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Rapid and Highly Sensitive Quantitative Analysis and Screening of Aflatoxins in Foods Using Liquid Chromatography Triple Quadrupole Mass Spectrometry

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Introduction

Aflatoxins (AFs) are the most harmful mycotoxins produced by the *fungi Aspergillus flavus* and *Aspergillus parasiticus* and can contaminate foods such as cereals and nuts. To reduce the risk of the ingestion from foods, analyses of the AFs are carried out in many countries. It is necessary to quantitate the total aflatoxin (B1, B2, G1, G2) in foods by the regulation in JAPAN. The conventional LC/MS method proposed by the Ministry of Health, Labour and Welfare of Japan has a total analysis time of 30 minutes. In this study, we examined two alternative high-throughput LC-MS/MS methods. The first optimized for sensitivity & quantification; the second, a rapid screening method, using UHPLC for the purpose of increasing work flow.

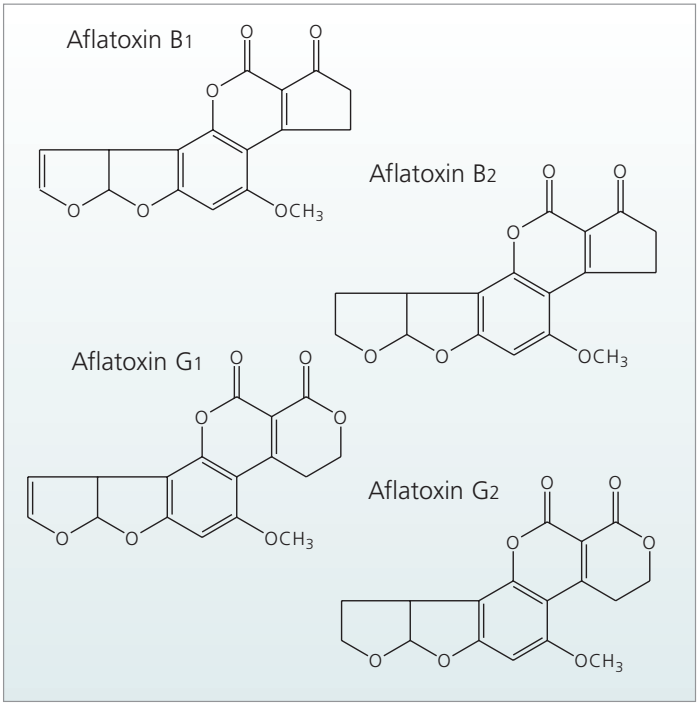

Fig. 1 Structure of Aflatoxins.

Materials and Methods

The system consisted of a SHIMADZU "Nexera" HPLC system, and "LCMS-8030" triple quadrupole mass spectrometer, equipped with an electrospray ionization (ESI) used in the positive ion MS/MS mode.
AFs standard solution were obtained from Biopure, MYCOTOXIN MIX5(AFLATOXINS) and Wako Chemicals, Aflatoxins Mixture Solution 1.
Sample preparation work flow (Fig. 2) shows how AFs in roast peanut was prepared by an immunoaffinity column (AFLAKING, HORIBA, JAPAN). Based on starting material 50g (roast peanut powder) spiked Aflatoxin B1 and G1(4 µg/Kg), B2 and G2 (1 µg/Kg) standard solution, final concentration of the sample solutions became: 2 µg/L B1 and G1, 0.5 µg/L B2 and G2.

