Determination of Fumonisins in Rodent Feed Using HPLC with Electrospray Mass Spectrometric Detection

Mona I. Churchwell, Willie Mae Cooper, Paul C. Howard, and Daniel R. Doerge*

National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, Arkansas 72079

A method based on liquid chromatography with electrospray ionization mass spectrometry was validated for analysis of low-level contamination of rodent feed by fumonisins B_1 (10−200 ppb) and also applied to the analysis of fumonisin B_2 and B_3. The quantitation limit for a fumonisin B_1 standard was 1.1 ppb using the protonated molecule signal (*m*/*z* 722), and similar sensitivity was observed for protonated molecules for fumonisins B_2 and B_3 in feed. To increase specificity of the mass spectrometric analysis, two fragment ions for fumonisin B_1 (*m*/*z* 352 and 334) were also acquired by using rapid sampling cone-skimmer potential switching in concert with acquisition of the respective ion. This permitted the use of ion intensity ratios for structural confirmation of fumonisin B_1 at levels >10 ppb. Intra-assay precision for fumonisin B_1 determination in feed (6−16% relative standard deviation) was comparable to inter-assay precision (7.7−16.0%) and appeared to be affected most by the variability in recovery of analytes from the immunoaffinity cleanup column. A survey of fumonisin contamination in rodent feed from 12 U.S. biomedical research institutions showed median contamination of 237 ppb and a range from 46 to 923 ppb of total fumonisin B congeners.

Keywords: *Fumonisin; electrospray; mass spectrometry; HPLC; rodent feed*

INTRODUCTION

Contamination of food and feed products derived from corn by fumonisin B congeners, produced by *Fusarium* species, has been well established (Shephard et al., 1996; Hopmans and Murphy, 1993). Liquid chromatography (LC)-based procedures are the standard for quantifying fumonisin levels in foods and animal feeds, and a collaborated method for fumonisins B_1, B_2, and B_3 in corn was recently accepted by the Association of Official Analytical Chemists (Sydenham et al., 1996). The procedure used aqueous methanolic extraction of ground corn, with solid phase extraction cleanup, followed by precolumn *o*-phthalic dialdehyde (OPA) derivatization and LC determination using fluorescence detection. Other uses of precolumn fluorogenic labeling by fluorenylmethyl chloroformate (Holcomb et al., 1993), OPA (Hopmans and Murphy, 1993), and naphthalene-2,3-dicarboxaldehyde (Ware et al., 1994) to quantify fumonisins in rodent feed have been reported previously. These procedures have minimum detection limits for fumonisin B_1 in rodent feed of 50−200 ppb, in part due to the presence of fluorescent interferences.

Fumonisin exposure in the diet of humans and food-producing animals is a toxicological and regulatory concern because of demonstrable toxicity to several animal species from pure fumonisin B_1 (Haschek et al., 1992; Riley et al., 1993; Kellerman et al., 1990; Gelderblom et al., 1991) and is associated with increased risk for esophageal cancer in humans (Sydenham et al., 1991; Rheeder et al., 1992). Due to the worldwide consumption of grain products containing fumonisins, ascertaining the effects on human health is critically important. Understanding the overall risk of fumonisins to humans requires not only accurate biological assessment of the toxicity and tumorigenicity in animal models but also accurate and sensitive analytical methods to establish the level of human exposure.

The long-term testing of fumonisin B_1 toxicity or carcinogenicity in rodents is dependent on feed of low, defined fumonisin content. A previous study observed undetectable or trace levels of fumonisin B_1 in 29 rodent feed samples using methodology with a minimum detectable limit of ca. 1 ppm (Chamberlain et al., 1993). In our preliminary rodent toxicity studies, low parts per million levels of fumonisin B_1 caused detectable alterations in sphingolipid biosynthesis as measured by the ratio of sphingoid bases (Howard et al., 1996). Because this endpoint is directly linked to the apoptotic hepato- and nephrotoxicity induced by fumonisin B_1 (Tolleson et al., 1996; Bucci and Howard, 1996), an association with long-term carcinogenic endpoints is likely. On the basis of these observations, a limit of 100 ppb of fumonisin B_1 and 200 ppb of total fumonisin B congeners (fumonisin B_1 + fumonisin B_2 + fumonisin B_3) was established for the untreated control feed for the ongoing studies at this institution. These maximum acceptable levels in feed were not compatible with the sensitivity provided by fluorogenic labeling/LC methods, even in conjunction with immunoaffinity (IAC) column cleanup.

On the basis of our previous work, we have optimized an LC−electrospray ionization (ESI)/MS method (Doerge et al., 1994) to achieve the sensitivity required for analysis of rodent feed. This approach is consistent with several recent publications that described the use of electrospray ionization mass spectrometry to analyze pure fumonisins (Musser, 1996), fumonisins in contaminated corn cultures (Plattner et al., 1996), and fumonisins B_1 and B_2 in corn-derived foods (Lukacs et al., 1996).

