

Extraction of Multiple Mycotoxins 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 a range of mycotoxins from wheat flour, wheat, maize and barley using ISOLUTE® Myco with LC-MS/MS.

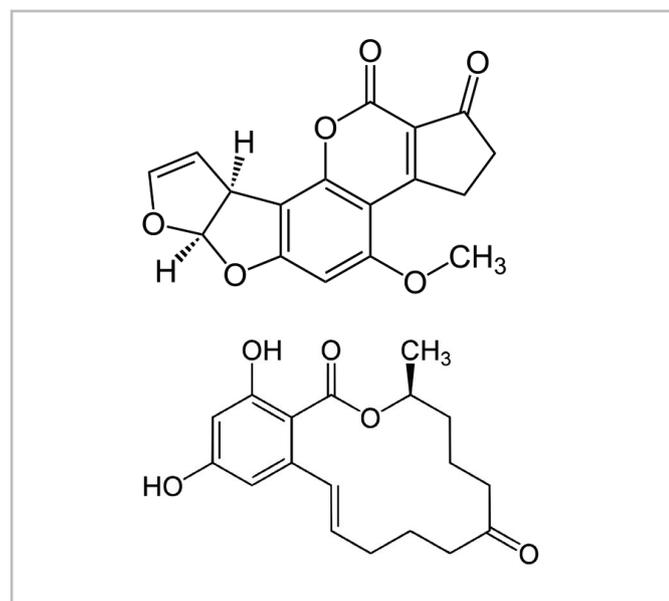


Figure 1. Structures of Aflatoxin B1 and Zearalenone

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 grain food crops.

The method described in this application note achieves high recoveries of all relevant mycotoxins 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 LCMSMS.

Analytes

Aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ergocryptine, ergocornine, ochratoxin A, fumonisin B1, zearalenone, T-2 mycotoxin, HT-2 mycotoxin

Sample Preparation Procedure

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

- Sample pre-treatment:**
1. Sample processing: Grind the sample (wheat, maize, barley, 50 g). Store ground sample in a sealed container at room temperature until required.
 2. Extraction: Mix the ground whole grain (or flour) sample (5 g) with 50% acetonitrile (aq) (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.

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)
Interference wash 2:	Wash the column with 10% acetonitrile (3 mL)
Drying:	Dry the column for 30 seconds at maximum vacuum
Elution 1:	Elute with 0.1% formic acid in acetonitrile (2 mL)
Elution 2:	Elute with methanol (2 mL)
Post elution:	The combined eluate is dried in a stream of air or nitrogen using a SPE Dry (35 °C, 20 to 40 L min ⁻¹) or TurboVap LV (15 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

Table 1: C 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
ergocornine	4.0
ergocryptine	4.5
fumonisin B1	5.4
HT-2	5.0
T-2	5.6
zearalenone	5.9
ochratoxin A	6.1

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 temperature:	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	4.2	fumonisin B1 1	-160	-12	-45	-15
720.2>562.3	4.2	fumonisin B1 2	-160	-12	-36	-15
317.2>131	4.7	zearalenone 1	-40	-4	-38	-15
317.2>175	4.7	zearalenone 2	-40	-4	-30	-15
317.2>255.1	4.7	zearalenone 3	-40	-4	-20	-15

