APPLICATION NOTE 65969

Quantification of 48 myco- and phytotoxins in cereal using Liquid Chromatography-Triple Quadrupole mass spectrometry

Author: Sanja Hofmann, Olaf Scheibner, Thermo Fisher Scientific, Dreieich, Germany

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Goals

To demonstrate a fast, easy and cost-effective approach for the determination of 48 myco- and phytotoxins in grain-based foods using a spiked matrix approach, using a Thermo Scientific™ Hypersil GOLD™ HPLC column, a Thermo Scientific™ UltiMate™ 3000 RS UHPLC system and a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer. The compounds are either legislated by the EU or of current interest.

Introduction

Mycotoxins are potentially toxic secondary metabolites produced by different species of fungi and therefore can occur in cereal, fruits, spices, and many other foods. Estimations are given that approximately 25% of all crops are contaminated with mycotoxins[1]. Many agencies throughout the world define regulatory limits for the most hazardous mycotoxins. With 11 regulated mycotoxins, the European Food Safety Authority (EFSA) has defined one of the most comprehensive panels and the lowest Maximum Residue Levels (MRLs). Exceptions are in China with zearalenone in wheat, corn and their products and Korea with deoxynivalenol in cereals.

Tropane alkaloids are a class of bicyclic [3.2.1] alkaloids. They are anticholinergic drugs and occur in henbane, thorne apple and atropa belladonna which are found in grain fields. The MRLs for this list of toxins have been specified in the EU regulations[2-6]. Compounds like ergot alkaloids, enniatins, citrinin and some others are either already regulated for feed or under discussion, so it is ideal to have a single method that is able to analyze all these compounds in one run. This technical note comprises 48 mycotoxins that are either regulated or under discussion for future regulation in the EU[7].

A method based on the use of a UltiMate 3000 RSLC UHPLC system coupled to a TSQ Quantis mass spectrometer was developed for the analysis of 48 myco- and phytotoxins in cereals. Sample preparation follows a fast, easy and cost-effective approach to securely capture all the target components. Extraction of cereals with a mixture of acetonitrile/water [von Bargen et al.] is followed by evaporation and sample reconstitution before analysis.

Experimental

Target analytes

A panel of 48 myco- and phytotoxins were optimized and their transitions, RT, CE and RFLens values are listed in Table 1.

Calibration standards

Calibration solutions were prepared by spiking matrix extracts with a mixture of standards. The individual standards were dried down and reconstituted in methanol/water (50/50, v/v). Thirteen calibration levels were used with a wide range from lowest at 0.004 to the highest at 4,000 ug/kg (Table 2). All standards were purchased from Romer Labs (Deutschland GmbH, Butzbach, Germany) with the exception of the four enniatins, scopolamin and sulochrin which were ordered from Merck/Sigma Aldrich, (Steinheim, Germany); ergocyptine from LGC Standards (Middlesex, UK), altenusin and phomopsin A from Biomol (Hamburg, Germany).

