Determination of Aflatoxins in Food by LC/MS/MS

Application

Food Safety

Authors

Masahiko Takino
Agilent Technologies
9-1 Takakura-Cho
Hachiouji-Shi, Tokyo
Japan

Toshitsugu Tanaka
Kobe Institute of Health
Department of Food Chemistry
4-6 Minatojima-nakamachi
Chuo-ku, Kobe
Japan

Abstract

A sensitive and selective analytical method for the determination of aflatoxin G1, G2, B1, and B2 residues in cereals using the Agilent G6410AA LC/MS Triple Quadrupole Mass Spectrometer was developed. This method uses simple sample preparation methods followed by LC/MS/MS. The limits of detection for all aflatoxins were less than 1 ng/mL in cereals.

Introduction

Aflatoxins (AFs) belong to a closely related group of secondary fungal metabolites. These mycotoxins are severely toxic metabolites produced mainly by *Aspergillus flavus* and *A. parasiticus*, and exposure to them can cause cancer in humans and livestock [1]. Based on epidemiological evidence, AFs have been classified as human liver carcinogens by the World Health Organization and by the U.S. Environmental Protection Agency. Thus, accurate determination of AFs is required to avoid human disease from AF exposure and to advance worldwide surveillance of food. Analysis of AFs in food products is routinely performed by thin-layer chromatography (TLC) and liquid chromatography (LC) with fluorescence detection (FD) in combination with both precolumn derivatization and postcolumn derivatization. The LC/FD technology is often used due to the high selectivity and sensitivity of these methods. Furthermore, hyphenated techniques such as LC coupled to mass spectrometric (MS) detection have been developed and applied in residual analysis of foods. The high selectivity and sensitivity of MS detection methods associated with the resolution of LC provide decisive advantages to perform qualitative as well as quantitative analysis of a wide range of molecules at trace levels. Several papers describing different kinds of MS methods for the analysis of AFs have been published [2-4.]

Experimental

Sample Preparation

The samples analyzed (peanuts, corn, nutmeg, and red pepper) were obtained from a local market and did not include any AFs. The extraction and cleanup steps for AFs were carried out according to validated methods reported by Tanaka [5]. Briefly, 20 g fine ground sample was poured into a 200-mL Erlenmeyer flask, followed by adding 40 mL acetonitrile-water (9:1, v/v) for corn and cereals. After shaking for 30 min, the mixed solution was centrifuged for 5 min at 1,650 g. The supernatant obtained was filtered through a glass microfiber GF/B grade filter (Whatman Interna-

tional Ltd, Maidstone, UK). A 5-mL portion of the filtrate was applied to a MultiSep number 228 cartridge column for the cleanup. After passing through at a flow rate of 1 mL/min, 2 mL of the first eluate was collected. The eluate was evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 1 mL methanol-water (4:6 v/v) containing 10 mM ammonium acetate.

Standard Preparation

Each of the standard reagents, aflatoxin G_2 (AFG_2), aflatoxin G_1 (AFG_1), aflatoxin B_2 (AFB_2) and aflatoxin B_1 (AFB_1), was dissolved in acetonitrile at 1 mg/mL and was stored at 4 °C in the dark until use. To prepare the working standard for LC/MS analysis, each AF stock solution was equally pipetted and transferred to a vial, and it was then diluted with the mobile phase. The final concentration of each AF was 1 ng/mL.

Chemicals

The standards AFG_2, AFG_1, AFB_2, and AFB_1 were obtained from Sigma Aldrich Japan (Tokyo, Japan). The purity of these compounds was greater than 99%. Ammonium acetate, toluene, HPLC-grade acetonitrile, and HPLC-grade methanol were obtained from Wako Chemical (Osaka, Japan). Water was purified in-house with a Milli-Q system (Millipore, Tokyo, Japan). The cartridge column of MultiSep number 228 was purchased from Showa Denko (Kanagawa, Japan).

LC/MS Instrument

The LC/MS/MS system used in this work consists of an Agilent 1200 Series vacuum degasser, binary pump, well-plate autosampler, thermostatted column compartment, the Agilent G6410 Triple Quadrupole Mass Spectrometer with an electrospray ionization (ESI) source. The objective of the method development was to obtain a fast and sensitive analysis for quantifying AFs in foods. For chromatographic resolution and sensitivity, different solvents and columns were optimized. It was found that a simple solvent system using water, methanol, ammonium acetate, and a 1.8-µm particle size C18 column worked very well.

