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Research Paper

UHPLC-ToF-MS method for determination of multi-mycotoxins in maize: Development and validation

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ABSTRACT

An Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC–ToF-MS) method has been developed for determination of nine mycotoxins, namely aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), ochratoxin A (OTA), zearalenone (ZEA), toxin T2 (T2) and fumonisins (FB_1 and FB_2) in maize. The method included a two-step extraction with acetonitrile 80% (v/v). After optimization, the analytical method was validated. The different concentrations tested take in account the Maximum Levels (ML) for maize (Commission Regulation EC no. 1881/2006) and good results for repeatability ($\%RSD_r \leq 15.4\%$), reproducibility ($\%RSD_R \leq 15.9\%$) and recovery (77.8–110.4%, except for AFG2 at 2 μg/kg which presented a recovery of 73.4%) were achieved. These met the performance criteria imposed by Commission Regulation (EC) no. 401/2006. The method was applied to twenty-two samples from Portuguese producers of maize. Fumonisins were the most frequently detected mycotoxins, but the levels do not exceed those imposed by European legislation.

1. Introduction

Maize (*Zea mays* L.) is a staple food in diet, responsible for proving more than one-third of the calories and proteins in some countries (Chulze, 2010). In 2016 the European Union (EU) production of grain maize and corn-cob-mix was around 21% of the total production of main cereals (301 million tonnes) (Eurostat, 2017).

Under a wide range of favourable environmental conditions (relative humidity, oxygen, proper temperature, physical damage and presence of fungal spores) and poor hygienic conditions, some fungi, mainly the genera *Aspergillus*, *Fusarium* and *Penicillium*, can produce secondary metabolites, known as mycotoxins, and contaminate food commodities such as maize, consumed by both humans and animals (Sforza et al., 2006; Abia et al., 2013; Anfossi et al., 2016). Therefore, the factors that affect mycotoxins production and dissemination can be categorized in physical (relative humidity, high temperature, insect's infestation), chemical (use of fungicides and/or fertilizers) and biological (base on the interaction between colonizing toxigenic fungal species and substrate) (Tola and Kebede, 2016). The contamination of commodities can occur in the different stages from the period before harvest, post harvest, storage, processing and post processing (Zheng et al., 2006). Climatic changes can also influence the occurrence of mycotoxins due to extreme rainfall and drought events which favour formation of deoxynivalenol and fumonisins, respectively (Miller, 2008). Mycotoxins are associated with a vast range of adverse health effects including carcinogenesis (Ostry et al., 2017), mutagenesis (Kim et al., 2016), hepatotoxicity (Li et al., 2018) , genotoxicity (Domijan et al., 2015) immunotoxicity (Hueza et al., 2014), cytotoxicity (Malekinejad et al., 2015), nephrotoxicity (Schulz et al., 2018), neurotoxicity (Malekinejad et al., 2015) and estrogenic effects (Fink-Grernmels, 1999; Vejdovszky et al., 2017). Mycotoxins can also induce immunosuppresion (Mohsenzadeh et al., 2016) or cause mycotoxicoses (Abia et al., 2013; Anfossi et al., 2016; Tola and Kebede, 2016; Zheng et al., 2006; Miller, 2008; Ostry et al., 2017; Kim et al., 2016; Li et al., 2018; Domijan et al., 2015; Hueza et al., 2014; Malekinejad et al., 2015; Schulz et al., 2018; Fink-Grernmels, 1999; Vejdovszky et al., 2017; Mohsenzadeh et al., 2016; Andrade et al., 2017) (Abia et al., 2013; Andrade et al., 2017). According to the International Agency for Research on Cancer (IARC), aflatoxins (produced from *Aspergillus* molds) are included in group 1 (carcinogenic to humans), FB1, FB2 (produced from *Fusarium* molds) and OTA (produced by *Penicillium* and *Aspergillus* molds) are included in the group 2B (possibly carcinogenic to humans) and ZEA (produced from *Fusarium* molds) and T2 (type A trichothecene,

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produced from *Fusarium* molds) are included in group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 1993a, b). Although those of group 3 are considered not carcinogenic, they can cause other adverse effects. For instance, ZEA can affect reproduction in mammals due to its estrogenic activity. In the past some aflatoxicosis outbreaks were reported (Krishnamachari et al., 1975; Ngindu et al., 1982; CDC, 2004, 2005). Apart from acute episodes, the prolonged exposure to mycotoxins can also increase the risk of other human diseases, such as infectious diseases (Antonissen et al., 2014).

