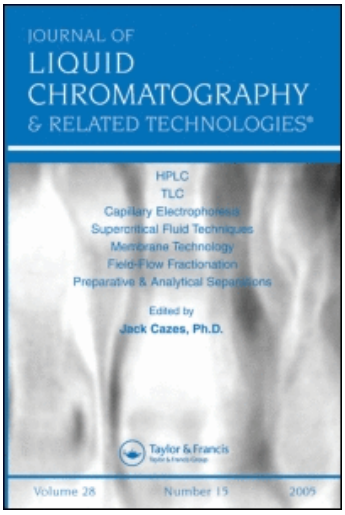

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A SIMPLE, SENSITIVE METHOD FOR DETECTION OF VOMITOXIN (DEOXYNIVALENOL) USING REVERSED PHASE, HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Vomitoxin, a trichothecene mycotoxin produced by Fusarium graminearum, can be measured at levels as low as 5-10 ppb in contaminated corn by reversed phase, high performance liquid chromatography on Partisil 10 ODS-2 eluted with acetonitrile-water and detection at 214 nm. Corn extracts were initially purified by preparative thin layer chromatography on silica gel. Optimal separations of vomitoxin from other components in a sample of contaminated corn were achieved with 25% or less acetonitrile in water in which vomitoxin elutes with $k' \geq 1.06$. Using 25% acetonitrile in water, the minimum detectable amount was 1.5 ng and the relationship between peak height and amount of vomitoxin was linear over the range 1-30 ng. Although resolution was greater when less acetonitrile was present in the eluant, sensitivity was somewhat lower.

INTRODUCTION

Vomitoxin (Fig. 1) (3α, 7α, 15-trihydroxy-12,13-epoxy-trichothec-9-ene-8-one; also called deoxynivalenol) is a

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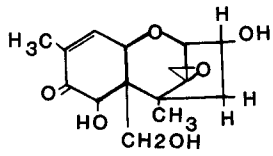

vomitoxin

FIGURE 1. Structure of vomitoxin (3α,7α,15-trihydroxy-12, 13-epoxy-trichothec-9-ene-8-one.

trichothecene mycotoxin produced by Fusarium graminearum (2,3). Recently, it has been identified in Fusarium-contaminated corn (4-6) and wheat (7) grown in the mid-western United States and has been shown to cause emesis and feed-refusal in swine (4,8,9) fed these contaminated grains.

A number of different physical methods have been described for detection and quantitation of vomitoxin (10-16). Except for thin layer chromatography, these methods require derivatization of vomitoxin prior to analysis. Only one report has been made to date describing the use of HPLC for analysis of vomitoxin and this describes a complicated detection system in which aliquots are collected after HPLC and analyzed by gas chromatography after derivatization (17). In this paper we describe a simple procedure in which vomitoxin in methanol-water extracts of contaminated corn is first purified by preparative thin layer chromatography and then measured by reverse-phased, high performance liquid chromatography.

EXPERIMENTAL

HPLC

The HPLC system used in this study included two Waters Model 6000A pumps, a Model 2000 solvent programmer, and a U6K injector. The injector was connected to a Whatman guard column (7 cm, ID = 2.1 mm) packed with Perisorb RP-18, 30-40 μm from EM Reagents. The column was a Partisil-10 ODS 2 from Whatman (25 cm, ID = 4.60 mm). The eluant was monitored by a Waters Model 441 UV detector operated at 214 nm. Acetonitrile was HPLC grade from Burdick and Jackson and water was purified on a Milli-Q-System with Organex-Q cartridge from Millipore. The water was degassed by autoclaving before use. Samples were dissolved in acetonitrile before injection.

Toxin

Vomitoxin used as a standard was a purified sample (mp 154°C) prepared in our laboratory by the method of Vesonder, et al (2,4). The UV spectra of vomitoxin were obtained on a Beckman DK2A ratio recording spectrophotometer.

Vomitoxin was also isolated from 50 g samples of contaminated corn by extraction with 200 ml of 85% aqueous MeOH. The filtered extract was evaporated to dryness, redissolved in a minimum amount of methanol, and ethyl acetate was added. The precipitate was discarded and the ethyl acetate solution evaporated to dryness. The resulting residue was triturated with hexane to

remove corn oils and then dissolved in 2 ml of ethyl acetate. An aliquot of the crude sample was further purified by preparative thin layer chromatography on a 20 x 20 cm plate containing a 0.5 mm layer of silica gel 60 (EM Reagents). The plate was eluted with benzene-ethyl acetate-formic acid (10:4:1) and, after development and drying, the band eluting at the same Rf as that of vomitoxin (spotted alongside the sample band and visualized by spraying with 20% $AlCl_3$ in water and heating at 120°C for 5 min). Absorbed components were eluted from the silica gel with methanol-chloroform (2:8) and after evaporation to dryness on a Büchi rotary evaporator, were redissolved in 1 ml acetonitrile for HPLC analysis. Mass spectra of trimethylsilyl-derivatized vomitoxin (6) in fractions isolated from HPLC were determined on a Finnegan mass spectrometer operated at 75 eV in the selected ion mode under computer control for the two ions characteristic of vomitoxin (m/e 422,407).

