2006

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Food Additives and Contaminants, August 2006; 23(8): 826–832

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International shipping of fumonisins from maize extracts on C_{18} sorbent

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(*Received 27 January 2006; revised 14 March 2006; accepted 15 March 2006*)

Abstract
Fumonisins are mycotoxins found in maize. In developing countries, the resources required for analysis are often lacking, and the shipping of maize between countries can be difficult since the importation of plant materials requires permits/inspection to prevent the entry of pests that frequently infest maize. A simple, safe and legal method for shipping maize extracts to the USA was needed to conduct a survey of fumonisins in Central America. The objective was to develop a method for isolating and shipping maize extracts for fumonisin analysis so as to facilitate a survey of fumonisin exposure. The results indicate that fumonisins in acetonitrile:water extracts of maize can be isolated on C_{18} cartridges, held for at least 3 days at 22°C and then an additional 4 days at 4°C before elution and analysis with no losses. This method allows the importation and analysis of maize samples from foreign locations without complications from international safety concerns.

Keywords: *Fumonisin B_1, Fusarium verticillioides, maize, liquid chromatography electrospray ionization mass spectrometry (LC/MS)*

Introduction

Fumonisins are mycotoxins produced by the fungus *Fusarium verticillioides* (formerly *F. moniliforme*). Fumonisin B_1 (FB_1) is the most abundant of the naturally occurring fumonisin congeners (Rheeder et al. 2002).

Fumonisins are a concern for maize producers, processors, consumers, and regulators. The occurrence and contamination of maize is worldwide and fumonisins are known to cause farm animal diseases including equine leucoencephalomalacia and porcine pulmonary oedema (Bolger et al. 2001, and references therein), are possible human carcinogens (International Agency for Research on Cancer (IARC) 2002) and are suspected risk factors for human neural tube defects (Marasas et al. 2004). There are currently several methods used to measure fumonisins in various matrices (Bolger et al. 2001, and references therein). One of the most common methods for quantitative analysis in maize is solvent extraction, solid-phase clean-up and *ortho*-phthalaldehyde plus 2-mercaptoethanol derivatization followed by high-performance liquid chromatography (HPLC) separation and quantification of the fluorescent FB_1 derivative (Shephard 1998, and references therein). Several liquid chromatography electrospray ionization-mass spectrometry (LC/MS) methods have also been developed that are very sensitive and do not require derivatization (Joseph 1996; Musser 1996; Plattner et al. 1996; Plattner 1999).

Exposure to fumonisins occurs through the consumption of maize and maize products. People living in areas of the world where maize consumption is high are at the greatest risk of consuming amounts of fumonisins that exceed the provisional maximal tolerable daily intake proposed by the World Health Organization (WHO)/Food and Agriculture Organization (FAO) Joint Expert Committee on Food Additives (JECFA) (Bolger et al. 2001). These include southern Africa, parts of China, Mexico,

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ISSN 0265–203X print/ISSN 1464–5122 online
DOI: 10.1080/02652030600699650

parts of South America and most of Central America (Marasas et al. 2004). Therefore, the greatest need for accurate exposure assessment, upon which risk management decisions are based, is in countries where the resources for accurate analysis and confirmation are often limited.

The present study was conducted to determine fumonisin stability when isolated from maize extracts on C_{18} solid-phase extraction cartridges. The specific objectives were (1) to develop and validate a method for extracting, isolating, and shipping maize extracts; and (2) to couple the method compatibly with a reproducible and sensitive analytical method.

Materials and methods

Materials and test agents

The analytical standards of FB_1, fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) were a gift from Ronald Plattner (USDA-ARS, Peoria, IL, USA) and the purity (>96%) was determined by the procedure of Plattner and Branham (1994). Working stock solutions at 50 and 5 $\mu g\,\mu l^{-1}$ containing FB_1, FB_2 and FB_3 were prepared in acetonitrile:water (1 : 1). Phytosphingosine (Pso) was obtained from Sigma Chemical Co. (# D2795; St Louis, MO, USA). A 0.010 $\mu g\,\mu l^{-1}$ Pso standard prepared from Pso stock (0.32 $\mu g\,\mu l^{-1}$ in ethanol) was used as an internal quality control check for instrument performance. C_{18} Sep-Pak® Cartridges were obtained from Waters (# WAT051910; Milford MA, USA).

