Development and validation of LC/MS/MS method for determination of mycotoxins

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INTRODUCTION

Mycotoxins (aflatoxins and ochratoxin A) are secondary toxic metabolites, that contaminate raw materials that are usually used in the preparation of products for human use. Presence of these contaminants in the preparation for human use, can causes various acute and chronic impacts on human health. Carcinogenicity, hepatotoxicity, nephrotoxicity, and endocrine disorders have been related to chronic exposure to low levels of mycotoxins.

MATERIAL AND METHODS:

Chemicals and Regents
Liquid standards of aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2 and ochratoxin A, as well as Immuno-affinity columns were supplied by R-biopharm (Germany).

Apparatus: Liquid chromatography was performed on LC/MS/MS system from Shimadzu.

Chromatographic Conditions and analyte transitions are given in Table 1 and Table 2

Column	Raptor Biphenyl 100 mm x 2.1 mm, particle size 2.7 µm, (Cat.No.980-18088)		
Guard Column	Raptor Biphenyl EXP Guard Column Cartridge 2.7 µm, 5 x 2.1 mm (cat.# 9309A0252)		
Mobile phase A	5 mM ammonium formate in water with 0,1% formic acid		
Mobile phase B	5 mM ammonium formate in methanol with 0,1% formic acid		
Time Program	Time (min.)	Flow (mL/min.)	%B
	2.20	0.45	30
	2.40	0.45	50
	8.20	0.45	70
	11.20	0.45	75
	12.20	0.45	90
	12.60	0.45	90
	12.61	0.45	75
	13.20	0.45	75
	13.21	0.45	30
	16	0.45	30
Oven Temp.	40oC		
Sample Temp.	15oC		
Inj. Volume	10 µL		
MS/MS	Shimadzu LCMS-8045		
Ion Mode	ESI+		

Table 1. Chromatographic conditions

Analyte	Precursor Ion	Product Ion Quantifier	Product Ion Qualifier
AfG2	331.0	189.2	313.2
AfG1	329.0	200.2	243.2
AfB2	315.1	287.2	243.2
AfB1	312.9	285.2	241.2
OchA	404.1	239.1	358.2

Table 2. Analyte transition

RESULTS:

Validation of the method
The calibration characteristics and validation parameters are shown in Table 3. Linearity of response was calculated as a ratio of peak areas of Aflatoxins B1, B2, G1, G2 and Ochratoxin A in standard solution vs. concentration in spiked samples in the concentration range of 0.1 – 5µg/L for Aflatoxin B1, B2, G1 and G2 and for Ochratoxin A from 1 – 50µg/L. Coefficient of correlation was greater than 0.999 for all mycotoxins.

Results from the limit of detection/limit of quantification for mycotoxins and precision and accuracy of the method are shown in Table 4 and Table 5.

	AfG2	AfG1	AfB2	AfB1	OchA
Linearity range	0.1 – 5 (µg/L)				1 – 50 (µg/L)
Determ. coef (r2)	0.999	0.999	0.999	0.999	0.999

Table 3. Characteristics of the linear regression analysis

Mycotoxin	Limit of detection (µg/kg)	Limit of quantification
AflG2	0,023	0,069
AflG1	0,017	0,053
AflB2	0,034	0,105
AflB1	0,027	0,082
OchA	0,329	0,997