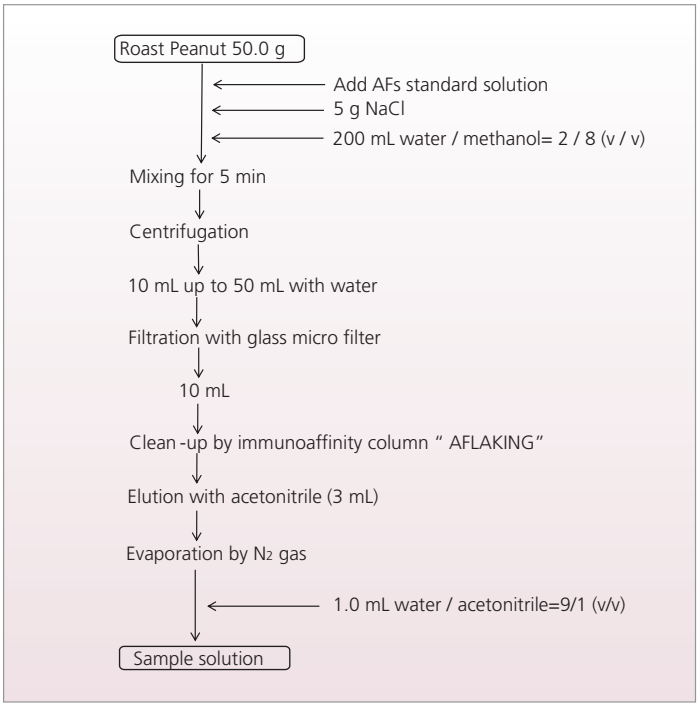

Fig. 2 Sample preparation work flow.

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Experiment

Analysis Standard Solution

The chromatogram and analytical conditions of the conventional method (Fig. 3, Table 1) were compared to and the high-speed method with using ultra-fast liquid chromatography (Fig. 4, Table 3). The flow rate was raised to 0.45 mL/min accelerating AFs elution to 4 minutes (operating back pressure 44-50 Mpa). MRM transitions summarized in Table 2.

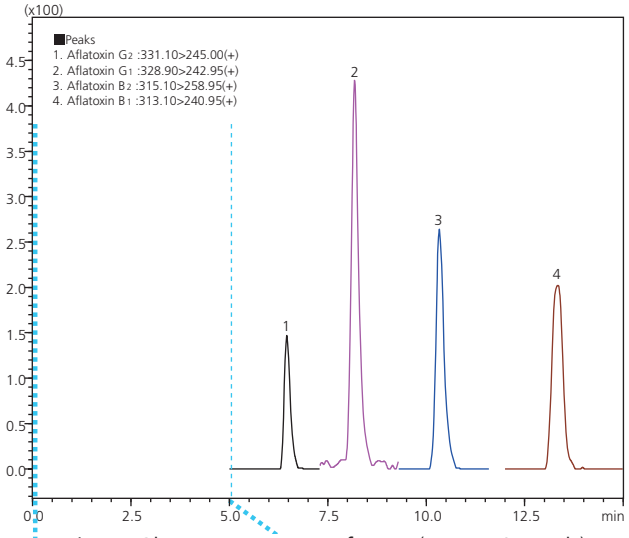

Fig. 3 Chromatograms of AFs (0.5 µg/L each).

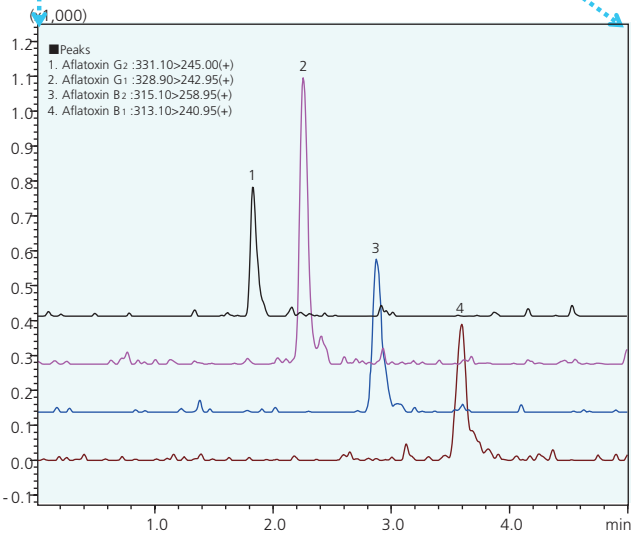

Fig. 4 Chromatograms of AFs (0.5 µg/L each).

Table 1 Analytical conditions (typical 30 minute method).

HPLC

Column:	Shim-pack FC-ODS (150 mm L. × 2.0 mm i.d., 3 µm)
Mobile Phase A:	10 mmol/L Ammonium acetate-water
Mobile Phase B:	Methanol
Gradient Program:	40%B(0-15 min) → 100%B (15.01-20 min) → 40%B (20.01-30 min)
Flow Rate:	0.2 mL/min
Column Temp. :	40°C
Injection Vol.:	6 µL

MS

Probe Voltage:	+4.5 kV ESI-Positive mode
Nebulizing Gas Flow:	3 L/min
Drying Gas Flow:	15 L/min
DL Temp.:	250°C
Heat Block Temp.:	400°C

Table 2 MRM Parameter.

