New Procedure for Fumonisins B_1 and B_2 in Corn and Corn Products by Ion Pair Chromatography with *o*-Phthaldialdehyde Postcolumn Derivatization and Fluorometric Detection

Makoto Miyahara,* Hiroshi Akiyama, Masatake Toyoda, and Yukio Saito

National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan

Fumonisins are a group of naturally occurring mycotoxins produced by *Fusarium moniliforme*. An analytical method for fumonisins B_1 and B_2 in corn and related foods has been developed that utilizes ion pair chromatography with a postcolumn reaction technique. Samples were prepared and purified by tandem solid phase extraction columns (SAX and C_{18}). The fumonisins were derivatized with *o*-phthaldialdehyde and *N*-acetyl-L-cysteine in an in-line reaction coil. Ion pair liquid chromatography with fluorescence detection is used for the separation and detection. Absolute recoveries of fumonisins ranged from 54 to 110% at 0.04 and 0.08 μg/g. The detection limit of this method is 20 ng. This procedure is relatively simple and provides a screening method for fumonisins in corn and related foods.

Keywords: *Determination; fumonisin; solid phase extraction; ion pair chromatography; o-phthaldialdehyde; postcolumn reaction; fluorometric*

INTRODUCTION

Our program for the development of analytical methods for food contaminants, which has included pesticides (Miyahara et al., 1991, 1992a,b, 1993a,b, 1994, 1995; Miyahara and Saito, 1994; Akiyama et al., 1995), has been expanded to include mycotoxins. This paper describes a method for fumonisins B_1 and B_2 (Figure 1). The levels of fumonisin B_1 in corn, milk, and other products depend upon the area of production (Bullerman and Tsai, 1994; Murphy et al., 1993; Pittet et al., 1992; Stack and Eppley, 1992; Sydenham et al., 1992a,b, 1993; Akiyama et al., 1994; Maragos and Richard, 1994). Several studies report the physiological effects of the fumonisins on animals and plants (Norred and Voss, 1994; Abbas et al., 1993; Hendrich et al., 1993). In addition, some work has demonstrated hepatotoxicity and renal toxicity in rats and proposed a mechanism of bioactivity based upon inhibition of sphingolipid metabolism (Riley et al., 1994). These studies suggest that fumonisins are also toxic mycotoxins for humans (Gelderblom et al., 1988; Norred and Voss, 1994). Therefore, many analytical procedures have been reported. For example, detection methods for fumonisins in foods include ELISA, GC−MS, HPLC (Pestka et al., 1994), and capillary zone electrophoresis (CZE) (Maragos, 1995). The detection limits are 200, 100, and 50 ng/g and 25 ng/mL, respectively. Fluorometric methods with HPLC are usually most sensitive, and one of them has been examined in interlabolatory studies (Holcomb et al., 1993a,b; Hopmans and Murphy, 1993; Scott and Lawrence, 1992; Shephard et al., 1990; Stack and Eppley, 1992; Sydenham et al., 1992a,b, 1993; Akiyama et al., 1995; Thiel et al., 1993). However, these methods require precolumn derivatization with a fluorescent reagent and subsequent analysis by HPLC. Several fluorescent derivatives have been studied. The fluorescamine derivative of fumonisin B_1 yields two peaks by HPLC (Holcomb et al., 1993a,b; Akiyama et al., 1994).

* Author to whom correspondence should be addressed (telephone 81-3-3700-1141; fax 81-3-3707-6950; e-mail mmiyaha@nihs.go.jp).

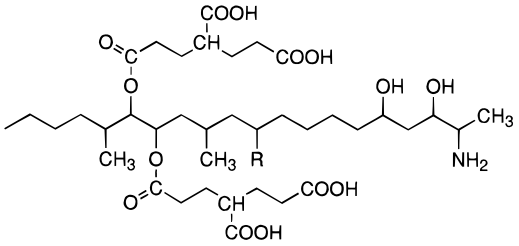

Figure 1. Structures of fumonisins: B_1, R = H; B_2, R = OH.