MATERIALS AND METHODS

Reagents and Materials. Authentic standards of fumonisins B_1, B_2, and B_3 were obtained from R. Eppley and S. Page, Center for Food Safety and Applied Nutrition, Food and

* Author to whom correspondence should be addressed [telephone (501) 543-7943; fax (501) 543-7720; e-mail DDOERGE@NCTR.FDA.GOV].

S0021-8561(97)00021-6

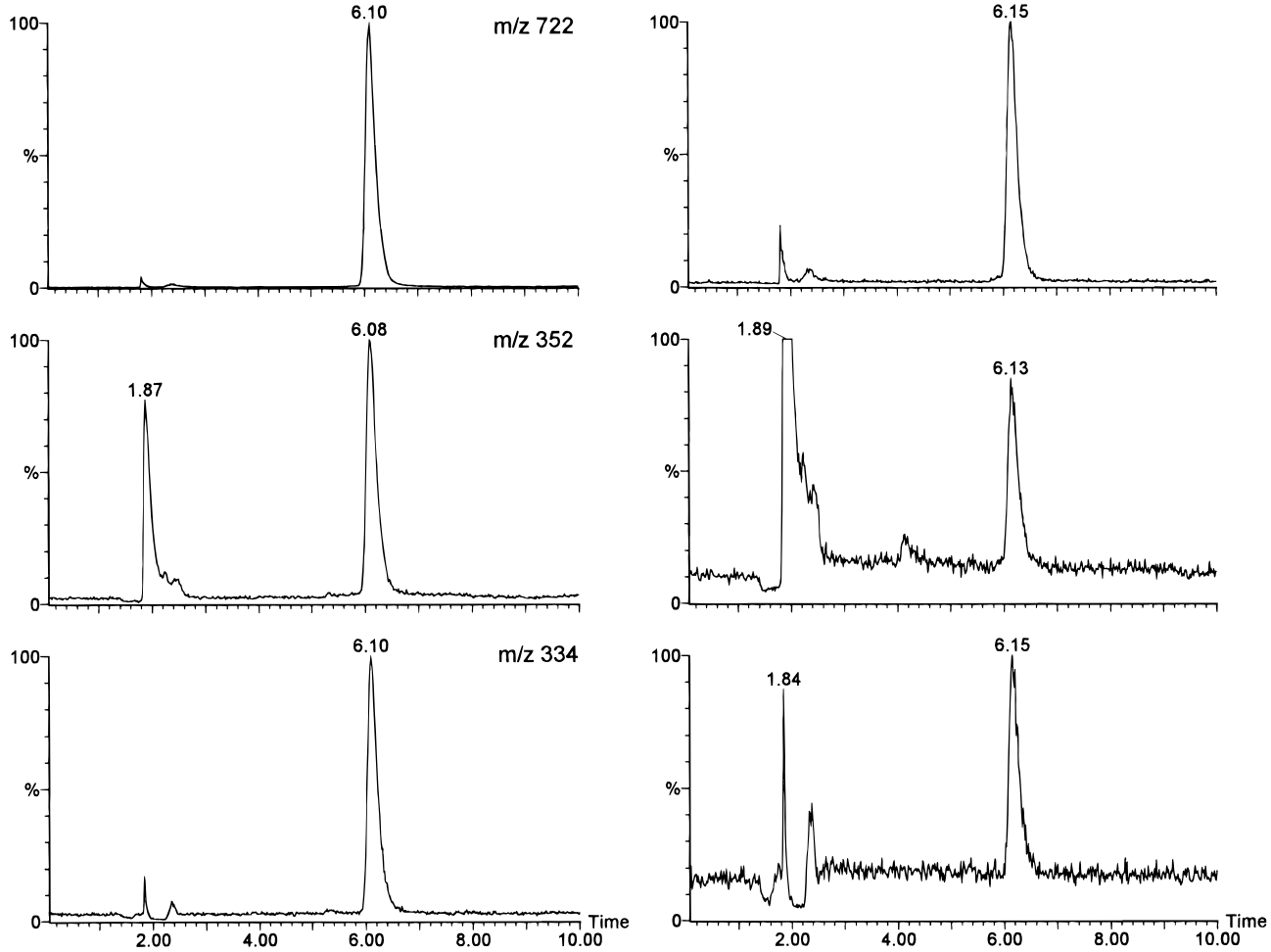

Figure 1. LC-ESI/MS chromatogram for protonated molecule and fragment ions for fumonisin B_1 in feed (right panel, 17 ppb) and the same feed sample spiked with an additional 100 ppb of authentic fumonisin B_1 (left panel).

