

Hongxia Li, Rebecca Cornelius, [Andy Stinson](#)

cpd_chem@labstat.com

Abstract

Many species of microorganisms, such as the food-borne mold *Aspergillus flavus*, produce aflatoxins which are acutely toxic. Regulatory reporting criteria for microbial analysis in tobacco products requires more specific and sensitive techniques for the analysis of aflatoxins in tobacco and tobacco products.

In the present study, we developed and validated a sensitive liquid chromatography-tandem mass spectrometry method for the quantitation of aflatoxins (B1, B2, G1, and G2) and ochratoxin A in tobacco, pouches, and, due to non-combustion temperatures, emissions from HTP (heated tobacco products). The samples were extracted with acetonitrile, and then injected on LC-MS/MS after dilution without further sample cleanup. A Poroshell Phenyl-hexyl column and EC-C18 column were used for the separation of the tobacco samples and HTP/pouches samples, respectively. The mobile phases utilized were 5 mM ammonium formate and 0.1% formic acid in water as mobile phase A, and 0.1% formic acid in methanol-acetonitrile mix (90:10) as mobile phase B. The detection was performed in MRM mode using an ESI source in positive mode. The samples were quantified using matrix-matched curves. The method showed good linearity ($R^2 > 0.99$), precision ($CV < 15\%$), and recoveries (70-120%) over the calibration range of 0.02-4 ng/mL for all the target analytes. The recoveries ranged from 75-120% at three fortified levels (low-, medium-, and high-fortification levels). The method quantitation limits for all aflatoxins and ochratoxin A were 2.0 ng/g, 4.0 ng/pouch, and 0.32 ng/cig for tobacco, tobacco-free (i.e., modern oral) pouches and HTP emissions, respectively. This simple, efficient, cost-effective, and sensitive method can be applied in monitoring tobacco and other plant or food matrices.

Methods

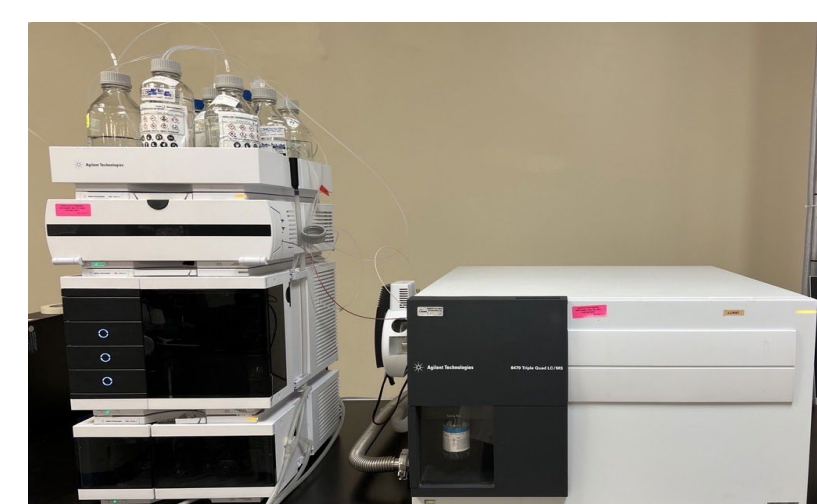
Sample preparation for non-emissions samples:

- Whole tobacco (1R6F filler) samples were weighed out, and whole pouches were cut in half (CPR1.1 and the tobacco-free nicotine pouches) and were both extracted in the acetonitrile.

Sample preparation for emissions

- Smoked 5 HTP cigarettes on an SM-450 smoking machine with a quartz pad and an impinger using intense smoking regime conditions
- Pads were extracted in acetonitrile and cleaned up using a SampliQ C18 SPE cartridge, then analyzed on the LC/MS/MS
- Impinger Solution was diluted with mobile phase A and analyzed on the LC/MS/MS

Instrument Method:



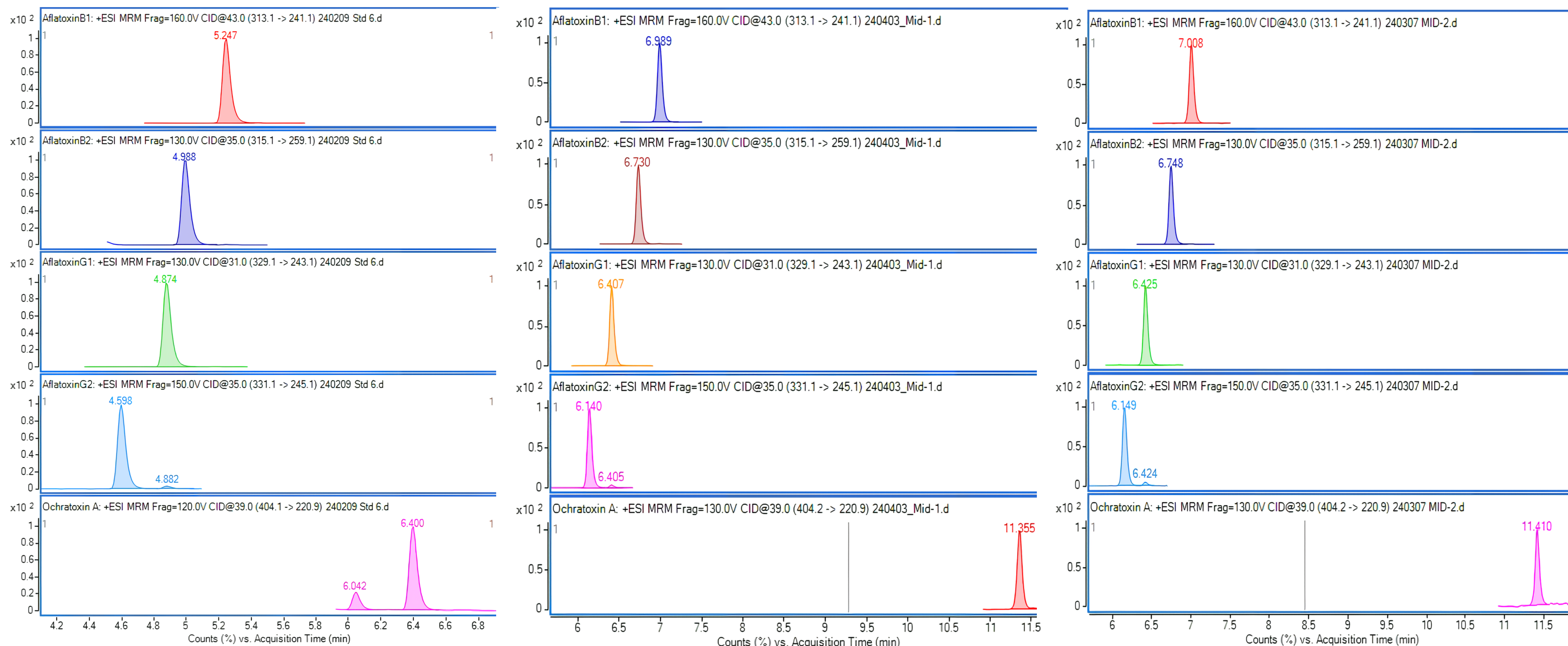
- LC-MS/MS (Agilent 6470 triple quadrupole)
- Column: Poroshell Phenyl-hexyl column (tobacco)
- Column: EC-C18 column (HTP and tobacco-free pouches)
- Mobile phase A: 5 mM ammonium formate and 0.1% formic acid in water
- Mobile phase B: 0.1% formic acid in methanol-acetonitrile mix (90:10)
- Flow rate: 0.5 ml/min
- Column temperature 55°C

Gradient:

Time (min)	Mobile Phase A%	Mobile Phase B%
0.0	70	30
1.00	70	30
4.00	30	70
6.00	20	80
6.50	5.0	95
10	5.0	95
10.5	70	30
14	70	30

- Run Time: 14 minutes
- MS/MS: MRM mode using an ESI source in positive mode
- Matrix-matched curves were used for some matrixes

Chromatograms and Results



Tobacco				
Compounds	LOQ (ng/g)	Results (ng/g)	% Recovery	% Accuracy
Aflatoxin B1	2.0	<LOD	110	5.6
Aflatoxin B2	2.0	<LOD	110	6.0
Aflatoxin G1	2.0	<LOD	108	5.3
Aflatoxin G2	2.0	<LOD	113	5.6
Ochratoxin A	2.0	<LOD	98.3	9.8

Tobacco-free Pouches				
Compounds	LOQ (ng/pouch)	Results (ng/pouch)	% Recovery	% Accuracy
Aflatoxin B1	4.0	<LOD	94.4	4.3
Aflatoxin B2	4.0	<LOD	94.3	3.7
Aflatoxin G1	4.0	<LOD	92.7	5.3
Aflatoxin G2	4.0	<LOD	94.6	4.4
Ochratoxin A	4.0	<LOD	106	11

Heated Tobacco products				
Compounds	LOQ (ng/cig)	Results (ng/cig)	% Recovery	% Accuracy
Aflatoxin B1	0.32	<LOD	100	9.5
Aflatoxin B2	0.32	<LOD	98.2	9.6
Aflatoxin G1	0.32	<LOD	97.8	8.7
Aflatoxin G2	0.32	<LOD	102	8.4
Ochratoxin A	0.32	<LOD	100	5.7

Summary & Conclusions

This is a simple, efficient, cost-effective, and sensitive method that can be applied in the monitoring of tobacco and other plant or food matrices for aflatoxins in accordance with quality and/or regulatory requirements.