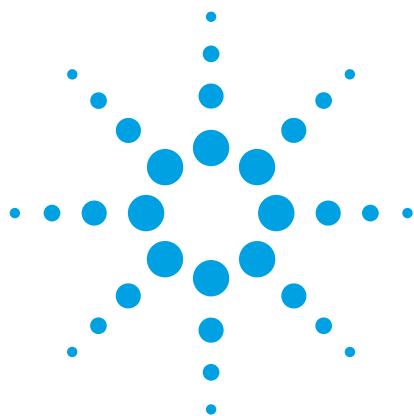




MS/MS Identification of Four Aflatoxins Using the Agilent 500 Ion Trap LC/MS

Application Note

Food Testing and Agriculture

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Abstract

A rapid and sensitive LC/MS/MS method for the detection and analysis of aflatoxins B1, B2, G1 and G2 provides detection down to 1 ppb. The 500 Ion Trap MS/MS capabilities result in the collection of clear, baseline resolved chromatographic peaks for target compound quantitation.

Introduction

Aflatoxins are a group of structurally related carcinogenic mycotoxins produced by *Aspergillus* spp. The most commonly found aflatoxins in food and feed are aflatoxins B1, B2, G1 and G2. Aflatoxin B1 has been classified as a Class 1 human carcinogen by the International Agency for Research on Cancer. Globally, there are significant regulations on the presence of aflatoxins in food and feed. The U.S. Federal Government action level for aflatoxins in food for human consumption or in dairy cow feed is 20 ppb.

The European Commission has set very low action levels for aflatoxins in cereals intended for direct human consumption or for ingredients in foodstuffs. These action levels are 4 ppb total aflatoxins and 2 ppb for aflatoxin B1. So far, these compounds have been extensively analyzed by HPLC. This study developed a rapid and sensitive LC/MS/MS method for the detection and analysis of aflatoxins B1, B2, G1 and G2.



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Instrumentation

The following instruments were used in this study:

- Agilent 500 Ion Trap LC/MS with ESI source
- Agilent 212-LC Binary Gradient LC/MS pumps
- Agilent Prostar 430 AutoSampler

Materials and Reagents

The aflatoxins used were purchased from Supelco, Bellefonte, PA. All other reagents were purchased from Sigma-Aldrich Co, St. Louis, MO.

HPLC conditions

Column:	Polaris C18-A, 150 × 3 mm, 5 μm (Agilent p/n A2000150X030)		
Solvent A:	Water		
Solvent B:	Methanol		
Injection volume:	10 μL		
LC program:	Time (min:sec)	%A	%B
	00:00	70	30
	01:00	70	30
	15:00	10	90
	15:01	0	100
	20:00	0	100
	20:01	70	30
	25:00	70	30
			Flow (μL/min)
			200
			200
			200
			200
			200
			200
			200

MS parameters

Ionization mode:	ESI Positive
API drying gas:	35 psi at 350 °C
API nebulizing gas:	40 psi
Needle:	5000 V
Shield:	600 V

Table 1. MS Segment Parameters

Segment	Analyte	Transition	Retention time (min)	Capillary voltage (V)	Excitation amplitude (V)	RF load %
1	Aflatoxin G2	331 → 280–320	10.45	75	1.36	82
1	Aflatoxin G1	329 → 280–320	11.12	75	1.35	82
2	Aflatoxin B2	315 → 280–320	11.68	75	1.29	82
2	Aflatoxin B1	313.2 → 280–320	12.30	75	1.28	82

Results and Discussion

This LC/MS/MS method separates aflatoxins G2, G1, B2 and B1, and provides excellent response for each of the analytes down to 1 ppb. The Polaris C18 column provides excellent chromatographic separation and reproducible retention times. Figure 1 shows the extracted ion chromatogram (EIC) of aflatoxin G2, G1, B2 and B1 at a concentration of 1 ppb.

