Performance and Application of a "Dilute-and-Shoot" LC-MS/MS Method for Determination of Mycotoxins in Food Products in São Paulo, Brazil

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***Abstract*—Mycotoxins are secondary metabolites produced by several species of fungi that occurs in agricultural commodities, which cause a great variety of toxic effects in vertebrates, including humans. In this study, a "dilute-and-shoot" method using ultra-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was evaluated and applied to assess the incidence of aflatoxins (AF) B_1, B_2, G_1 and G_2, fumonisins (FB) B_1 and B_2, ochratoxin A (OTA), zearalenone (ZEA) and deoxynivalenol (DON) in samples of corn meal (*N* = 4), corn flour (*N*=2), wheat flour (*N*=4), rice (*N*=14) and bean (*N*=12) collected in small-scale farms from São Paulo, Brazil. The performance parameters (aparent recovery, matrix effect, extraction recovery, linearity, limit of quantification and limit of detection) were satisfactory, and the most frequent mycotoxin detected in food samples was FB (7.11-316.04 μg/kg) below the Brazilian regulations. DON was detected in all samples of wheat flour (162.49-324.66 μg/kg) along with FB in 3 samples, and in one sample of bean (46.52 μg/kg). ZEA was detected in 2 samples of rice (4.90-6.78 μg/kg). Results indicated low incidences of mycotoxin in the products evaluated, although the co-occurrence of FB and DON warrants concern about their incidence in wheat flour in Brazil.**

***Index Terms*—aflatoxins, ochratoxin A, *Fusarium* toxins, occurrence, LC-MS/MS, analysis**

I. Introduction

Mycotoxins are secondary metabolites produced by fungi species that develop naturally in food products, which cause a great variety of toxic effects in vertebrates, including humans [1]. Exposure to toxins occurs predominantly by the ingestion of contaminated food, especially cereals and grains such as corn, wheat, and peanut, among others [2]. The most common toxigenic fungi found in Brazilian cereals include species from the genera *Aspergillus* and *Fusarium* [3]-[5].

The aflatoxins are produced by *Aspergillus* species, mainly *A. flavus*, *A. parasiticus* and *A. nomius* [2]. The main aflatoxin compounds are B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), although AFB_1 has the higher acute, mutagenic and carcinogenic effects [3]. In Brazil, *A. ochraceus* (formerly *A. alutaceus*) is the main producing species of ochratoxin A (OTA), a toxin that interferes with the synthesis of macromolecules in the cells of the renal parenchyma, including DNA, RNA and proteins [1]. The *Fusarium* toxins are mycotoxins produced by several species in the genus, including the fumonisins, trichothecenes, and zearalenone (ZEA), among others [6]. Fumonisins are produced mainly by *Fusarium verticillioides*, being fumonisins B_1 (FB_1) and B_2 (FB_2) the most prevalent in grains under natural conditions, although FB_1 is the most toxic compound [7]. FB_1 has been associated with animal diseases such as equine leucoencephalomalacia and porcine pulmonary edema [2]. ZEA is an estrogenic substance derived from resorcylate acid, produced by several *Fusarium* species, e.g., *F. roseum (F. graminearum)*, *F. culmorum* and *F. equisetum*, among others [7]. Deoxynivalenol (DON), also called vomitoxin, is a class B trichothecene mainly produced by *F. roseum*, which provokes nausea, diarrhea, reduced nutritional efficiency, gastrointestinal tract injuries and weight loss in animals [8].

Taking into account the risks posed by mycotoxins to the human health, several countries have determined tolerance limits for these toxins in food products. Brazilian regulations for mycotoxins were revised in 2011, with the introduction of maximum permitted levels (MPL) for aflatoxins (sum of AFB_1, AFB_2, AFG_1 and AFG_2), OTA, fumonisins (sum of FB_1 and FB_2), ZEA and DON in several food products [9]. Since the adoption of action levels for mycotoxins, a few reports in Brazil indicated high incidence rates (up to 100%) of fumonisins in corn products [4]. In another study [3], corn products showed 42% positive samples for aflatoxins at levels ranging from 0.05 to 8.3 μg/kg (sum of AFB_1, AFB_2, AFG_1 and AFG_2). Bean kernels had the highest incidence (75% positive samples) of aflatoxins, but at low levels (0.025 to 0.042 μg/kg, sum of all fractions) [3]. In Paraná state, DON was detected in 66% of wheat grains samples, with a mean level of 1,895 μg/kg [10]. The data presented in those studies confirm that individual mycotoxins have

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been frequently detected in Brazilian cereals. Importantly, two or more mycotoxins may be present in the food as a consequence of multiple fungi contamination, or when one species produces more than one type of mycotoxin (e.g., *Fusarium* species) [7]. However, there is very little information on the simultaneous occurrence of mycotoxins in food products in Brazil.