Dry shelled maize used to determine fumonisin extraction efficiency with FB_1, FB_2 and FB_3 spiked samples and as a source of diluent in the stability studies (to be described below) was ground (2 mm) using a Romer Mill (Romer Labs, Union, MO, USA) and extracted and analysed as described below. Two lots of 'clean maize' (CM1 and CM2) and two lots of heavily contaminated maize (HCM1 and HCM2) were prepared. CM1 contained 0.138 ± 0.013, 0.040 ± 0.001, and $0.013 \pm 0.003\,\mu g\,g^{-1}$ maize ($n = 3$) of FB_1, FB_2 and FB_3, respectively; CM2 contained 0.0398 ± 0.0066, 0.0284 ± 0.0036 and $0.0105 \pm 0.0006\,\mu g\,g^{-1}$ maize ($n = 5$) of FB_1, FB_2 and FB_3, respectively.

Dry maize heavily contaminated with *Fusarium verticillioides* and showing varying signs of maize-ear rot was obtained from North Carolina, USA, in 1999. The HCM was hand shelled and segregated so as to contain predominately intact kernels. HCM1 contained 46.8 ± 3.2, 13.5 ± 0.1, and $10.7 \pm 0.3\,\mu g\,g^{-1}$ maize ($n = 3$) of FB_1, FB_2 and FB_3, respectively; HCM2 contained 53.6 ± 5.1, 17.0 ± 0.9, and $11.9 \pm 0.4\,\mu g\,g^{-1}$ maize ($n = 5$) of FB_1, FB_2 and FB_3, respectively. The HCM was used to obtain extracts to use for determining the stability of fumonisins isolated on C_{18} cartridges and for other analyses to determine the compatibility of the C_{18}-cartridge eluates with electrospray ionization (ESI) ion-trap mass spectrometry (LC/MS) as described below.

Liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) of fumonisins

Fumonisins were chromatographically separated essentially as described by Plattner (1999), except that the column was equilibrated for 7 min at 0.2 ml min^{-1} with 30% solvent A (97% acetonitrile : 2% water : 1% formic acid) and 70% solvent B (2% acetonitrile : 97% water : 1% formic acid) and then at injection a gradient began, where 'A' was increased to 60% at 15 min and then 90% at 20 min, and at 22 min the composition was returned to 30% 'A'. The MS detector was tuned in a manner similar to that described previously (Plattner 1999). However, three separate scan segments were used and ($m/z = 722.3$, 706.3 and 318.2) timed so as to isolate FB_1, FB_2 and FB_3, and Pso. The collision energy was 32%.

Quantification was accomplished using external standardization and instrument performance was monitored by spiking extracted samples with Pso. The correlation coefficient (r^2) of the standard curve for each fumonisin was >0.99. The Pso working standard used as an internal quality control check for instrument performance among samples was added to each standard and sample vial so that the final Pso concentration was 0.1 ng Pso/µl of sample. Pso is naturally present in maize kernels. However, after clean-up on C_{18} cartridges, the endogenous levels are much less than the amount of Pso spiked into each sample vial.

Fumonisin extraction procedure and isolation on C_{18} cartridges

The maize extraction procedure and isolation on C_{18} cartridges was similar to that described by Rice et al. (1995). Briefly, dry shelled maize (>1 kg) was ground using a Romer Mill and then carefully mixed 5-g samples were placed in flasks and 25 ml of acetonitrile:water (1 : 1, v/v) were added, mixed, and the pH adjusted to 4.5 with 1 N HCl. The mixture was placed on a reciprocating shaker at high speed and extracted for 16 h at 21–23°C. After 16 h the samples were filtered and then 2 ml of the extract were diluted with 6 ml of water. Up to 20 cartridges were prepared as described by Rice et al. (1995) at one time before loading samples. It is critical not to allow the cartridges to run dry of solvent between rinses since air drying of the C_{18} sorbent will result in the loss of binding capacity.