Table 4. LOD / LOQ of mycotoxins

Concentration added	Measured concentration (µg/L)[a]	
AfG2		Recovery
1.5 (µg/L)	1.215 ± 0.96%	81.0%
2.0 (µg/L)	1.892 ± 0.82%	94.6%
5.0 (µg/L)	4.321 ± 0.78%	86.42%
AfB2		Recovery
1.5 (µg/L)	1.228 ± 0.87%	81.86%
2.0 (µg/L)	1.927 ± 0.58%	96.35%
5.0 (µg/L)	4.283 ± 0.72%	85.66%
AfG1		Recovery
1.5 (µg/L)	1.214± 0.58%	80.9%
2.0 (µg/L)	1.940± 0.82%	97.0%
5.0 (µg/L)	4.696± 0.85%	93.9%
AfB1		Recovery
1.5 (µg/L)	1.334± 0.71%	88.93%
2.0 (µg/L)	1.938± 0.49%	96.9%
5.0 (µg/L)	4.900± 0.57%	98.0%
OchA		Recovery
15 (µg/L)	14.09± 0.86%	93.93%
20 (µg/L)	20.93± 0.93%	104.6%
50 (µg/L)	50.59± 1.03%	101.18%

Table 5. Precision and accuracy of the method

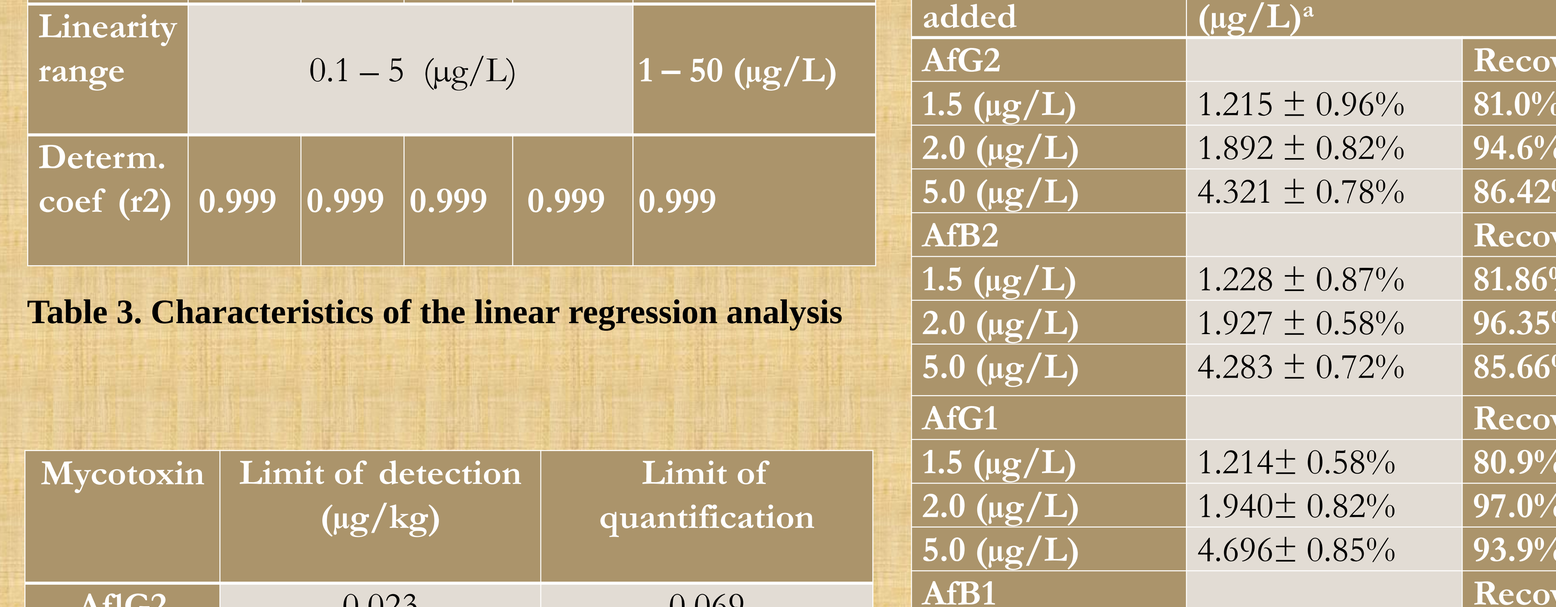

Figure 1. Typical chromatograms of aflatoxins

CONCLUSION A novel LC/MS/MS method was developed and validated for determination of aflatoxins and ochratoxin A in cannabis flowers and extracts