Derivatives formed with *o*-phthaldialdehyde (OPA) (Sydenham et al., 1992a,b) and 4-fluoro-7-nitrobenzofurazan (NBD-F) (Scott and Lawrence, 1992) are very sensitive for detection but unstable (the half-lives of OPA and NBD-F are 1−2 and 20 min, respectively). As a result of their instability, the data obtained by the precolumn derivatization with those reagents are not accurate when using manual sample preparation with an autosampler. The (9-fluorenylmethyl)chloroformate (FMOC) derivative, which can be resolved with gradient elution HPLC (Holcomb et al., 1993b), has the disadvantage of a lengthy chromatographic procedure. Naphthalene dicarboxaldehyde (NDA) derivatives are very sensitive and stable enough to utilize autoinjectors, but the derivatization procedure requires a rather long reaction time (15 min), a high temperature (60 °C), and an extremely toxic reagent (NaCN) (Bennett and Richard, 1994). The 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) derivative is also stable but needs a long reaction time (40−45 min) (Akiyama et al., 1995). Compounds that form derivatives with carboxylic acid groups in the fumonisins with DBD-F require a very long reaction time (6 h) and a high temperature (60 °C). The sensitivity of CZE is the as same as that of HPLC fluorometry, but it requires a very long reaction time (90 min) and a high temperature (60 °C) in a pH 9.5 buffer solution. Consequently, fumonisins decompose easily in these conditions (heat and high pH) (Hendrich et al., 1993). This results in the variable reproducibility (57−104% at 60 °C for 90 min and 67−85% at 24 °C for 180 min) for the analysis of fumonisin B_1 in horse serum (Maragos, 1995) and risks faultily

0021-8561/96/1444-0842$12.00/0

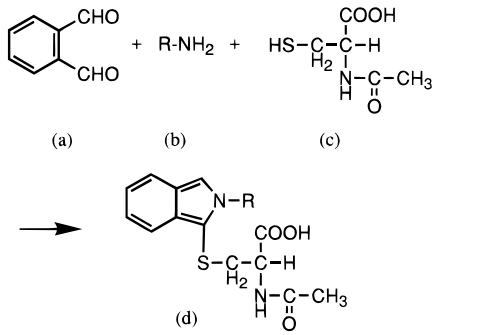

Figure 2. Schematic diagram of derivatization reaction: a, *o*-phthaldialdehyde; b, fumonisin; c, *N*-acetyl-L-cysteine; d, fluorescent derivative.

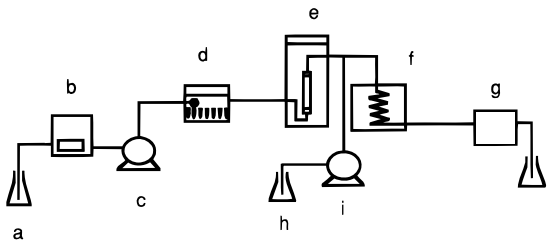

Figure 3. Schematic diagram of the postcolumn derivatization system with the HPLC: a, mobile phase; b, degasser; c, pump; d, autosampler; e, column and column oven; f, reaction box and reaction coil; g, fluorescence detector; h, reaction solution (buffer + OPA + *N*-acetyl-L-cysteine); i, pump.

negative results at low fumonisin levels in complex matrixes (Akiyama et al., 1995). Fumonisin B_1 and B_2 levels in milk were reduced by 7 and 13%, respectively, by heating to 90 °C for 30 min (Maragos and Richard, 1994). Thus, fumonisins (Figure 1) would readily be hydrolyzed under the derivatization conditions, which are stronger conditions (pH 8−10, 60−90 °C, 15−90 min) than those Maragos examined. Therefore, with precolumn derivatization procedures there is always the risk of false negative results; postcolumn derivatization is therefore more desirable.

For routine determination of many food samples, a simple, safe, and rapid automated derivatization procedure is required to accommodate a large number of samples. There have been no reported studies on fumonisin analysis using ion pair chromatography and postcolumn derivatization techniques (Figure 2).

To expedite analysis time, obtain reproducible results, and automate the detection process, we have developed an ion pairing chromatographic separation with postcolumn derivatization with OPA and subsequent fluorometric detection.

EXPERIMENTAL PROCEDURES

Apparatus. A schematic diagram of the postcolumn HPLC system used is shown in Figure 3.

(a) The instruments consisted of a Tosoh Model CM pump for high-performance liquid chromatography with a fluorescence detector (Shimadzu Model 350 monitor, which was operated at 336 nm for excitation and at 460 nm for emission), an autosampler (Tosoh Model AS8020), a degasser (Tosoh Model SD8022), and a column oven (Tosoh Model CO8010). An ODS column [300 mm × 4.7 mm i.d., 5 mm; TSK gel 80 (Tosoh Co.)] was used with a mixture of pH 2.5 phosphate buffer ($^1/_{15}$ M potassium dihydrogen phosphate + phosphoric acid + 1 mM sodium lauryl sulfate) and acetonitrile (1:1) as mobile phase at 1 mL/min. The column oven was maintained isothermally at 40 °C. No mixing chamber was required for this process. Injections were performed by an autosampler with a completely filled 40-mL injection loop. Data were processed with a Shimadzu Model CR5A data processor.