The diluted samples were slowly loaded onto the cartridges taking care not to allow the cartridges to run dry of solvent. Each cartridge was rinsed sequentially with water followed by 10% acetonitrile in water and then the residual liquid was gently removed from the cartridge using an air filled syringe. The ends of the C_{18} cartridges were wrapped in Parafilm® (American National Can, Greenwich, CT, USA) and then the entire cartridge was wrapped in aluminium foil and individually labelled, and all cartridges were taped onto and wrapped together in brown wrapping paper. To determine how long fumonisins were stable while bound on the C_{18} cartridges, fumonisin-loaded C_{18} cartridges were stored at 22°C for 72 h to simulate 'express' shipping conditions and then for an additional 4 days at 4°C to simulate a worst-case scenario for a delay after receipt at the destination and elution of the fumonisins with 2 ml of 70% acetonitrile in water.

This protocol was chosen to approximate the possible conditions to be encountered during the shipping of loaded C_{18} cartridges from Guatemala City to Athens, GA, USA. Aliquots of the eluates in 70 : 30 acetonitrile:water (v/v) were diluted with formic acid (1.73% in water) so that the final concentration was acetonitrile:water (30 : 70) with 1% formic. Samples were centrifuged at 15 000 rcf and 1 ml was transferred to sample vials that contained 10 µl of a 10 ng µl^{-1} Pso internal standard to make a final concentration of 0.1 ng Pso µl^{-1} of sample. For precision data (per cent coefficient of variation, CV), 1 kg of a sample of clean maize (CM2) and heavily contaminated maize (HCM2) were ground, mixed and 5 g samples ($n = 5$) were extracted, isolated on C_{18} cartridges and analysed as described above.

Spiked maize sample preparation

To determine the extraction efficiency of the method, a total of 1 ml of acetonitrile:water (1 : 1) or the working stock solutions of FB_1, FB_2, and FB_3 in 1 : 1 acetonitrile : water were added to 125 ml flasks containing 5 g of ground CM1 (six flasks for vehicle and each working solution), thoroughly mixed and air dried at 40°C for 16 h. Maize samples were occasionally mixed during drying to ensure uniform distribution of the fumonisins. The target concentration in the ground maize spiked with pure fumonisins was 5 µg of each fumonisin g^{-1} maize. Recoveries of total fumonisins were determined by comparison of the extracts of spiked CM1 to the original working solutions diluted into extracts of CM1 to compensate for matrix effects. The results were corrected for the natural contamination of CM1 with fumonisins.

Preparation of maize extracts for stability and binding studies and for the determination of matrix effects

Five 5-g samples of HCM1 and five 5 g samples of CM1 were extracted. The extract of CM was used to prepare dilutions of the HCM1 that were 0.25 µg FB_1 ml^{-1} (equivalent to extracts of maize with 1.25 mg kg^{-1} FB_1) and 1.75 µg FB_1 ml^{-1} (equivalent to 8.75 mg kg^{-1}). Extracts of HCM1 were also spiked with FB_1, taking into account the natural contamination, so as to approximate maize samples with very high levels of fumonisins (300 mg kg^{-1}). These spiked extracts were used to determine if the C_{18} cartridges were able quantitatively to bind and release fumonisins from maize samples that contained very high levels of fumonisins. The HCM used in these studies also contained FB_2 and FB_3 and the ratio of $FB_1 : FB_2 : FB_3$ was approximately 1 : 0.3 : 0.1. The pooled extracts of HCM and CM used for determining stability and binding were diluted and analysed by LC-MS/MS to determine the exact final concentrations in the maize extracts.

In order to determine if the C_{18} eluates had any effects on the ability to quantitate fumonisin, CM2 extracts were subjected to clean-up on the C_{18} cartridges and then the eluates, processed as described above, were spiked with known amounts of the FB_1, FB_2, FB_3 and Pso solutions used for preparing analytical standards (final concentrations 0.01, 0.1 and 1 ng µl^{-1}). A total of four samples were prepared at each concentration, and the calculated amounts were compared with those of standards prepared in the initial mobile phase (30% 'A' and 70% 'B').