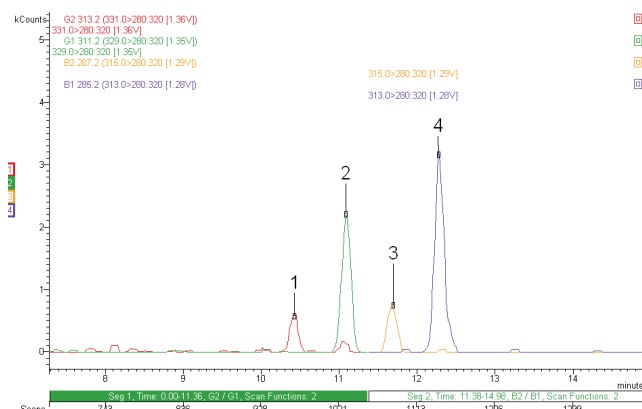
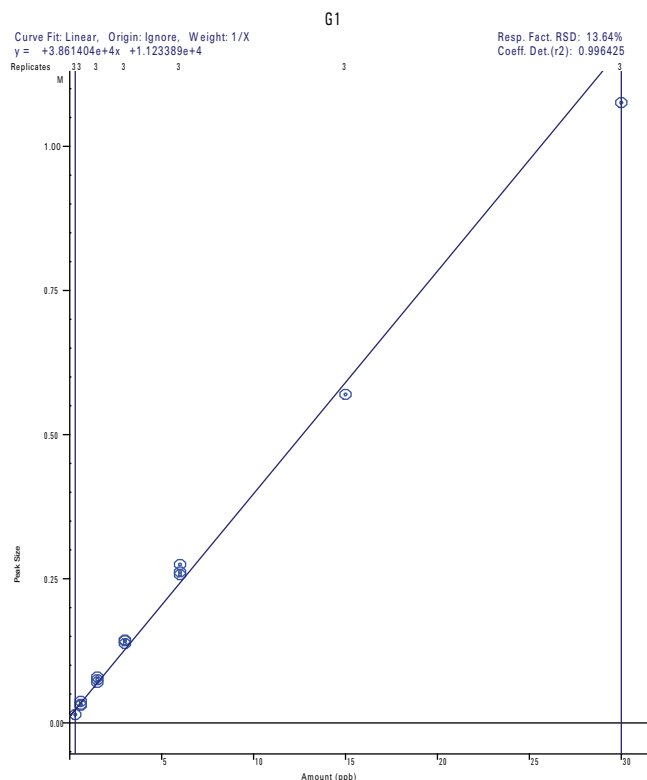


Figure 1. EIC of (1) aflatoxin G2: m/z 331.0 > 313.2; (2) aflatoxin G1: m/z 329.0 > 311.2, (3) aflatoxin B2: m/z 315.0 > 285.2, and (4) aflatoxin B1: m/z 313.0 > 285.1

Calibration curves were found to be linear from 1 to 50 ppb, with %RSD values of 15.62% for aflatoxin G2, 13.64% for aflatoxin G1, 12.65% for aflatoxin B2 and 10.44% for aflatoxin B1. Figure 2 shows the calibration curve for aflatoxin G1.

File: ...atoxins\afatoxin_02-msms 2seg@500,000 200ul 1uscan@1hz cal_2.mth
Detector: 500-MS Mass Spec, Address: 56



Conclusion

The MS/MS capabilities of the Agilent 500 Ion Trap LC/MS allow for isolation of desired precursor ions followed by collision induced dissociation (CID) and characteristic product ion spectra, resulting in the collection of clear, baseline resolved chromatographic peaks for target compound quantitation. This method is fast, rugged and sensitive.

1. Commission regulation (EC) No 2174/2003 / (EC) No 466/2001 as regards aflatoxins.
2. U.S. Food and Drug Administration Industry Activities Staff Booklet August 2000.
3. European Commission Council directive 96/23/EC/SANCO/1805/2000.

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