Table 1. SRM-transitions for the different analytes

Compound	RT [min]	Ion species	Precursor *m/z*	Quantifier *m/z*	Collision energy	Qualifier 1 *m/z*	Collision energy	Qualifier 2 *m/z*	Collision energy	RF lens [V]
3+15-Acetyl-Deoxynivalenol	5.56	[M+H]+	339.09	230.98	12.16	261.13	10.57	137.00	11.44	150
Aflatoxin B1	6.67	[M+H]+	313.06	285.07	23.08	241.00	37.22	270.03	27.40	243
Aflatoxin B2	6.42	[M+H]+	315.06	287.07	26.07	243.01	39.68	271.08	32.22	224
Aflatoxin G1	6.20	[M+H]+	329.01	243.07	26.98	200.04	41.31	215.07	32.97	245
Aflatoxin G2	5.92	[M+H]+	331.05	313.04	24.33	189.04	42.30	285.01	27.93	233
Aflatoxin M1	5.96	[M+H]+	329.06	273.07	25.20	228.97	40.97	259.07	24.97	177
Agroclavine	5.26	[M+H]+	239.10	183.05	19.44	208.10	18.87	198.01	18.87	148
alpha-Zearalenol	8.43	[M-H]-	319.00	275.14	20.96	173.96	27.02	159.93	30.89	249
Altenuene	6.76	[M+H]+	293.08	257.08	14.55	275.05	10.23	239.05	21.18	146
Altenusin	7.09	[M-H]-	289.00	245.00	16.71	230.00	21.41			176
Alternariol	7.53	[M-H]-	257.05	213.00	22.85	214.97	25.35	146.99	32.63	225
Alternariol-methylether	8.88	[M-H]-	271.05	255.99	22.25	228.04	29.14	212.95	36.84	211
Atropin	4.68	[M+H]+	290.20	124.08	23.99	93.00	31.50	91.00	40.33	178
Beauvericin	9.84	[M+H]+	784.36	134.00	53.82	244.05	28.96	262.08	26.64	299
Citrinin	7.26	[M+H]+	251.10	233.00	18.26	205.00	30.17	202.96	32.67	141
Deoxynivalenol	3.89	[M+H]+	297.04	249.07	10.23	231.07	13.53	175.04	19.86	146
Diacetoxyscripenol	6.94	[M+H]+	367.09	307.07	10.23	349.05	10.23	229.05	12.73	135
Enniatin A	10.04	[M+H]+	682.48	210.08	27.21	100.07	51.89	228.26	28.69	299
Enniatin A1	9.96	[M+H]+	668.40	210.08	26.49	196.08	25.54	228.19	27.14	299
Enniatin B	9.74	[M+H]+	640.40	196.07	26.68	214.14	26.42	314.21	35.70	299
Enniatin B1	9.85	[M+H]+	654.40	196.01	26.68	210.15	25.51	314.15	35.86	299
Ergocornine	6.78	[M+H]+	562.30	268.14	25.20	208.07	42.91	223.08	36.23	278
Ergocorninine	7.15	[M+H]+	562.30	544.25	15.76	305.05	27.67	277.14	28.16	218
Ergocristine	7.21	[M+H]+	610.30	268.16	26.23	208.08	43.66	348.10	25.62	299
Ergocristinine	7.40	[M+H]+	610.30	592.29	14.89	305.05	28.88	325.08	27.25	227
Ergocryptine	7.20	[M+H]+	576.30	268.15	25.77	208.07	44.23	223.08	36.84	299
Ergocryptinine	7.38	[M-H2O+H]+	558.31	348.14	19.82	291.08	22.97	305.00	24.18	299
Ergosine	6.62	[M+H]+	548.30	208.07	40.82	223.08	33.62	268.16	24.52	233
Ergosinine	6.48	[M-H2O+H]+	530.30	223.15	28.73	263.15	27.67	277.05	22.36	299

Compound	RT [min]	Ion species	Parent ion	Quantifier	Collision energy	Qualifier 1	Collision energy	Qualifier 2	Collision energy	RF lens [V]
Fumagillin	9.22	[M+H]+	459.20	131.00	29.75	177.02	15.19	215.05	13.60	190
Fumonisin B1	7.95	[M+H]+	722.50	334.01	42.72	352.50	37.98	704.39	29.90	239
Fumonisin B2	8.73	[M+H]+	706.30	336.22	38.89	688.45	27.63	354.15	34.64	221
Fumonisin B3	8.39	[M+H]+	706.39	336.22	38.93	688.32	29.22	354.30	33.01	299
Fusarenon X	4.68	[M+H]+	355.09	229.07	16.56	175.00	22.09	277.00	12.39	136
HT-2-toxin	7.79	[M+Na]+	447.20	345.08	19.25	285.07	20.84	255.13	23.04	230
Moniliformin	0.87	[M-H]-	96.85	41.03	13.18					76
Nivalenol	3.09	[M+HCOO]-	357.04	281.16	10.23	311.13	10.23	45.00	13.15	126
Ochratoxin A	8.64	[M+H]+	404.00	238.97	24.18	358.07	14.78	220.97	14.78	184
Patulin	5.26	[M+H]+	155.00	128.00	26.60	101.00	31.84	74.97	45.71	241
Phomopsin A	5.82	[M+H]+	789.20	226.00	40.86	323.00	27.10	452.08	19.97	299
Scopolamin	3.93	[M+H]+	304.10	138.07	21.26	156.13	16.52	102.93	37.49	151
Sterigmatocystin	8.74	[M+H]+	325.10	310.00	24.71	281.00	38.09	253.06	44.95	224
Sulochrin	6.71	[M+H]+	333.20	209.07	10.23	151.04	22.28	135.93	43.74	112
T-2 toxin	8.27	[M+Na]+	489.21	245.13	28.05	327.04	24.10	387.05	22.36	265
Tentoxin	7.95	[M-H]-	413.18	271.16	16.10	141.00	20.05	369.24	18.49	226
Wortmannin	6.98	[M+H]+	429.05	355.13	10.23	313.00	14.40	295.00	17.39	122
Zearalenone	8.60	[M-H]-	317.09	175.00	24.03	131.07	29.30	273.16	19.21	232