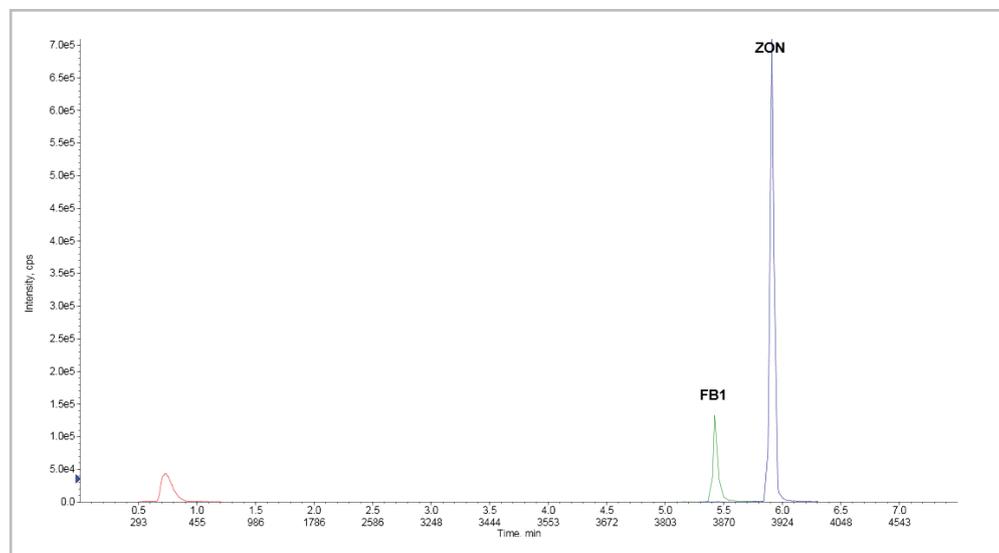


Figure 2: Extracted ion chromatograms in negative ion mode using ISOLUTE Myco protocol at 50 µg kg⁻¹ from wheat

Table 3. Positive Ion Mode - MRM Parameters

MRM transition	RT	Compound ID	DP, V	EP, V	CE, V	CXP, V
331.1>313.1	2.9	aflatoxin G2 1	100	10	33	12
331.1>245.1	2.9	aflatoxin G2 2	100	10	41	12
331.1>257.1	2.9	aflatoxin G2 3	100	10	41	12
329>243.1	3.1	aflatoxin G1 1	80	10	37	12
329>200	3.1	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
562.4>268.1	3.4	ergocornine 1	80	10	32	12
562.4>223.2	3.4	ergocornine 2	80	10	43	12
562.4>305.1	3.4	ergocornine 3	80	10	33	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
576.3>223.1	3.7	ergocryptine 1	90	10	43	12
576.3>268.1	3.7	ergocryptine 2	90	10	33	12
576.3>305.1	3.7	ergocryptine 3	90	10	35	12
442.2>263.1	4.1	HT-2 toxin 1	50	12	18	12
442.2>215.1	4.1	HT-2 toxin 2	50	12	18	12
484.2>305.1	4.4	T-2 toxin 1	60	10	18	12
484.2>215.1	4.4	T-2 toxin 2	60	10	17	12
484.2>185.1	4.4	T-2 toxin 3	60	10	28	12
404.1>239	4.8	ochratoxin A 1	165	10	32	12
404.1>221	4.8	ochratoxin A 2	165	10	47	12
404.1>102	4.8	ochratoxin A 3	165	10	84	12

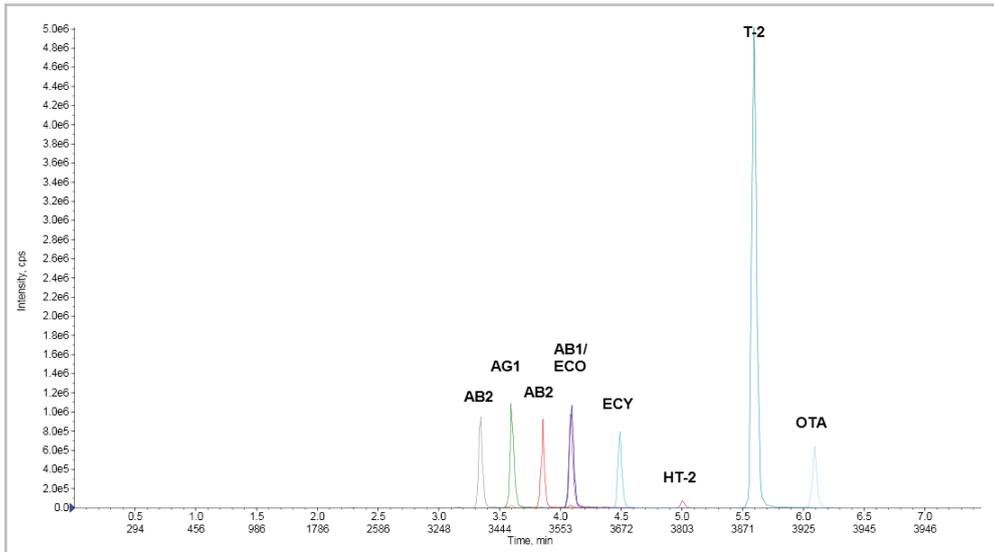


Figure 3. Extracted ion chromatograms in positive ion mode using ISOLUTE Myco protocol at $5 \mu\text{g kg}^{-1}$ (aflatoxins and ochratoxin A) and $50 \mu\text{g kg}^{-1}$ (others) from wheat grain

Validation Criteria

Method linearity was determined using matrix-matched calibration standards in six replicates over a minimum of five levels (the majority were determined with seven levels); the ranges are shown below.