Compound	Transition	Pause time (ms)	Dwell time (ms)	CE (V)	Resolution (Q1,Q3)
Aflatoxin B1	313.10 > 240.95	3	100	-40	Unit
Aflatoxin B2	315.10 > 258.95	3	100	-33	Unit
Aflatoxin G1	328.90 > 242.95	3	100	-30	Unit
Aflatoxin G2	331.10 > 245.00	3	100	-32	Unit

Table 3 Analytical conditions (optimized fast method).

HPLC

Column:	Shim-pack XR-ODS II (100 mm L. ×2.0 mm i.d., 2.2 µm)
Mobile Phase A:	10 mmol/L Ammonium acetate-water
Mobile Phase B:	Methanol
Gradient Program:	40%B (0-4.5 min)→ 100%B(4.51-6.5 min)→ 40%B (6.51-12 min)
Flow Rate:	0.45 mL/min
Column Temp.:	50°C
Injection Vol.:	6 µL

MS

Probe Voltage:	+4.5 kV ESI-Positive mode
Nebulizing Gas Flow:	3 L/min
Drying Gas Flow:	15 L/min
DL Temp.:	250°C
Heat Block Temp.:	400°C

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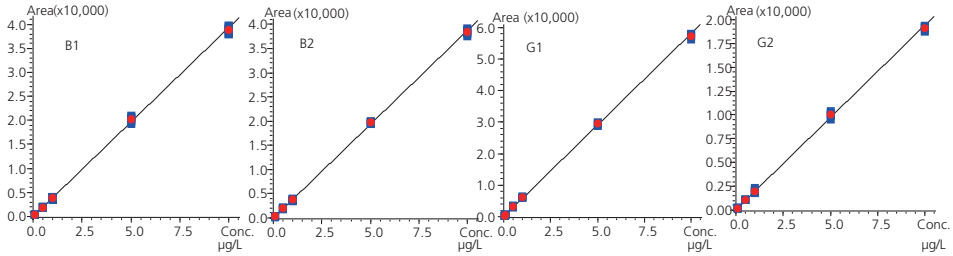

Fig. 5 Callibration curves AFs (linearity beyond R^2=0.999 was acquired).

Table 4 L.O.Q. and Linearity (n=6).

Compound	L.O.Q. (µg/L)	Linearity	R^2
Aflatoxin B1	0.1	0.1-10 µg/L	0.9995372
Aflatoxin B2	0.1	0.1-10 µg/L	0.9997556
Aflatoxin G1	0.05	0.05-10 µg/L	0.9994336
Aflatoxin G2	0.1	0.1-10 µg/L	0.9992275

Effect of Column Temperature

Column temperature was optimized in order to accelerate compound elution without compromising peak shape or intensity. Finally a column temperature of 50°C was chosen (Fig. 6).