In the past decade, analytical methods based on the "dilute-and-shoot" approach and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have been developed for the simultaneous analysys of mycotoxins in foods [11]. In the present study, the performance of a "dilute-and-shoot" LC-MS/MS analytical method was evaluated, aiming to determine the levels ofaflatoxins, OTA, fumonisins, ZEA and DON in food products susceptible to mycotoxin contamination in the state of S ão Paulo, Brazil.

II. Material and Methods

A. Mycotoxin Standards

The standards of AFB_1, AFB_2, AFG_1, AFG_2, OTA, FB_1, FB_2, ZEA and DON were purchased from Sigma-Aldrich (St Louis, MO, USA). Isotopically labeled standards [$13C_{17}$]-AFB_1 (Sigma-Aldrich, St. Louis, MO, USA), [$13C_{20}$]-OTA, [$13C_{34}$]-FB_1, [$13C_{18}$]-ZEA and [$13C_{15}$]-DON (Biopure, Romer Labs, Tulln, Austria) were also used as internal standards. Individual standard solutions (10 μg/mL) of either labeled or unlabeled mycotoxins were prepared in water/acetonitrile (1:1, v/v), and used to prepare two standard working solutions of mixed mycotoxins, as follow: one mixture containing unlabeled standards of AFB_1, AFB_2, AFG_2, AFG_2, OTA, ZEA, FB_1, FB_2 at 100 ng/mL and DON at 750 ng/mL, and a second mixture of the labeled standards of [$13C_{17}$]-AFB_1, [$13C_{20}$]-OTA, [$13C_{34}$]-FB_1, [$13C_{18}$]-ZEA at 5 ng/mL and [$13C_{15}$]-DON at 100 ng/mL. All solutions were stored in freezer at -20°C, and before use they were kept in the dark until room temperature was reached.

For the preparation of all curves, 80 μL of solutions of each calibration point and 20 μL of the labeled standard working solution in water/acetonitrile (9:1) were added into amber vials provided with glass inserts so that the final concentrations of the internal standards in the vials were 1.0 ng/mL of [$13C_{17}$]-AFB_1, [$13C_{20}$]-OTA, [$13C_{34}$]-FB_1, 50 ng/mL of [$13C_{18}$]-ZEA and 100 ng/mL of [$13C_{15}$]-DON.

B. Evaluation of Method Performance

The analytical method was evaluated in terms of aparent recovery (R_A), matrix effects (SSE) and extraction recovery (R_E). Additionaly, linearity, limit of quantification (LOQ) and limit of detection (LOD) were also evaluated. RA, SSE and RE parameters were calculated according to the equations (1), (2) and (3), respectively [12].

$$R_A\ (\%) = 100 \times slope_{spiked\ sample} / slope_{liquid\ standard} \quad (1)$$

$$SSE\ (\%) = 100 \times slope_{spiked\ extract} / slope_{liquid\ standard} \quad (2)$$

$$R_E\ (\%) = 100 \times R_A / SSE \quad (3)$$

Recovery assays were performed for each type of food product susceptible to mycotoxin contamination and regulated in Brazil [6]. Triplicate blank samples (1.0 g) of finelly milled corn meal, corn flour, wheat flour, rice and bean were spyked at six concentration levels of the unlabeled mycotoxins. For the 3 highest points, 160 μL, 120 μL and 80 μL of the working solution containing the mixture of mycotoxin standards (prepared as described in item II.A) were added to fortify the samples. For the 4 lowest points, the working solution was diluted (1:4), and volumes of 80 μL, 40 μL and 8 μL were added. This resulted in the concentrations of 4.0, 3.0, 2.0, 1.0, 0.5, 0.25 and 0.05 ng/mL for AFB_1, AFB_2, AFG_2, AFG_2, OTA, ZEA, FB_1, FB_2 and 30.0, 22.5, 15.0, 7.5, 3.75, 1.87 and 0.37 ng/mL for DON. The samples were vortexed and conditioned in the dark at room temperature and uncapped for 12 h to allow solvent evaporation and absorption of the analytes by the food matrix. Next, the samples were shaken 2 times for 30 min. in a horizontal shaker (Tecnal, Piracicaba, Brazil) to homogenate the samples.

