Liquid Chromatography/Thermospray/Mass Spectrometry Analysis of Beauvericin†

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Beauvericin (BEA) is a bioactive hexadepsipeptide coproduced by the same *Fusarium* species that produce fumonisin mycotoxins. BEA is insecticidal and antimicrobial, and, being lipophilic, has the potential to bioaccumulate. Its mammalian toxicity has only recently been characterized, and sensitive analytical methodology to analyze for BEA is lacking. On-line thermospray mass spectrometry (LC/TSP/MS) was used to analyze BEA. A reversed-phase column and a mobile phase that consisted of CH_3CN/0.4 M HCO_2NH_4 were used to resolve BEA. Positive ion spectra yielded signals at *m/z* 785 $(M + H)^+$, *m/z* 802 $(M + NH_4)^+$, and *m/z* 823 $(M + K)^+$. Standard curves plotted in the range 10−100 ng for the ions *m/z* 785, 802, and 823 had coefficients of determination (r^2) of 0.996, 0.992, and 0.999. The lowest detectable limit was 1 ng at a S/N of 5:1. The developed LC/TSP/MS method was used successfully to analyze *Fusarium proliferatum*-fermented cracked corn containing BEA and spiked meat samples.

Keywords: *Beauvericin; fumonisins; thermospray; Fusarium moniliforme*

INTRODUCTION

Beauvericin (BEA), a bioactive cyclic hexadepsipeptide (Hamill et al., 1969), has come under scrutiny because it is coproduced by several fumonisin-producing *Fusarium* species. The recently discovered fumonisin mycotoxins are suspected to be nongenotoxic carcinogens possibly involved in some human esophageal cancers (Rheeder et al., 1992; Gelderblom et al., 1991). They are etiological agents of many animal diseases; however, they are water-soluble and when dosed orally are rapidly eliminated, mainly through the feces (Smith and Thakur, 1996; Shepard et al., 1992).

Beauvericin was discovered as the toxic principle produced by the potent insect pathogenic fungus *Beauveria bassiana* (Hamill et al., 1969; Ovchinnikov et al., 1971; Grove and Pople, 1980). It is moderately antimicrobial, manifesting its toxicity by catalyzing the translocation of cations (cationophoric) across the lipid bilayer in the direction of the electrochemical gradient (Benz, 1978; Steinrauf, 1985). Macchia et al. (1995) have reported that BEA is toxic to several mammalian cell lines, which may suggest a role in human toxicoses from contaminated grains. Structurally, BEA ($C_{45}H_{57}N_3O_9$) consists of repeating D-α-hydroxyisovaleryl-*N*-methyl-L-phenylalanine units (see Figure 1) and has a molecular weight of 784 (Hamill et al., 1969). Recently, researchers have discovered the natural occurrence of BEA in corn infested with fumonisin-producing *Fusarium moniliforme, Fusarium proliferatum*, and *Fusarium subglutinans* isolates (Logrieco et al., 1993; Plattner and Nelson, 1994; Bottalico et al., 1995).

The role of BEA in animal toxicoses remains unknown; however, it is known to trigger apoptosis via calcium-activated endonucleases causing cytolysis accompanied by internucleosomal DNA fragmentation (Ojcius et al., 1991). Because many of these *Fusarium* spp. are present in corn, human and animal exposure to BEA seems plausible. Furthermore, BEA's potential to bioaccumulate could translate into a carry-over problem in meats intended for human consumption, via livestock fed BEA-contaminated grain.

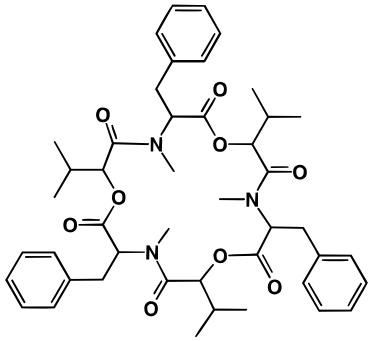

Figure 1. Chemical structure of the beauvericin mycotoxin.