Table 2.

Compound	Concentration Level [µg/kg]												
3+15-Acetyl-Deoxynivalenol	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Aflatoxin B1	0.016	0.032	0.08	0.16	0.32	0.8	1.6	3.2	8	16	32	80	160
Aflatoxin B2	0.005	0.01	0.025	0.05	0.1	0.25	0.5	1	2.5	5	10	25	50
Aflatoxin G1	0.014	0.029	0.072	0.143	0.286	0.715	1.43	2.86	7.15	14.3	28.6	71.5	143
Aflatoxin G2	0.004	0.009	0.022	0.044	0.088	0.22	0.44	0.88	2.2	4.4	8.8	22	44
Aflatoxin M1	0.02	0.04	0.1	0.2	0.4	1	2	4	10	20	40	100	200
Agroclavine	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
alpha-Zearalenol	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Altenuene	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Altenusin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Alternariol	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Alternariolmethylether	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Atropin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Beauvericin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Citrinin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Deoxynivalenol	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Deoxynivalenol-3-glucoside	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Diacetoxyscirpenol	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Enniatin A	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Enniatin A1	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Enniatin B	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Enniatin B1	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Ergocornine	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Ergocorninine	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Ergocristine	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Ergocristinine	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Ergocryptine	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000

Ergocryptinine	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Ergosine	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Ergosinine	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Fumagillin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Fumonisin B1	0.4	0.8	2	4	8	20	40	80	200	400	800	2000	4000
Fumonisin B2	0.4	0.8	2	4	8	20	40	80	200	400	800	2000	4000
Fumonisin B3	0.4	0.8	2	4	8	20	40	80	200	400	800	2000	4000
Fusarenon X	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
HT-2 Toxin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Moniliformin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Nivalenol	0.2	0.4	1	2	4	10	20	40	100	200	400	1000	2000
Ochratoxin A	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Patulin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Phomopsin A	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Scopolamin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Sterigmatocysin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Sulochrin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
T2-Toxin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Tentoxin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Wortmannin	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000
Zearalenone	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500	1000

Sample preparation

Samples of polenta (maize) and wheat flour (5 g) were weighed into separate 50 mL extraction tubes. The addition of 20 mL acetonitrile/water (80/20, v/v) was followed by horizontal shaking at 100 rpm for 60 min and centrifugation at 2500 rpm for 5 min at room temperature. Supernatant was filtered through 0.45 µm PTFE disposable syringe filter. A 200 µL aliquot of the filtered supernatant was spiked with a mix of mycotoxins from Table 1[8] and then transferred into a HPLC vial and dried down with nitrogen to complete dryness. The sample was reconstituted with 1000µL methanol/water (50/50, v/v) for LC-MS/MS analysis.

Liquid chromatography

A chromatographic method of 15 minutes was used for the analysis of the mycotoxins implemented on a UltiMate 3000 RSLC UHPLC system consisting of a binary pump, a column oven and an autosampler. Chromatographic conditions are described in Table 3.

