Extraction of Multiple Mycotoxins From Cereal Based Infant Food Using ISOLUTE® Myco prior to UHPLC MS/MS Analysis

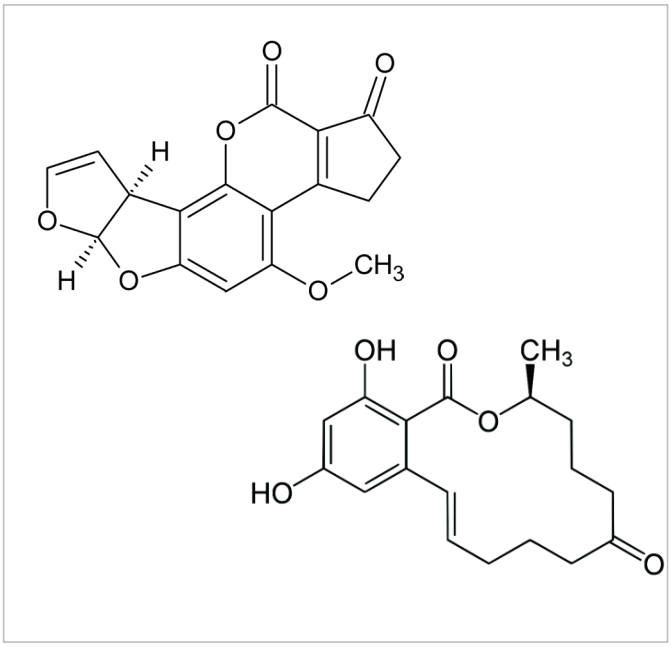

Figure 1. Structures of Aflatoxin B1 and Zearalenone

Introduction

This application note describes a Solid Phase Extraction (SPE) protocol for the extraction of a range of mycotoxins from cereal based infant food using ISOLUTE® Myco with UHPLC-MS/MS.

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 cereal based infant food.

The method described in this application note achieves high recoveries of relevant mycotoxins from a cereal based infant food with low %RSDs and LOQs.

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, fumonisin B1, zearalenone, HT-2, T-2 mycotoxin.

Sample Preparation Procedure

Format: **ISOLUTE® Myco 60 mg/3 mL (Tabless), part number 150-0006-BG**

Sample pre-treatment:

1. Sample processing: Grind the sample (50 g, Cow&Gate® Sunny Start Baby Wheat Flakes were used in this application note) with a burr-grinder or equivalent device. Store the ground sample in a sealed container at room temperature until required.
2. Extraction: Mix the ground sample (5 g) with NaCl (1g) and 60% methanol (aq) (25 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 (7 mL), transfer to a new 50 mL centrifuge tube and dilute with water (35 mL). Centrifuge diluted extract at 3000 g for a further 10 minutes.

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 (2 x 2.5 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 (2 x 2.5 mL).

Interference wash 2: Wash the column with 10% acetonitrile (2 x 2.5 mL)

Drying: Dry the column for 30 seconds at maximum vacuum, 2 bar/29 psi

Elution 1: Elute with 0.1% formic acid in 60% aqueous acetonitrile (2 mL)

Elution 2: Elute with 0.1% formic acid in methanol (2 mL)

Post Elution: The eluate is dried in a stream of air or nitrogen using a SPE Dry (45 °C, 20 to 40 L min^{-1}) or TurboVap® LV (1.2 bar at 45 °C for 80 min). Reconstitute in 0.1 % acetic acid in 1:1:8 methanol:acetonitrile:H_2O (v/v/v). Syringe-filter using a 0.2 µm PTFE membrane prior to analysis.

UHPLC Conditions

Instrument: Shimadzu Nexera UHPLC (Shimadzu Europe Gmbh)

Column: Kinetex XB-C18 50 x 2.1 mm 1.7 µm dp (Phenomenex, Macclesfield UK)

Mobile Phase: A: 1 mM ammonium acetate, 0.5% acetic acid
B: 1mM ammonium acetate, 0.5% acetic acid in 95% methanol (aq)

Flow rate: 0.6 mL min^{-1}

Injection: 20 µL

Gradient: Initial 20 % B, hold 0.8 min
linear ramp to 73 % B in 5 min
linear ramp to 100 % B in 1.2 min, hold 1.0 min
linear ramp to initial conditions in 0.2 min
hold 1.0 min, total run time 8.2 min

Column Temperature: 40 °C

Sample Temperature: 15 °C

Table 1. Typical retention times for a range of mycotoxins using the UHPLC-MS/MS method described.

Compound	Retention Time (min)
aflatoxin G2	2.8
aflatoxin G1	3.0
aflatoxin B2	3.3
aflatoxin B1	3.5
HT-2	4.3
fumonisin B1	4.8
T-2	4.9
zearalenone	5.3
ochratoxin A	5.4

MS Conditions

Ions were selected in order to achieve maximum sensitivity, and the MS was operated in dual polarity (+ve/-ve switching) mode, using multiple reaction monitoring.