C. Sample Preparation

Extraction procedures were performed using a "dilute-and-shoot" approach as described by [11] Sulyok et al. (2007), with minor modifications. One gram of milled sample of each food product was weighed in 15-mL Falcon Tubes in duplicate, and 4 mL of acetonitrile:water:acetic acid (80:20:0.1) was added to the tubes. After vortexing for 1 min, the mixtures were shaken in a horizontal shaker (Tecnal, Piracicaba, Brazil) for 60 min. Next, the tubes were vortexed and shaken again for 30 min. The samples were then centrifuged (Quimis, Brazil) at 1,700 g for 5 min. The supernatant was filtered in PTFE 0.22 μm membrane filters (Millex, Millipore Corp.). An aliquot of 80 μL of these solutions were transferred to glass inserts inside vials and mixed with 20 μL of the internal standards working solution (prepared as described in item II.A). The final extracts were vortexed and reserved for LC-MS/MS determinations

D. Liquid Chromatographic and Mass Spectrometry Conditions

The final extracts were injected into a Waters Acquity I-Class Ultra-Performance LC system (Waters, Milford, MA, USA) equipped with a BEH C_{18} column (2.1 × 50 mm, 1.7 μm) and coupled to a Xevo TQ-S mass spectrometer (Waters, Milford, MA, USA). The column was kept at 40°C during analysis, and samples were maintained at 15°C. Ten microliters of final extracts of samples and standards were injected. Elution gradient was accomplished in a mobile phase composed by water (eluent A) and acetonitrile (eluent B), both containing 0.1% of acetic acid and ammonium acetate 5 mM. After an initial period of 0.5 min at 98% eluent A, the percentage of eluent B was linearly raised to 30% over 4.5 min (5.0 min). Then, eluent B was increased to 96% over 2.0 min, followed by a hold time of 0.5 min (7.5 min). After that,

the percentage of eluent B was reduced to 2% over 2 min (8.0 min), and the column re-equilibrated to the initial conditions for 2.0 min. Total chromatographic run time was 10 min, and the mobile phase flow rate was maintained at 0.6 mL/min.

The mass spectrometer was operated in MRM mode using electrospray ionization in positive and negative ion mode, with a capillary voltage of of 3.00 kV for the positive mode and 2.00 kV for the negative, source temperature of 150 ℃, and desolvation temperature of 500 ℃. Desolvation gas flow and cone gas flow were maintained at 800 L/h and 150 L/h, respectively. Cone voltage, collision energy, and MRM transitions (major precursor ion > fragment ion) were manually optimized. Quantification and confirmatory MRM transitions for mycotoxins and corresponding optimal mass spectrometric parameters used in the LC-MS/MS analysis are presented in Table I, along with the mass, molecular ion, cone voltage and collision energy values used for each mycotoxin analyzed. Data collection and processing were performed using software MassLynx version 4.1.