Table 3. LC-conditions and gradient

LC Conditions	
Injection volume	10 µL
Column temperature	40°C
Flow rate	300 uL/min
Analytical column	Thermo Scientific™ Hypersil GOLD™ C18 column, 100mm x 2.1 mm, 1.9 µm
Run time	15 min
Tray temperature	10°C
Wash needle solvent	50% methanol in water
Sample loop size	100 µL
Mobile phases	A: Water + 0.1% FA B: Methanol + 0.1% FA
LC gradient	0 min 5% B 0.5 min 5% B 7 min 70% B 9 min 100% B 12 min 100% B 12.1 min 5% B 15.0 min 5% B

Mass spectrometry

Compounds were analyzed on a TSQ Quantis triple quadrupole mass spectrometer equipped with a Thermo Scientific™ OptaMax NG™ source and a HESI probe. Data were acquired in timed-SRM mode. All SRM transitions for precursor, qualifier and quantifier ions were individually optimized for each target analyte by direct infusion of a working standard solution (100 ng/mL) using syringe pump infusion. MS conditions were set as in Table 4.

Table 4. MS settings

MS settings	
Ionization mode	Heated electrospray (HESI)
Scan type	Timed-SRM
Polarity	Positive/negative switching
Spray voltage in positive mode	3500 V
Spray voltage in negative mode	2500 V
Sheath gas	30 arbitrary units (Arb)
Auxillary gas	6 Arb
Sweep gas	1 Arb
Ion transfer tube temperature	325 °C
Vaporizer temperature	350 °C
CID gas pressure	1.5 mTorr
Cycle time	0.5 s
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7
Chromatographic peak width (s)	10
Chrom filter	Yes

Method evaluation

Linearity was evaluated by collecting calibration curve data (n=5) on each day of analysis and obtained by plotting the peak area against the concentrations of the calibration standards. Calibration was performed in linear mode with a 1/X weighting. The matrix effect was evaluated by comparing spiked solvent standards with the spiked matrix-matched calibrations. Within batch precision was obtained in terms of percentage coefficient of variation (%CV) and accuracy as the bias of the result expressed in percentage for the 13 spiked levels with 5 replicates prepared and analyzed in one batch.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software.

Results and discussion

The calibration curves were obtained by external calibration for concentrations from the LOQ up to a concentration of 1000-4000 µg/kg (except for aflatoxins) (Table 5). The LOQ was defined as the lowest concentration in the studied concentration range for which percentage coefficient of variation (%CV) was equal to or less than 20% and percentage difference between calculated amount and the specified amount (%Diff) was less than ±30% for all 5 replicates. LOD was defined as a signal-to-noise ratio greater than 3. Table 6 presents these results for each compound. Representative chromatograms at the LOQ are reported in Figure 1.

The removal of acetonitrile from the sample (as described in the Sample Preparation) prior to injection into the LC-MS system is a crucial step to obtain a low percentage coefficient of variation (%CV).

The averaged matrix effect based on the comparison of the signal obtained from matrix-matched standards to standards at the same concentration was between 43% and 222% dependent on the individual compounds. The results are corrected for the precision and accuracy assays within the concentration ranges tested in this work. Intra-assay and inter-assay accuracy in %bias and precision in %CV were found to be below 20%. The complete results of this evaluation are found in Table 5.

For legislated mycotoxins (FBs, DON, ZEN), all LOQs were lower than the Maximum Residue Limits (MRLs) established by the European Union (Commission Regulation (EC) N 401/2006) and worldwide.

Table 5. Method performance: LODs and LOQs (µg/kg) in solvent, wheat and maize matrix compared to MRL from EU legislation.