Therefore, the occurrence of mycotoxins is monitored and Maximum Levels (ML) are regulated by different regulatory bodies worldwide to assure food safety (Commission regulation No, 1881/2006; Commission Recommendation, 2013). At a global scale, the Joint Expert Committee on Food Additives (JEFCA), a scientific advisory board of World Health Organization (WHO) and Food and Agriculture Organization (FAO) is responsible for the evaluation of risks associated with mycotoxins. In the European Union, the European Food Safety Authority (EFSA) scientifically addresses the issues related with mycotoxins and advices the European Commission. Due to the restrictive legislation of mycotoxins in food, sensitive and precise detection methods are demanded allied with low analysis time. Due to the heterogeneous distribution of mycotoxins in cereals and other commodities, it is of utmost importance to implement correct sampling procedures, to obtain reliable results (Zheng et al., 2006). In the past a vast number of analytical systems have been used to determine mycotoxins in food and feed, from immunochemical-based techniques to chromatographic methods (Turner et al., 2009; Bankole et al., 2010; Huang et al., 2014) (Turner et al., 2009; Bankole et al., 2010; Huang et al., 2014). Nowadays LC-MS is the methods that presents more advantages due to the high selectivity, no need of derivatization, simple sample preparation procedures and no need of clean-up, besides simultaneous quantification of multi-mycotoxins at reasonable low cost. However, it is important to assure that the ionization technique (Electrospray Ionization - ESI, Atmospheric Pressure Chemical Ionization - APCI, Atmospheric Pressure Photoionization -APPI) is constant and able to lower matrix effects and ion suppressions (Sforza et al., 2006).

The main goal of this paper was to develop and validate a multi-mycotoxin UHPLC-ToF-MS method to determine aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), ochratoxin A (OTA), zearalenone (ZEA), toxin T2 (T2) and fumonisins (FB_1 and FB_2) in maize. Validation was carried out in order to meet the criteria of performance of analytical methods established by Commission Regulation EC no, 401/2006. Results from the analysis of 22 different samples of maize from Portuguese producers were presented and compared with ML according to the Commission Regulation EC no, 1881/2006 (Table S1).

2. Materials and methods

2.1. Chemicals and reagents

Methanol, acetonitrile (both HPLC gradient grade) and formic acid were purchased from Merck (Darmstadt, Germany). Water was purified by Milli-Q plus system from Millipore (Molsheim, France). Mycotoxins standards and internal standard (zearalenone, ZAN) were purchased from Sigma–Aldrich (Madrid, Spain) and were dissolved in acetonitrile (AFB_2, AFG_1, ZEA, T2 and ZAN), methanol (AFB_1, AFG_2 and OTA) or acetonitrile:water (50:50, v/v) (FB_1 and FB_2). Stock solutions were prepared with a concentration of 1 mg/mL, except T2, which presented a concentration of 2.5 mg/mL. AFB_1, AFB_2, AFG_1, AFG_2, OTA, ZEA, T2, FB_1 and FB_2 were supplied from Sigma- Aldrich (Table S2). These stock solutions were subsequently used to prepare different working solutions for calibrations. All standard solutions were stored in amber vials in the dark at - 20 °C, for at least 2 years, and before use, they were kept at room temperature for 15 min.

Certified reference materials MA1750-1/CM and MA1764/CM from Test Veritas (Padova, Italy) were used to evaluate accuracy of the method.

2.2. Samples and sampling procedure

Twenty-two samples of maize from 2018 harvest were provided by InovMilho (Portuguese National Competence Center for Maize and Sorghum Cultures) for quantification of multi-mycotoxins. These samples were intended for human consumption and were collected from the field experimentation trials located in the Coruche region of Portugal from September to October 2018. Each test portion (5 kg) corresponds to a trial modality and was hand collected after thorough mixing of several incremental samples taken from random field place locations. The laboratory samples have been homogenized by grinding (Retsch rotor mill SK 300 with sieve of trapezoid holes of 1.00 mm) the entire test portion (5 kg) and the flours were mixed thoroughly for guarantee complete homogenisation as possible. From each sample, three sub-samples of 50 g each were placed in separate sterile sample collection tubes and preserved at −20 °C until analysis. No further processing of the samples was done.