TABLE I. MASS SPECTROMETRY CONDITIONS USED FOR MYCOTOXIN ANALYSES IN FOOD PRODUCTS

Mycotoxin	Mass (g/mol)	Molecular ion	Transition (*m/z*)	Cone voltage (V)	Collision energy (V)
Aflatoxin B_1	312.28	$[M+H]^+$	312.7 > 284.9 [a]	94	36
			312.7 > 241.1 [b]	94	22
$[^{13}C_{17}]$-Aflatoxin B_1	329.15	$[M+H]^+$	330.3 > 301.5	94	20
Aflatoxin B_2	314.29	$[M+H]^+$	314.7 > 259.0 [a]	2	28
			314.7 > 287.0 [b]	2	26
Aflatoxin G_1	328.27	$[M+H]^+$	328.9 > 243.0 [a]	2	26
			328.9 > 199.5 [b]	2	38
Aflatoxin G_2	330.29	$[M+H]^+$	330.9 > 245.0 [a]	56	28
			330.9 > 188.9 [b]	56	40
Ochratoxin A	403.08	$[M+H]^+$	404.0 > 238.9 [a]	35	22
			404.0 > 357.9	35	12
$[^{13}C_{20}]$-Ochratoxin A	423.67	$[M+H]^+$	424.2 > 250.0	30	20
Fumonisin B_1	721.84	$[M+H]^+$	722.5 > 334.0 [a]	50	40
			722.5 > 352.1 [b]	50	30
$[^{13}C_{34}]$-Fumonisin B_1	755.58	$[M+H]^+$	756.6 > 374.4	50	30
Fumonin B_2	70.83	$[M+H]^+$	706.5 > 336.2 [a]	50	35
			706.5 > 318.3 [b]	50	40
Zearalenone	318.15	$[M-H]^-$	317.1 > 175.1 [a]	50	23
			317.1 > 130.9 [b]	50	33
$[^{13}C_{18}]$-Zearalenone	336.23	$[M-H]^-$	335.1 > 185.1	50	31
Deoxinyvalenol	296.32	$[M+H]^+$	397.3 > 249.1 [a]	6	10
			397.3 > 231.1 [b]	6	14
$[^{13}C_{15}]$-Deoxynivalenol	311.21	$[M+H]^+$	312.1 > 98.7	30	35

[a] Transitions used for quantification; [b] Transitions used for confirmation.

In order to evaluate the matrix effects of each food product, individual blank samples of corn meal, corn flour, wheat flour, rice and beans were extracted exactly as described in item II.C. The resulting raw blank stracts were used to prepare matrix-matched standard curves for each product, which were generated by 7 concentration points in triplicates. SSE parameters were stablished by comparing the matrix calibration curve with the corresponding curve prepared in solvent (water/acetonitrile 9:1). Eighty µL of the spiked raw extracts were combined with 20 µL of the IS working solutions for injection into the LC-MS/MS system. By using this procedure, the following mycotoxin levels were achieved in each extract: 4.0, 3.0, 2.0, 1.0, 0.5, 0.25, 0.05 ng/mL for AFB_1, AFB_2, AFG_2, AFG_2, OTA, ZEA, FB_1, FB_2, and 30.0, 22.5, 15.0, 7.5, 3.75, 1.87, and 0.37 ng/mL for DON. LOD and LOQ values were calculated from the matrix matched calibration curves using signal to noise ratios (S/N) of minimum 3 and 10 for each qualifier MRM transition, respectively. The method characteristics were determined using internal and external calibrations, aiming to verify the efficiency of internal standards to compensate the matrix effects [13]. To quantify the mycotoxins, the ratio of sample peak area to internal standard peak area was calculated and the final concentration was determined through the corresponding calibration line.

E. Sampling Procedures and Application of the Method

Sampling procedures for collection of food products were performed in small-scale farms from the surroundings of Pirassununga and Descalvado, located in the State of S ão Paulo, Brazil. Samples of corn meal (N = 4), corn flour (N = 2), wheat flour (N = 4), rice (N = 14) and bean (N = 12) availabe and stored in the households of each farm were collected in June 2016. All food products were commercially available, and had been purchased by the farmers and stored in the households prior to sample collection. Samples were packed in polyethylene bags and sent immediately to the laboratory, where they were homogenized, finely milled and submitted to analysis for mycotoxins by using the same analytical method as described in items IIC-D.

III. RESULTS AND DISCUSSION

The method performance parameters (concentration range, LOQ, LOD, R_A using external or internal calibration, SSE and R_E) determined in the food products are presented in Table II. Overall, the calculated LOQ

values for individual mycotoxins varied between 0.8 to 18.8 μg/kg, which were much lower than the MPLs for mycotoxins in foods established by the Brazilian regulations [9]. These results confirm that the method evaluated was suitable for routine determination of all the target mycotoxins in samples of corn meal, corn flour, wheat flour, rice and bean.