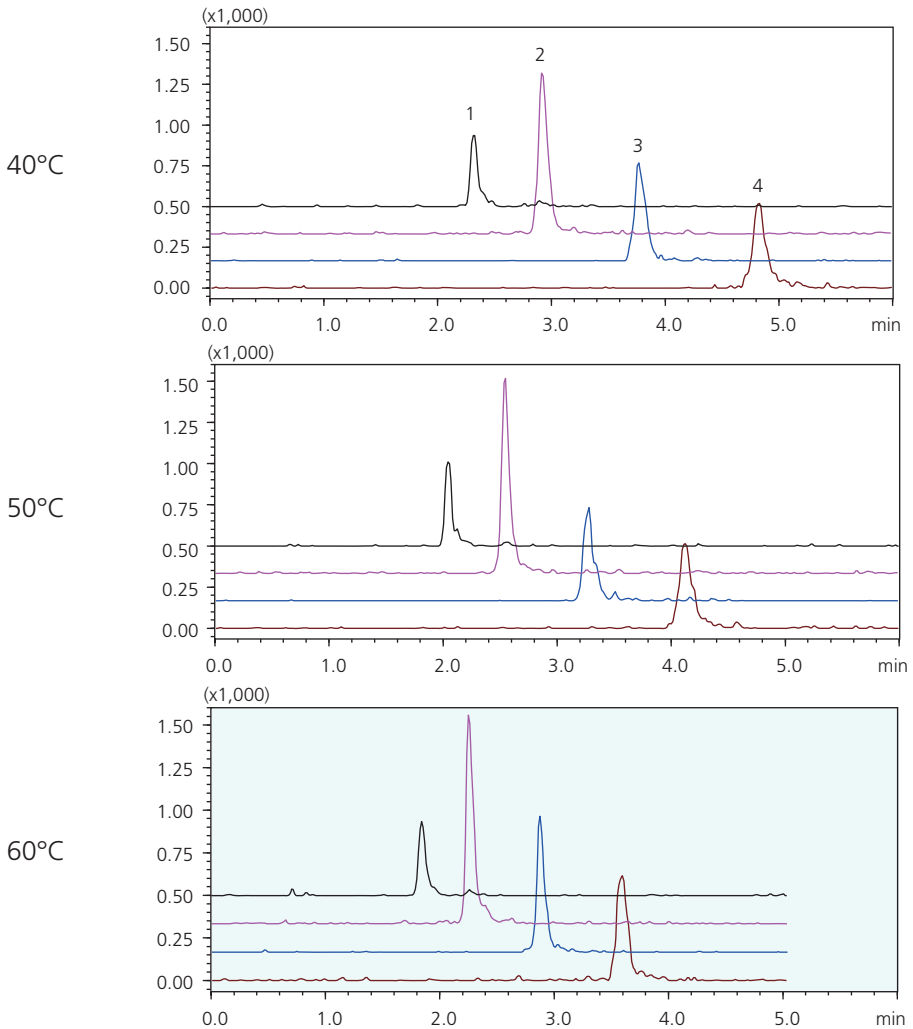

Fig. 6 Chromatographic optimization of column temperature.

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Effect of ESI Probe Position

Further optimisation was achieved through optimization of ESI probe position ranging from -2 mm to +3 mm from the central position (Fig. 7). Chromatographic comparison (Fig. 8) illustrates both peak intensity and level of noise are influenced by probe position. Optimization required highest S/N and minimum noise at +1 mm (Fig. 9 & 10).
[Noise was calculated by ASTM method with 3 blocks of 0.5 min around each

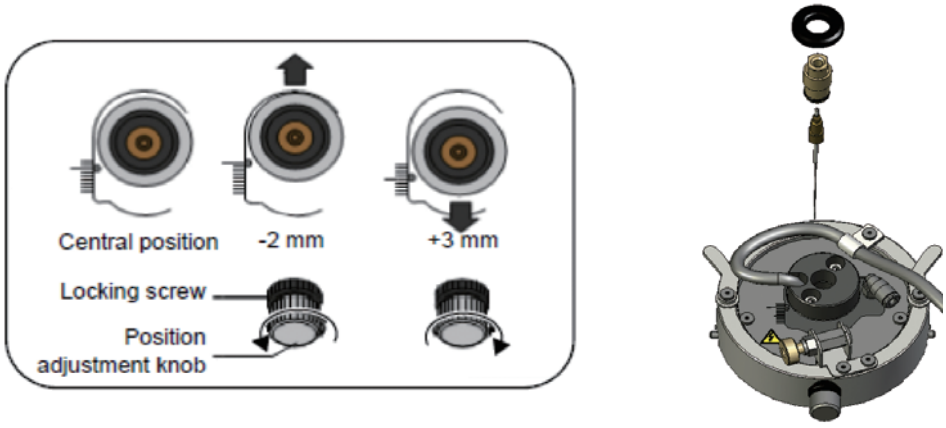

Fig. 7 ESI probe of LCMS-8030.