RESULTS AND DISCUSSION

The UV maximum of vomitoxin in ethanol has been reported as λ max = 218 nm with an ε = 4500 (16). In 50% aqueous acetonitrile vomitoxin had an ε = 5600 at 214 nm, the wavelength used for HPLC detection. This absorbance was not affected significantly by acid (acetonitrile concentration remaining the same) but showed a 30% decrease when the solvent was changed from 50% to 5% aqueous acetonitrile. These data suggest that the sensi-

tivity of detection of vomitoxin will increase with higher acetonitrile content in the eluting solvent mixture.

The retention times and capacity ratios (k') for vomitoxin eluted with different mixtures of acetonitrile and water are given in Table 1. As can be seen, k' and peak retention time increase as acetonitrile content decreases. Using water only, vomitoxin did not elute within 30 min. Significant peak broadening was noted when acetonitrile content of the eluting mixture was less than 15%. Optimal resolution and sensitivity was experienced using acetonitrile-water ratios ranging from 1:6 to 1:3. No peak tailing of the standard was noted even with low

TABLE I. Elution Parameters of Vomitoxin in Acetonitrile-Water on a C_{18}-Bonded Silica Column (Partisil-10 ODS-2).

Percent acetonitrile	Retention time (t_r) (sec)	k'*
70	118	0.56
60	123	0.56
50	132	0.55
40	127	0.69
30	161	1.00
25	170	1.06
20	227	2.00
15	316	2.94
10	769	7.15
5	1026	8.06
0	>1800	>20

* k' = (Ve - Vo)/Vo where Vo = void volume and Ve = volume of solvent required for peak elution; flow rate 1.5 ml/min.

levels of acetonitrile; peak shape also was satisfactory. The peak for vomitoxin standard (eluted with acetonitrile-water (1:3)) was sufficiently sharp (Fig. 2) to allow detection of as little as 1.5 ng per injection.

Using the solvent system, acetonitrile-water (1:3), the relationship between peak height (Fig. 2) and peak area and the amount of vomitoxin injected was almost linear over the range 1-30 ng (Fig. 3). Using decreased sensitivity settings on the detector, this range could be extended to 300 ng per injection with only slight deviation from linearity. Precision and reproducibility of peak area and retention time was good with coefficients of variation (20 injections) of 2.0 for peak area and 1.5

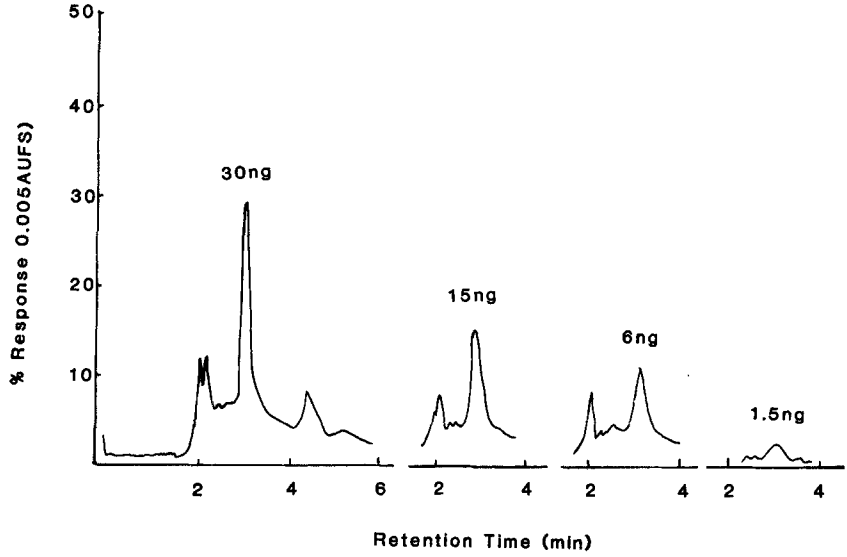

FIGURE 2. HPLC resolution of vomitoxin on a Whatman Partisil-10-ODS 2 column eluted at 1.5 ml/min with acetonitrile-water (1:3) with detection at 214 nm.

