3. Analyte recovery and limit of quantitation data for a range of mycotoxins from brazil nut using the ISOLUTE® Myco protocol

Analyte	r^2	LOQ / µg kg^{-1}		%RSD$_r$		Recovery %	
Brazil nut		**Target**	**Actual**	**Target**	**Actual**	**Target**	**Actual**
aflatoxin B1	0.9982	2	1	40	3.5	50 to 120	97
aflatoxin B2	0.9978	2	1	40	3.5	50 to 120	105
aflatoxin G1	0.9978	2	1	40	2.8	50 to 120	98
aflatoxin G2	0.9982	2	1	40	7.0	70 to 110	105
ochratoxin A	0.9991	3	1	40	3.5	70 to 110	90

Table 4. Analyte recovery and limit of quantitation data for a range of mycotoxins from peanut using the ISOLUTE® Myco protocol

Analyte	r^2	LOQ / µg kg^{-1}		%RSD_r		Recovery %	
Peanut		**Target**	**Actual**	**Target**	**Actual**	**Target**	**Actual**
aflatoxin B1	0.9960	2	2	40	2.8	50 to 120	103
aflatoxin B2	0.9939	2	2	40	6.7	50 to 120	113
aflatoxin G1	0.9973	2	1	40	7.2	50 to 120	103
aflatoxin G2	0.9993	2	1	40	2.7	70 to 110	100
ochratoxin A	0.9993	3	1	40	5.3	70 to 110	101

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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