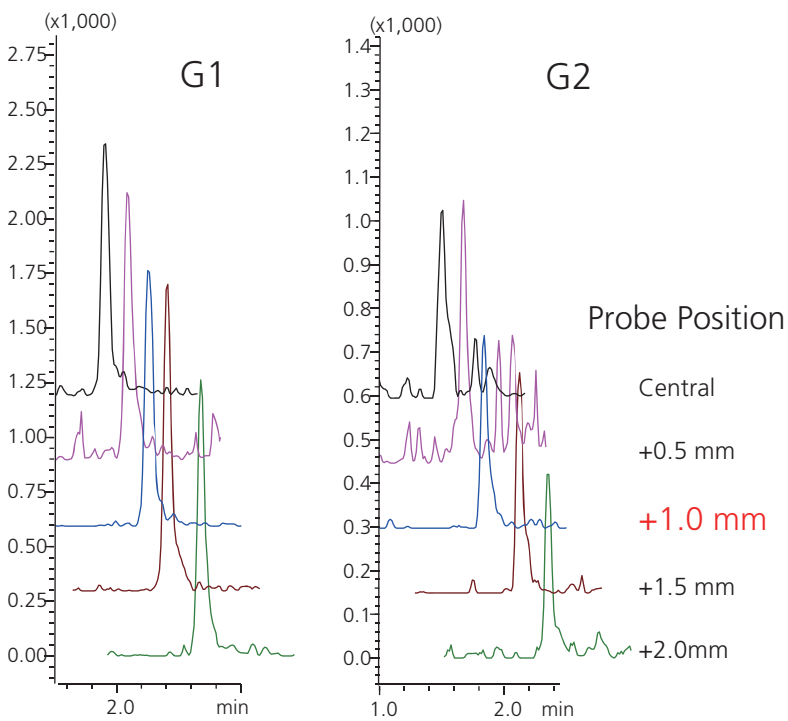

Fig. 8 Chromatograms comparison of a ESI probe position difference.

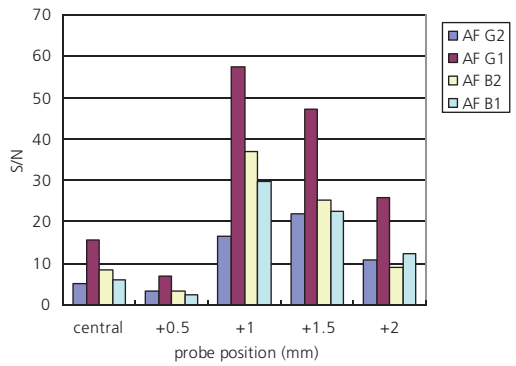

Fig. 9 S/N of probe position difference.

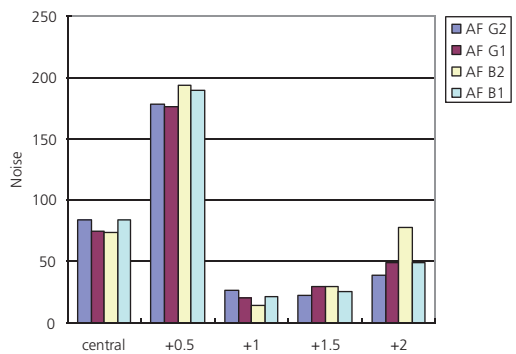

Fig. 10 Noise of probe position difference.

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Results

Analysis of Roast Peanut Matrix

The recovery test of AFs spiked into the roast peanut powder was performed in duplicate experiments. Overlaid chromatograms of spiked AFs in roast peanut matrix to un-spiked were shown (Fig. 11). Solid line - spiked compared to dotted line - un-spiked. Interference peaks were not detected in un-spiked samples.
Quantitation results using external standard method show the recovery rate was in the range of 69-86% (Table 5). This relatively low recovery rate is a known problem when extracting AFs with a solvent from the powder of a roast peanut. Further method development is underway to increase recovery rate.