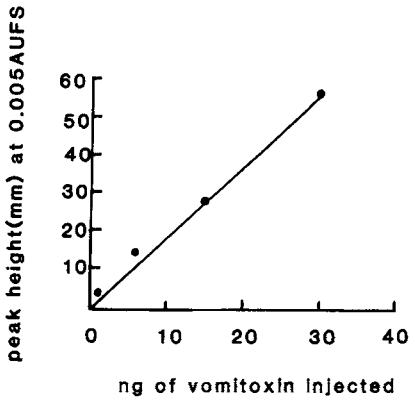

FIGURE 3. Linearity, peak height vs. quantity of vomitoxin, at 214 nm, eluted with $CH_3\overline{CN}$-H_2O (1:3). (See Figure 2.)

for retention time respectively, using the solvent ratio of 1:3 acetonitrile-water. Mean sensitivity for vomitoxin detection was 1.9 mm/ng of peak height for this solvent system. Detection sensitivity was somewhat higher in systems containing a higher ratio of acetonitrile and somewhat lower in systems containing a lower ratio.

To check whether contaminated corn would contain interfering compounds with retention times overlapping that of vomitoxin, an extract of corn infected with Fusarium graminearum NRRL 5883 (2) was prepared under conditions identical to those which would be used in an analytical isolation of vomitoxin from corn. This mixture was fractionated by preparative thin layer chromatography. Upon HPLC (elution at 1.0 ml/mm, CH_3CN-H_2O 1:4) only the TLC fraction (R_f = 0.14 to 0.18) co-eluting with vomitoxin standard showed a peak (Fig. 4a) eluting at 5.3 min. Upon

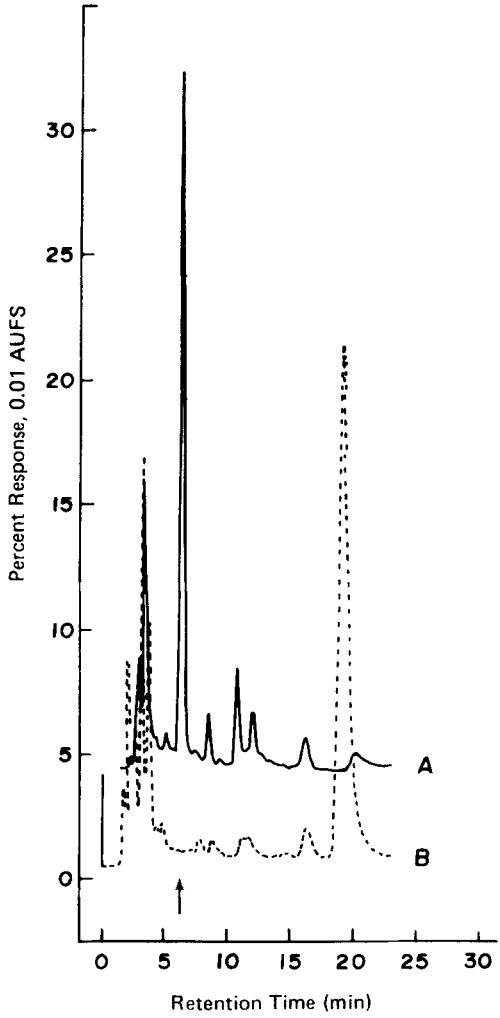

FIGURE 4A HPLC chromatogram of the vomitoxin fraction from TLC of an extract of F. graminearum contaminated corn. (see text for details). Elution was at 1.0 ml/min with CH_3CN-H_2O (1:4).

FIGURE 4B HPLC chromatogram of the material migrating at the same R_f as vomitoxin from TLC of an extract of non-contaminated corn, prepared under the same conditions as those for Fig. 4A.

co-injection of standard and sample the peak height was increased without affecting peak shape indicating co-migration of standard with the vomitoxin in the sample. Furthermore, the vomitoxin peak in the HPLC of extracts from three different samples of contaminated corn was collected and analyzed by gas liquid chromatography-mass spectrometry. The quantitative data obtained by this method was in close agreement with that obtained by HPLC. Extracts of corn not contaminated with F. graminearum were prepared under identical conditions to those for contaminated corn. No peak was found which overlapped that of vomitoxin (Fig. 4b).

These results show that amounts as low as 2-5 ng of vomitoxin can be determined using HPLC and detection at 214 nm. Vomitoxin is only slightly retained on reversed phase silica columns using high ratios of acetonitrile to water. However, using preparative TLC followed by HPLC of crude extracts of contaminated corn good separation of vomitoxin from other components in the corn can be achieved. The method described here permits rapid and sensitive quantitative analysis of vomitoxin without prior derivatization.

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