No rapid and sensitive method seems to be available for the analysis of BEA, a molecule that may become an emerging concern in human and animal mycotoxicoses. A rapid method of sensitive analysis for BEA is especially important to fumonisin toxicity research, because co-occurrence would complicate results. Analysis of BEA involves either thin-layer chromatography (TLC) or high-pressure liquid chromatography (HPLC) using reversed-phase columns with subsequent ultraviolet (UV) detection (Gupta et al., 1991; Logrieco et al., 1993; Plattner and Nelson, 1994). The use of acetonitrile (UV cutoff = 190 nm) and methanol (UV cutoff = 210 nm) as the mobile phase solvents has made sensitive analysis of BEA difficult because the molecule has a UV_{max} of about 192 nm and only a weakly absorbing shoulder at 210 nm. Furthermore, extensive cleanup involving liquid−liquid extractions and/or solid−liquid extractions is required before HPLC/UV analysis (Gupta et al., 1991; Logrieco et al., 1993; Plattner and Nelson, 1994).

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On-line thermospray mass spectrometry (LC/TSP/MS) offers many advantages over conventional HPLC detectors. Unequivocal identification and high sensitivity can be achieved from unit resolution quadrupole mass spectrometers with the help of retention time, selected-ion monitoring (SIM), and deuterium-exchange experimentation (Barcelo, 1988, 1989; Voyksner et al., 1990). Lower signal-to-noise (S/N) ratios, associated with mass selective detection, minimize sample cleanup, consequently saving sample preparation time and the use of expensive solvents.

Nonvolatile smaller molecules (200−1000 Da) such as BEA that do not develop multiple charges on ionization are conducive to routine LC/TSP/MS analysis because of the interface's ability to handle high LC flow rates. Comprehensive discussions on the use of LC/TSP/MS in the analysis of biologically significant compounds are well documented in the literature (Yergey et al., 1989). In the work described here, a simplified cleanup procedure, and the applicability of LC/TSP/MS with the discharge-on, fragmentor-on, in acetonitrile/0.4 M ammonium formate buffer, using the positive-ion mode will be demonstrated for the analysis of BEA-containing culture material and BEA-spiked corn and beef samples.

EXPERIMENTAL PROCEDURES

Chemicals. Acetonitrile (HPLC grade), hexane, and ammonium formate were obtained from Fisher Scientific Co. (Fair Lawn, NJ). Deuterium oxide and the BEA standard (5 mg, lot 54H0197) were obtained from Sigma Chemical Co. (St Louis, MO). This was diluted serially in CH_3CN to yield stock solutions containing 0.1, 0.3, 0.5, and 1.0 μg/mL BEA. Standard curves were plotted using these dilutions (0, 10, 30, 50, and 100 ng injected) and evaluated for linearity by determining the coefficient of determination (r^2). Three major ions were selected for quantitative work: *m/z* 785, 802, and 823.

Samples. A sample of *F. proliferatum* (M-6993)-inoculated cracked corn culture material known to contain high amounts of fumonisin B_1 (FB_1) was sent to this laboratory for analyses of BEA (courtesy of Dr. Patricia Murphy, Department of Foods and Nutrition, Iowa State University, Ames, IA). For analysis, 5 g of culture material was blended with 20 mL of CH_3CN/H_2O (90:10, v/v) in a Waring blender for 3 min, allowed to stand for 10 min, and blended again for 3 min.

The slurry was filtered through a Whatman No. 4 filter paper and evaporated to dryness under vacuum in a Büchi rotary evaporator (Brinkman Inst., Westbury, NY). The residue obtained was redissolved in 1 mL of CH_3CN and transferred using a Pasteur pipet to a 3 mL capacity glass concentration vial. The 1 mL extract then was partitioned (two times) with 2 mL of hexane. The upper hexane layer was discarded using a Pasteur pipet, and the lower acetonitrile layer was filtered through a 0.2 μm nylon syringe filter. The extract was stored in a Teflon-lined amber-colored vial at −4 °C.

A ground beef sample was obtained from the Department of Animal Sciences and Industry's meat processing facility. Two 5 g portions of the ground beef sample were used for analysis. One 5 g portion served as the blank to monitor base levels of naturally occurring BEA, whereas the other 5 g portion of ground beef was spiked to contain 1 μg/mL of BEA.

The spiking was achieved by pipetting 500 μL of the BEA stock solution (10 μg/mL) onto the surface of the ground beef sample (5 g) placed in a blender jar. To ensure dispersion of spiked standards into the beef matrix, the spiked-beef sample was allowed to stand for 5 min before the extraction. The spiked-beef sample then was treated as described above.

Corn samples (5 g) obtained from the Department of Animal Sciences and Industry's feed-processing facility were spiked similarly (i.e., to contain 1 μg/g BEA). A blank (unspiked) corn sample was treated similarly to establish baseline levels of naturally occurring BEA. All extracts were filtered through 0.2 μM nylon syringe filters and stored in Teflon-lined, amber-colored vials at −4 °C.