Drug Administration (Washington, DC). HPLC-grade solvents and analytical-grade reagents, purchased from commercially available sources, and MilliQ water (Millipore Co., Milford, MA), were used for sample preparation, extraction, and analysis. FumoniTest immunoaffinity columns were obtained from Vicam Co. (Somerville, MA). Rodent feed (NIH-31) was obtained from Ralston Purina Co. (St. Louis, MO). NIH-31 feed is an open formula chow that contains approximately 20% by weight whole corn.

Safety Precautions. Fumonisins interfere with lipid metabolism in a number of organs and are toxic to many animal species. Solid fumonisins are electrostatic, and the spread of fine particles through the air as dust is common. Weighing of all fumonisins for preparation of standards was performed in a sealed glovebox that was subsequently decontaminated using water rinsing.

Sample Preparation and Extraction. NIH-31 feed was used for all analyses except those acquired from other institutions (eight types of feed from five manufacturers). Feed samples were prepared and extracted essentially as described by the FumoniTest manufacturer using a single lot of NIH-31 feed. According to the manufacturer's specifications, the cartridges have a capacity for up to 2 μg of total fumonisins in feed extracts. Approximately 2 kg of commercial feed pellets was ground and thoroughly mixed in a V-blender for 30 min. A 10 g portion was extracted with 40 mL of acetonitrile/water (50/50) in a 250 mL blender cup for 2 min. A 20 mL aliquot of this extract was centrifuged at approximately 5000*g* for 5 min, and then 2 mL of the extract was added to 8 mL of dilution solution (2.5% sodium chloride and 0.5% sodium bicarbonate in water) and the diluted sample again centrifuged for 5 min. The entire 10 mL sample was passed through the FumoniTest column. The column was then washed with 5 mL of dilution solution followed by 5 mL of water, and the fumonisin B congeners were eluted with 3.5 mL of methanol. The eluate plus 300 μL of water was evaporated to about 300 μL and then brought up to 1 mL with water. This procedure results in 0.5 g feed equivalent in 1 mL of solution that is ready for injection. Spiked feed samples were prepared according to the same procedure with the fumonisins added to the feed immediately before the acetonitrile/water extraction.

Liquid Chromatography. The LC separation was performed with a GP40 gradient pump (Dionex, Sunnyvale, CA) using an Ultracarb 3 ODS (20) column (2.0 × 150 mm, 3 μm, Phenomenex, Torrance, CA) and an isocratic mobile phase consisting of 55% 0.1% formic acid and 45% acetonitrile, at a flow rate of 0.2 mL/mn. A Dionex Model AS3500 autosampler equipped with a 100 μL loop was used to inject 25 μL aliquots onto the LC column.

Mass Spectrometry. A Platform II single quadrupole mass spectrometer (Micromass, Altrincham, U.K.) equipped with an atmospheric pressure ionization source and electrospray interface was used for the mass spectrometry analyses. The total LC column effluent was delivered into the ion source (150 °C) through the electrospray capillary (3.3 kV), using nitrogen as the nebulizing and drying gas. Positive ions were acquired in selected-ion monitoring mode (dwell time 0.3 s, span 0.02 u, and interchannel delay time 0.03 s) while the sampling cone-skimmer voltage was varied between 40 and 70 V to produce in-source CID simultaneously with the acquisition of the corresponding selected ion. In-source CID produces fragment ions in the intermediate pressure region between the atmospheric pressure ion source and the quadrupole mass analyzer. Fragmentation occurs by collisions with neutral molecules in the transport region in a manner analogous to that observed with the inert gas present in the collision cell of a triple quadrupole mass spectrometer. The primary difference is the inability to maintain as precise pressure