LC Conditions

Instrument:	Agilent 1200 HPLC
Column:	ZORBAX Extend C18, 100 mm × 2.1 mm, 1.8 µm (p/n 728700-902)
Column temp:	40 °C
Mobile phase:	A = 10 mM ammonium acetate in water B= Methanol 40% A/60% B
Flow rate:	0.2 mL/min
Injection volume:	5 µL

MS Conditions

Instrument:	Agilent 6410 LC /MS Triple Quadrupole
Source:	Positive ESI
Drying gas flow:	10 L/min
Nebulizer:	50 psig
Drying gas temp:	350 °C
V_{cap}:	4000 V
Scan:	*m/z* 100 – 550
Fragmentor:	Variable 100 V
MRM ions:	Shown in Table 1
Collision energy:	Shown in Table 1

LC/MS/MS Method

Quantitative analysis was carried out using MRM mode. The parameters for MRM transitions are shown in Table 1.

Table 1. Data Acquisition Parameters of MRM Transitions for Each Aflatoxin

No	Mycotoxins	RT (min)	Molecular weight	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)
1	Aflatoxin G_2	5.21	330	331	245	30
2	Aflatoxin G_1	6.61	328	329	243	30
3	Aflatoxin B_2	8.44	314	315	259	30
4	Aflatoxin B_1	10.89	312	313	241	30

Results and Discussion

Optimization of MRM Transitions

Determination of the optimal MRM transitions for each aflatoxin was carried out using single-MS full-scan mode followed by product ion scan mode using taflatoxin standard mixtures at 1 ug/mL. Mass spectra of these standard mixtures in full scan mode and product ion scan mode are shown in Figures 1 and 2. The mass spectrum of each aflatoxin by full-scan mode exhibited the protonated molecule $[M+H]^+$ as the base peak ion. These ions were selected as precursor ions for MRM mode. The optimum collision voltage is compound dependent. To establish the optimum collision voltage, this parameter was varied from 5 to 40 V using a step size of 5V. As shown in Figure 2, a distinct optimum in the intensity of the product ion of each AF was observed at 30 V. The product ions that indicated the highest intensity were *m/z* 245 (AFG_2), 243 (AFG_1), 259 (AFB_2), and 241 (AFB_1), respectively. On the basis of the above results, the collision voltage was set to 30 V.

Table 1 shows the parameters of MRM mode of each aflatoxin.