(b) The derivatization apparatus consisted of a reaction coil at 40 °C with a 5-m stainless steel reaction coil (0.25 mm i.d.). The reagent flow rate was 0.5 mL/min.

(c) Capillary electrophoresis of fumonisins for purity testing was conducted with a Waters Model 4000E capillary electrophoresis system. The system was equipped with a 60-cm silica capillary (75 mm i.d.) and operated at 20 kV positive power supply. The UV monitor was operated at 185 nm. The electrophoresis buffer consisted of 25 mM borate. The pH was adjusted with sodium hydroxide (pH 9.2). A 30-nL of sample was injected using the siphoning method (10 cm, 30 s). The maximum setting was chosen because it is the most sensitive to impurities.

(d) Solid phase extraction was performed using a vacuum manifold, Supelco Model 5-7030, Bellefonte, PA.

Reagents and Other Materials. (a) Fumonisin standards included fumonisins B_1, approximately 98%, and B_2, approximately 95% (purities were checked against peak area ratios of HPLC chromatogram determined at 200 nm). The standards were purchased from Sigma, St. Louis, MO.

(b) All inorganic reagents for analysis were of Japanese Industrial Standards (JIS) extra pure grade. These may be compatible with ACS grade. Water was of HPLC grade (Ciba Merck Co.).

(c) A solid phase extraction cartridge (SPE), Sep-Pak tC_{18} (sorbent quantity, 1 g; tube size, 6 mL), Waters Co., was used for extraction. The Sep-Pak was washed with 10 mL of methanol and 10 mL of 1% potassium chloride before use.

(d) A solid phase extraction for strong anion exchange cartridge (SAX, sorbent quantity, 1 g; tube size, 6 mL), Bond Elut, Varian, Harbor City, CA, was used for further purification of C_{18} eluate. The cartridge was washed with 20 mL of 70% methanol before use.

(e) Membrane filters of 5 μm (Microprep disk membrane filter, Bio-Rad Co., Hercules, CA) were used for sample filtration. The filters were washed with water (10 mL) before use.

(f) The reaction solution for derivatization was prepared by dissolving 800 mg of *o*-phthaldialdehyde and 1 g of *N*-acetyl-L-cysteine in 1 L of the reaction buffer.

(g) Reaction buffer consisted of 0.384 M sodium carbonate, 0.216 M boric acid, and 0.108 M potassium sulfate.

Samples. Corn and corn product samples were purchased at retail stores in Setagaya, Tokyo.

Sample Preparation. A 50-g sample was ground thoroughly in a homogenizer. The ground sample (5 g) was extracted with 200 mL of 50% aqueous acetonitrile solution. For a recovery test, standard mixture solution was added to the ground sample. The mixture was filtered through a 5-μm filter. A 20-mL portion of the filtrate was centrifuged at 3000 rpm (2000*g*) for 10 min at 10 °C. Four milliliters of supernatant was diluted with 10 mL of 1% potassium chloride. The mixture was applied to a conditioned C_{18} cartridge. The cartridge was washed with 6 mL of 0.5% potassium chloride solution, 5 mL of 10% acetonitrile in water, and finally 2−3 mL of water. Fumonisins were eluted by 7 mL of methanol. The eluate was diluted with 3 mL of water. The mixture was loaded onto a conditioned SAX cartridge. The cartridge was washed with 20 mL of 70% methanol in water and then 6 mL of methanol. The sample was eluted with 10 mL of 0.5% trifluoroacetic acid in methanol. The fumonisins were stable at room temperature (a 4 ppm standard solution in 1% trifluoroacetic acid in methanol was kept overnight at room temperature; the fumonisin B_1 level dropped to 81%, but the fumonisin B_2 level did not drop). The eluate was evaporated using nitrogen gas. Then, the remaining residue was dissolved in 400 mL of methanol.

Recovery Test. Spiked sample was analyzed exactly as described above. Recovery is defined as the ratio of recovered fumonisins to spiked fumonisins.

Caution: Fumonisins and some organic solvents used in this study are suspected carcinogens. Handle them with care.