Analytes	Working Range, $\mu\text{g kg}^{-1}$ ($\mu\text{g } \mu\text{L}^{-1}$ on column)
aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ochratoxin A	0.67 to 66.7 (0.1 to 10)
fumonisin B1, zearalenone, deoxynivalenol, ergocornine, ergocryptine	13.3 to 1333 (2 to 200)
T-2 toxin, HT-2 toxin	13.3 to 800 (2 to 120)

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

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 chromatograms in figures 2 and 3 demonstrate chromatography at $5 \mu\text{g kg}^{-1}$ (aflatoxins and ochratoxin A) and $50 \mu\text{g kg}^{-1}$ for all other analytes from a spiked extraction of 10 g ground wheat. Good linearity was achieved for all analytes in all the different matrices as demonstrated in the example charts shown in figures 4 and 5.

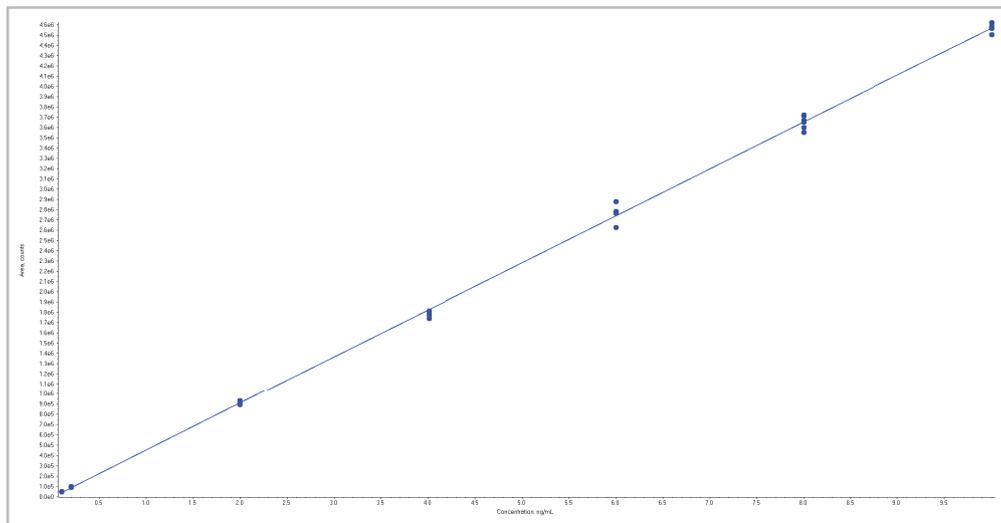


Figure 4: Calibration curve for aflatoxin B1 from ground wheat using the ISOLUTE Myco protocol from 0.1 – 10 ngmL⁻¹

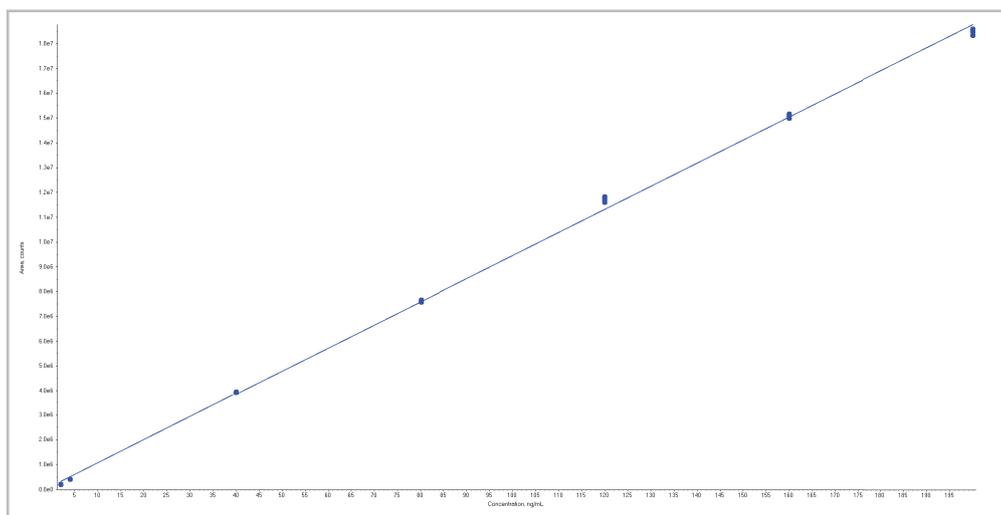


Figure 5. Calibration curve for T2 from ground wheat using the ISOLUTE® Myco protocol from 5 – 200 ngmL⁻¹