TABLE II. METHOD PERFORMANCE PARAMETERS DETERMINED IN CORN FLOUR [A]

	Conc. Range (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	R_{Aext}	R_{Aint}	SSE_{int}	R_E
AFB_1	1.25-20	0.8-1.0	0.4-0.5	133-141	101-108	92-99	109-110
AFB_2	1.25-20	0.8-1.0	0.4-0.5	90-112	96-106	93-103	102-107
AFG_1	1.25-20	0.8-1.0	0.4-0.5	61-93	89-109	95-107	94-102
AFG_2	1.25-25	0.9-1.0	0.4-0.6	57-80	92-103	95-97	94-108
OTA	1.25-20	1.0-1.1	0.5-0.7	86-143	97-106	92-99	102-109
FB_1	2.5-25	2.5-2.8	0.9-1.0	153-231	99-109	95-102	97-111
FB_2	2.5-25	2.0-2.5	0.7-0.9	148-211	91-101	90-99	96-102
DON	18.7-150	18.5-18.8	5.8-6.1	33-60	93-103	89-109	93-105
ZEA	0.25-20	0.3-0.6	0.12-0.23	42-72	97-105	95-105	98-107

[a] Expressed as minimum-maximum values; LOQ: Limit of quantification; LOD: Limit of detection; R_A: Apparent recovery; SSE: Matrix effects; R_E: Extraction recovery; AF: aflatoxin; FB: fumonisin; OTA; ochratoxin A; ZEA: zearalenone; DON: deoxynivalenol.

The use of external and internal calibrations was efficient for compensating the matrix effects on the method performance. The overall values obtained for the apparent recovery using external calibration (R_{Aext}) varied markedly among the different mycotoxins and food products, ranging from 33% to 143% (Table II). However, the addition of internal standards efficiently compensated the matrix effect, since the overall R_{Aint} values ranged from 92 to 109%. The recovery of the extraction procedure (R_E) was calculated by the ratio between the R_{Aint} values obtained through the fortified matrices before the extraction, and the SSE_{int} values obtained through the fortified matrices after extraction, both using internal calibration. As shown in Table II, recovery values were adequate for the target mycotoxins in all evaluated matrices (93-111%). Finally, the calculated linearity (r^2) of the analytical curves were > 0.99. The satisfactory performance parameters obtained for the analytical method enable its application for the simultaneous determination of mycotoxins in the food products analyzed.

TABLE III. MYCOTOXIN LEVELS IN FOOD PRODUCTS FROM SMALL-SCALE FARMS IN THE STATE OF SÃO PAULO, BRAZIL

Mycotoxin	*n* (%)	Variation (μg/kg)	Mean ± DP (μg/kg)	MPL (μg/kg)
Corn meal (*N* = 4)				
Aflatoxins [a]	0 (0)	-	-	20
Ochratoxin A	0 (0)	-	-	10
Fumonisins [b]	4 (100)	69.44 - 131.92	103.06 ± 29.43[a]	1500
Zearalenone	0 (0)	-	-	150
Deoxynivalenol	0 (0)	-	-	750
Corn flour (*N* = 2)				
Aflatoxins [a]	0 (0)	-	-	20
Ochratoxin A	0 (0)	-	-	10
Fumonisins [b]	2 (100)	7.11 - 48.74	27.92 ± 29.44[a]	1500
Zearalenone	0 (0)	-	-	150
Deoxynivalenol	0 (0)	-	-	750
Wheat flour (*N* = 4)				
Aflatoxins [a]	0 (0)	-	-	5
Ochratoxin A	0 (0)	-	-	10
Fumonisins [b]	3 (75)	11.54 - 316.04	109.19 ± 179.23[a]	NRE
Zearalenone	0 (0)	-	-	100
Deoxynivalenol	4 (100)	162.49-324.66	244.54 ± 90.55	750
Rice (*N* = 14)				
Aflatoxins [a]	0 (0)	-	-	5
Ochratoxin A	0 (0)	-	-	10
Fumonisins [b]	0 (0)	-	-	NRE
Zearalenone	2 (14)	4.90 - 6.78	5.84 ± 1.33	100
Deoxynivalenol	0 (0)	-	-	750
Bean (*N* = 12)				
Aflatoxins [a]	0 (0)	-	-	5
Ochratoxin A	0 (0)	-	-	10
Fumonisins [b]	0 (0)	-	-	NRE
Zearalenone	0 (0)	-	-	NRE
Deoxynivalenol	1 (8)	-	46.52 ± 0	NRE

n: Samples showing concentrations above the limit of determination (LOD) – refer to Table II for LOD values; MPL: Maximum permitted level adopted by Brazilian regulation [9]; NRE: No regulation established; [a] Sum of aflatoxins B_1 , B_2, G_1 and G_2; [b] Sum of fumonisins B_1 and B_2.