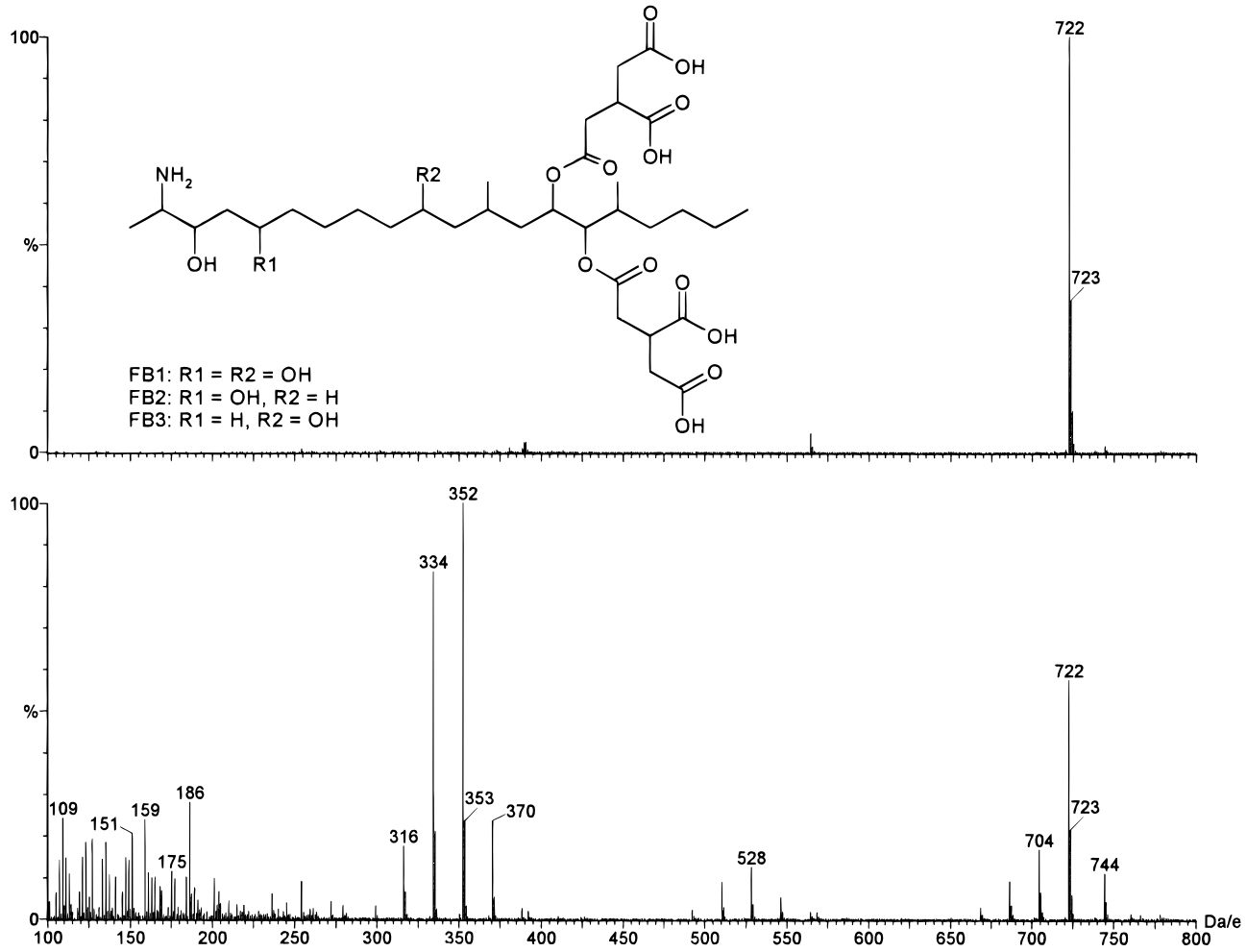

Figure 2. ESI mass spectrum of fumonisin B_1 at different sampling cone-skimmer potentials. The spectrum in the top panel was acquired at 40 V and that in the bottom panel at 70 V.

control as is possible in a collision cell. In most cases, identical fragment ions are observed regardless of the CID technique used (Doerge and Bajic, 1992; Doerge et al., 1996a,b). The protonated molecules for fumonisin B_1 (*m*/*z* 722) and fumonisins B_2 and B_3 (both *m*/*z* 706) were acquired at 40 V, while two fragment ions for fumonisin B_1 (*m*/*z* 352 and 334) were acquired at 70 V.

A 10−20% decrease in mass spectrometric responses of standards and sample extracts was often observed during analysis of many feed samples throughout the day. This was presumably the result of undefined changes in source or probe conditions. To compensate for these changes, feed sample injections were bracketed in the autosampler method with injections of an authentic standard containing an amount of fumonisin B_1 equivalent to 100 ppb that had been passed through an IAC column. The standard responses before and after a set of four feed samples were averaged, and this response was used to normalize responses throughout the entire sample set. This procedure had the effect of compensating for losses in analyte through the IAC step and changes in instrument response throughout an analysis. These complications could be obviated through the use of labeled internal standards. Deuteriated fumonisin B_1 has been prepared (Plattner and Branham, 1994) and used for food analysis (Lucacs et al., 1996), although a commercial source is not yet available.

RESULTS AND DISCUSSION

A previous paper from this laboratory described the determination of fumonisin B congeners in corn meal using LC−ESI/MS (Doerge et al., 1994). Because the solid phase extraction procedures used were insufficient to remove significant mass spectral interferences, this method was further optimized to yield the enhanced sensitivity and specificity required for analysis of rodent feed as reported here. Specifically, the interferences from the feed matrix were substantially reduced through the use of off-line immunoaffinity chromatography cleanup, a high-efficiency isocratic reversed phase analytical separation that improved peak shape, and rapid sampling cone-skimmer potential switching that was used to acquire simultaneously signals for protonated molecule and fragment ions of fumonisin B_1 along with protonated molecules for fumonisins B_2 and B_3. Figure 1 shows the selected ion chromatograms from feed contaminated with a low level of fumonisin B_1 (ca. 15 ppb) and the same feed sample spiked with additional fumonisin B_1 at 100 ppb. The detection limit determined for fumonisin B_1 standards, using the protonated molecule signal at *m*/*z* 722, was estimated to be equivalent to 0.3 ppb (s/n = 3), and the limit of quantification (s/n = 10) was equivalent to 1.1 ppb. Endogenous levels of fumonisins in feed always exceeded 10 ppb, so minimum detectability was never a constraint.