2.3. Extraction procedure for UHPLC-ToF-MS

About 2 g of maize flour (2.0 ± 0.1 g) was weighted in 50 ml polypropylene tubes. Internal standard (zearalanone) was added (100 μl from a 10 μg/mL). Subsequently, maize was extracted with 10 mL of acetonitrile 80% (v/v) for 1 h at 110 rpm using a Kotterman 4010 Orbital shaker (Uetze/Hänigsen, Germany). After centrifugation at 3000 rpm for 10 min, the supernatant was removed to another Falcon tube and samples were re-extracted with the same volume of acetonitrile 80% (v/v) for 1 h. After centrifugation (3000 rpm, 10 min), supernatants were collected. For analysis of fumonisins, one mL of the extract was diluted with 1 mL of ultra-pure water, filtered through a PVDF mini-uniprep™ and injected into the UHPLC-ToF-MS system. For the analysis of the other mycotoxins, 8 mL of the extract was transferred to a 15 mL Falcon tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was redissolved with 1 mL of acetonitrile 40% (v/v), vortexed for 30 s, filtered through a PVDF mini-uniprep™ and injected into the UHPLC-Tof-MS system.

2.3.1. Spiking experiment

To determine the recovery of the target analytes, spiking experiments were performed. Calibration standards were prepared by spiking blank sample of maize (2 g) with 6 different concentrations of multi-mycotoxins standard solution prepared in acetonitrile 80% (v/v), thoroughly mixed and kept at ambient temperature in the dark for 30 min. Afterwards extraction was performed as described in sub-Section 2.3.

2.4. LC–ToF-MS parameters

Detection and quantification was performed with a Nexera X2 Shimadzu UHPLC coupled with a 5600+ ToF-MS detector (SCIEX, Foster City, CA) equipped with a Turbo Ion Spray electrospray ionization source working in positive mode (ESI+). In terms of chromatographic conditions a column Zorbax Eclipse Plus C18 (2.1 × 50 mm, 1.8 μm) was used and kept at 30 °C, the autosampler was maintained at 10 °C to refrigerate the samples and a volume of 20 μL of sample extract was injected in the column. The mobile phase consisted of 0.1% formic acid [a] and acetonitrile [B] with a flow rate of 0.5 mL/min and with the following gradient program: 0–12 min from 90% to 30% [A]; 12–13 min from 30% to 10% [A] and kept until 14 min; back to 90% [A] from 14 to 15 min until the end of the run (total of 17 min). In terms of mass spectrometry the acquisition was performed in full-scan from 100 to 750 Da using the Analyst® TF (SCIEX, Foster City, CA) software and with the following settings: ion source voltage of 5500 V; source temperature 575 °C; curtain gas (CUR) 30 psi; Gas 1 and Gas 2 of 55 psi; declustering potential (DP) 100 V. Every 10 injections the ToF-MS detector was calibrated in the mass range of the method, to guarantee the accurate mass resolution.