All analytes extracted using the ISOLUTE Myco protocol achieved the limits of quantities and recovery required by the current European standards for mycotoxin analysis as shown in tables 4, 5 and 6.

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

Analyte	r ²	LOQ / µg kg ⁻¹		%RSD _r		Recovery %	
		Target	Actual	Target	Actual	Target	Actual
Wheat							
aflatoxin B1	0.9994	2	0.67	40	3.0	50 to 120	96
aflatoxin B2	0.9995	2	0.67	40	5.6	50 to 120	102
aflatoxin G1	0.9990	2	0.67	40	3.7	50 to 120	99
aflatoxin G2	0.9998	2	1.33	40	3.3	70 to 110	110
ochratoxin A	0.9995	3	1.33	40	5.9	70 to 110	88
T-2 toxin	0.9996	50	13.3	40	3.8	60 to 130	102
HT-2 toxin	0.9987	100	26.7	40	19.0	60 to 130	106
fumonisin B1	0.9997	1000	26.7	30	2.8	60 to 120	100
zearalenone	0.9996	50	26.7	40	1.8	60 to 120	73
ergocornine	0.9997	N/A	13.3	N/A	5.9	N/A	96
ergocryptine	0.9996	N/A	13.3	N/A	4.2	N/A	76

Table 5. Analyte recovery and limit of quantitation data for a range of mycotoxins from maize grain using the ISOLUTE Myco protocol

Analyte	r ²	LOQ / µg kg ⁻¹		%RSD _r		Recovery %	
		Target	Actual	Target	Actual	Target	Actual
Maize							
aflatoxin B1	0.9994	2	0.67	40	4.2	50 to 120	94
aflatoxin B2	0.9988	2	0.67	40	2.6	50 to 120	96
aflatoxin G1	0.9995	2	0.67	40	3.3	50 to 120	97
aflatoxin G2	0.9993	2	1.33	40	2.4	70 to 110	95
ochratoxin A	0.9997	3	1.33	40	3.8	70 to 110	72
T-2 toxin	0.9992	50	13.3	40	2.4	60 to 130	99
HT-2 toxin	0.9989	100	13.3	40	4.5	60 to 130	97
fumonisin B1	0.9993	1000	26.7	30	2.6	60 to 120	100
zearalenone	0.9995	50	26.7	40	2.8	60 to 120	71
ergocornine	0.9995	N/A	13.3	N/A	2.0	N/A	78
ergocryptine	0.9995	N/A	13.3	N/A	1.1	N/A	77

Table 6. Analyte recovery and limit of quantitation data for a range of mycotoxins from maize grain using the ISOLUTE Myco protocol

Analyte	r ²	LOQ / µg kg ⁻¹		%RSD _r		Recovery %	
		Target	Actual	Target	Actual	Target	Actual
Barley							
aflatoxin B1	0.9996	2	1.33	40	5.0	50 to 120	100
aflatoxin B2	0.9995	2	0.67	40	4.3	50 to 120	99
aflatoxin G1	0.9992	2	1.33	40	2.1	50 to 120	99
aflatoxin G2	0.9989	2	1.33	40	3.4	70 to 110	98
ochratoxin A	0.9990	3	2.00	40	4.5	70 to 110	96
T-2 toxin	0.9981	50	13.3	40	8.5	60 to 130	96
HT-2 toxin	0.9988	100	20.0	40	8.8	60 to 130	100
fumonisin B1	0.9995	1000	13.3	30	2.0	60 to 120	84
zearalenone	0.9995	50	26.7	40	8.7	60 to 120	96
ergocornine	0.9996	N/A	13.3	N/A	2.2	N/A	82
ergocryptine	0.9997	N/A	13.3	N/A	2.5	N/A	85

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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