Figure 2 shows the mass spectra of fumonisin B_1 at two sampling cone-skimmer potentials. At low voltage (40 V), the soft ionization and minimal CID yield a spectrum consisting of the protonated molecule (*m*/*z* 722). At 70 V, characteristic fragment ions were formed that resulted from successive losses of 0−3 water units from the protonated molecule (*m*/*z* 722, 704, 686, and

Table 1. Extraction Efficiency for Fumonisins Spiked into Feed[a]

fumonisin	recovery (mean ± SD)
fumonisin B_1 ($n = 11$)	68.9 ± 11.4
fumonisin B_2 ($n = 3$)	60.4 ± 2.7
fumonisin B_3 ($n = 15$)	74.3 ± 9.2

[a] Feed was spiked with 100 ppb of the fumonisin indicated, and replicate samples were extracted and analyzed.

668) and the molecule minus one (*m*/*z* 546, 528, 510, and 492) or two (*m*/*z* 370, 352, 334, and 316) tricarballylic side chains (Δm = 176 Da). To increase the specificity of the selected ion monitoring method, along with its inherent enhanced sensitivity, signals for the protonated molecule and the two most intense fragment ions (*m*/*z* 334 and 352) for fumonisin B_1 were acquired using a rapid cone voltage switching method previously described (Doerge et al., 1996a,b). Figure 1 shows the responses for the selected ions in a feed sample. In this method, the protonated molecule and fragments are acquired under conditions that maximize the respective signal and provide ion intensity ratios to use for confirmation of structure simultaneous with high sensitivity detection. The intensity for fragment ions signals at any cone voltage could not be increased above about 20% of that observed for the protonated molecule at low voltage. This resulted in deviation of ion intensity ratios determined for very low levels of fumonisin B_1. For this reason, the detection limit for structural confirmation of fumonisin B_1 was estimated to be 10 ppb. Ion intensity ratios, compared for fumonisin B_1 in an authentic standard and in contaminated feed, showed accurate agreement (within 10%, not shown). The ion corresponding to the protonated molecule for fumonisin B_2 and fumonisin B_3 (*m*/*z* 706) was added to the method to perform multicomponent analysis. Addition of this ion had minimal effect on the sensitivity for fumonisin B_1.

The protonated molecule signal intensities observed for fumonisins B_1, B_2, and B_3 were similar (relative slope of calibration curves 1.0, 0.91, and 1.56, respectively). Furthermore, recovery of standards through the IAC cleanup column was similar for the three compounds (see Table 1). The chromatogram in Figure 3 shows responses for fumonisin B congeners present in a contaminated feed sample. The amounts of fumonisin B congeners present in this sample were determined using the relative responses listed above to be 104, 36, and 9 ppb, respectively. Although not determined precisely, detection limits for fumonisins B_2 and B_3 are similar to that determined for fumonisin B_1 on the basis of the observed slopes from calibration curves of standards.

Method performance was investigated using the method of standard additions, i.e., addition of a known amount of standard to a sample. Feed contaminated with a very low level of fumonisins was spiked with various amounts of authentic fumonisin B_1, and precision was determined by replicate extraction and analysis of samples at many spike levels. Table 2 shows the intra-assay precision determined from spiking feed with various levels of fumonisin B_1. Similar results were obtained from two other experiments (see below). Table 1 also shows that the variability associated with the analysis probably arises from variability in recovery from the IAC column. While the MS responses from fumonisin B_1 standards injected directly showed small variability (RSD = 2%), responses for the same amount

