



APPLICATION NOTE

Liquid Chromatography / Mass Spectrometry

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Analysis of Aflatoxins by UHPLC with MS/MS Detection

Introduction

Aflatoxins are some of the most carcinogenic mycotoxins known and include aflatoxins B1, B2, G1 and G2. They are produced by toxigenic strains of *Aspergillus flavus*, *Aspergillus nominus*, and *Aspergillus parasiticus* fungi after feed, crop or

harvest exposure to moisture or warm temperatures. Aflatoxin B1 is considered to be the most genotoxic of the mycotoxins, and, when ingested by cows, is converted to aflatoxin M1. Though less potent than B1, M1 has been shown to cause liver cancer in certain animals¹.

One of the strictest regulatory levels for any aflatoxin has been set forth by the European Union (EU), having established a stringent control limit of 0.05 ppb for M1 in milk². This limit is currently significantly lower than the Food and Drug Administration's (FDA's) limit of 0.5 ppb¹.

For aflatoxin analysis, HPLC with fluorescence (FL) detection is commonly used along with online post-column derivatization, to enhance B1 and G1 sensitivity. However, chromatographers prefer to avoid any derivatization requirements. With this in mind, the work presented herein demonstrates the effective chromatographic separation of aflatoxins B1, B2, G1, G2 and M1 by UHPLC-MS/MS, achieving the 0.05-ppb control limit for M1 without the need for derivatization.

Experimental

Hardware/Software

For the chromatographic separations, a PerkinElmer UHPLC system was used with a PerkinElmer QSight® 210 MS/MS detector. All instrument control, analysis and data processing was performed using the Simplicity 3Q™ software platform.

Method Parameters

The LC and MS/MS method parameters are shown in Table 1 and Table 2, respectively.

Table 1: LC Method Parameters.

Column	PerkinElmer Brownlee C18, 3 µm, 2.1x100-mm (Part# N9303551)
Mobile Phase:	17:23:60 acetonitrile/methanol/water with 0.1% formic acid and 5-mM ammonium formate
Analysis Time:	4 min
Flow Rate:	0.6 mL/min. (~4300 psi maximum pressure)
Oven Temp.:	35 °C
Injection Volume:	20 µL
Diluent:	Water

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade

A 20-µg/mL (20-ppm) aflatoxin B1, B2, G1 and G2 standard solution in acetonitrile was obtained from Sigma-Aldrich, Inc® (Allentown, PA). A 10-µg/mL aflatoxin M1 standard solution in acetonitrile was also obtained from Sigma-Aldrich. A 10-ppb aflatoxin working standard was then prepared by transferring 50 µL of the B1/B2/G1/G2 stock solution and 100 µL of the M1 stock solution to a 100-mL volumetric flask and diluting to the mark with water. For calibration, aflatoxin concentrations of 1.00, 0.50, 0.25, 0.1 and 0.05 ppb were prepared via serial dilution with water.

Results and Discussion

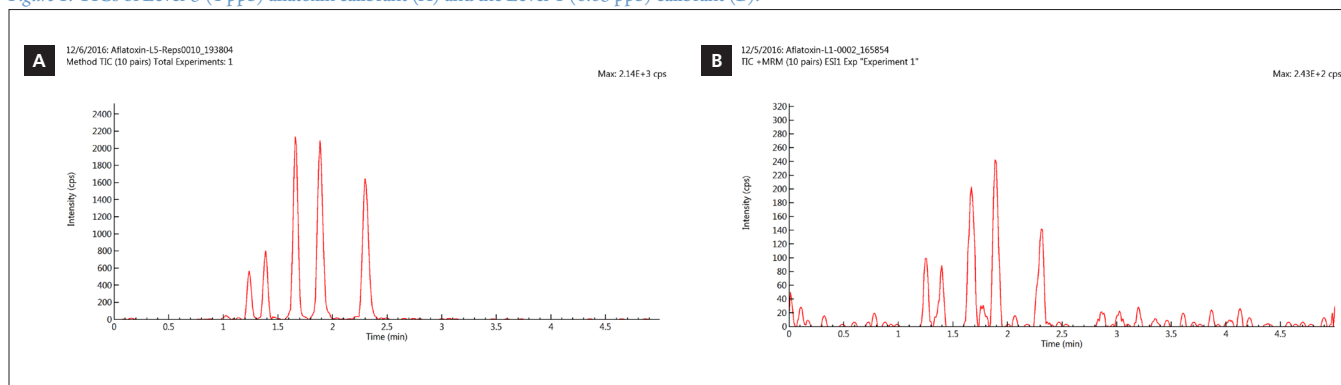
Figure 1 shows the total-ion chromatograms (TICs) of the Level-5 (1 ppb) aflatoxin calibrant (A) and the Level-1 (0.05 ppb) aflatoxin calibrant (B), demonstrating the isocratic separation of all five analytes in under three minutes.

Table 2. MS/MS Parameters.