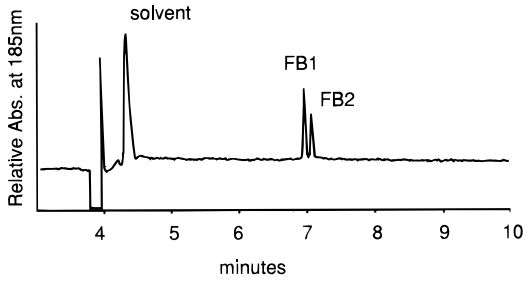

Figure 4. Electropherogram of 1 ng each of fumonisins B_1 and B_2 standards.

RESULTS AND DISCUSSION

Purity of Standard Fumonisins. Isolation and structural elucidation of the fumonisins are relatively recent achievements (Cawood et al., 1991; Bezuidenhout et al., 1988). The structure of fumonisin B_1 has been determined (Shier et al., 1995). The absolute configurations are now under study (Kraus et al., 1992; Caldas et al., 1994). Fumonisin standards were obtained from Sigma with no certification of purities. Fumonisins have low UV absorbance; therefore, the purities were checked using HPLC and capillary electrophoresis (Figure 4) by injecting 1 ng of the standard mixture-(B_1:B_2 = 1:1). From the electropherograms and chromatograms (not shown), the purities were calculated as described under Experimental Procedures. The resolving capability of capillary electrophoresis is equivalent to more than 300 000 theoretical plates. Electrophoresis is an excellent analytical technique for checking the purity of unknowns. Fumonisins are amenable to electrophoretical analysis because the molecule includes many hydroxyl and carboxyl groups. The detection limit (3 times the baseline noise) of both fumonisins for capillary electrophoresis was 0.1 ng. As a result of Maragos (1995) using the fluorometric technique and this experiment being based on the UV spectrometric technique, the method used in our experiments is 4000 times less sensitive than Maragos's method (0.025 pg). No major peaks were observed other than the solvent peaks in both the electropherogram and the chromatogram of the HPLC. Thus, it was concluded that the standard mixture is adequate.

On-Line Derivatization Conditions. To establish postcolumn reaction conditions, temperature, reaction coil length, and flow rate were examined by introducing fumonisin B_1 samples without the separation column. As shown in Figures 5 and 6, a 5-m reaction coil at 60 °C with a flow rate of 0.2 mL/min is the optimum. A long coil results in a reduced peak intensity of the fumonisin B_1 as shown in Figure 6. The derivatization solution consists of OPA with *N*-acetyl-L-cysteine in a pH 9 boric buffer. This reagent system was previously established for amino acid analysis with postcolumn derivatization (Bohlen and Mellet, 1979; Shimadzu, 1986). The reaction solution mixture is much more stable than that with 2-mercaptoethanol. The conditions for postcolumn derivatization are similar to the standard procedure for amino acid analysis but required minor adjustments because of the mobile phase. The postcolumn conditions with the analytical column are described under Experimental Procedures. There are many papers on the precolumn derivatization with OPA and 2-mercaptoethanol (Sydenham et al., 1993). Those systems are also very rapid but less accurate because of the instability of the derivatives without proper operation. Therefore, sample recovery always had to

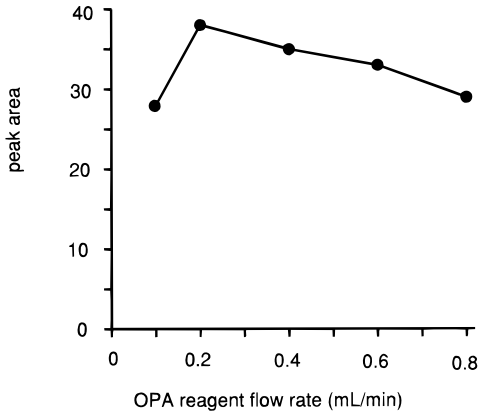

Figure 5. Effect of postcolumn reagent flow rate on the peak intensity (fumonisin B_1): coil length, 5 m; temperature, 60 °C; mobile phase flow rate of HPLC, 1 mL/min.

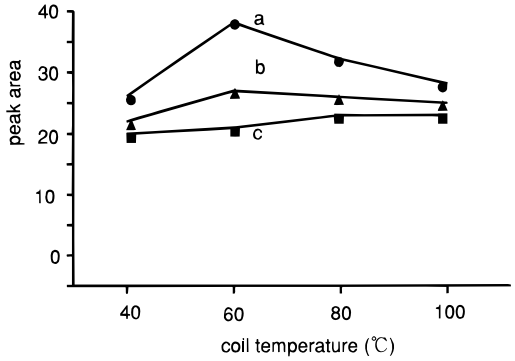

Figure 6. Effects of oven temperature and coil length on the peak intensity (fumonisin B_1) by HPLC with postcolumn derivatization: a, 5 m of coil length; b, 10 m; c, 2.5 m.

