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

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INTRODUCTION

Medicinal products based on Cannabis sativa in traditional medicine, have been used for thousands of years in the treatment of different diseases.[1] Although, there is a lack of evidence-based medical information that can prove potential benefit of the therapy with medicinal cannabis preparations, recently, an increasing number of pharmacists had to supply cannabis preparations to individual patients prescribed by their physicians.[2]

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.[3] Presence of these contaminants in herbal drugs, used in the preparation of products for human use, can causes various acute and chronic impacts on human health.[4] Carcinogenicity, hepatotoxicity, nephrotoxicity, and endocrine disorders have been related to chronic exposure to low levels of mycotoxins.[5] Therefore, the existence of an analytical method with which the concentration of these metabolites (impurities) can be monitored is very important.

MATERIAL AND METHODS

Chemicals and Regents

Liquid standards of aflatoxin B1 (AfB1), Cat.No.TSL-104-10, aflatoxin B2 (AfB2), Cat.No.TSL-105-10, aflatoxin G1 (AfG1), Cat.No.TSL-106-10, aflatoxin G2 (AfG2), Cat.No.TSL-107-10 and ochratoxin A (OchA), Cat.No.TSL-504-5 were supplied by R-biopharm (Germany). Other chemicals and reagents used in this work were LC/MS grade provided from Fisher Chemicals (UK).

Immuno-affinity columns were obtained from R-biopharm (Germany), Cat.No.RBRP112B.

Apparatus

Liquid chromatography was performed on LC/MS/MS system (LC - 30AD series) equipped with MS/MS detector (8045 series) from Shimadzu.

Chromatographic Conditions

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

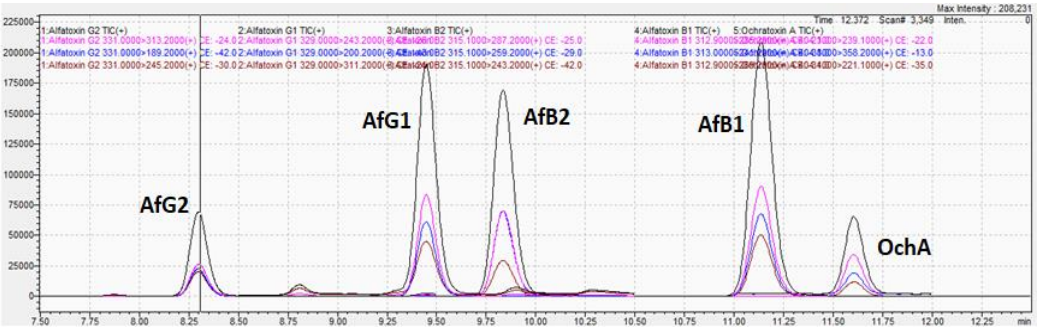

Figure 1. Typical chromatograms of the AfG2, AfG1, AfB2, AfB1 and OchA

RESULTS

Validation of the method

The calibration characteristics and validation parameters of the proposed method are shown in Table 3. Linearity of response was calculated as a ratio of peak areas of Aflatoxines 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.

	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
CCα* (%)	4.32	3.84	4.55	3.95	3.87

*CCα – Decision limit (max. allowed 5%)

Table 3. Characteristics of the linear regression analysis

Figure 1 shoes typical chromatograms of the AfG2, AfG1, AfB2, AfB1 and OchA.

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.

Mycotoxin	**Limit of detection** (µg/kg)	**Limit of quantification** (µg/kg)
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. Limit of detection / Limit of quantification of mycotoxins

Concentration added	Measured concentration (µg/L)[a]	
AfG2	Mean (µg/L)± RSD	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	Mean (µg/L)± RSD	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	Mean (µg/L)± RSD	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	Mean (µg/L)± RSD	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	Mean (µg/L)± RSD	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

CONCLUSION

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

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