High-Performance Liquid Chromatography. Analyses were done using a Hewlett-Packard (HP) Series II, 1090M HPLC (Hewlett-Packard, Palo Alto, CA), fitted with a Rheodyne 7125 injector (Rheodyne Inc., Cotati, CA) and having a 100 μL loop. Chromatographic separation was achieved using a HP ODS Hypersil (100 mm × 2.1 mm, 5 μm) column equilibrated at a temperature of 40 °C. Absorbance wavelength was determined using a photodiode array UV−vis detector connected to the HPLC.

The mobile phases were acetonitrile (A) and 0.4 M ammonium formate buffer (B) mixtures pumped in a gradient of 50% A (3 min hold) to 80% A at the end of 3.1 min. The flow rate was 0.8 mL/min. The 0.4 M ammonium formate buffer was prepared daily in deionized water (HPLC grade) and filtered through a 0.45 μm nylon filter. The mobile phase components were degassed by helium sparging for 10 min before use.

Thermospray and Mass Spectrometer. Mass spectral analyses were done using a Hewlett-Packard 5989A quadrupole mass spectrometer connected to the HPLC via an HP thermospray interface (TSP). A Model 59970C processor was used for data acquisition and processing. The MS was operated in the filament-off, discharge-on mode, with the fragmentor voltage set at 200 V. The ion source operated at a temperature of 350 °C, and the quadrupoles were maintained 100 °C. The TSP stem temperature was set at 65 °C to produce a TSP tip temperature of 180 ± 5 °C. Linear scanning between *m/z* 350 and 850 resulted in scan rates of 0.84 scan cycle/s. SIM used in the quantitative analysis resulted in 7.4 ± 0.4 scan cycles/s.

The instrument was tuned manually using a 10 μg/mL BEA stock solution prepared in acetonitrile/0.4 M ammonium formate buffer (80:20, v/v) and pumped at a flow rate of 0.8 mL/min without the column. Sensitivity was enhanced by adjusting the ion optics to yield maximum abundance for the ion *m/z* 802. A BEA standard (1 μg/mL) was used before and after analysis of each sample to check for standard curve variation. This was done because absolute intensity variations of 30−40% have been reported for routine LC/TSP/MS analysis (Yergey et al., 1989).

Deuterium Exchange Studies. The sandwich slug technique described by Chi and Baker (1992) was used to characterize the peaks seen in the mass spectra. A 200 μL injection loop was used to perform the sandwich slug technique. Fifty microliters of 10 μg/mL BEA stock solution (standard dissolved in CD_3OD) was sandwiched between two 75 μL slugs of CD_3OD/0.4 M ammonium formate in D_2O and injected into the nondeuterated carrier stream. A shift in the spectral lines between the spectra obtained from nondeuterated versus deuterated solvents would indicate the number of chemically changeable functional groups (Siegel, 1988). The aim of this procedure was to confirm the identity of BEA and its adducts by the corresponding spectral shift.

RESULTS AND DISCUSSION

Figure 2 shows the linear scan mass spectrum of the BEA standard. The base peak is *m/z* 802 $(M + NH_4)^+$, the protonated molecular ion is seen at *m/z* 785 $(M + H)^+$, and the ion *m/z* 823 is suspected to be an $(M + K)^+$ adduct. No major fragmentation occurs, which is typical of LC/TSP/MS analysis because it is a soft ionization technique (Barcelo, 1988, 1989; Yergey et al., 1989). Even though the ion source was maintained at a temperature of 350 °C, there was no indication of pyrolysis. The probable identities of the ions observed were determined by the isotope exchange studies (Figure 3). The base peak (ion *m/z* 802), which occurs at M + 18, is an adduct that can be formed by the addition of either H_2O (FW = 18) or NH_4^+ (FW = 18).

Injecting the sandwich slug containing the deuterated solvents enriches the ion source plasma with ND_4^+ ions

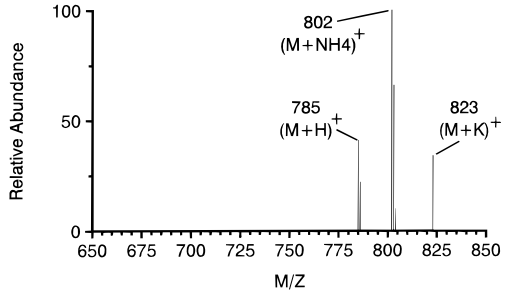

Figure 2. Positive ion, discharge-on, linear scan mass spectrum of BEA standard (50 ng) resolved on a C_{18} column using mobile phases containing acetonitrile (A) and 0.4 M ammonium formate buffer (B) mixtures and pumped in a gradient of 50% A (3 min hold) to 80% A at the end of 3.1 min.