	LOD/LOQ			Maximum residue limit defined by EC 1881/2006	
Analyte	Solvent (µg/kg)	Maize (µg/kg)	Flour (µg/kg)	Maize [µg/kg]	Cereals [µg/kg]
3+15-Acetyl-Deoxynivalenol	4/10	1/4	1/10	no limit	no limit
Aflatoxin B1	0.16/0.8	0.32/0.8	0.16/1.6	2	2
Aflatoxin B2	0.1/0.25	0.1/1.0	0.1/2.5	4 (sum of 4)	4 (sum of 4)
Aflatoxin G1	0.072/0.286	0.286/1.43	0.286/1.43	4 (sum of 4)	4 (sum of 4)
Aflatoxin G2	0.22/0.44	0.88/2.2	0.44/0.88	4 (sum of 4)	4 (sum of 4)
Aflatoxin M1	0.1/0.2	0.2/1	0.1/2	no limit	no limit
Agroclavine	0.1*/0.2	0.1*/0.5	0.1*/0.5	no limit	no limit
alpha-Zearalenol	50/200	50/100	10/50	no limit	no limit
Altenuene	2/5	2/5	1/5	no limit	no limit
Altenusin	5/5	5/10	5/20	no limit	no limit
Alternariol	5/20	5/10	1/50	no limit	no limit
Alternariol-methylether	1/5	0.2/1	0.2/1	no limit	no limit
Atropin	0.1*/0.5	0.1*/0.2	0.1*/0.2	1	1
Beauvericin	0.1*/0.1*	0.1/0.5	0.1*/0.2	no limit	no limit
Citrinin	0.1*/0.5	0.1*/0.1	0.1*/0.5	no limit	no limit
Deoxynivalenol	40/40	4/40	10/40	no limit	no limit
Diacetoxyscripenol	10/20	10/20	10/20	no limit	no limit
Enniatin A	0.1*/0.2	0.1*/0.5	0.1*/20	no limit	no limit
Enniatin A1	0.1*/0.2	0.1*/0.5	0.1*/20	no limit	no limit
Enniatin B	0.1*/0.2	0.1*/0.5	0.1*/20	no limit	no limit
Enniatin B1	0.1*/0.2	0.1*/0.5	0.1*/20	no limit	no limit
Ergocornine	0.2*/0.4	0.2*/1	0.2*/20	no limit	100 (sum)
Ergocorninine	0.2*/0.4	0.2*/0.4	0.2*/100	no limit	100 (sum)
Ergocristine	0.2*/1	0.2*/1	0.2*/100	no limit	100 (sum)
Ergocristinine	0.2*/0.4	0.2*/1	0.2*/100	no limit	100 (sum)
Ergocryptine	0.2*/1	0.2*/1	0.2*/100	no limit	100 (sum)
Ergocryptinine	0.4/2	0.2*/2	0.2*/40	no limit	100 (sum)
Ergosine	0.2*/0.4	0.2*/0.4	0.2*/40	no limit	100 (sum)
Ergosinine	1/4	0.4/1	0.2*/40	no limit	100 (sum)
Fumagillin	2/5	10/10	10/10	no limit	no limit
Fumonisin B1	0.4*/40	0.4*/2	0.4*/4	800 (sum)	1000 (sum)
Fumonisin B2	0.4*/40	0.4*/2	0.4*/8	800 (sum)	1000 (sum)
Fumonisin B3	0.4*/8	0.4*/2	0.4*/80	no limit	no limit
Fusarenon X	10/20	10/20	4/10	no limit	no limit
HT-2-toxin	5/5	2/5	0.1/2	no limit	50 (sum + T-2)
Moniliformin	0.1*/1	0.5/10	0.1*/5	no limit	no limit
Nivalenol	4/10	4/10	4/100	no limit	no limit
Ochratoxin A	0.2/0.2	0.1*/0.5	0.1*/0.5	3	3
Patulin	50/50	50/100	20/50	no limit	no limit
Phomopsin A	1/2	0.5/1	0.2/0.5	no limit	no limit
Scopolamin	0.1*/0.2	0.1*/0.2	0.1*/0.2	1	1
Sterigmatocystin	0.1*/0.5	0.1*/0.1	0.1*/0.5	no limit	no limit
Sulochrin	0.2/0.5	0.1*/0.5	0.1*/1	no limit	no limit
T-2 toxin	0.1/1	0.1*/0.5	0.2/1	no limit	50 (sum + HT-2)
Tentoxin	1/10	1/5	0.5/20	no limit	no limit
Wortmannin	1/5	2/10	1/100	no limit	no limit
Zearalenone	2/20	0.2/20	0.1/20	100	50