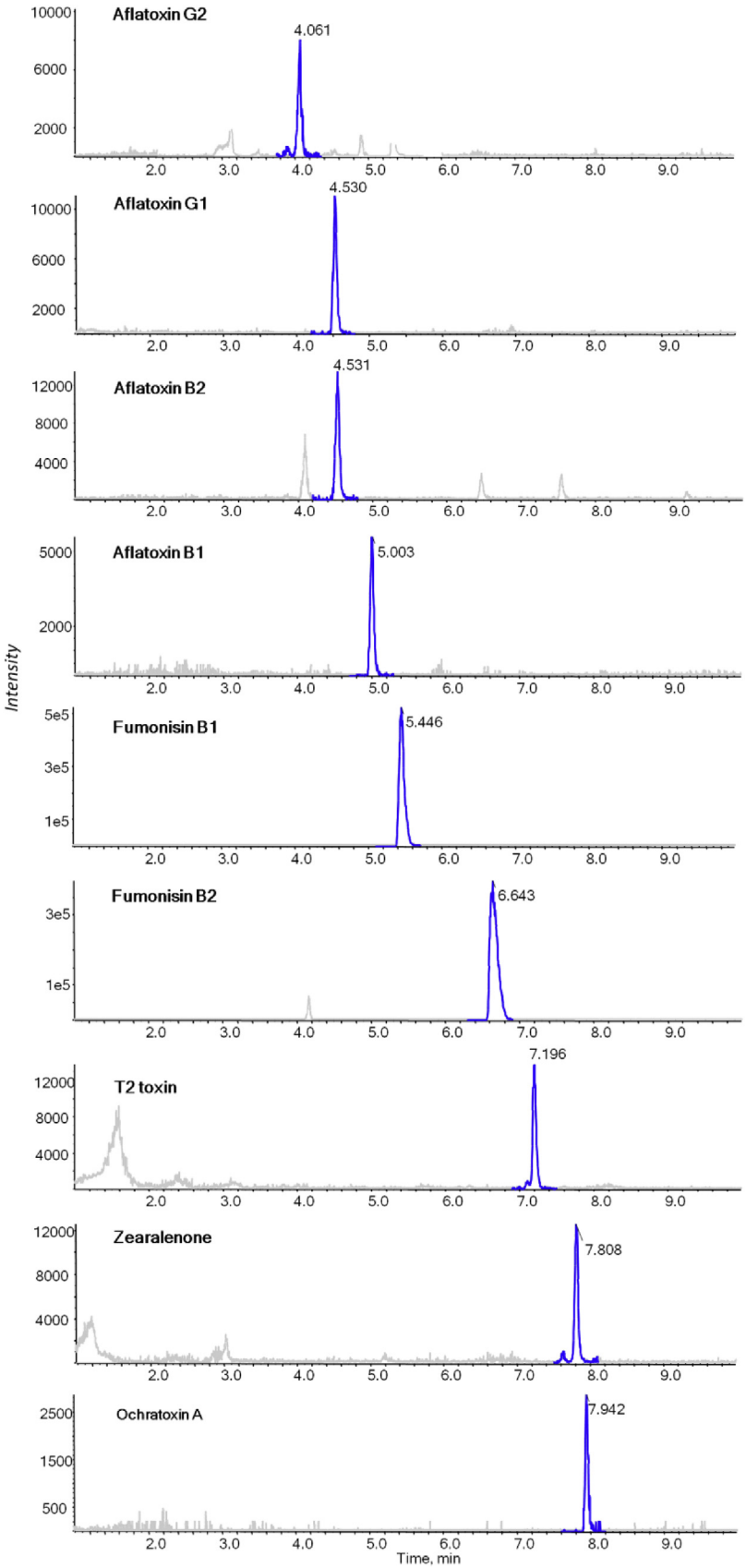

Fig. 1. Chromatogram of a blank maize sample spiked with 2 μg/kg of AFB1, 4 μg/kg of AFB2, AFG1 and AFG2, 3 μg/kg of OTA, 1000 μg/kg of FB1 and FB2, 200 μg/kg of ZEA and T2.

2.5. Identification of mycotoxins

The identification and data processing were made through the PeakView™ and MultiQuant™ (SCIEX, Foster City, CA) softwares.

In terms of identification criteria three parameters were used: maximum relative retention time deviation (ΔRRT) of 2.5%; difference in the isotope pattern with a tolerance of 10% and exact mass deviation (Δm) with a tolerance of 5 ppm. The isotope match is presented automatically by the PeakView™ software although for the other criteria the following equations were used:

Equation (1): Relative Retention Time (RRT)

$$RRT = \frac{RT_{analite}}{RT_{internal\ standard}}$$

Where $RT_{analite}$ is the retention time of the analite, and the $RT_{internal\ standard}$ is the retention time of the internal standard (zearalanone).

Equation (2): Deviation of RRT (ΔRRT)

$$\Delta RRT\ (\%) = \left(\frac{RRT_{spiked\ samples} - RRT_{standard}}{RRT_{standard}}\right) x100$$

Equation (3): Deviation of exact mass (Δm)

$$\Delta m\ (ppm) = \left(\frac{\text{Exact mass} - \text{Detected mass}}{\text{Exact mass}}\right) x\ 10^6$$

2.6. Validation of LC–ToF-MS method

The method was validated by the determination of concentration range, linearity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and intra-laboratory reproducibility) and accuracy (using recovery assays).