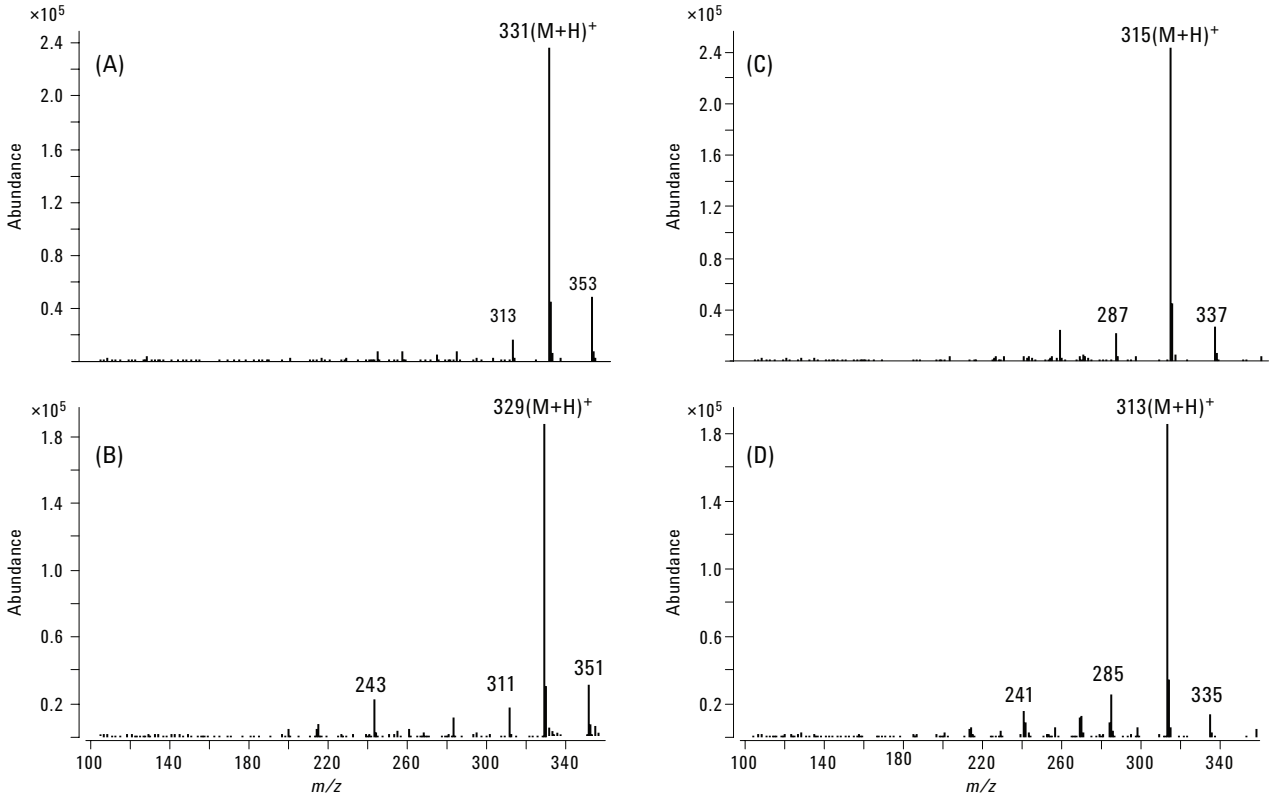

Figure 1. Mass spectra of four aflatoxins standard in single-MS full-scan mode at 1 µg/mL (A): aflatoxin G_2, (B): aflatoxin G_1, (C): aflatoxin B_2, and (D): aflatoxin B_1.