Instrument:	AB Sciex Triple Quad 5500 (Warrington, UK)
Source:	Turbo-V ESI
Desolvation temp.:	500 °C
Curtain gas:	30 psi
Spray voltage:	+5.0 kV / -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
720.2>157	4.8	fumonisin B1 1	-160	-12	-45	-15
720.2>562.3	4.8	fumonisin B1 2	-160	-12	-36	-15
317.2>131	5.3	zearalenone 1	-40	-4	-38	-15
317.2>175	5.3	zearalenone 2	-40	-4	-30	-15
317.2>255.1	5.3	zearalenone 3	-40	-4	-20	-15
MRM detection window 60 s / target scan time 0.25 s / settling time 50 ms / scan pause 5 ms						

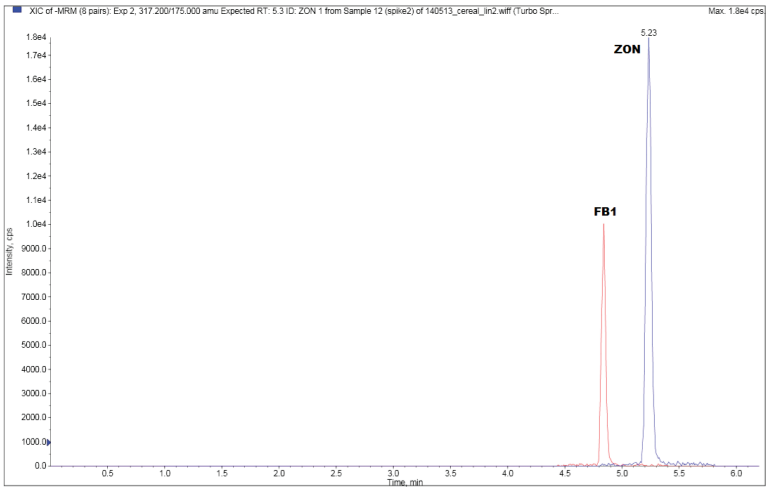

Figure 2. Extracted ion chromatograms in negative ion mode using ISOLUTE® Myco protocol at 100 µg kg^{-1} from infant cereal food.

Table 3. Positive Ion Mode - MRM Parameters

MRM transition	RT	Compound ID	DP, V	EP, V	CE, V	CXP, V
331.1>313.1	2.8	aflatoxin G2 1	100	10	33	12
331.1>245.1	2.8	aflatoxin G2 2	100	10	41	12
331.1>257.1	2.8	aflatoxin G2 3	100	10	41	12
329>243.1	3.0	aflatoxin G1 1	80	10	37	12
329>200	3.0	aflatoxin G1 2	80	10	53	12
315.1>287	3.3	aflatoxin B2 1	100	10	35	12
315.1>259.1	3.3	aflatoxin B2 2	100	10	40	12
315.1>243.1	3.3	aflatoxin B2 3	100	10	51	12
313.1>285	3.5	aflatoxin B1 1	100	10	31	18
313.1>241.1	3.5	aflatoxin B1 2	100	10	49	18
313.1>185	3.5	aflatoxin B1 3	100	10	65	18
442.2>263.1	5.0	HT-2 toxin 1	50	12	18	12
442.2>215.1	5.0	HT-2 toxin 2	50	12	18	12
484.2>305.1	4.9	T-2 toxin 1	60	10	18	12
484.2>215.1	4.9	T-2 toxin 2	60	10	17	12
484.2>185.1	4.9	T-2 toxin 3	60	10	28	12
404.1>239	5.4	ochratoxin A 1	165	10	32	12
404.1>221	5.4	ochratoxin A 2	165	10	47	12
404.1>102	5.4	ochratoxin A 3	165	10	84	12
MRM detection window 60 s / target scan time 0.2 s / settling time 50 ms / scan pause 5 ms						

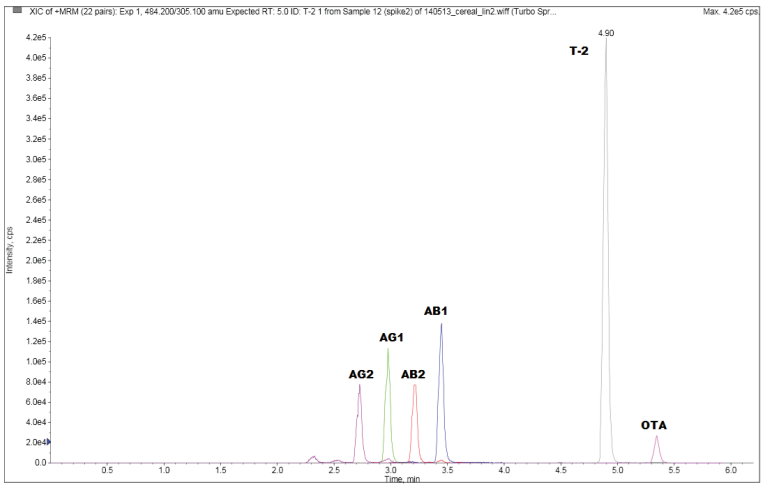

Figure 3. Extracted ion chromatograms in positive ion mode using ISOLUTE® Myco protocol at 10 µg kg^{-1} (aflatoxins and ochratoxin A) and 100 µg µg kg^{-1} (T-2) from infant cereal food.