LoD and LoQ were determined as the concentration that originates a signal-to-noise ratio (S/N) ≥3 and ≥ 10, respectively. For the determination of repeatability (RSD_r) and intra-laboratory reproducibility (RSD_R), blank samples of maize were spiked at different levels (n=6) take in account the ML of each mycotoxin. In the case of RSD_R extraction was carried out in different days by different operators.

Accuracy of the method was evaluated using recovery experiments and certified reference materials.

3. Results and discussion

3.1. Development and validation of UHPLC-ToF-MS method

OTA and ZEA could be studied under positive and negative mode electrospray ionization (ESI+ and ESI-). However, the other studied mycotoxins were determined in ESI + mode, consequently, this mode was selected in order to determine all the studied mycotoxins. OTA and mainly ZEA presented higher sensitivity in the negative mode (data not shown). Huang et al. (2014), have also report this for OTA.

The developed method included a two-step extraction with acetonitrile 80% (v/v), without clean-up. Soleimany et al. (2012) used a similar method but with a single extraction and addition of 1% acetic acid to the extraction solvent.

Excellent peak resolution of the nine mycotoxins was achieved in a 10 min chromatographic run (Fig. 1). The optimized method was validated according to the criteria defined by Commission Regulation (EC) No, 401/2006 which establishes the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Identification criteria, described in section 2.5., were always evaluated. In the experiments carried out for validation purposes, ΔRRT deviation was always lower than 0.3%, except for FB1 and FB2 which was lower than 0.6%. Moreover, isotopic pattern deviation always met the defined criterion and Δm was always lower than 2 ppm, except for FB1 which was lower than 2.1 ppm.

Table 1
Linearity and sensitivity of UHPLC-ToF-MS method for the simultaneous determination of nine mycotoxins.

Mycotoxin	Linear range (μg/kg)	Calibration curve parameters			LoD (μg/kg)	LoQ (μg/kg)
		a	b	r^2		
AFB1	1.0–8.0	9093.4	579.45	0.9961	0.5	1
AFB2	1.0–16	10665	2747.5	0.9962	0.5	1
AFG1	1.0–16	10402	- 3099.2	0.9947	0.5	1
AFG2	1.0–16	2543.4	2967	0.9789	1	2
FB1	125–2000	279.72	- 4748.2	0.9978	62.5	125
	750–4000	216.56	72007	0.9834		
FB2	125–4000	252.78	- 3297.7	0.9988	62.5	125
OTA	1.5–12	2405.6	1393.4	0.9851	0.75	1.5
T2	25–400	256.5	−426.2	0.9786	10	25
ZEA	50–400	207.42	−579.11	0.9928	25	50
	100–800	173.79	6426.6	0.9876		

Linearity was evaluated by matrix matched calibration curves in different ranges for different mycotoxins (see Table 1). Correlation coefficients (r^2) of calibration curves were always higher than 0.979, indicating suitability to quantify mycotoxins in the selected calibration range. Table 2 shows the results of recovery, repeatability and reproducibility for the different mycotoxins in a blank maize sample spiked at 6 levels. Good recoveries were achieved, between 92.4 and 105.1% for a spiking level between 1 and 8 μg/kg for AFB1, between 85.2 and 105.3% for a spiking level between 1 and 16 μg/kg for AFB2, between 79.8 and 110.4% for a spiking level between 1 and 16 μg/kg for AFG1 and between 73.4 and 108.8% for a spiking level between 2 and 16 μg/kg for AFG2. Regarding fumonisins, FB_1 presented a recovery level between 82.3 and 104.2% while FB2 a recovery between 87.1 and 104.8%, both in the range 125–4000 μg/kg. In what concerns to OTA recovery ranged from 82.9 to 109.6% between 1.5 and 12 μg/kg, T2 from 95.1 to 105.5% between 100 and 800 μg/kg and ZEA from 85.9 to 103.7% between 50 and 800 μg/kg. The recoveries of the methods were all within the appropriated range of the Commission Regulation EC No, 401/2006 criteria. Two CRM were analysed in order to evaluate accuracy of the method. Both CRM were maize samples, one was contaminated with AFB_1, AFB_2, AFG_1, AFG_2, FB_1 and FB_2, while the other was contaminated just with ZEA. For this study it wasn't possible to evaluate a CRM contaminated with OTA or T2. Comparison between the assigned contamination levels of the CRMs and the measured values by UHPLC-ToF-MS is presented in Table 3 and shows excellent agreement for the evaluated mycotoxins (aflatoxins, fumonisins and ZEA).