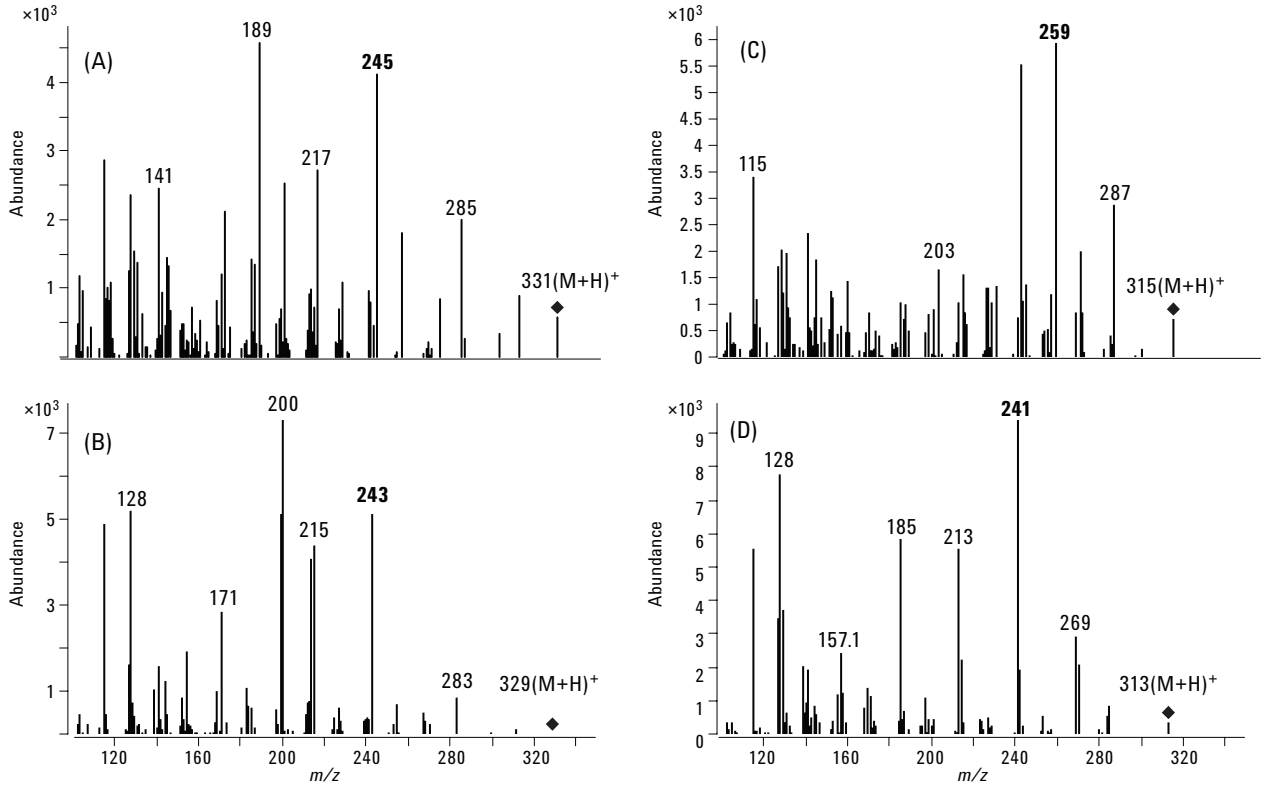

Figure 2. Mass spectra of four aflatoxins standard in product ion scan mode at 1µg/mL (A): aflatoxin G_2, (B): aflatoxin G_1, (C): aflatoxin B_2, and (D): aflatoxin B_1.

The MRM chromatogram of each aflatoxin at 0.1 ng/mL is shown in Figure 3. These show excellent signal-to-noise (S/N) ratios for all aflatoxins. The limit of detection (LOD) for each aflatoxin was determined using an S/N ratio of 3 with this MRM chromatogram and is shown in Table 2. To evaluate the linearity of the calibration curves, various concentrations of aflatoxin standard solutions ranging from 0.1 ng/mL to 100 ng/mL were analyzed. As shown in Figure 4 and Table 2, the linearity was very good for all aflatoxins with correlation coefficients (r^2) greater than 0.999.

