Extraction of Aflatoxin M1 From Infant Formula Using ISOLUTE® Myco SPE Columns prior to LC-MS/MS Analysis

This application note describes a Solid Phase Extraction (SPE) protocol for the extraction of aflatoxin M1 (AM1) internally standardized with aflatoxin B2 (AB2) from infant formula using ISOLUTE® Myco SPE columns with LC MS/MS.

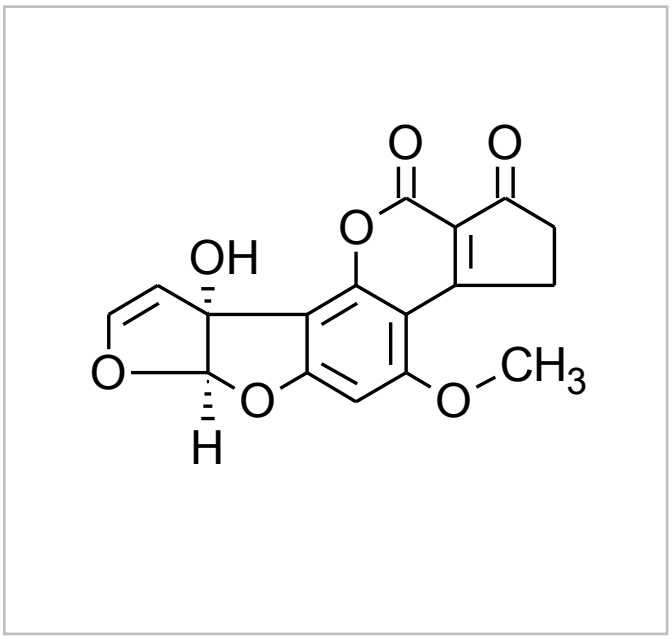

Figure 1. Structure of Aflotoxin M1

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 aflatoxin M1 found in infant formula.

The method described in this application note achieves high recoveries of aflatoxin M1 from infant formula with %RSDs and LOQs that all meet the requirements set in European regulations for its measurement.

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 M1, aflatoxin B2 (internal standard).

Sample Preparation Procedure

Format:	ISOLUTE® Myco 60 mg/3 mL (Tabless), part number 150-0006-BG
Sample Pre-treatment	1. Sample processing: reconstitute the infant formula according to the manufacturer's recommendations using 1% formic acid (aq) as the solvent. Add a small volume of AB2 at an appropriate concentration (e.g. 18 µL x 100 ng mL^{-1} AB2 / 36 mL formula = 50 ng L^{-1})
	2. Extraction: shake the reconstituted formula vigorously by hand for 30 seconds. Place the sample tube in an ultrasonic water bath and sonicate for 20 minutes. Centrifuge the sample tube at 4000 g for 10 minutes.
	3. Work-up: Spoon off and discard the upper cream layer.

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 (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 (5 mL)
Interference wash 2:	Wash the column with 10% acetonitrile (5 mL)
Drying:	Dry the column for 5 minutes at maximum vacuum, 2 bar/29 psi
Interference wash 3:	Wash the column with hexane (5 mL)
Drying:	Dry the column for 5 minutes at maximum vacuum, 2 bar/29 psi
Elution:	Elute with 0.1% formic acid in acetonitrile (2 mL)
Post elution:	The 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 (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: 1mM 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. Typical retention times for AM1 and AB2 using the LC-MS/MS method described

Compound	Retention Time (min)
Aflatoxin M1	3.4
Aflatoxin B2	3.8

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Mass Spectrometry Conditions

Ions were selected in order to achieve maximum sensitivity. The MS was operated in positive 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:	+0.5 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
329.0>273.3	3.4	Aflatoxin M1 1	80	10	32	12
329.0>229.2	3.4	Aflatoxin M1 2	80	10	52	12
329.0>301.1	3.4	Aflatoxin M1 3	80	10	26	12
315.1>287.0	3.8	Aflatoxin B2 1	100	10	35	12
315.1>259.1	3.8	Aflatoxin B2 2	100	10	40	12
315.1>243.1	3.8	Aflatoxin B2 3	100	10	51	12

MRM detection window 60 s / target scan time 0.1 s / settling time 50 ms / scan pause 5 ms