* LOD is the lowest concentration that was measured

Table 6: Linear range, correlation coefficient and matrix effect

	Solvent				Wheat					Maize				
	Linear range [µg/kg]	R^2	%RSD @ LOD	%CV @ LOD	Linear range [µg/kg]	R^2	%RSD @ LOD	%CV @ LOD	ME%	Linear range [µg/kg]	R^2	%RSD @ LOD	%CV @ LOD	ME%
3+15-Acetyl-Deoxynivalenol	10-2000	0.9985	8.25	8.93	10-2000	0.9949	9.6	6.01	201	10-1000	0.9964	10.89	6.59	170
Aflatoxin B1	0.8-160	0.999	11.68	12.18	1.6-80	0.9944	12.66	10.85	58	0.8-160	0.993	7.39	4.11	48
Aflatoxin B2	0.25-50	0.9939	10.45	9.84	2.5-50	0.9914	10.71	7.89	62	1-50	0.994	15.47	8.7	43
Aflatoxin G1	0.286-144	0.9989	12.99	16.72	1.43-140	0.9918	8.5	7.41	58	1.43-50	0.9976	9.69	7.57	49
Aflatoxin G2	0.44-44	0.9977	11.23	9.65	0.88-44	0.9952	7.76	5.36	60	2.2-44	0.9947	9.3	5.08	56
Aflatoxin M1	0.2-200	0.9987	10.23	8.83	2-200	0.9987	6.89	4.68	92	1-200	0.9967	10.31	6.4	95
Agroclavine	0.2-1000	0.999	8.14	9.88	0.5-1000	0.9952	6.57	5.23	118	0.5-1000	0.9985	6.16	4.69	75
alpha-Zearalenol	50-1000	0.9846	16.54	11.96	50-1000	0.99	5.4	3.93	152	100-1000	0.9886	10.86	7.74	71
Altenuene	5-1000	0.9981	4.81	5.03	5-500	0.9939	14.85	10.78	161	5-1000	0.997	5.57	4.26	118
Altenusin	5-500	0.9887	13.02	9.8	20-1000	0.9972	11.67	10.15	86	10-1000	0.9966	8.64	6.67	86
Alternariol	20-1000	0.9937	3.42	3.31	10-500	0.9924	12.02	11.49	199	10-500	0.9945	16.92	12.09	128
Alternariol-methylether	5-1000	0.998	8.54	9.23	1-1000	0.9942	10.12	5.9	161	1-500	0.9972	12.08	7.45	132
Atropin	0.5-1000	0.997	1.6	5.58	0.2-1000	0.9976	2.58	1.81	147	0.2-1000	0.9982	5.36	4.26	125
Beauvericin	0.1*-1000	0.9991	1.96	1.75	0.2-500	0.9964	11.39	5.6	77	0.5-500	0.9969	12.21	2.12	66
Citrinin	0.5-1000	0.9858	6.73	6.34	0.2-200	0.9953	8.56	5.26	143	0.5-200	0.9986	2.61	2.26	90
Deoxynivalenol	40-2000	0.9973	2.74	2.9	40-2000	0.9939	10.67	4.17	53	40-2000	0.9896	5.12	1.66	48
Diacetoxyscripenol	20-2000	0.9975	11.38	8.95	20-2000	0.9916	8.74	4.93	129	20-1000	0.9924	9.78	8.74	106
Enniatin A	0.2-1000	0.9997	2.94	3.04	0.2-1000	0.9989	9.53	3.59	87	0.5-1000	0.9975	6.85	4.33	80
Enniatin A1	0.2-1000	0.9993	5.94	7.34	0.5-500	0.9993	13.06	2.24	88	0.5-1000	0.9983	2.88	2.01	80
Enniatin B	0.2-1000	0.9995	7.11	9.67	0.5-500	0.9986	19.54	1.29	85	0.5-1000	0.9977	5.85	2.03	79
Enniatin B1	0.2-1000	0.9994	5.03	6.19	1-500	0.9984	18.09	3	87	0.5-1000	0.998	9.89	4.97	83
Ergocornine	0.4-2000	0.9988	7.96	11.58	1-2000	0.9996	18.78	15.93	103	1-2000	0.9991	4.95	4.23	151
Ergocorninine	0.4-1000	0.9929	14.14	15.86	1-1000	0.9907	6.19	4.73	232	0.4-400	0.9965	8.55	6.09	123
Ergocristine	1-1000	0.9971	12.06	12.99	1-1000	0.9913	12.96	8.96	107	1-400	0.9971	11.91	9.52	139
Ergocristinine	1-1000	0.995	9.38	8.6	1-1000	0.9891	5.1	3.43	81	1-1000	0.9963	7.48	6.57	99
Ergocryptine	1-1000	0.9959	9.07	9.98	1-400	0.9879	19.72	15.06	109	1-1000	0.9917	9.84	7.85	135
Ergocryptinine	2-1000	0.9969	6.72	8.3	2-1000	0.9935	5.72	4.2	209	2-1000	0.9969	5.16	4.73	118
Ergosine	0.4-2000	0.998	5.76	6.24	0.4-400	0.9932	13.57	8.354	144	0.4-1000	0.9982	4.68	2.78	139
Ergosinine	4-400	0.997	8.82	8.74	2-400	0.997	12.68	9.58	140	2-2000	0.9956	10.82	9.66	154