Analysis of samples collected in Guatemala, extracted, isolated on C_{18} sorbent and shipped

In order to test the procedure in a 'real-world' situation, samples were purchased or received as a courtesy from the shop owners of large grain shops in Guatemala City (Department of Guatemala, $n = 16$). Each sample comprised approximately 1 kg of dry shelled maize and was placed in individual bags and delivered to the Instituto de Nutricion de Centro America y Panama, where it was stored dry at –20°C until the extraction of fumonisins. Each collected sample was ground using a Romer Mill and then carefully mixed, and a 5 g sample was extracted and the fumonisins isolated on C_{18} cartridges as described above. The C_{18} cartridges were shipped by express mail and were received 2 days after extraction and isolation. Upon receipt, the samples were refrigerated (4°C) and then on day 3 were eluted and analysed by LC-MS/MS.

Statistical analysis

Statistical analysis was performed using Sigma Stat software (Jandel Scientific, San Rafael, CA, USA). One-way analysis of variance (ANOVA) was used followed by tests for post-hoc multiple comparisons where appropriate. All data were expressed as mean ± standard deviation (SD), and differences among means were considered significant if the probability (p) was <0.05.

Results and discussion

Under the chromatographic conditions used, FB_1, FB_2, FB_3 and Pso were easily resolved and confirmed by their mass spectra. The approximate retention times for FB_1, FB_2, FB_3 and Pso were 8.5, 11.5, 10.5 and 17 min, respectively. Based on the peak areas, the response factors for the three fumonisins were similar (Figure 1). The lower detection limit for FB_1, FB_2 and FB_3 was less than 0.012 ng injected (equivalent to 0.007 $\mu g\,g^{-1}$ in maize), and the response was linear up to about 400 ng injected (equivalent to 233 $\mu g\,g^{-1}$ in maize), after which the response was no longer linear (Figure 1). When amounts greater than 12 ng were injected, fumonisins were detected in subsequent solvent blanks (Figure 1). This has been reported previously and is attributed to the tendency of fumonisins to be reversibly adsorbed by the column-packing material (Plattner et al. 1996). The fact that the Pso response was constant over the entire range of injected fumonisins suggests that the decreased response seen at the highest levels of injected fumonisins was not a result of overloading the capacity of the HPLC column, but most likely a consequence of the concentration of ions entering the ion trap being so great that the ion trap overfilled, leading to space charging, with the net result being a drop off in linearity. Nonetheless, the LC-MS/MS method using the LCQ™ Duo was linear over four orders of range, which is more than sufficient to allow the accurate quantification of fumonisins in naturally contaminated maize.

Pso is a natural component of plant tissues and low levels were detected in the C_{18}-cartridge eluates of both CM and HCM (Figure 2). The HCM contained significantly more Pso than the CM, which would not be surprising in growing plants, but which is unexpected in ungerminated maize kernels. Fumonisins are inhibitors of ceramide synthase in maize seedling and cause the accumulation in roots of both Pso and sphinganine, which is the precursor of Pso (Riley et al. 1996). Regardless, the amount of Pso added to C_{18}-cartridge eluates was nine to 34 times greater than the contribution from endogenous Pso when injected. Under the conditions optimized for FB_1, the Pso response is less than that for the fumonisins, but it is still linear over a wide concentration range (Figure 2, inset). The addition of the Pso has proven useful when monitoring instrument performance. For example, partial plugging of the heated capillary, forward movement of the fused silica sample tube in the ESI needle, or build up of residual materials on the tube lens and skimmer.