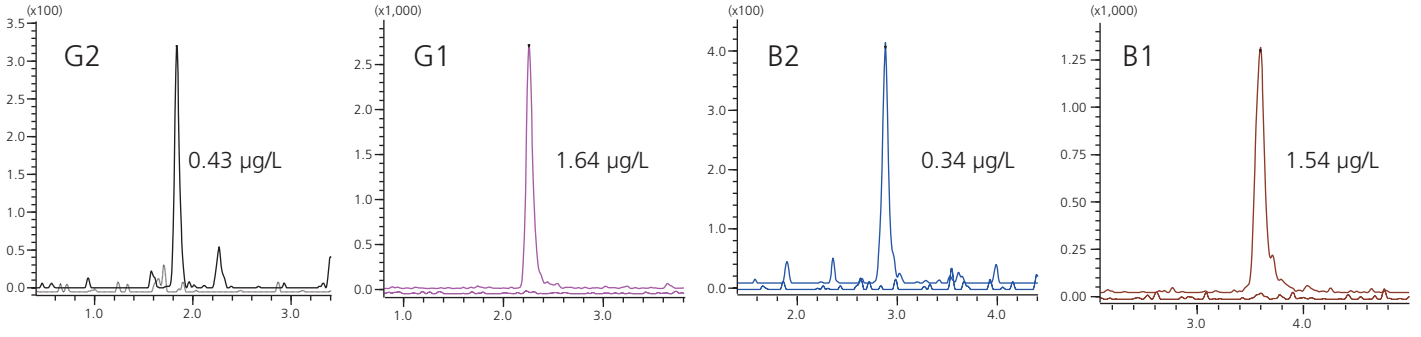

Fig. 11 Chromatograms of AFs in roast peanut matrix.

Table 5. Results of quantity analysis.

	Aflatoxin B1		Aflatoxin B2		Aflatoxin G1		Aflatoxin G2	
	Conc. (µg/L)	Recovery (%)	Conc. (µg/L)	Recovery (%)	Conc. (µg/L)	Recovery (%)	Conc. (µg/L)	Recovery (%)
Sample1	1.54	75	0.34	69	1.64	82	0.43	86
Sample2	1.44	70	0.34	69	1.64	82	0.37	74

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Ultra High-speed Method For Screening Analysis

Further improvements to speed of analysis were made using a reduced column particle size and length (1.6 µm, Shim-pack XR-ODS III 50 mm × 2.0 mm). With these conditions, AFs eluted within 2 minutes with L.O.Q. of 0.5 µg/L. This ultra high-speed analysis could prove useful when screening many samples.

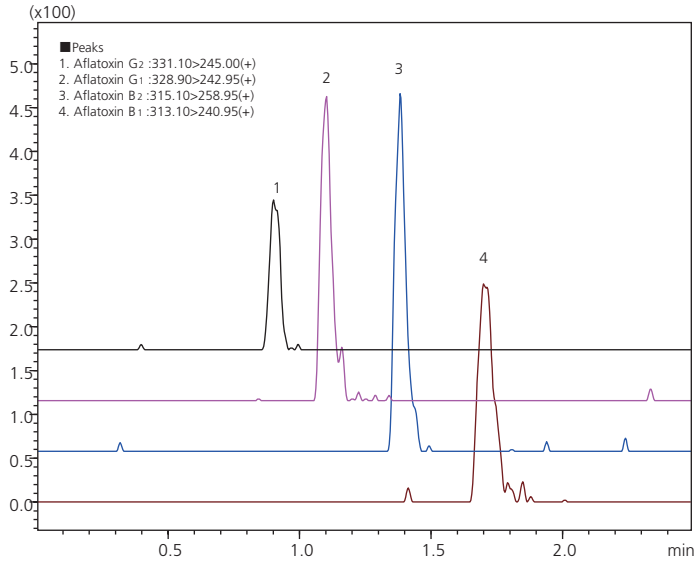

Fig. 12 Chromatograms of AFs (0.5 mg/L each).

Table 6. Analytical conditions.

HPLC

Column:	Shim-pack XR-ODS III (50 mm L. ×2.0 mm i.d., 1.6 µm)
Mobile Phase A:	10 mmol/L Ammonium acetate-water
Mobile Phase B:	Methanol
Gradient Program:	40%B(0-2.25 min)→ 100%B(2.26-3.25 min)→ 40%B(3.26-6.00 min)
Flow Rate:	0.45 mL/min
Column Temp. :	50°C
Injection Vol.:	6 µL

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Probe Voltage:	+4.5 kV ESI-Positive mode
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Aflatoxin G2	331.10 > 245.00	1	50	-32	Unit

Conclusion

Analysis of AFs in the roast peanut was studied.

- Accelerated method was developed due to capacity of ultra high pressure liquid chromatography (Nexera).
- New choice of Shim-pack column enabled faster elution times.
- Two high-speed methods were developed eluting AFs within 4 and 2 min.
- Column temperature and ESI probe position were important conditions of AFs analysis.
- Immunoaffinity column was useful for cleanup from the roast peanut matrix.
- The results of recovery test was 69-86%.

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