Fumagillin	5-1000	0.9992	9.53	8.81	5-200	0.995	14.99	8.77	70	10-500	0.9982	10.61	8.46	57
Fumonisin B1	40-2000	0.9944	1.53	2.86	2-4000	0.9999	8.77	5.15	196	2-2000	0.9994	11.24	2.36	213
Fumonisin B2	40-4000	0.99156	4.86	6.83	4-4000	0.9984	7.08	4.27	163	2-4000	0.9994	2.56	0.84	179
Fumonisin B3	4-4000	0.9964	4.47	4.7	4-4000	0.9957	9.45	5.68	163	2-4000	0.9994	13.6	4.86	167
Fusarenon X	20-2000	0.9985	10.33	11.01	20-2000	0.9945	9.52	7.17	144	20-2000	0.9961	13.44	9.07	109
HT-2-toxin	5-1000	0.9982	13.95	13.76	2-500	0.9937	12.5	8.49	258	5-500	0.9966	12.6	6.25	134
Moniliformin	2-1000	0.9976	11.19	8.65	5-1000	0.995	6.31	2.91	96	10-1000	0.9954	1.95	0.85	88
Nivalenol	10-2000	0.9965	12.22	13.92	10-2000	0.9911	8.53	6.4	159	10-200	0.9934	12.87	6.4	139
Ochratoxin A	0.5-1000	0.9981	12.05	13.27	1-1000	0.9966	7.43	5.71	148	0.5-1000	0.9987	8.36	7.03	134
Patulin	50-1000	0.9972	13.22	6.06	50-1000	0.9927	9.94	6.23	113	100-1000	0.9856	11.04	9.63	80
Phomopsin A	2-1000	0.9984	15.53	19.05	0.5-1000	0.9983	9.73	8.03	223	1-1000	0.9989	5.43	4.74	222
Scopolamin	0.2-1000	0.9994	5.42	6.47	0.2-1000	0.9986	10.57	6.64	138	0.2-1000	0.999	4.36	3.28	134
Sterigmatocystin	0.5-1000	0.9982	9.17	9.46	0.5-500	0.9875	8.01	6.16	124	0.2-200	0.9948	8.71	6.72	81
Sulochrin	0.5-1000	0.998	14.08	11048	1-1000	0.9912	13.45	11.36	118	0.5-500	0.9953	9.25	7.1	78
T-2 toxin	1-1000	0.9986	17.69	16.96	1-200	0.9984	10.38	7.17	245	0.5-200	0.9967	14.47	9.32	142
Tentoxin	10-500	0.9987	10.95	10.15	20-500	0.9935	10.28	10.18	145	5-200	0.992	10.29	8.41	117
Wortmannin	5-500	0.9821	13.16	14.46	10-500	0.9842	16.34	11.68	99	10-200	0.9843	5.73	5.31	48
Zearalenone	20-1000	0.9971	13.81	15.41	20-1000	0.9948	11.66	11.31	142	20-1000	0.9954	14.27	11.46	96