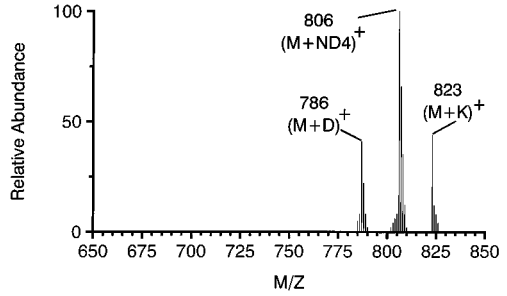

Figure 3. Positive ion, discharge-on linear scan mass spectrum of BEA standard in CD_3OD (0.5 μg) sandwiched between two 75 μL slugs of CD_3OD/0.4 M ammonium formate in D_2O, resolved on a C_{18} column using nondeuterated mobile phases containing acetonitrile (A) and 0.4 M ammonium formate buffer (B) mixtures and pumped in a gradient of 50% A (3 min hold) to 80% A at the end of 3.1 min.

(and $HCOO^-$ ions) resulting from the solvation of NH_4-HCOOH in D_2O/CD_3OD. The acidic protons on the electronegative nitrogen are exchanged readily with deuterium atoms, facilitating ND_4^+ production in the plasma. The base peak (ion *m/z* 806) in the deuterium-enriched ion source plasma was observed at M + 22 mass units. This spectral shift of 22 mass units can be attributed only to the formation of an $(M + ND_4)^+$ adduct or [N(14) + D_4(8) = 22] and not to the formation of an $(M + D_2O)^+$ adduct or [D_2(4) + O(16) = 20]. This confirmed the identity of the base peak (ion *m/z* 802) as an adduct resulting from formation of $(M + NH_4)^+$. Because the BEA cage consists of both electronegative and electropositive atoms (carbonyl oxygens and carbon atoms), the BEA molecule is able to cage small charged molecules (Steinrauf, 1985), such as the ammonium ion.

The deuterated molecular ion (*m/z* 786) shifted by 1 mass unit [M(784) + D(2) = 786] and was confirmed to be an $(M + D)^+$ adduct. The $(M + D)^+$ adduct also was observed by Sheil et al. (1990) when BEA was analyzed by Fourier transform ion cyclotron resonance spectrometry. The observed mass spectral shifts using the deuterated solvents indirectly indicated the absence of acidic protons in BEA, because the BEA itself did not contribute to spectral shift (Blum et al., 1976; Hunt and Sethi, 1980).

No spectral shift was observed in the ion at *m/z* 823 $(M + 39)^+$ during deuterium exchange experimentation. An addition of 39 mass units can be attributed to the presence of a caged potassium cation (K^+), which is a distinct possibility because BEA is a cationic ionophore (Steinrauf, 1985). The ion *m/z* 823 was assigned as an $(M + K)^+$ adduct, and the presence of a caged K^+ was proven by atomic absorption spectroscopy. A 100 μg/mL standard BEA solution (in CH_3CN) was analyzed via atomic absorption spectrometry (absorption wavelength 76.5 nm) to indicate the presence of potassium. This result confirmed the presence of K^+ in the 100 μg/mL BEA standard solution. The source of K^+ could be either the BEA purification process or leaching from the glassware.

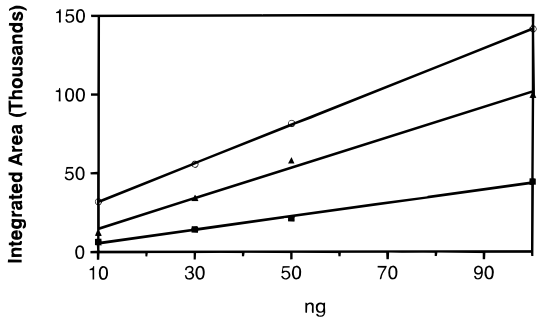

Figure 4. Standard curves for BEA in the range 10−100 ng injected. Coefficients of determination are 0.996 (■) for ion *m/z* 785 $(M + H)^+$, 0.992 (▲) for ion *m/z* 802 $(M + NH_4)^+$, and 0.999 (○) for ion *m/z* 823 $(M + K)^+$. Data were obtained in selected-ion monitoring mode, using a C_{18} column and mobile phases containing acetonitrile (A) and 0.4 M ammonium formate buffer (B) mixtures and pumped in a gradient of 50% A (3 min hold) to 80% A at the end of 3.1 min.