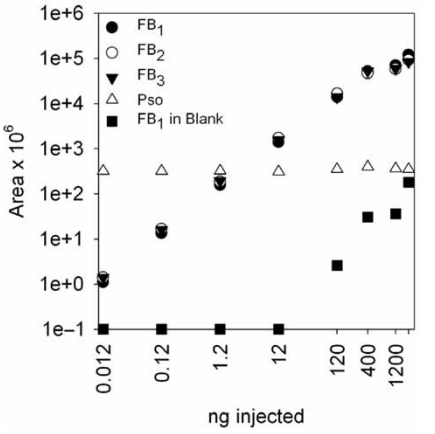

Figure 1. Quantitation of fumonisins B_1 (FB_1), FB_2 and FB_3 by liquid chromatography coupled with tandem mass spectrometry detection (LC-MS/MS) and carry over of FB_1 in the solvent blanks injected following each sample concentration (a 20-µl injection ranging from 0.0006 to 100 ng μl^{-1}). Also shown is the response of the phytosphingosine (Pso) at 0.1 ng μl^{-1} injected with each sample. The standards were prepared by adding known amounts of fumonisin stock solutions to 1 ml of the initial mobile phase (30% A : 70% B).

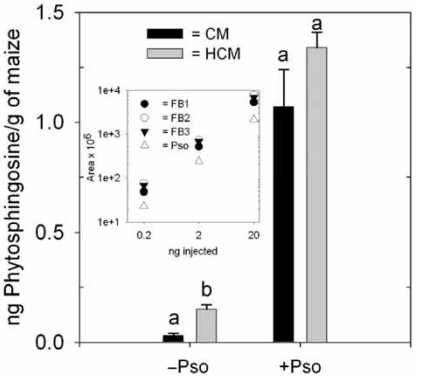

Figure 2. Phytosphingosine levels in clean maize (CM) and heavily contaminated maize (HCM) extracts after clean-up on C_{18} cartridges and prepared for injection without added phytosphingosine (–Pso) and with added phytosphingosine (+Pso), as described in the Materials and methods. Values are the mean ± SD ($n = 3$ extracts) and means within treatments with differing letter superscripts were significantly different ($p < 0.05$). The inset shows the response (peak areas) for the injection of standards containing various amounts of fumonisins (FB_1, FB_2, FB_3) and phytosphingosine (Pso).

The eluate from the C_{18} cartridges did not interfere with the LC/MS detection of fumonisins (Figure 3). There was no significant difference between the amounts of FB_1 quantified in the sample solvent (standards) and in the C_{18}-cartridge eluates of CM spiked with the same amounts of fumonisins (Figure 3). In addition, while instrument performance was often adversely affected (plugging of the heated capillary) after multiple injections of the CM extracts that were not cleaned-up on the C_{18} cartridges, the C_{18}-cartridge eluates of the CM had no appreciable effect on instrument performance after 16 sequential injections (Table I). More recently, up to 40 samples have been injected sequentially with no apparent adverse effect on instrument performance. The precision analysis for FB_1 using C_{18}-cartridge eluates of extracts (5 g samples; $n = 5$) of maize containing an average of 0.04, 1.23, 9.1 and 53.6 $\mu g\,g^{-1}$ showed that the coefficient of variation (per cent of SD) was 17, 5, 3 and 9%, respectively. The results were similar for FB_2 and FB_3 (data not shown).

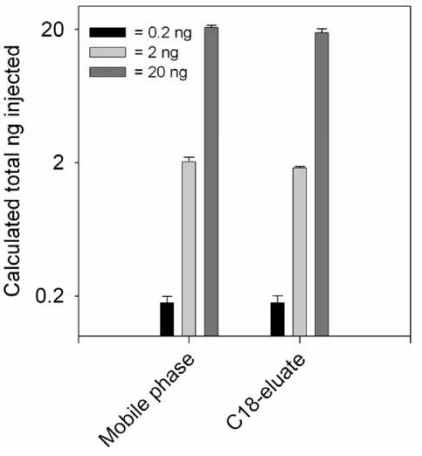

Figure 3. Calculated total injected nanograms of FB_1 in samples ($n = 4$) dissolved in the initial mobile phase or in C_{18}-cartridge eluates so as to contain 0.2, 2.0 or 20 ng total FB_1 in a 20-µl injection. Similar results were obtained for FB_2, FB_3 and Pso (data not shown). Values are means ± SD.