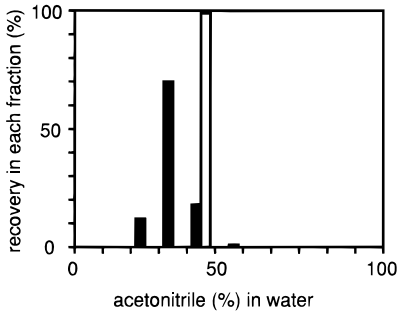

Figure 7. Elution profile of standard mixture of fumonisins B_1 and B_2 from C_{18} cartridge under stepwise gradient elution: black bar, fumonisin B_1; blank bar, fumonisin B_2. Each fraction volume was 1.5 mL.

be re-evaluated, and it was difficult to identify problems in chromatography and the cleanup procedure. This postcolumn derivatization system resolves those problems. The reproducibility of the HPLC system with postcolumn derivatization equipment is adequate for the analytical procedure.

Analytical Conditions. The sample was purified through tandem solid phase extraction (SPE) cartridges. To obtain reproducible recoveries of fumonisin B_2 and improve sensitivity of the method, the reported procedure (Holcomb et al., 1993) was modified. The elution profile of fumonisins from the initial C_{18} column by stepwise gradient elution is shown in Figure 7. The C_{18} cartridge is washed with 3−6 mL of 0.5% potassium chloride solution in 20% acetonitrile without loss of fumonisins. Figure 7 suggests that fumonisins could elute from 20 to 50% acetonitrile in water. However, elution with 50% acetonitrile in water did not give

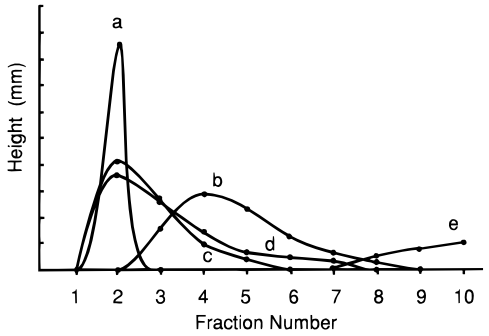

Figure 8. Salt effects on elution profiles of fumonisin B_2: a, 30% acetonitrile; b, 20% acetonitrile containing 0.5% KCl; c, 20% acetonitrile; d, 10% acetonitrile; e, 10% acetonitrile containing 0.001% KCl. Each fraction volume was 1.5 mL.

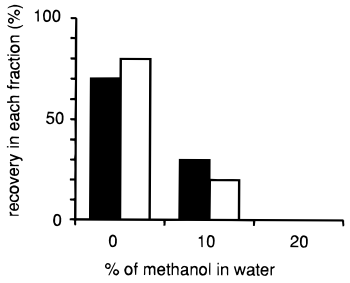

Figure 9. Elution profile of fumonisins B_1 and B_2 from SAX cartridge under stepwise gradient elution: black bar, fumonisin B_1; blank bar, fumonisin B_2. Each fraction volume was 1.5 mL.

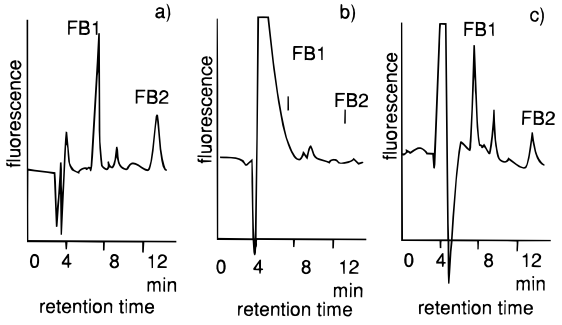

Figure 10. Liquid chromatogram of the standard (40 ng) (a), control sample (b), and spiked sample (at 0.04 ppm) of corn (c) by HPLC with postcolumn derivatization. Negative peak near 4 min was due to residual methanol in the sample. Reaction solution flow rate: 0.5 mL/min at 40 °C with 5 m coil (stainless steel, 0.25 mm i.d.).