Chromatographically, the compound had a retention time of 4.40 ± 0.1 min and was eluted using a strong mobile phase composition and therefore was retained strongly on the reversed-phase column. This property is advantageous, because aqueous impurities can be washed off the column, thereby reducing sample cleanup procedures. Furthermore, effects from matrix interference in the ion source plasma were negligible, because the chromatographic conditions precluded coelution of interfering compounds. Thus, BEA eluted in an area devoid of matrix interference, enhancing the S/N.

Quantitative analyses were based on standard curves obtained by plotting the integrated areas of the ion currents *m/z* 785, 802, and 823 (generated by SIM) versus the amount of BEA injected. The lowest detectable limit was 1 ng (injected) at an S/N of 5:1. In the range of 10−100 ng (injected), the standard curves were linear. The coefficients of determination (r^2) of the standard curves for the ions *m/z* 785, 802, and 823 were 0.996, 0.992, and 0.999, respectively (Figure 4).

Figure 5a shows the normalized total ion current and extracted ion current (*m/z* 785, 802, and 823) chromatographs of the spiked-beef sample. Most of the impurities were eluted between 0.4 and 3.0 min (Figure 5a), resulting in a clean mass spectrum (Figure 5b). Negligible matrix interference was an important factor in determining the amount of recovered BEA in the spiked-meat sample, because the ion current generated at 4.5 min was due almost entirely to the ions *m/z* 785, 802, and 823. Because the ion current was not shared by coeluting interfering compounds and their ions, as seen from the blank meat sample, reproducibility was enhanced (Figure 5c). On the basis of the standard curves, the amount of BEA recovered from the spiked-beef sample was calculated to be 0.92 μg/mL, which translated to an extraction efficiency of 92%. No BEA was detected in the blank meat sample (Figure 5d).

Figure 6a shows the normalized total ion current and extracted ion current (*m/z* 785, 802, and 823) chromatographs of the corn sample spiked to contain 1 μg/mL of BEA. Because the nonpolar impurities were removed by hexane partitioning in the cleanup step and the