Validation Criteria

Method linearity was determined using matrix-matched calibration standards in four replicates over five levels with a 1/x weighting; 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	0.1 to 15 (0.033 to 5.00)
zearalenone, T-2 toxin	1 to 150 (0.333 to 50)
fumonisin B1	20 to 150 (6.67 to 50)

Note: no linearity data was generated for HT-2

LOQ was determined from the lowest pre-SPE spike meeting EU repeatability and recovery criteria. Where no criteria were specified the LOQ were estimated by correlation to similar analytes.

Repeatability (%RSD_r) was determined from single acquisitions of 5 SPE replicates of a single sample extraction.

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

Results

The extracted ion chromatograms in figures 2 and 3 demonstrate chromatography at 2 µg kg^{-1} (aflatoxins and ochratoxin A) and 20 µg kg^{-1} for all other analytes from a spiked extraction of 5 g ground infant cereal. Linearity is demonstrated in the example charts shown in **figures 4 and 5**.

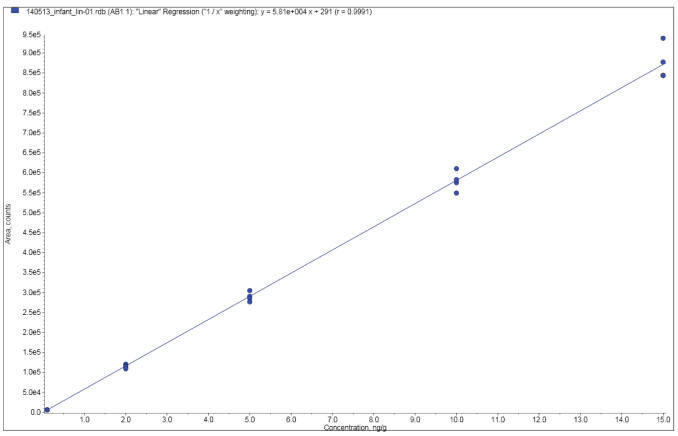

Figure 4. Calibration curve for AB1

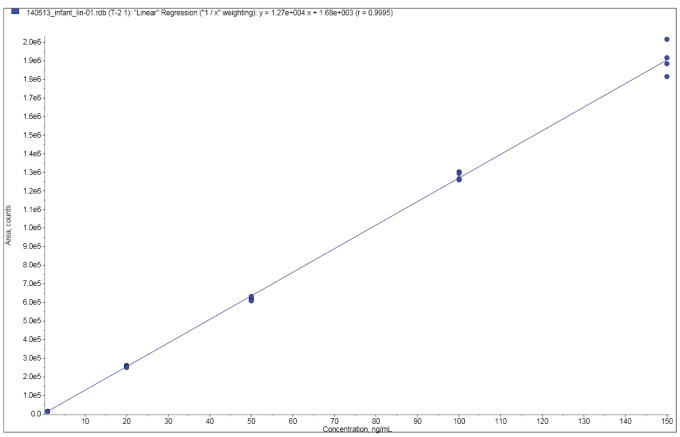

Figure 5. Calibration curve for T-2 toxin

The analytes extracted using the ISOLUTE® Myco protocol achieved the limits of quantities and recovery demonstrated in **Table 4**. These are tabulated alongside the current European standards for mycotoxin analysis.

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

Analyte	r^2	LOQ / µg kg^{-1}		%RSD_r		Recovery %	
Infant Cereal		**Target**	**Actual**	**Target**	**Actual**	**Target**	**Actual**
aflatoxin B1	0.9991	0.1	0.2	40	10.8	50 to 120	77.7
aflatoxin B2	0.9991	1.0	0.2	20	4.8	70 to 110	79.7
aflatoxin G1	0.9986	1.0	0.2	20	5.3	70 to 110	71.3
aflatoxin G2	0.9992	1.0	0.2	20	3.7	70 to 110	65.4
ochratoxin A	0.9964	0.5	0.2	40	10.9	50 to 120	92.7
T-2 toxin	0.9995	50	2.0	40	3.3	60 to 130	98.4
HT-2 toxin	N/A	100	2.0	40	3.3	60 to 130	90.6
fumonisin B1	0.9892	200	2.0	30	9.8	60 to 120	97.7
zearalenone	0.9968	20	2.0	25	8.1	70 to 120	97.5

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/120V	1
C103199	TurboVap® LV, 220/240V	1

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