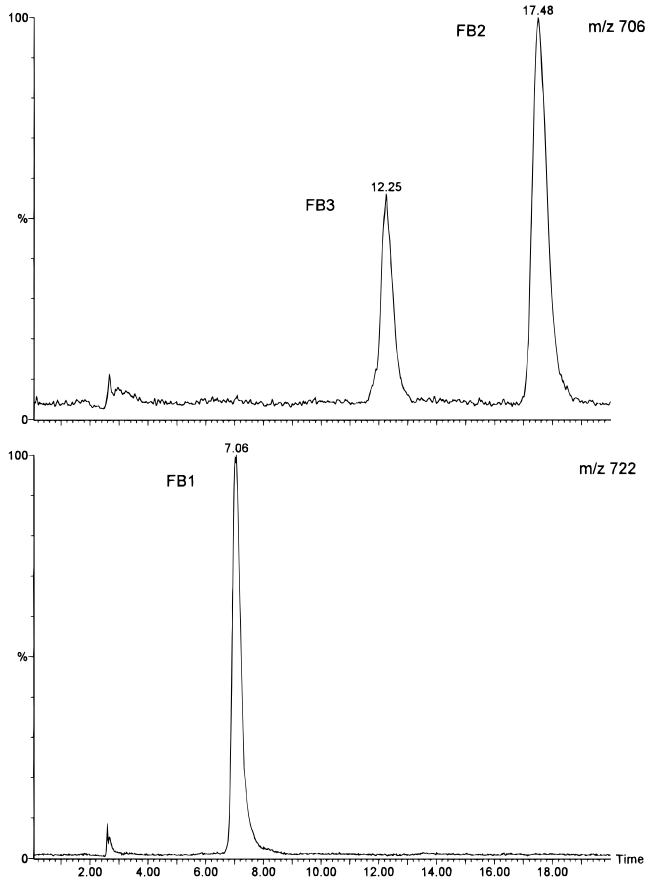

Figure 3. LC-ESI/MS chromatograms for fumonisins in feed. Responses for the respective protonated molecules (fumonisin B_1 = 722 Da; fumonisin B_2 = fumonisin B_3 = 706 Da) in contaminated feed are shown as indicated. The feed sample contained 104, 36, and 9 ppb of fumonisin B_1, B_2, and B_3, respectively.

Table 2. Intraassay Precision for Analysis of Fumonisins in Feed[a]

sample	RSD (%)
fumonisin B_1 standard (100 ppb) ($n = 4$)	2.2
fumonisin B_1 standard through IAC (100 ppb) ($n = 4$)	7.7
feed containing 17 ppb of fumonisin B_1 ($n = 4$)	14.9
feed spiked with 10 ppb of fumonisin B_1 ($n = 4$)	16.2
feed spiked with 50 ppb of fumonisin B_1 ($n = 4$)	9.9
feed spiked with 100 ppb of fumonisin B_1 ($n = 4$)	11.5
feed spiked with 200 ppb of fumonisin B_1 ($n = 4$)	5.8
feed spiked with 100 ppb of fumonisin B_2 ($n = 3$)	4.5
feed spiked with 100 ppb of fumonisin B_3 ($n = 9$)	11.6

[a] Feed containing 17 ppb of fumonisin B_1 was spiked with additional fumonisin as indicated and extracted in replicate on a single day.

of fumonisin B_1 applied to and subsequently eluted from the IAC column showed much higher variability (RSD = 8%). This variability was similar to that observed for a feed sample spiked with a comparable amount of fumonisin B_1 (RSD = 10%), which was extracted and subjected to IAC cleanup. As the total amount of fumonisin B_1 increased, the variability showed a declining trend. Similar precision was observed for feed spiked with fumonisin B_3 (see Table 1), although the fumonisin B_2 data represent a limited sample set. These results are similar to those reported by Ware et al. (1994), who observed RSD of approximately 10% for fumonisins B_1 and B_2 when using the same commercial IAC columns for cleanup of corn spiked with 50−1000 ppb of fumonisin B_1 (Ware et al., 1994).

Table 3. Interassay Precision for Analysis of Fumonisin B_1 Spiked into Feed[a]

	nominal fumonisin B_1				
	0 ppb	10 ppb	50 ppb	100 ppb	200 ppb
mean ± SD	17.0 ± 1.3	10.0 ± 1.6	40.0 ± 5.3	97.7 ± 10.7	198.7
RSD	7.7	16.0	13.3	11.0	

[a] Rodent feed containing 17 ppb of fumonisin B_1 was spiked with the amounts of authentic fumonisin B_1 indicated on two or three different dates and the total fumonisin B_1 content determined as described under Materials and Methods. The endogenous fumonisin content was determined from the unspiked feed, and the nominal amount of fumonisin B_1 was calculated by subtracting the endogenous component from the total amount observed.

Table 4. Survey of Fumonisins in Rodent Feed from Different U.S. Biomedical Research Institutions[a]

source	date collected	fumonisin B_1	fumonisin B_2	fumonisin B_3	total
A	July 9, 1996	635.1	180.8	52.1	868.0
B	–	229.6	67.5	19.6	316.7
C	–	663.7	204.2	54.7	922.6
D	May 14, 1996	104.9	46.0	9.6	160.5
E	Aug 1, 1996	32.3	11.2	2.6	46.1
F	June 10, 1996	42.0	14.7	3.6	60.3
G	July 3, 1996	61.7	16.3	4.2	82.2
H	Feb 1, 1996	286.3	108.1	24.3	418.7
I	Jan 2, 1996	219.3	95.9	19.9	335.1
J	June 22, 1996	144.8	53.0	12.5	210.3
K	July 31, 1996	179.4	60.0	17.2	256.6
L	July 31, 1996	59.9	23.0	ND	82.9

[a] Fumonisin concentrations in ppb are listed for 12 feed samples from 5 manufacturers that were analyzed once. Values shown are corrected for recovery through the IAC step.