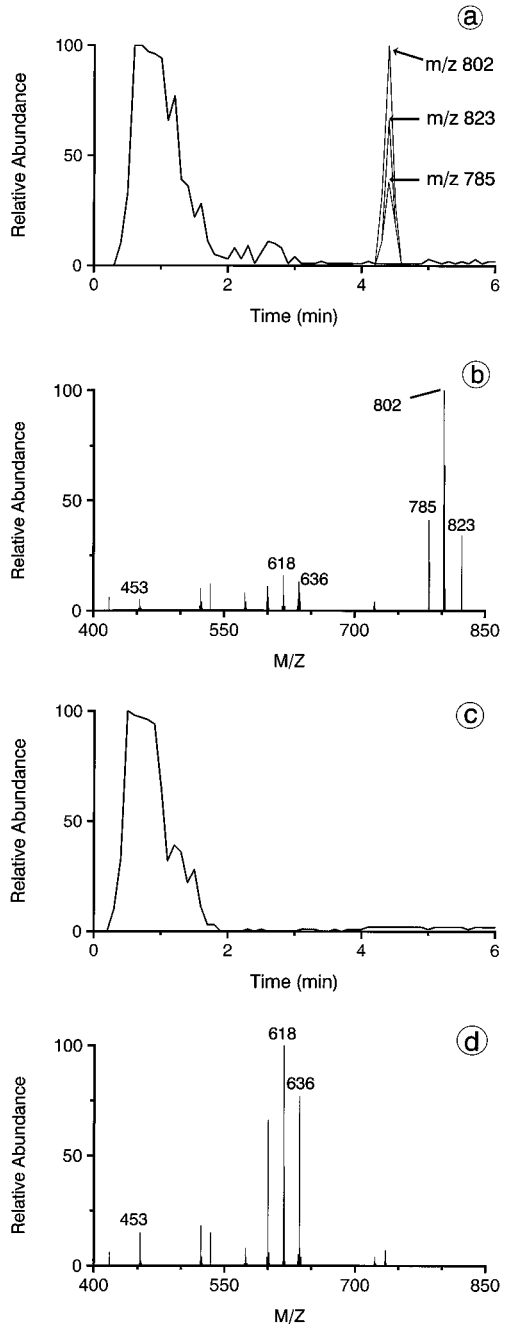

Figure 5. Positive ion, discharge-on, normalized (a) total ion current and the extracted ion chromatographs at 4.5 ± 0.2 min of beef spiked to contain 1 μg/g BEA, (b) linear scan mass spectrum collected at 4.5 ± 0.2 min of beef spiked to contain 1 μg/g BEA, (c) total ion current of blank beef sample denoting absence of matrix interference, and (d) linear scan mass spectrum collected at 4.5 ± 0.2 min of blank beef sample; all samples resolved on a C_{18} column using mobile phases containing acetonitrile (A) and 0.4 M ammonium formate buffer (B) mixtures and pumped in a gradient of 50% A (3 min hold) to 80% A at the end of 3.1 min.

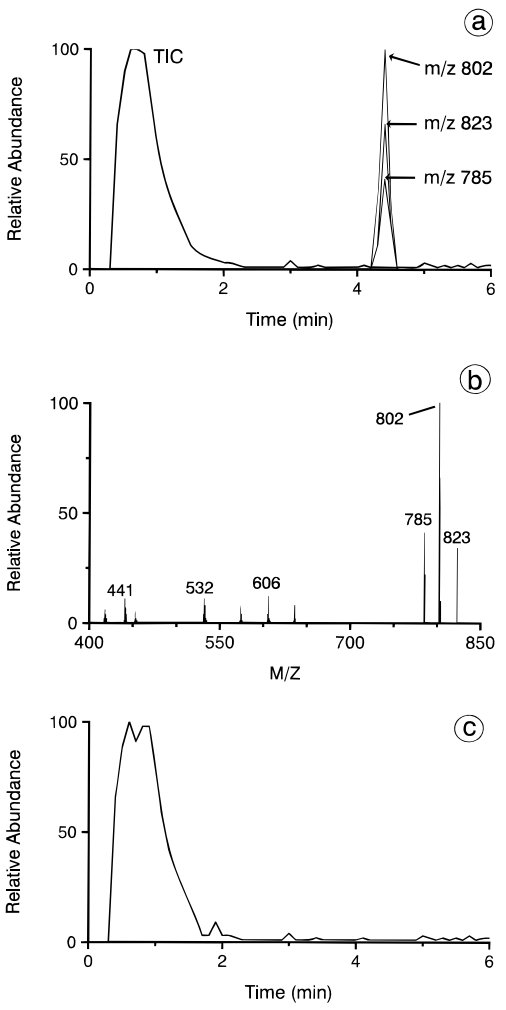

Figure 6. Positive ion, discharge-on, normalized (a) total ion current and the extracted ion chromatographs at 4.5 ± 0.2 min of corn spiked to contain 1 μg/g of BEA, (b) linear scan mass spectrum collected at 4.5 ± 0.2 min of corn spiked to contain 1 μg/mL BEA, and (c) total ion current of blank corn sample denoting absence of matrix interference; all samples resolved on a C_{18} column using mobile phases containing acetonitrile (A) and 0.4 M ammonium formate buffer (B) mixtures and pumped in a gradient of 50% A (3 min hold) to 80% A at the end of 3.1 min.

initial chromatographic conditions (50% aqueous, 0.0−3.1 min) facilitated elution of most polar impurities, BEA eluted in an area devoid of matrix interference (Figure 6a,b). On the basis of the standard curves, the amount of BEA recovered from the spiked-corn sample was 0.96 μg/g, which translated to an extraction efficiency of 96%. No BEA was detected in the blank corn sample (Figure 6c).

Figure 7a shows the normalized total ion current and extracted ion (*m*/*z* 785, 802, and 823) current chromatographs of the *F. proliferatum*-fermented corn sample. Although a large number of impurities eluted between 0.4 and 3.5 min, BEA can be identified easily because it eluted in an interference-free region of the mass spectrum (Figure 7b). Due to the high amount of BEA in the sample, an aliquot was diluted to ensure quantitation within the linear dynamic range of the standard curves. On the basis of the standard curves, the *F. proliferatum*-fermented corn sample was calculated to contain 330 μg/g of naturally occurring BEA. The culture medium was analyzed separately for the presence of FB_1−*o*-phthaldialdehyde fluorescence derivative by HPLC and was found to contain 2335 μg/g of FB_1 (Thakur and Smith, 1996). The co-occurrence of FB_1 and BEA in *F. proliferatum*-culture material was shown previously by Plattner and Nelson (1994) and Bottalico et al. (1995).