Repeatability of the method was evaluated by the Relative Standard Deviation RSD_r. RSD_r was between 1.1 and 9.3% for AFB_1 (between 1 and 8 μg/kg); 2.4 and 8.7% for $_{AFB2}$ (between 1 and 16 μg/kg); 1.2 and 8.8% for AFG_1 (between 1 and 16 μg/kg); 3.4 and 9.9% for AFG_2 (between 2 and 16 μg/kg). For FB_1 was between 1.1% and 4.0% and for FB_2 between 1.2 and 6.3%, both in the range 125–4000 μg/kg. For OTA was between 2.0 and 15.4% in the range 1.5–12 μg/kg, for T_2 between 5.3 and 15.4% in range 25–400 μg/kg and for ZEA was between 3.0 and 8.0% in the range 50–800 μg/kg.

Reproducibility was evaluated by the Relative Standard Deviation RSD_R at 3 different days of analysis, different concentration levels and with different operators and values were considered acceptable. LoD and LoQs are shown in Table 1 and are sensitive enough to meet the requirement imposed by EU regulations for the ML of mycotoxins in maize, except for babyfood. LoDs are the same or lower than those reported by Spanjer et al. (2009) in maize slurry, for AFB_1 (0.5 μg/kg), AFB_2 (1 μg/kg), AFG_1 (1 μg/kg), OTA, (1 μg/kg), FB_1 (100 μg/kg), FB_2 (100 μg/kg) and T_2 (25 μg/kg). Our results only indicated higher LoDs for AFG_2 and ZEA.

Table 2
Results of the validation for different mycotoxins in a blank maize sample, including recovery, repeatability and reproducibility at different spiking levels (n=6, at each spiking level).

Mycotoxin	Ion	Retention time (min)	Spiked level (μg/kg)	Rec. (%)	RSD_r (%)	RSD_R (%)
AFB1	313.07066 [M+H]+	5.01	1.0	92.4	9.33	9.85
			1.5	95.4	2.50	
			2.0	96.9	6.34	5.94
			3.0	103.5	2.08	
			4.0	105.1	3.71	4.73
			8.0	98.7	1.14	
AFB2	315.08631 [M+H]+	4.53	1.0	85.2	6.97	
			2.0	97.1	8.74	9.25
			3.0	95.9	2.57	
			4.0	97.0	5.06	5.06
			6.0	105.3	2.81	
			8.0	104.6	2.85	2.49
			16.0	98.5	2.36	
AFG1	329.06558 [M+H]+	4.53	1.0	110.4	1.54	
			2.0	103.4	8.80	6.10
			3.0	98.4	3.64	
			4.0	93.7	3.13	4.23
			6.0	102.4	3.13	
			8.0	100.6	1.17	3.61
			16.0	79.8	1.84	
AFG2	331.08123 [M+H]+	4.05	2.0	73.4	3.38	15.87
			3.0	86.2	9.85	
			4.0	96.4	8.01	10.28
			6.0	108.8	7.04	
			8.0	106.3	6.16	8.74
			16.0	98.2	5.92	
FB1	722.39575 [M+H]+	5.34	125.0	95.8	4.0	6.84
			250.0	104.2	3.3	
			500.0	100.5	2.1	
			750.0	97.9	2.4	
			1000.0	99.8	1.1	5.16
			1500.0	101.3	1.9	
			2000.0	99.6	3.5	7.21
			4000.0	82.3	2.0	
FB2	706.40081 [M+H]+	6.47	125.0	87.1	6.3	14.0
			250.0	104.8	3.7	
			500.0	100.0	2.3	
			750.0	98.4	3.3	
			1000.0	101.8	1.2	9.2
			1500.0	100.9	1.3	
			2000.0	99.3	1.6	11.5
			4000.0	94.1	4.5	
OTA	404.08954 [M+H]+	7.95	1.50	82.9	15.4	14.5
			2.25	91.2	9.3	
			3.00	92.4	6.9	9.59
			4.50	109.6	2.0	
			6.00	109.5	3.1	5.03
			12.0	97.3	4.4	
T2	489.2095 $[M+Na]^+$	7.20	25.0	99.3	5.33	
			50.0	100.5	15.4	
			100.0	105.5	8.67	15.1
			150.0	101.0	7.02	
			200.0	98.3	9.13	8.77
			300.0	95.1	12.3	
			400.0	102.7	8.55	14.3
ZEA	319.154 [M+H]+	7.82	50.0	101.6	7.99	
			100.0	103.7	5.51	8.5
			150.0	98.5	6.56	
			200.0	100.5	5.23	3.8
			300.0	96.6	3.82	
			400.0	101.8	3.01	6.5
			800.0	85.9	3.17	