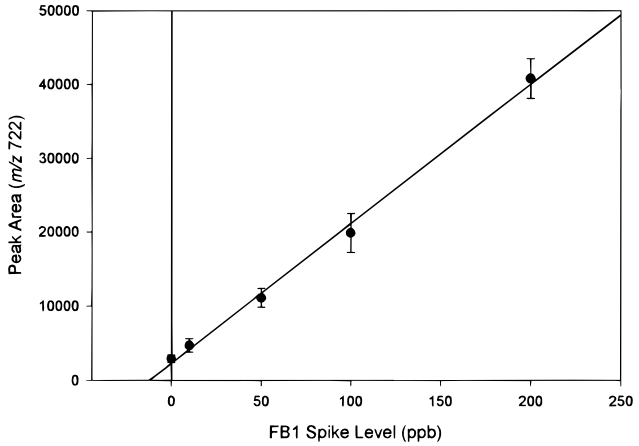

Figure 4. Standard addition plot for fumonisin B_1 in feed. The feed sample shown in Figure 1 was spiked with various amounts of fumonisin B_1, and the responses were determined in quadruplicate for each level. The points plotted are the means ± standard deviation, the correlation coefficient is >0.99, and the amount of fumonisin B_1 determined in the unspiked feed was 15.3 ppb.

Inter-assay precision was determined from the replicate standard addition experiments. Feed samples were processed on three separate occasions, and a typical curve is shown in Figure 4. The slopes of the response curves (correlation coefficient > 0.99 in all cases) showed some day-to-day variability (16% RSD), which is probably due in part to differences in MS source/probe conditions that can affect responses. However, the accuracy of spike analysis, obtained in the separate experiments shown in Table 3, was not related to the changes in mass spectrometer sensitivity and was always within 80% of the nominal fumonisin B_1 content. Moreover, the inter-assay (Table 3) and intra-assay (Table 1) precision values were similarly ≤16% for all spike levels. Thus, the data demonstrate the validation of this procedure for analysis of fumonisin B_1 in feed between 10 and 200 ppb with the observed precision and accuracy.

The effect of using different mass spectrometer systems was also investigated. An entire sample set comprised of standards, spiked feed samples, and contaminated corn samples was extracted and analyzed as described under Materials and Methods, and then aliquots were shipped for a second analysis using the same chromatography with a different single-quadrupole mass spectrometer (Navigator, Finnigan Manchester, U.K.). The determined fumonisin values agreed closely, and similar precision of analysis was observed.

This methodology was used to survey the fumonisin B content in rodent feed used during 1996 in biomedical research institutions around the United States. Table 4 shows the results. The median values were approximately 162 ppb for fumonisin B_1 and 236 ppb for total fumonisin B content. The contamination ranged from 32 to 663 ppb for fumonisin B_1 and from 46 to 923 ppb for total fumonisin B congeners. The significance of this level of contamination in the feed of rodents on long-term toxicity tests is at present unclear and must await the results of a 2-year bioassay of fumonisin B_1 that is being conducted at our institute and currently scheduled to end in 1997.

The results presented here show that LC with ESI/MS is a robust technique for quantifying trace levels of fumonisins in corn-derived feed. Current studies are in progress to determine the minimal levels of fumonisins required to affect sphingolipid biosynthesis and the role of the fumonisins in cancer etiology. The high degree of sensitivity and specificity of determination using LC separation and ESI/MS with selected ion monitoring detection also suggests that this method may be valuable in screening feed and foodstuffs for contamination by not only fumonisins B_1, B_2, and fB_3, but also other potentially toxic fumonisin species (e.g., the acetylated and hydrolyzed fumonisin B series).

ABBREVIATIONS USED

CID, collision-induced dissociation; ESI/MS, electrospray ionization mass spectrometry; IAC, immunoaffinity chromatography; OPA, *o*-phthalic dicarboxaldehyde; RSD, relative standard deviation.

ACKNOWLEDGMENT

We gratefully acknowledge the contribution of Drs. Robert Eppley and Sam Page, U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, for providing fumonisin standards, and the assistance of Dr. Steve Bajic, Finnigan Manchester, in performing the interinstrument comparison.

LITERATURE CITED

Bucci, T. J.; Howard, P. C. Effect of fumonisin mycotoxins in animals. *J. Toxicol., Toxin Rev.* **1996**, *15*, 293−302.

Chamberlain, W. J.; Voss, K. A.; Norred, W. P. Analysis of commercial laboratory rat rations for fumonisin B_1, a mycotoxin produced on corn by *Fusarium moniliforme*. *Contemp. Top.* **1993**, *32*, 26−28.

Doerge, D. R.; Bajic, S.; Analysis of Pesticides Using Atmospheric Pressure Chemical Ionization Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 663−666.

Doerge, D. R.; Howard, P. C.; Bajic, S.; Preece, S. W. Determination of fumonisins using on-line liquid chromatography coupled to electrospray mass spectrometry. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 603−606.