The high recovery efficiency associated with the method is not surprising, considering the simple cleanup procedure. All of the sample extract (filtrate) was concentrated, the resulting residue was redissolved, and the nonpolar impurities were removed by partitioning with hexane (BEA is insoluble in hexane and 100% water). The chance of target analyte loss was low,

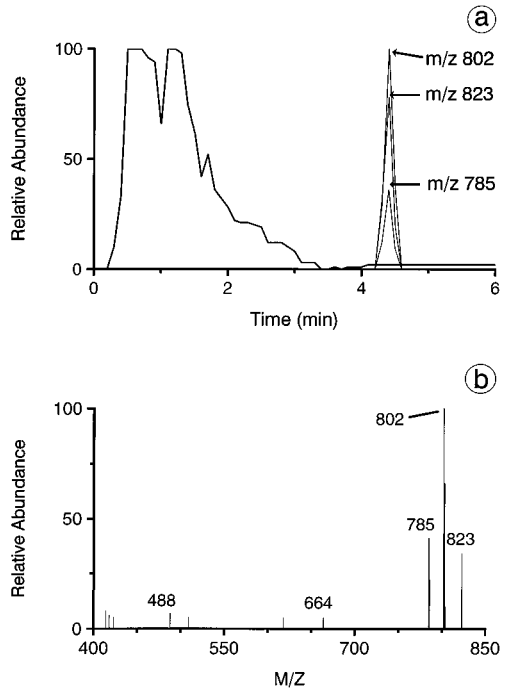

Figure 7. Positive ion, discharge-on, normalized (a) total ion current and the extracted ion chromatographs at 4.5 ± 0.2 min of *F. proliferatum*-fermented cracked corn sample containing 330 μg/g BEA and (b) linear scan mass spectrum collected at 4.5 ± 0.2 min of *F. proliferatum*-fermented cracked corn sample; all samples resolved on a C_{18} column using mobile phases containing acetonitrile (A) and 0.4 M ammonium formate buffer (B) mixtures and pumped in a gradient of 50% A (3 min hold) to 80% A at the end of 3.1 min.

because no limiting partition coefficients were present to undermine the extraction procedure. However, further experimentation is needed to prove the reproducibility of the developed extraction procedure, keeping in mind that it was developed only for mass selective detection of BEA. It must be reiterated that the emphasis of research was on detection of BEA from biologically diverse matrices, and it was not our goal to develop an extraction procedure for BEA.

CONCLUSION

On-line LC/TSP/MS was used to measure and verify the presence of BEA in three biologically diverse samples, namely *F. proliferatum*-fermented cracked corn, beef, and corn. Retention time and the presence of three distinct ions in the mass spectra enabled unequivocal identification of BEA. By taking advantage of the properties of reversed-phase chromatography and mass selective detection, we were able to simplify the cleanup procedure. The minimal sample cleanup procedure ensured against target analyte loss and, coupled with chromatography, permitted mass spectra acquisition devoid of matrix interference. Thus, LC/TSP/MS offers a practical method of BEA analysis in both food and biological matrices, with added advantages of minimal sample preparation and no chemical manipulation.

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LITERATURE CITED

Barcelo, D. Application of thermospray liquid chromatography/mass spectrometry for determination of organophosphorus pesticides and trialkyl and triaryl phosphates. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 363−369.

Barcelo, D. Effect of ammonium formate as ionizing additive in thermospray liquid chromatography-mass spectrometry for the determination of triazine, phenylurea, and chlorinated phenoxyacetic acid herbicides. *Org. Mass Spectrom.* **1989**, *24*, 219−224.

Benz, R. Alkali ion transport through lipid bilayer membranes mediated by enniatin A and B and beauvericin. *J. Membrane Biol.* **1978**, *43*, 367−394.

Blum, W.; Schlumpf, E.; Liehr, J. G.; Richter, W. J. On-line hydrogen-deuterium exchange in capillary gas chromatography-chemical ionization mass spectrometry (GC-CIMS) as a means of structure analysis in complex mixtures. *Tetrahedron Lett.* **1976**, *7*, 565−568.

Botallico, A.; Logrieco, A.; Ritieni, A.; Moretti, A.; Randazzo, G.; Corda, P. Beauvericin and fumonisin B1 in preharvest *F. moniliforme* maize ear rot in Sardinia. *Food Addit. Contam.* **1995**, *12*, 599−607.