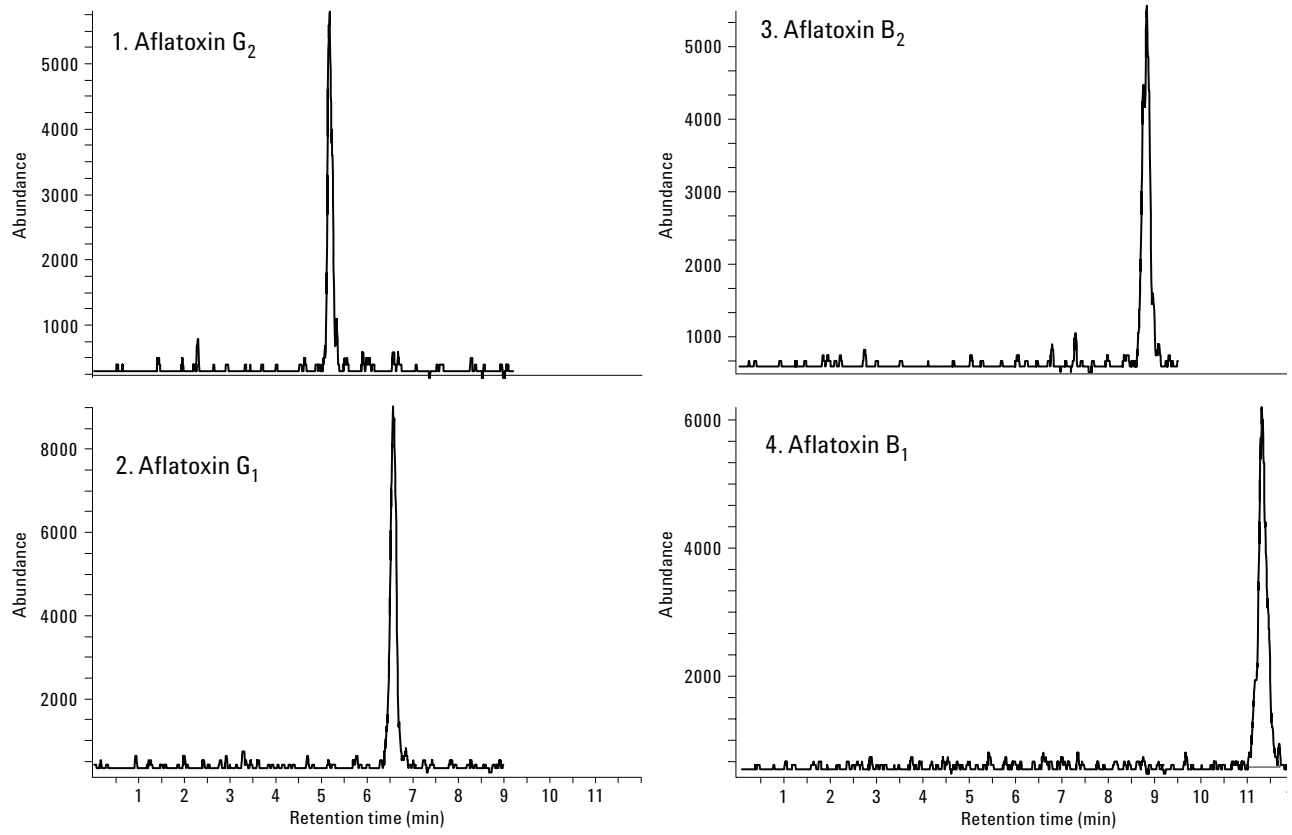

Figure 3. MRM chromatograms of four aflatoxin standards at 0.1 ng/mL in MRM mode.

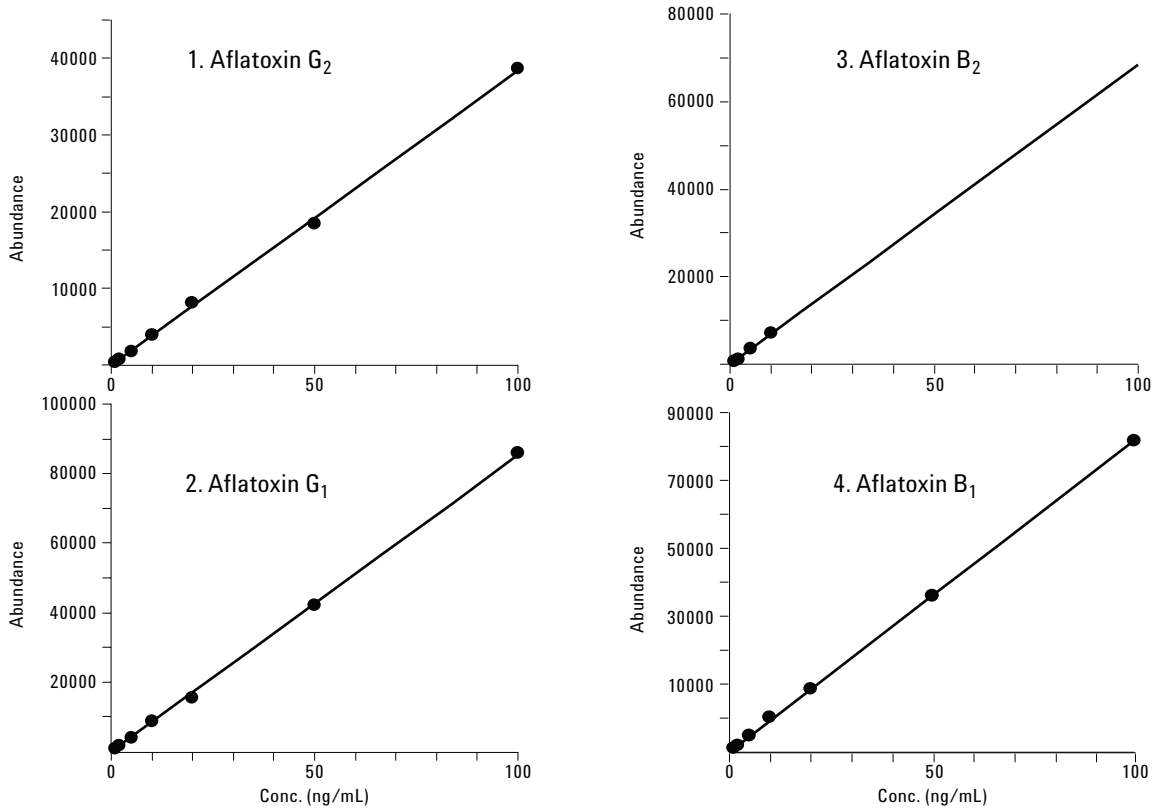

Figure 4. Calibration curves of four aflatoxins ranged from 0.1 ng/mL to 100 ng/mL.