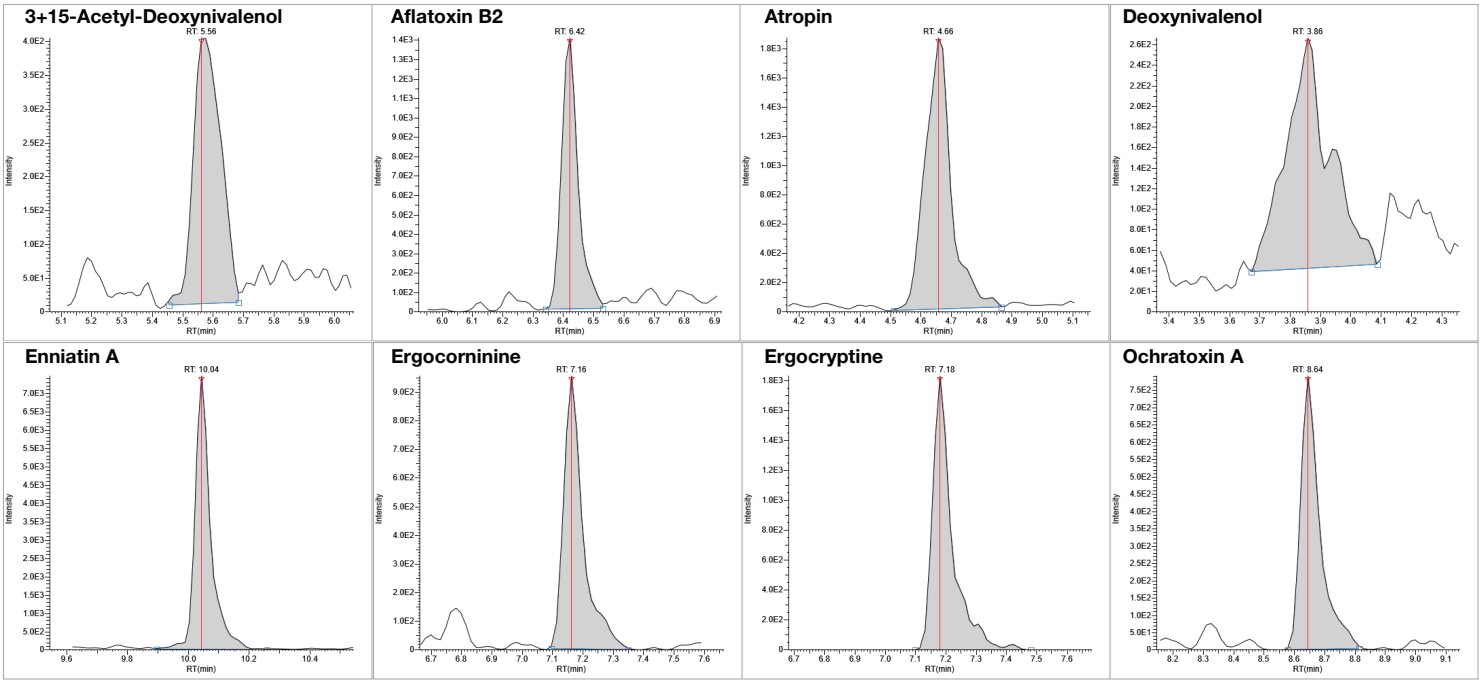

Figure 1. Representative chromatograms at LOQ.

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Conclusion

An HPLC method coupled to a triple quadrupole mass spectrometer was implemented for the quantification of 48 mycotoxins plus phytotoxins in cereal and maize. Sample preparation is simple, fast and economical.

This method is suitable for the quantitation of all 11 legislated mycotoxins worldwide and 37 more that are either already legislated in feed or are prospects for further legislations. MRLs have been reached for all regulated compounds with good precision and reproducibility on five replicates at the limit of detection (see Table 6).

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