Table 1. Results of Recovery Tests

sample	spiking level (ppm)	fumonisin B_1 av[a] (%)	fumonisin B_1 CV%	fumonisin B_2 av[a] (%)	fumonisin B_2 CV%
corn 1	0.04	54	25	79	18
corn 1	0.08	80	5	61	16
corn flour	0.04	100	13	95	17
corn flour	0.08	78	4	57	10
sweet corn	0.04	110	8	79	9
sweet corn	0.08	78	4	57	10
corn 2	0.08	94	10	73	16
corn 3[b]	0.65	83	10	87	7

[a] Means of triplicate determinations. [b] Direct determination of spiked fumonisins in diluted supernatant without cleanup.

reproducible recoveries of fumonisin B_2 from the cartridge. To obtain reproducible recoveries of fumonisin B_2, eluting conditions were examined. As shown in Figure 8, the elution profile of fumonisin B_2 is changed by the concentration of potassium chloride and also illustrates that the elution volume of fumonisin B_2 is changed by eluting conditions. Therefore, if potassium chloride residue is present in the cartridge, the recovery of fumonisin B_2 from the C_{18} cartridge will vary. Washing is important to get reproducible recovery from the C_{18} cartridge. This washing process also helps to reduce solid matter and water-soluble matter that would foul the SPE during the SAX cartridge cleanup. The reproducibility of the C_{18} cartridge is not adequate if used improperly. To improve the recoveries and reproducibility, affinity columns for cleanup may be a further study.

Purification with SAX is very sensitive to the flow rate of loading solvent. It should be less than 1 mL/min (0.8−1 mL). The elution profile is shown in Figure 9. This illustrates that if the methanol concentration in the water is higher than 20%, fumonisins will be retained in the SAX column. However, the retention volume will change as minor experimental conditions change (i.e. flow rate and pH of loading solvent, eluting solvent, etc.). To avoid the loss of fumonisins and wash out lipophilic materials, 70% methanol and 100% methanol are used. The elution flow rate of 1% trifluoroacetic acid in methanol is important and should be less than 1 mL/min. This purification procedure effectively removes insoluble materials in the eluate from the C_{18} cartridge. However, the tandem cartridge purification procedure still requires about 40 min to obtain reproducible results.

To separate fumonisins from interfering compounds, ion pair chromatography was examined using an ODS column. To suppress ionizations of the carboxyl groups, an acidic buffer was used. The mobile phase consisted of 0.06 M potassium dihydrogen phosphate adjusted to the optimum pH with phosphoric acid, 1 mM sodium lauryl sulfate, and 50% acetonitile. Using the conditions described in the previous section, fumonisin standards do not give sharp peaks. The flow rate of the reaction solution was adjusted to 0.5 mL/min to improve the shapes. To reduce background noise, the temperature of the reaction coil was lowered to 40 °C. Under these conditions, a representative chromatogram of standards at 40 ng (a), control corn sample (b), and spiked sample (c) is shown in Figure 10. Linearity of the calibration curve from 18 to 160 ng was achieved.

Recovery was tested at 0.08 and 0.04 ppm fumonisin standards in corn samples that did not contain any fumonisins. The recoveries for fumonisins B_1 and B_2 at 0.04 and 0.08 ppm ranged from 54 to 110% as shown in Table 1. The recoveries of fumonisin B_2 were poorer than those of fumonisin B_1. The recoveries from the C_{18} cartridge reflect these results. Corn sample 3 was not purified with the cartridges. Using this postcolumn system, the diluted extract could have been analyzed at high levels of fumonisins in corn samples. Unfortunately, after about 10 determinations, the HPLC column had to be washed with methanol. Improvements of the extraction processes are under study in our laboratory to eliminate the need for cleanup.

In conclusion, the postcolumn determination system has been established as useful and sensitive. Conventional analysis time is approximately 1−2 h. However, this technique was successful in reducing analysis time by 33−66% to 40−45 min. The detection limits, which are defined by the ACS committee, are 0.08 ppm for fumonisins B_1 and B_2. The signal to noise ratio was 10. The procedure presented here is adequate for the screening of fumonisins in foods.

ACKNOWLEDGMENT

This paper was presented at the 108th Annual AOAC International Meeting, September 12−15, 1994, Portland, OR. We are grateful to Dr. C. R. Warner of the U.S. Food and Drug Administration for reviewing the manuscript and to Dr. Russel B. Poe at the National Institute of Health Sciences, Tokyo, and his wife, Ms. Susan A. Poe, for useful advice.

LITERATURE CITED

Abbas, H. K.; Duke, S. O.; Tanaka, T. Phytotoxicity of fumonisins and related compounds. *J. Toxicol. Toxin Rev.* **1993**, *12*, 225−251.