Table 2. Linearity and LODs of Four Aflatoxins

No	Mycotoxins	r^2	LOD (ng/mL)
1	Aflatoxin G_2	0.9999	0.025
2	Aflatoxin G_1	0.9992	0.020
3	Aflatoxin B_2	0.9999	0.025
4	Aflatoxin B_1	0.9993	0.020

The matrix effect of this method was investigated by using cereal and corn extracts spiked with mycotoxin standards at 0.2 ng/mL. Typical MRM chromatograms of cereal and corn extract are shown in Figures 5 and 6, respectively. There were no additional peaks from sample matrix in either food when compared with the mycotoxin standard mixture. These results indicate that MRM mode has very high selectivity.

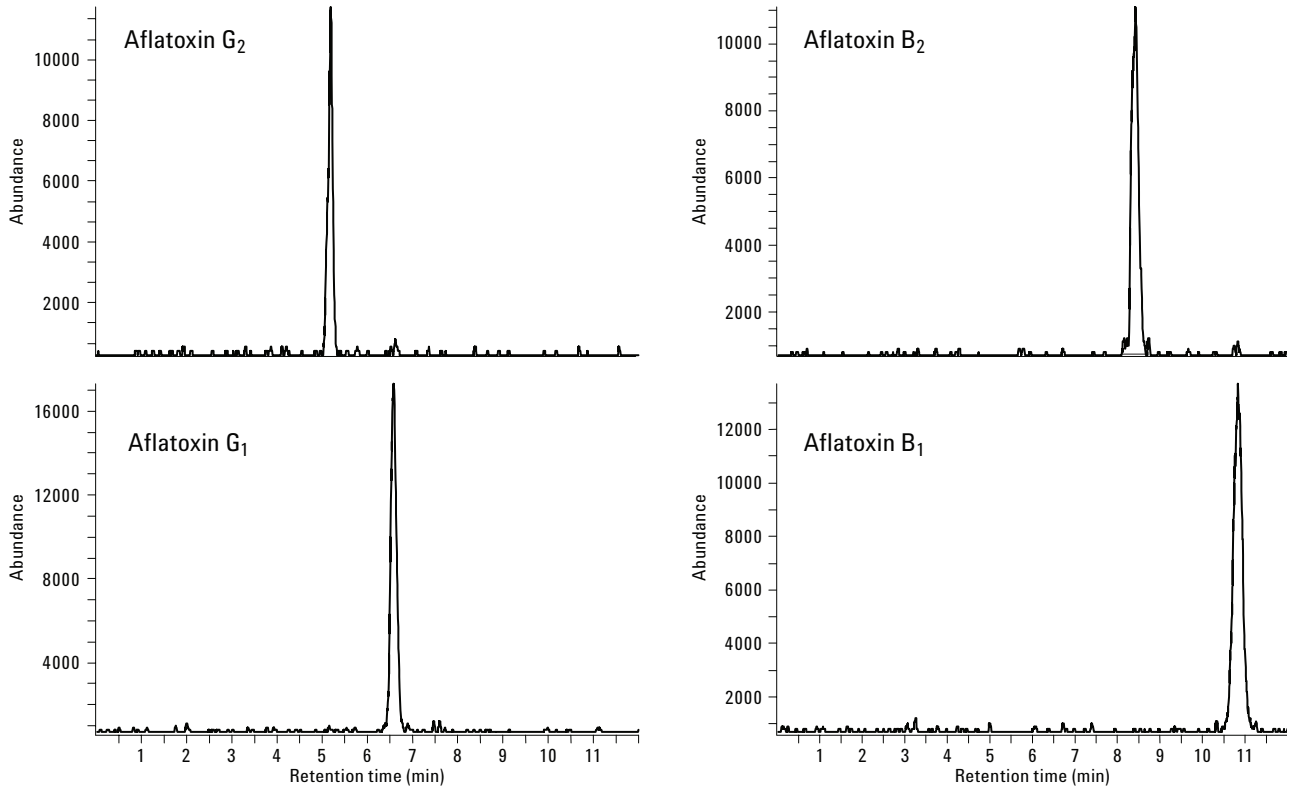

Figure 5. MRM of four aflatoxins in cereal extract spiked at 0.2 ng/g.