Doerge, D. R.; Churchwell, M. I.; Rushing, L. G.; Bajic, S. Confirmation of gentian violet and its metabolite leucogentian violet in catfish muscle using LC-APCI/MS. *Rapid Commun. Mass Spectrom.* **1996a**, *10*, 1479−1484.

Doerge, D. R.; Churchwell, M. I.; Holder, C. L.; Rowe, L.; Bajic, S. Detection and confirmation of β-agonists in bovine retina using LC-APCI/MS. *Anal. Chem.* **1996b**, *68*, 1918−1923.

Gelderblom,W. C. A.; Kriek, N. P. J.; Marasas, W. F. O.; Thiel, P. G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1, in rats. *Carcinogenesis* **1991**, *12*, 1247−1251.

Haschek, W. M.; Motelin, G.; Ness, D. K.; Harlin, K. S.; Hall, W. F.; Vesonder, R. F.; Peterson, R. E.; Beasley, V. R. Characterization of fumonisin toxicity in orally and intravenously dosed swine. *Mycopathologia* **1992**, *117*, 83−96.

Holcomb, M.; Thompson, H. C.; Hankins, L. J. Analysis of fumonisin B_1 in rodent feed by gradient elution HPLC using precolumn derivatization with and fluorescence detection. *J. Agric. Food Chem.* **1993**, *41*, 764−767.

Hopmans, E. C.; Murphy, P. A. Detection of fumonisin B_1, B_2, and B_3 and hydrolyzed fumonisin B_1 in corn-containing foods. *J. Agric. Food Chem.* **1993**, *41*, 1655−1658.

Howard, P. C.; Muskhelishvili, M.; Dooley, K. L.; Bolon, B.; Warbritton, A.; Voss, K. A.; Lorentzen, R. J.; Bucci, T. J. Hepatic and renal apoptosis and sphingolipid changes in F344 rats and $B_6C_3F_1$ mice chronically fed the corn fungal toxin fumonisin B_1. *Proc. Am. Assoc. Cancer Res.* **1996**, *37*, 791.

Kellerman, T. S.; Marasas, W. F. O.; Thiel, P. G.; Gelderblom, W. C. A.; Cawood, M.; Coetzer, J. A. W. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. *Onderstepoort J. Vet. Res.* **1990**, *57*, 269−275.

Lukacs, Z.; Schaper, S.; Herderich, M.; Schreier, P.; Humpf, H. U. Identification and determination of fumonisin FB_1 and FB_2 in corn and corn products by high-performance liquid chromatography-electrospray-ionization tandem mass spectrometry. *Chromatographia* **1996**, *43*, 124−128.

Plattner, R. D.; Branham, B. E. Labeled fumonisins: production and use of fumonisin B_1 containing stable isotopes. *J. Assoc. Off. Anal. Chem. Int.* **1994**, *77*, 525−532.

Rheeder, J. P.; Marasas, W. F. O.; Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; van Schalkwyk, D. J. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* **1992**, *82*, 353−357.

Riley, R. T.; An, N.-H.; Showker, J. L.; Yoo, H.-S.; Norred, W. P.; Chamberlain, W. J.; Wang, E.; Merrill, Jr., A. H.; Montelin, G.; Beasley, V. R.; Haschek, W. M. Alteration of tissue and serum sphinganine to sphingosine ratio: An early biomarker of exposure to fumonisin-containing feeds in pigs. *Toxicol. Appl. Pharmacol.* **1993**, *118*, 105−112.

Shephard, G. S.; Thiel, P. G.; Stockenstrom, S.; Sydenham, E. W. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. Assoc. Off. Anal. Chem. Int.* **1996**, *79*, 671−687.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G.; Marasas, W. F. O.; Stockenstrom, S. Fumonisin contamination of commercial corn-based human foodstuffs. *J. Agric. Food Chem.* **1991**, *39*, 2014−2018.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G.; Stockenstrom, S.; Snijman, P. W. Liquid chromatographic determination of fumonisin B_1, B_2, and B_3 in corn: AOAC-IUPAC collaborative study. *J. Assoc. Off. Anal. Chem. Int.* **1996**, *79*, 688−696.

Tolleson,W. H.; Sheldon, W. G.; Thurman, J. D.; Bucci, T. J.; Howard, P. C. The mycotoxin fumonisin induces apoptosis in cultured human cells and in livers and kidneys of rats. In *Fumonisins in Foods*; Jackson, L., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; pp 237−250.

Ware, G. M.; Umrigar, P. P.; Carman, A. S.; Kuan, S. S. Evaluation of Fumonitest immunoaffinity columns. *Anal. Lett.* **1994**, *27*, 693−715.

Received for review January 3, 1997. Revised manuscript received April 10, 1997. Accepted April 28, 1997.[⊗] This work was supported in part by Interagency Agreement 224-93-0001 between the NCTR and the National Institute for Environmental Health Sciences/National Toxicology Program.

JF970021L

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1997.