Chi, H. T.; Baker, J. K. Use of deuterium-hydrogen exchange to characterize the fragmentation pathways of arteether and its metabolites in a thermospray mass spectrometer. *Org. Mass Spectrom.* **1992**, *27*, 12−17.

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.

Grove, J. F.; Pople, M. A. The insecticidal activity of beauvericin and enniatin complex. *Mycopathologia* **1980**, *70*, 103−105.

Gupta, S.; Krasnoff, S. B.; Underwood, N. L.; Renwick, J. A. A.; Roberts, D. W. Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans*. *Mycopathologia* **1991**, *115*, 185−189.

Hamill, R. L.; Higgens, C. E.; Boaz, H. E.; Gorman, M. The structure of beauvericin, a new depsipeptide antibiotic toxic to *Artemia salina*. *Tetrahedron Lett.* **1969**, *49*, 4255−4258.

Hunt, D. F.; Sethi, S. K. Gas-phase ion/molecule isotope-exchange reactions: methodology for counting hydrogen atoms in specific organic structural environments by chemical ionizations mass spectrometry. *J. Am. Chem. Soc.* **1980**, *102*, 6953−6963.

Logrieco, A.; Moretti, A.; Ritieni, A.; Chelkowski, J.; Altomare, C.; Botallico, A.; Randazzo, G. Natural occurrence of beauvericin in preharvest *F. subglutinans* infected corn ears in Poland. *J. Agric. Food Chem.* **1993**, *41*, 2149−2152.

Macchia, L.; Di Paola, R.; Fornelli, F.; Nenna, S.; Moretti, A.; Napoletano, R.; Logrieco, A.; Caiaffa, M. F.; Tursi, A.; Bottalico, A. Cytotoxicity of beauvericin to mammalian cells. *Abstract International Seminar on Fusarium*, Martina Franca, Italy, May 9−13, 1995; p 72.

Ojcius, D. M.; Zychlinsky, A.; Zheng, L. M.; Young, J. D-E. Ionophore-induced apoptosis: role of DNA fragmentation and calcium fluxes. *Exp. Cell Res.* **1991**, *197*, 43−49.

Ovchinnikov, Y. A.; Ivanov, V. T.; Mikhaleva, I. I. The synthesis and some properties of beauvericin. *Tetrahedron Lett.* **1971**, *2*, 159−162.

Plattner, R. D.; Nelson, P. E. Production of beauvericin by a strain of *Fusarium proliferatum* isolated from corn fodder for swine. *Appl. Environ. Microbiol.* **1994**, *60*, 3894−3896.

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.

Sheil, M. M.; Guihaus, M.; Derrick, P. J. Collision-activated decomposition of peptides by Fourier transform ion cyclotron resonance spectrometry. *Org. Mass Spectrom.* **1990**, *25*, 671−680.

Shepard, G. S.; Thiel, P. G.; Alberts, J. F.; Gelderblom, W. C. A. Fate of a single dose of the ^{14}C-labelled mycotoxin, fumonisin B_1 in rats. *Toxicon* **1992**, *30*, 768−770.

Siegel, M. M. Hydrogen-deuterium exchange studies utilizing a thermospray mass spectrometer interface. *Anal. Chem.* **1988**, *60*, 2090−2095.

Smith, J. S.; Thakur, R. A. Occurrence and fate fumonisins in beef. In *Fumonisins in Food*; Jackson, L. S., DeVries, J. W., Bullerman, L. B., Eds.; Plenum Press: New York, 1996; pp 39−55.

Steinrauf, L. K. Beauvericin and other ennatins. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Dekker: New York, 1985; pp 140−171.

Thakur, R. A.; Smith, J. S. Determination of fumonisins B_1 and B_2 and their major hydrolysis products in corn, feed, and meat, using HPLC. *J. Agric. Food Chem.* **1996**, *44*, 1047−1052.

Voyksner, R.; Pack, T.; Smith, C.; Swaisgood, H.; Chen, D. Techniques for enhancing structural information from HPLC/MS. In *Liquid Chromatography/Mass Spectrometry*; Brown, M. A., Ed.; American Chemical Society: Washington, DC, 1990; pp 14−39.

Yergey, A. L.; Edmonds, C. G.; Lewis, S.; Vestal, M. L. *Liquid Chromatography/Mass Spectrometry—Techniques and Applications*; Plenum Press: New York, 1989.

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