The co-elution of matrix components can cause matrix effect and affects the ionization efficiency of the analytes (Huang et al., 2014). The signal suppression-enhancement (SSE) was used to evaluate matrix effect of the mycotoxins in maize. SSE was calculated as follows:

SSE(%)= (matrix-matched calibration slope/standard calibration slope)* 100.

Table 3
Comparison between the assigned contamination levels of the certified reference materials (maize) and the measured value by UHPLC-ToF-MS.

Certified control material	Mycotoxin	Assigned contamination level (μg/kg)	Satisfactory range (μg/kg)	Measured value (μg/kg)
MA1750–1/CM	AFB1	9.34	5.23–13.4	8.66
	AFB2	0.42	0.24–0.60	0.52
	AFG1	1.57	0.88–2.26	1.06
	AFG2	traces		
	AFB1+AFB2+AFG1+AFG2	11.5	4.32–18.8	10.2
	FB1	2545	1272–3817	2370
	FB2	608	399–818	482
	FB1+FB2		1714–5143	2852
MA1764/CM	ZEA	190	112–269	199

It was considered signal enhancement, when SSE>100%, inexistence of matrix effect when SSE=100% and signal suppression when SSE<100%. Signal suppression was found for AFB_1 (SSE= 95.2%), AFB_2 (87.7%) and AFG_2 (87.8%). In the case of ZEA, matrix effect was negligible (101.5%). However, it was found signal enhancement for FB_1 (SSE= 121.4%), FB_2 (SSE= 129.3%) and T2 (SSE= 112.9%). For OTA this effect was prominent (194.1%) and it has already been reported for other authors, for instance Huang et al. (2014) have reported for OTA an SSE% in the range of 173–177% for raw, liquid and powder milk.

3.2. Occurrence of mycotoxins in maize

FB_1 and FB_2 were detected in maize samples collected in September–October 2018 (Fig. 2). Table 4 compiles the results of these samples for FB_1 and FB_2. All the samples were negative for the other mycotoxins under study. The values of replicates are shown in order to conclude about the homogeneity of the sub-samples analysed (n=3). After a careful homogenisation process, following the established EU guidelines, results allow concluding that sub-samples were very homogeneous. Moreover, any of the samples exceeded EU ML for maize (Table 4).

In the last 2-3 decades, numerous studies have reported Fusarium infested crops (Placinta et al., 1999; Sulyok et al., 2010; Pereira et al., 2014). In a study carried out by Doko et al. (1995) different maize genotype grown in different countries of Europe and Africa where compared. At the time, Portugal was indicated as one of the studied countries with more occurrences of positives (100%) ranging from 90 to 4450 μg/kg. Unfortunately, in this study it was not established the relationship among genotype, area and season.

Abia et al. (2013) have reported the occurrence of mycotoxins in food commodities from Cameroon. These found a mean concentration of 508 μg/kg for FB_1 and 149 μg/kg for FB_2 for 37 samples of maize. In a study carried out by Soleimany et al. (2012), the levels of FB_1 in maize meal from Malaysian markets were in the range 48.2–209.3 μg/kg while for FB_2 was in the range 58.7–113.5 μg/kg. In 2016, Hove et al. (2016) reported that 95% of the maize (n=95) samples analysed (from Zimbabwe) were positive for FB_1 (mean = 242 μg/kg) and 31% for FB2 (mean = 120 μg/kg). In Brazil, a recent study reported 100% of maize samples (n=148) for FB_1 and FB_2 (62.4–66,274 μg/kg) (Oliveira et al., 2017). High frequency (>81%) of maize samples, from Côte d'Ívoire, contaminated with FB1 + FB2 was also reported (Manizan et al., 2018). Shephard et al. (2019) recognized that the high maize consumption in Eastern Cape Province and other parts of Africa contributes for the high exposure to fumonisins and the promotion of a diverse diet can lighten this issue.