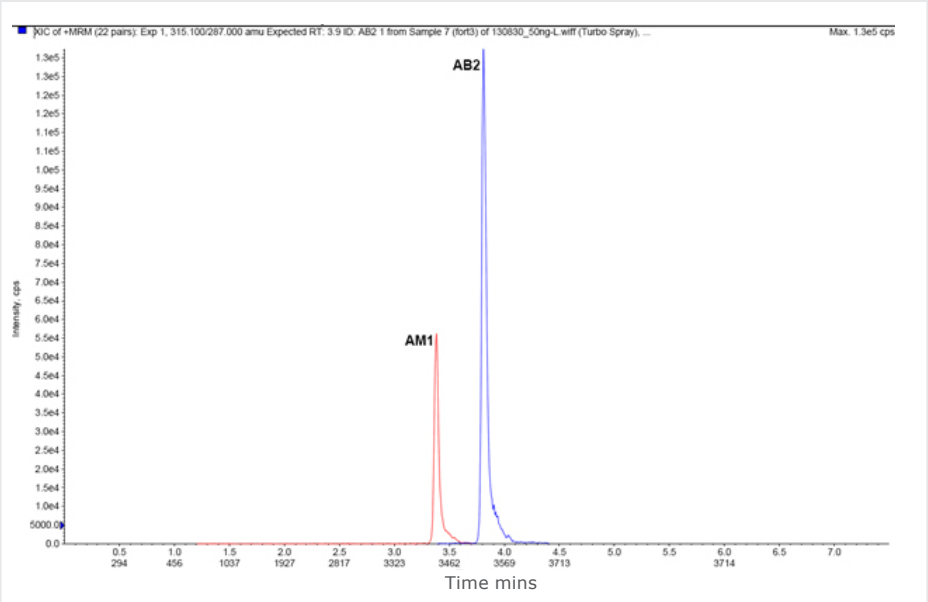

Figure 2. Extracted ion chromatograms in positive ion mode using ISOLUTE Myco protocol at 50 ng L^{-1} (aflatoxin M1 and aflatoxin B2 (internal standard)) from reconstituted infant formula

Validation Criteria

Method linearity was determined using internally standardized matrix-matched calibrants in six replicates over eight levels; the ranges are shown below.

Analytes	Working Range, ng L^{-1} (fg μL^{-1} on column)
Aflatoxin M1	2 to 100 (10 to 500)
Aflatoxin B2 (internal standard)	40 (200)

LOQ was determined from the lowest matrix-matched standard meeting EU repeatability and recovery criteria.

Repeatability (%RSDr) was determined from single acquisitions of 4 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 close to the analytical LOQ.

Results

The extracted ion chromatograms in figure 2 demonstrates chromatography at 50 ng L^{-1} (aflatoxin M1, aflatoxin B2) from a spiked extraction of 6 g dried infant formula reconstituted with 36 mL 1% formic acid (aq). Good linearity was achieved for aflatoxin M1 as demonstrated in the example chart shown in **Figure 3**.

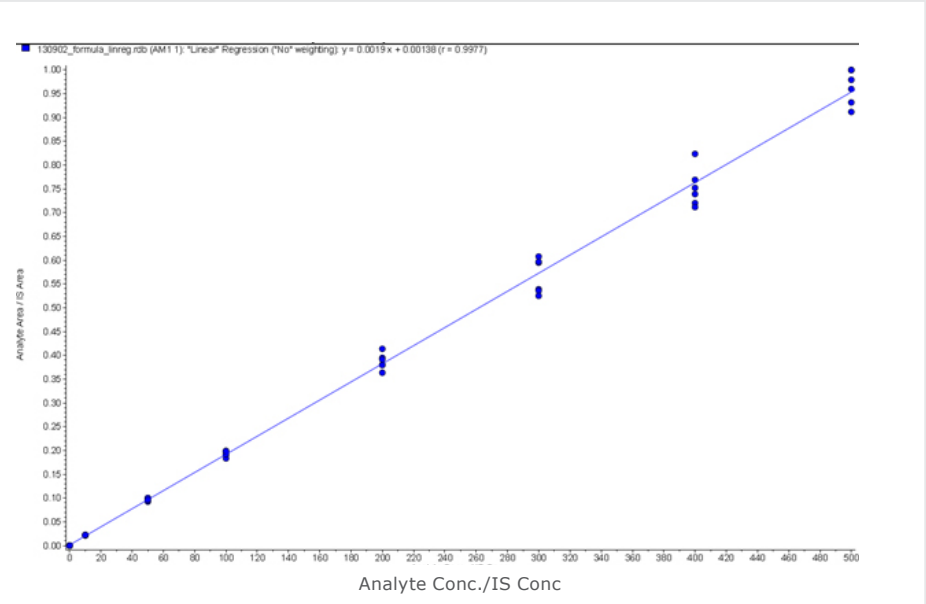

Figure 3. Internally standardized calibration curve for aflatoxin M1 from reconstituted infant formula using the ISOLUTE Myco protocol from 2 – 100 ng L^{-1} (aflatoxin B2 at 40 ng L^{-1})

Aflatoxin M1 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 **Table 3**.

Table 3. Analyte recovery and limit of quantitation data for aflatoxin M1 from reconstituted infant formula using the ISOLUTE Myco protocol

Analyte	r^2	LOQ / ng kg^{-1}		%RSD_r		Recovery %	
		Target	Actual	Target	Actual	Target	Actual
Aflatoxin M1	0.9977	25	2	20	6.4	70 to 110	90

Ordering Information

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

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