Akiyama, H.; Miyahara, M.; Toyoda, M.; Saito, Y. Comparison of several fluorescence HPLC methods for fumonisin analysis. *Bull. Natl. Inst. Health Sci.* **1994**, *112*, 112−117.

Akiyama, H.; Miyahara, M.; Toyoda, M.; Saito, Y. Liquid chromatographic determination of fumonisin B_1 and B_2 in corn by precolumn derivatization with 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F). *J. Food Hyg. Soc. Jpn.* **1995**, *36*, 77−81.

Bennett, G. A.; Richard, J. L. Liquid chromatographic method for analysis of the naphthalene dicarboxaldehyde derivative of fumonisins. *J. AOAC Int.* **1994**, *77*, 501−506.

Bezuidenhout, S. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Horak, R. M.; Marasas, W. F. O.; Spiteller, G.; Vleggaar, R. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *J. Chem. Soc., Chem. Commun.* **1988**, 122−124.

Bohlen, P.; Mellet, M. Automated fluorometric amino acid analysis: the determination of proline and hydroxyproline. *Anal. Biochem.* **1979**, *94*, 313−321.

Bullerman, L. B.; Tsai, W. Y. J. Incidence and levels of *Fusarium moniliforme, Fusarium proliferatum* and fumonisins in corn and corn-based foods and feeds. *J. Food Prot.* **1994**, *57*, 541−546.

Caldas, E. D.; Jones, A. D.; Ward, B.; Winter, C. K.; Gilchrist, D. G. Structural characterization of three new AAL toxins produced by *Alternaria alternata* f. sp. *iycopersici*. *J. Agric. Food Chem.* **1994**, *42*, 327−333.

Cawood, M. E.; Gelderblom, W. C. A.; Vleggaar, R.; Behrend, Y.; Thiel, P. G.; Marasas, W. F. O. Isolation of the fumonisin mycotoxins: a quantitative approach. *J. Agric. Food Chem.* **1991**, *39*, 1958−1962.

Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. fumonisins−novel mycotoxins with cancer-promoting activity produced by *fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806−1811.

Hendrich, S.; Miller, K. A.; Wilson, T. M.; Murphy, P. A. Toxicity of *Fusarium proliferatum*-fermented nixtamalized corn-based diets fed to rats: effect of nutritional status. *J. Agric. Food Chem.* **1993**, *41*, 1649−1654.

Holcomb, M.; Sutherland, J. B. M.; Chiarelli, P.; Korfmacher, W. A.; Thompson, , H. C., Jr.; Lay, J. O., Jr.; Hankins, L. J.; Cernigliat, C. E. HPLC and FAB mass spectrometry analysis of fumonisins B_1 and B_2 produced by *Fusarium moniliforme* on food substrates. *J. Agric. Food Chem.* **1993a**, *41*, 357−360.

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

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

Kraus, G. A.; Applegate, J. M.; Reynolds, D. Synthesis of analogs of fumonisin. *J. Agric. Food Chem.* **1992**, *40*, 2331−2332.

Maragos, C. M. Capillary zone electrophoresis and HPLC for the analysis of fluorescin isothiocyanate-labeled fumonisin B_1. *J. Agric. Food Chem.* **1995**, *43*, 390−394.

Maragos, C. M.; Richard, J. L. Quantitation and stability of fumonisins B_1 and B_2 in milk. *J. AOAC Int.* **1994**, *77*, 1162−1167.

Miyahara, M.; Saito, Y. Determination of bromide ions in food by unsuppressed ion chromatography with ultraviolet detection after microwave digestion in sealed PTFE vessel. *J. Agric. Food Chem.* **1994**, *42*, 1126−1131.

Miyahara, M.; Sasaki, K.; Suzuki, T.; Saito, Y. Expanded coagulating cleanup procedures for simultaneous gas chromatographic determination of organophosphorus pesticides in crops and fruits. *Chem. Pharm. Bull.* **1991**, *39*, 1055−1058.

Miyahara, M.; Suzuki, T.; Saito, Y. Multi-residue methods for some pesticides in lanolin by capillary gas chromatography with detection by electron capture, flame photometric, mass spectrometric, and atomic emission techniques. *J. Agric. Food Chem.* **1992a**, *40*, 64−69.

Miyahara, M.; Suzuki, T.; Saito, Y. Capillary gas chromatographic determination of captafol in vegetables, fruits and grains. *J. Agric. Food Chem.* **1992b**, *40*, 1150−1153.