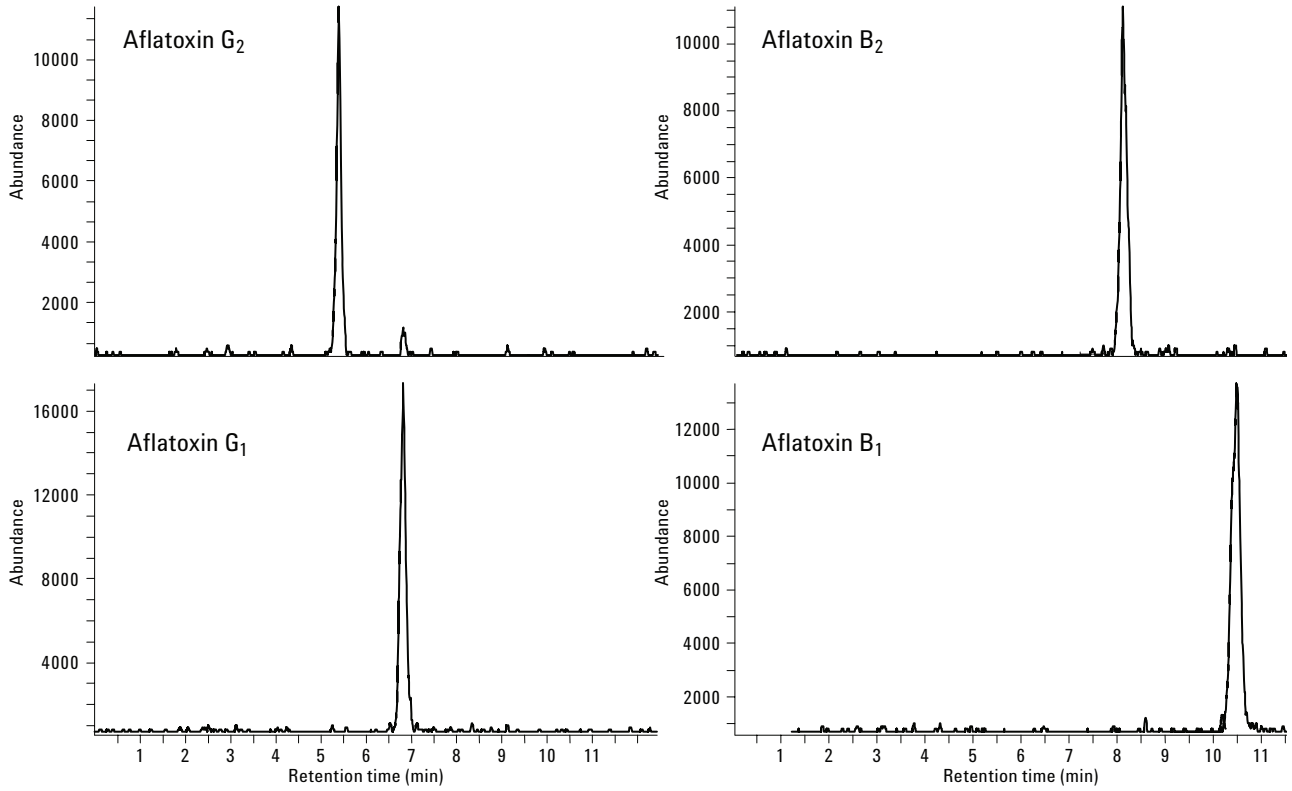

Figure 6. MRM of four aflatoxins in corn extract spiked at 0.2 ng/g.

Furthermore, the change on the peak intensity of each aflatoxin by the sample matrix was investigated by comparison with the peak intensity of aflatoxin standards. As these results show in Table 3, the relative intensity of each pesticide ranged from 88 to 96%. Thus, matrix effects such as ion suppression may be insignificant and it is possible to use external standards instead of matrix-matched standards.

Table 3. Relative Intensity of Each Aflatoxin in Sample Extracts

No	Mycotoxins	Relative intensity (%)	
		Cereal	Corn
1	Aflatoxin G_2	88	91
2	Aflatoxin G_1	92	94
3	Aflatoxin B_2	93	96
4	Aflatoxin B_1	97	95

Conclusions

The multi-aflatoxin method by LC/MS/MS described here was suitable for the determination of four aflatoxins in cereal and corn extract due to its high sensitivity and high selectivity. Another advantage of this method is that ion suppression was not observed for all food samples studied. Thus, it may eliminate the need for matrix-matched standards, which makes analysis more tedious for samples from different origins.

References

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