4. Concluding remarks

It is of utmost importance to control mycotoxins in food chain due to the severity of adverse health effects, from toxic acute to chronic, in both animals and humans. The update of legislation is also important to meet

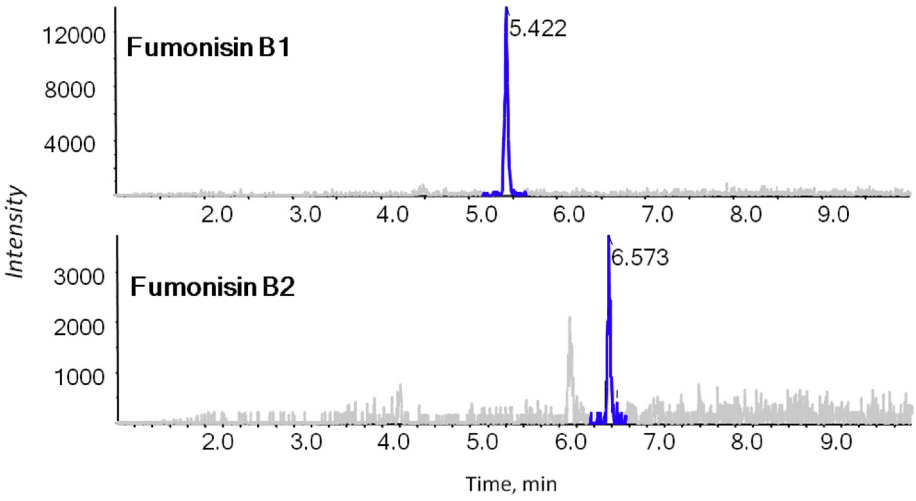

Fig. 2. Chromatogram of a maize sample contaminated with both FB1 and FB2.

Table 4
Results of the twenty two samples of maize by UHPLC-ToF-MS for FB1 and FB2.

Mean ± SD (μg/kg)	Samples #	
	FB1	FB2
1	<LoQ	<LoD
2	<LoQ	<LoD
3	<LoQ	<LoD
4	<LoD	<LoD
5	848 ± 65.3	196 ± 8.2
6	<LoD	<LoD
7	<LoD	<LoD
8	<LoD	<LoD
9	141 ± 4.1	<LoQ
10	<LoD	<LoD
11	<LoD	<LoD
12	<LoQ	<LoD
13	446 ± 54.8	162 ± 9.7
14	301 ± 42.4	102 ± 10.8
15	486 ± 63.8	<LoQ
16	280 ± 24.2	<LoQ
17	419 ± 32.1	158 ± 14.7
18	<LoD	<LoD
19	<LoQ	<LoD
20	134 ± 17.7	<LoD
21	<LoD	<LoD
22	<LoD	<LoD

LoQ – 125 μg/kg; LoD- 62.5 μg/kg.

the advances of high-resolution analytical techniques and to assure the protection of individuals. The analytical UHPLC-ToF-MS method developed and validated in maize is an excellent tool to monitor the levels of mycotoxins in this cereal and its application was demonstrated in real samples. Moreover, future work is important in order to include other mycotoxins in the method (e.g. HT2 toxin and deoxynivalenol) evaluate the types of genotypes of maize that can be resistant to the infection caused by fungi, namely species of *Fusarium*, in order to prevent the development of mycotoxins. Other mitigation strategies are related with the agronomic practices and treatments during the storage of cereal grains in order to decontaminate them (James and Zikankuba, 2018). Finally, it is greatly recommended the educational intervention, the dissemination of good management practices from the field to the agroindustry maize chain in order to reduce the exposure to food contaminated with mycotoxins.

Conflict of interests

Authors declare there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2019.07.001.

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