Table III presents the incidence and levels (range and mean values) of aflatoxins, OTA, fumonisins, ZEA and DON found in samples of corn meal, corn flour, wheat flour, rice and bean collected in small-scale farms from the state of São Paulo, Brazil. MPL values adopted in Brazil for aflatoxins, OTA, fumonisins, ZEA and DON are also presented in Table III. Aflatoxins or OTA were not detected in any sample, and although fumonisins, ZEA and DON were quantified in several samples, none exceeded the MPL values adopted in Brazil [9].

Fumonisins (FB_1 and FB_2) had the highest incidence in the food products, being detected in 100% samples of corn meal and corn flour at mean levels of 103.06 ± 29.43 μg/kg and 27.92 ± 29.44 μg/kg, respectively (sum of FB_1 and FB_2). These results are consistent with those reported in a previous survey conducted in São Paulo state in 2015 [4], in which FB_1 was found in 60% samples of corn-based products at a mean level of 360.4 μg/kg. However, the fumonisin concentrations found in corn meal in the present work were lower than those described previously (mean level of FB_1: 422.9 μg/kg) [4]. Although the number of samples analyzed was low, our results may indicate that manufacturers of corn products have improved their control practices regarding fumonisin contamination.

FB_1 and FB_2 were also detected in 75% of samples of wheat flour, at a mean concentration of 109.19 ± 179.23 μg/kg (sum of fractions), and all samples of this food product had DON (mean level: 244.54 ± 90.55 μg/kg), as shown in Table III. The high incidence of FB_1 and FB_2 in wheat flour was surprising, since there is no MPL established for fumonisins in this product in Brazil. Accordingly, the maximum level of fumonisins found in wheat flour (316.04 μg/kg) was higher than the highest level determined in corn meal (131.92 μg/kg). Importantly, the co-occurrence of fumonisins and DON was observed in 3 samples (75%) of wheat flour (data not shown), which warrants concern about the incidence of those toxins in wheat flour in Brazil.

The simultaneous occurrence of *Fusarium* toxins in wheat flour has been described elsewhere. DON, ZEA and other *Fusarium* toxins (e.g., nivalenol, 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol) were detected in the majority of 348 samples of wheat flour marketed in Hebei province of China, although DON was the most prevalent, being detected in 91.4% of samples analyzed [14]. The mean concentration reported for DON (240 μg/kg) [14] was similar to the level described in the present study. In a recent survey conducted in the Brazilian states of São Paulo, Paraná and Rio Grande do Sul Brazil [15], ZEA and DON were found in 39% and 55% of wheat grain samples, respectively, with overall mean levels of 79.78 μg/kg and 795.2 μg/kg, respectively, which is much high than the values reported in our work. In the present study, DON was also detected in one sample (8.3%) of bean, as shown in Table III. Although the concentration found was low (46.52 μg/kg), the fact that bean is among the most important food items consumed in Brazil indicate the need for adoption of MPL for DON in this product.

ZEA was detected in only 2 samples (14.3%) of rice, at a mean concentration of 5.84 ± 1.33 μg/kg (Table III). There is little information on the incidence of ZEA in rice in Brazil, although a recent study aiming to evaluate the presence of ZEA, AFB_1 and DON in rice from southern regions in Brazil indicated that only ZEA was significantly detected in 60% of samples at levels of 90.56 to 126.31 μg/kg [16]. Moreover, 36% of the samples analyzed were higher than the tolerance limit established by Brazilian regulations [16]. However, our results are similar to those reported in white rice from South Korea [17], in which ZEA was detected in 8.8% of samples at levels of 4.0-11.5 μg/kg.

IV. Conclusion

The performance parameters of the analytical method were satisfactory, hence enabling its application for the simultaneous determination of all mycotoxins regulated in Brazil in corn meal, corn flour, wheat flour, rice and bean. By using the method in 36 samples of food products, high incidences of fumonisins in corn products along with DON in wheat flour were observed, although at concentrations below the Brazilian tolerance limits. Results indicated a low dietary exposure to mycotoxins in the small-scale farms evaluated, although the co-occurrence of fumonisins and DON warrants concern about their incidence in wheat flour in Brazil.

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