Miyahara, M.; Suzuki, T.; Saito, Y. Silica gel chromatographic cleanup procedure for organochlorine pesticide analysis with capillary gas chromatography. *J. Agric. Food Chem.* **1993a**, *41*, 221−226.

Miyahara, M.; Suzuki, T.; Saito, Y. Pesticide removal efficiencies of soybean oil process. *J. Agric. Food Chem.* **1993b**, *41*, 731−735.

Miyahara, M.; Okada, Y.; Takeda, H.; Aoki, G.; Kobayashi, A.; Saito, Y. Multiresidure procedures for determination of pesticides in foods using capillary gas chromatographic, flame photometric and mass spectrometric techniques. *J. Agric. Food Chem.* **1994**, *42*, 2795−2802.

Miyahara, M.; Toyoda, M.; Ushijima, K.; Nose, N.; Saito, Y. Volatile halogenated hydrocarbons in foods. *J. Agric. Food Chem.* **1995**, *43*, 320−326.

Murphy, P. A.; Rice, L. G.; Ross, P. F. Fumonisin B_1, B_2, and B_3 content of Iowa, Wisconsin, and Illinois corn and corn screenings. *J. Agric. Food Chem.* **1993**, *41*, 263−266.

Norred, W. P.; Voss, K. A. Toxicity and role of fumonisins in animal diseases and human esophageal cancer. *J. Food Prot.* **1994**, *6*, 522−527.

Pestka, J. J.; Azcona-Olivera, J. I.; Plattner, R. D.; Minervini, F.; Doko, M. B.; Visconti, A. Comparative assessment of fumonisin in grain-based foods by ELISA, GC-MS, and HPLC. *J. Food Prot.* **1994**, *57*, 169−172.

Pittet, A.; Parisod, V.; Schellenberg, M. Occurrence of fumonisins B_1 and B_2 in corn-based products from the Swiss market. *J. Agric. Food Chem.* **1992**, *40*, 1352−1354.

Riley, R. T.; Voss, K. A.; Yool, H. S.; Gelderblom, W. C. A.; Merrrill, Jr., A. H. Mechanism of fumonisin toxicity and carcinogensis. *J. Food Prot.* **1994**, *6*, 528−535.

Scott, P. M.; Lawrence, G. A. Liquid chromatographic determination of fumonisins with 4-fluoro-7-nitrobenzofurazan. *J. AOAC Int.* **1992**, *75*, 829−833.

Shephard, G. S.; Sydenham, E. W.; Thiel, P. G.; Gelderblom, W. C. A. Quantitative determination of fumonisins B_1 and B_2 by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.* **1990**, *13*, 2077−2087.

Shier, W. T.; Abbas, H. K.; Badria, F. A. Complete structures of the shingsine analog mycotoxins fumonisin B_1 and AAL toxin T_A: absolute configuration of the side chains. *Tetrahedron Lett.* **1995**, *10*, 1571−1574.

Shimadzu. Amino acid analysis by gradient methods, HPLC Appreciation Report 2, CA190-176; Shimadzu Technical Bulletin; Kyoto, 1986.

Stack, M. E.; Eppley, R. M. Liquid chromatographic determination of fumonisins B_1 and B_2 in corn and corn products. *J. AOAC Int.* **1992**, *75*, 834−837.

Sydenham, E. W.; Marasas, W. F. O.; Shephard, G. S.; Thiel, P. G.; Hirooka, E. Y. Fumonisin concentrations in Brazilian feeds associated with field outbreaks of confirmed and

suspected animal mycotoxicoses. *J. Agric. Food Chem.* **1992a**, *40*, 994−997.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G. Liquid chromatographic determination of fumonisins B_1, B_2, and B_3 in foods and feeds. *J. AOAC Int.* **1992b**, *75*, 313−318.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G.; Marasas, W. F. O.; Rheeder, J. P.; Sanhueza, P. C. E.; Gonzalez, H. H. L.; Resnik, S. L. Fumonisins in Argentinian field-trial corn. *J. Agric. Food Chem.* **1993**, *41*, 891−895.

Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; Schalkwyk, D. J. Study of the reproducibility characteristics of a liquid chromatographic method for the determination of fumonisins B_1 and B_2 in corn: IUPAC collaborative study. *J AOAC Int.* **1993**, *76*, 36−367.

Received for review June 19, 1995. Revised manuscript received November 20, 1995. Accepted December 19, 1995.[⊗]

JF9503669

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1996.