The recoveries of FB_1, FB_3 and FB_2 from maize samples ($n = 6$) spiked with fumonisins at a level equivalent to 5 µg of each fumonisin g^{-1} maize were 57% ± 3.7%, 63.5% ± 4.5% and 68.9% ± 5.3% for FB_1, FB_3 and FB_2, respectively. The recoveries from the spiked maize samples were quantified using standards prepared from stock solutions dissolved in unspiked maize extracts and corrected for their natural fumonisin content. The mean recovery of FB_2 was significantly ($p \leq 0.05$) greater than that of FB_1 and FB_3 This may be attributed to the fact that FB_1 is more polar than FB_2, which has one less hydroxyl group, and presumably FB_2 is less polar than FB_3, as evidenced by the fact that the elution of FB_3 and FB_2 occur at approximately 48 and 54% of acetonitrile, respectively, in the mobile phase based on their retention times. These differences in polarity could result in slight differences in the extractability of the fumonisins into the acetonitrile : water extraction solvent. However, it is also possible that the heating of the spiked maize samples to 40°C for 16 h resulted in FB_1 being more

Table I. Results of the analysis of 16 maize samples collected from markets in Guatemala City, then extracted and isolated on C_{18} cartridges and express-mailed to the USA for elution and analysis by liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS). The final concentration for each fumonisin is corrected for the extraction efficiencies.

Sample number	FB_1 ($\mu g\,g^{-1}$)	FB_2 ($\mu g\,g^{-1}$)	FB_3 ($\mu g\,g^{-1}$)	($FB_1 + FB_2 + FB_3$)	Pso[a] ($ng\,ml^{-1}$)
1	13.49	3.51	1.35	1.36	0.090
2	1.76	0.33	0.17	1.28	0.082
3	2.53	0.42	0.17	1.23	0.088
4	3.16	0.76	0.28	1.33	0.099
5	0.81	0.14	0.06	1.25	0.111
6	6.65	1.17	0.74	1.29	0.121
7	7.02	1.34	0.83	1.31	0.110
8	1.42	0.27	0.10	1.26	0.074
9	1.04	0.14	0.06	1.19	0.074
10	5.81	1.49	0.43	1.33	0.094
11	0.48	0.07	0.02	1.20	0.121
12	0.99	0.20	0.11	1.31	0.110
13	19.34	7.26	1.83	1.47	0.110
14	0.12	0.03	0.02	1.25	0.114
15	0.60	0.11	0.04	1.25	0.122
16	0.56	0.12	0.03	1.26	0.104
Mean	4.11	1.08	0.39	1.29	0.102
SD	(5.40)	(1.87)	(0.54)	(0.07)	(0.016)

[a] Concentration of phytosphingosine spiked into each vial was 0.1 $ng\,\mu l^{-1}$ and was used as an indicator of instrument performance.

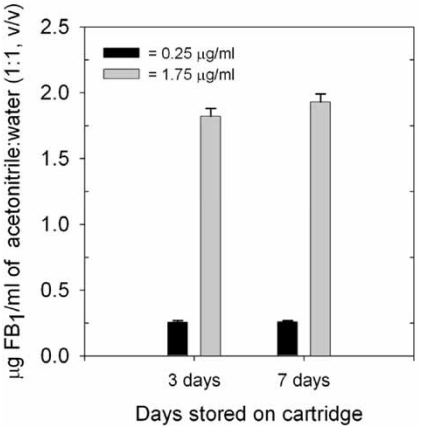

Figure 4. Stability of FB_1 extracted from maize and then loaded onto C_{18} cartridges and stored at 22°C for 3 days ($n = 5$/concentration) and then an additional 4 days at 4°C ($n = 5$/concentration) for a total of 7 days. The concentrations of fumonisin in the solutions loaded on the cartridges were 0.25 and 1.75 μg ml^{-1}, which were equivalent to maize contaminated at 1.25 and 8.75 μg FB_1 g^{-1} maize, respectively. The amount of FB_1 in the eluate following the solid-phase clean-up was compared with the FB_1 content of the original solutions used for loading the columns. The eluates containing the high concentration of FB_1 were diluted with the initial mobile phase before analysis. Values are the mean ± SD.