Ionization mode: ESI, positive						
Drying gas (nitrogen): 75 Lpm; Source temp.: 425 °C; HSID Temp.: 320 °C; Electrospray V1: 5000 V						
MRM Transitions (amu)						
Aflatoxin	Quantifier Ion	Qualifier Ion	EV	CCL2	CE(V)	Dwell Time (msec)
B1 :	313.0/285.0	313.0/213.0	30	-100	-35	50
B2 :	315.0/287.0	315.0/259.0	30	-100	-32	50
G1 :	329.0/243.2	329.0/200.0	25	-100	-38	50
G2 :	331.0/245.0	331.0/257.0	25	-100	-36	50
M1 :	329.0/273.0	329.0/259.0	30	-100	-30	100

EV=Entrance voltage; CCL2=Collision cell lens 2 voltage; CE(V)=Collision energy

Figure 1: TICs of Level-5 (1 ppb) aflatoxin calibrant (A) and the Level-1 (0.05 ppb) calibrant (B).



As shown in Figure 2, chromatographic repeatability was confirmed via eight replicate injections of the Level-5 calibrant, demonstrating exceptional reproducibility. The retention time %RSD for all analytes was $\leq 0.06\%$.

Figure 2. Overlay of eight replicates of the Level-5 (1 ppb) calibrant.

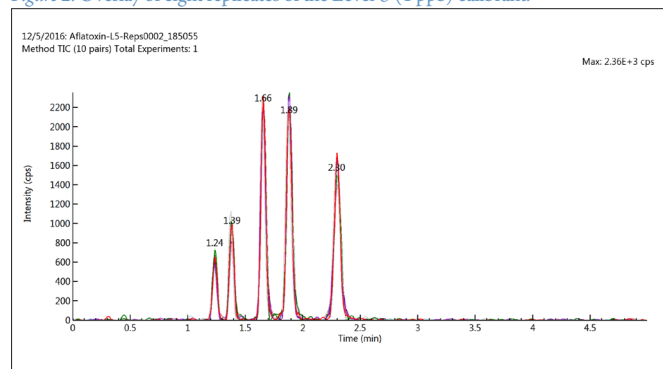
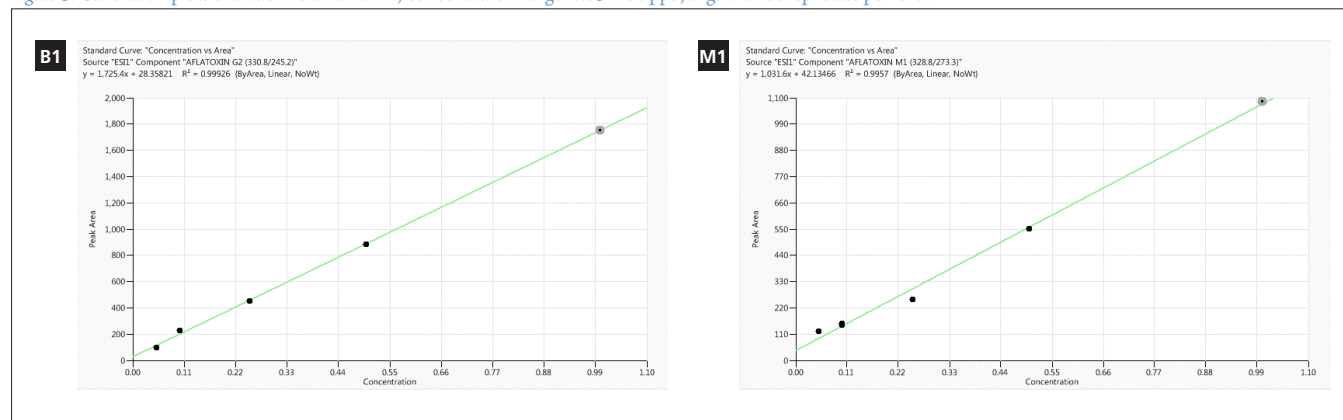


Figure 3 shows example calibration plots for aflatoxins B1 and M1. Calibration linearity was greater than $R^2 = 0.995$ for all analytes.

Based upon a S/N of $>10/1$, the limit of quantitation (LOQ) for aflatoxin M1 was calculated to be 0.014 ppb. This level is far below the EU's regulated control limit of 0.05 ppb.

Figure 3. Calibration plots of aflatoxins B1 and M1; concentration range: 0.05-1.00 ppb; avg. of three replicates per level.



Conclusion

This work demonstrated the effective separation and quantitation of B1, B2, G1, G2 and M1 aflatoxins using a PerkinElmer UHPLC system with Q-Sight MS/MS detector, obviating the need for derivatization. The results exhibited exceptional linearity for each aflatoxin over the tested concentration range. Quantitation was afforded down to below 0.05 ppb for all aflatoxins and the 0.014-ppb LOQ for aflatoxin M1 confirmed that the EU's 0.05-ppb control limit in milk is easily achievable.

References

1. FDA Compliance Policy Guide, under Inspections, Compliance, Enforcement, and Criminal Investigations; CPG Section 527.400 Whole Milk, Lowfat Milk, Skim Milk - Aflatoxin M1
2. Commission Regulation (EU) No 165/2010. Amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs as Regards Aflatoxins. Official Journal of the European Union, Feb 26, 2010, pp L 50/8 – L 50/12.