Sensitive Femtogram Determination of Aflatoxins B_1, B_2, G_1, and G_2 in Food Matrices using Tandem LC-MS/MS

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Introduction

Aflatoxins are a group of mycotoxins produced as metabolites by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (1). They can be found in various foods including grains, nuts, and spices (2). There are four major naturally occurring aflatoxins: B_1, B_2, G_1 and G_2 (Fig 1). Exposure to them can cause cancer in humans and live stock. Reliable and sensitive analytical methods for the determination of aflatoxins are required to safe-guard our food supply.

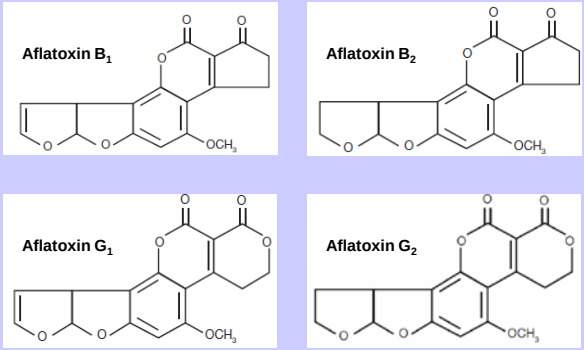


Fig 1. Structures of aflatoxins B_1, B_2, G_1 and G_2

Objectives

To develop a robust sample preparation and sensitive analytical method for the determination and confirmation of major aflatoxins in cereals and nuts.

Materials and Methods

LC/MS/MS Instrumentation

An Agilent 1200 series rapid resolution LC and a 6460 series Triple Quadruple MS/MS system equipped with Agilent Jet Stream thermal focussing electrospray ion source was used (Fig 2). Aflotoxins were detected using electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode. MassHunter Workstation software was used for identification and quantitation.

Aflatoxin standards and foods

Purified aflatoxin standards (B_1, B_2, G_1 and G_2) were obtained from Sigma-Aldrich. Aflatoxin-free corn flour, wheat, peanut and walnut samples obtained from a local grocery store were used for recovery studies.

Fig 2. Agilent LC/MS/MS system

HPLC conditions

Column:	Agilent Zorbax Eclipse C18, 4.6 x 50 mm, 1.8 micron		
Flow rate:	0.6 mL/min		
Column temp:	40°C		
Injection volume:	5 µL		
Mobile phase:	A: 10 mM ammonium acetate in water, B: methanol		
Gradient:	Time (min)	A (%)	B (%)
	0	95	5
	5	0	100
	6	0	100

MS conditions

Drying gas temp:	325°C	Sheath gas temp:	350°C
Gas flow rate:	10 L/min	Sheath gas flow:	11 L/min
Nebulizer pressure:	50 psi	EMV:	400 V
Capillary voltage:	4000 V		

Optimization of ionization and data acquisition parameters

The fragmentor voltage and collision energy for the MS/MS analysis were optimized for more specific and sensitive detection of aflatoxins (Table 1).

Table 1. Data acquisition parameters of MRM transitions for each aflatoxin

Aflatoxin	Retention time (min)	Fragmentor voltage (V)	Molecular weight	Precursor ion (m/z)	Product ions (m/z)	Collision energy (V)
B_1	4.68	130	312.1	313.1	285.1	20
					241.0	35
					269.1	25
B_2	4.57	130	314.1	315.1	287.1	25
					259.1	25
					243.0	40
G_1	4.40	130	328.1	329.1	243.1	25
					311.1	20
					283.0	20
G_2	4.26	130	330.1	331.1	313.1	25
					245.1	30
					285.1	25

Sample preparation and recovery studies

- Corn flour, ground wheat, peanut and walnut samples (10 g each) spiked with a mixture of 4 aflatoxin standards, each at 5 and 25 ppb.
- Extracted using 40 mL acetonitrile-water (84:16, v/v) for 30 min with shaking at room temperature.
- Extract clean-up using either C_{18} powdered adsorbent material (ODS SPE bulk sorbent, Agilent) or MycoSep 226 multi-functional column (Romer).
- Aliquots (0.4 mL) of the cleaned up extracts were diluted with 0.6 mL 10 mM ammonium acetate in water.
- The sample was then centrifuged at 14,000 x g for 3 min prior to LC-MS/MS analysis.
- Each food matrix and spike level was conducted in 7 replicates

Results and Discussion

The use of precursor and product ions in MS analysis allowed for sensitive detection and confirmation of all four aflatoxins (Fig 3). The standard curves for all 4 aflatoxins showed a good linearity from 0.1 to 100 ppb with R^2 greater 0.9999 (Fig 4 & 5). Recoveries were between 85-110% for each of the aflatoxins for all four spiked food matrices, with the MycoSep clean-up method slightly better than the C18 one for walnut samples (Fig 6). A limit of detection was determined to be <0.15 µg/kg and limit of quantitation <0.5 µg/kg for all four sample matrices.

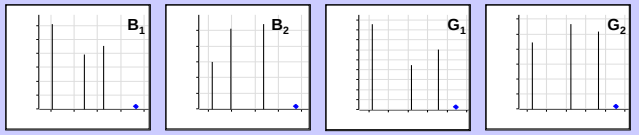


Fig 3. Mass spectra of aflatoxins B_1, B_2, G_1 and G_2.

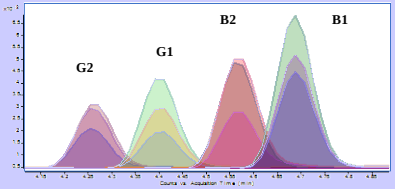


Fig 4. LC/MS/MS chromatogram of aflatoxin B_1, B_2, G_1 and G_2 standards at 1 ppb

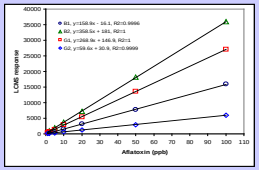
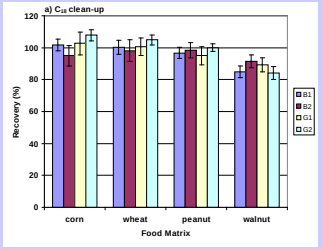


Fig 5. Standard curve of aflatoxins from 0.1 to 100 ppb



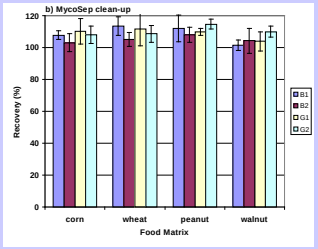


Fig 6. Recovery of aflatoxin B_1, B_2, G_1 and G_2 from food matrices using a) C_{18} or b) MycoSep clean-up

Conclusions

An LC/MS/MS method has been developed for the analysis and confirmation of aflatoxins B_1, B_2, G_1 and G_2 in cereals and nuts, with a detection limit of less than 1 ppb.

References

1) Microhim Acts 153, 2006, 101-108
2) Rapid Commun. Mass Spectrum, 2009; 23: 3-11

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