tightly bound to compounds in the maize matrix compared with the binding of FB_2 and FB_3. The average recoveries in this study were less than earlier studies using methanol:water (3 : 1, v/v), where mean recoveries ranged from 76 to 87% (Sydenham et al. 1996), and acetonitrile : water (1 : 1, v/v), where mean recoveries ranged from 81 to 100% for FB_1, FB_2 and FB_3 (Rice et al. 1995). Nonetheless, using acetonitrile:water extraction in the present study, the coefficients of variation (expressed as SD as a per cent of the mean) were less than 10%, which indicated that the method was reproducible and the compatibility of the acetonitrile with the subsequent isolation on C_{18} cartridges and the desire to avoid methylation of the fumonisins during storage and shipping led the authors to prefer acetonitrile as an extraction solvent.

Once loaded onto C_{18} cartridges, fumonisins from maize extracts are stable for at least 3 days at 22°C and an additional 4 days at 4°C at fumonisin concentrations, which were equivalent to 1.25 and 8.75 mg kg^{-1} FB_1 in maize, respectively (Figure 4). The mean recoveries of FB_1, FB_2 and FB_3 from the C_{18} cartridges ranged from 102% ± 5% to 110% ± 11% of what was loaded onto the cartridges (data for FB_2 and FB_3 not shown). The recoveries were very close to the predicted recoveries based on the analysis of the maize extracts. In the early stages of developing the protocol for loading the C_{18} cartridges, it was discovered that it is critical that columns are not allowed to become dry during the equilibration with solvents before the maize extracts are loaded. If the column is allowed to become dry, then the binding capacity is reduced and recoveries will not be quantitative. According to the manufacture (Waters, Milford MA, USA), the reduced recoveries are due to the hydrophobic collapse of the bonded phase of the C_{18} cartridge. When the C_{18} cartridges were loaded with maize extracts containing FB_1 at a concentration equivalent to maize contaminated at 300 mg kg^{-1}, 98% ± 3.2% ($n = 3$) of the FB_1 was recovered in the eluate, which indicates that extracts of naturally contaminated maize samples would seldom, if ever, be able to overload the fumonisin-holding capacity of the C_{18} cartridge.

All 16 samples collected from the markets in Guatemala City contained detectable levels of FB_1, FB_2 and FB_3 (Table I). The total fumonisins ($FB_1 + FB_2 + FB_3$) in the samples ranged from 0.17 to 28.4 mg kg^{-1}. The 16 samples were loaded in the autosampler and injected in the sequence shown in Table I. The levels of Pso showed no trend indicative of decreasing instrument performance over the course of the sequence. All the samples obtained at the markets in Guatemala City were intended to be sold for human consumption and were harvested between November 2004 and January 2005 from various locations. The results show that fumonisins are present in maize consumed in Guatemala.

In conclusion, a simple, safe and legal method for shipping fumonisins from extracts of maize bound to C_{18} cartridges for fumonisin analysis has been developed and is compatible with analysis by ion-trap LC/MS. While strong anion exchange columns are also frequently used to bind fumonisins, and may also serve as a way to ship samples, the advantage of the C_{18} cartridge is that it can also be used to isolate hydrolysed fumonisins (Plattner 1999), which are frequently found in maize products consumed in Guatemala (Meredith et al. 1999; Palencia et al. 2003). The results indicate that fumonisins in acetonitrile : water extracts of maize can be isolated on C_{18} cartridges, held for at least 3 days at 22°C and then an additional 4 days at 4°C before elution and analysis with no losses. This method will facilitate the importation and analysis of a large number of maize samples from distant foreign locations and will avoid complications arising from international safety concerns and obviates the requirement for US Animal Plant Health Inspection Service permits. This approach may also be useful for shipping samples into other countries where the importation of plant materials is restricted by law. In addition, the eluates from the C_{18}

cartridges are compatible with analysis by ESI ion-trap LC/MS.

Acknowledgement

The authors thank Mrs A. Jency Showker for expert technical assistance and good humour. The work was supported by the US Department of Agriculture (USDA) FAS Grant No. X01-4510-62-751071-4, by the International Life Science Institute of North America Technical Committee on Food Toxicology and Safety Assessment, and by the Pan American Health Organization.

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