Multitoxin Screening Method for *Fusarium* Mycotoxins in Grains

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A screening method has been developed for the major *Fusarium* mycotoxins. It allows for analysis of trichothecenes derived from nivalenol (NIV), deoxynivalenol (DON), scirpentriol (Sctol), and T-2 tetraol (T-2tol) as the parent alcohols. The toxins are extracted with acetonitrile–methanol–water (80:5:15), and primary purification on a cation-exchange/alumina–carbon column is followed by hydrolysis and then, after neutralization, by a further purification on a carbon–Celite minicolumn. Quantification is by HPLC/UV (NIV and DON) or GC/ECD (NIV, DON, Sctol, T-2tol). Recoveries of parent alcohols and representative ester derivatives spiked into wheat and maize extracts at 0.5 μg/g equiv were in the range 55–103%. Levels of 0.05 μg/g or less are detectable by the method. The method gives improved stability of extracts after hydrolysis compared to an existing method; hence, results are more reliable and consistent. The hydrolysis step may be eliminated for analysis of individual trichothecenes. Estimated concentrations of trichothecenes (as parent alcohols) in maize samples commonly increased when analyzed with hydrolysis relative to analyses performed without hydrolysis. This suggested the common occurrence of trichothecene esters as well as the parent alcohols in naturally contaminated samples. An additional part of the method allows for cleanup of a second aliquot of extract using a C_{18} solid-phase column. This portion produces separate fractions suitable for the analysis of moniliformin and zearalenone, both by HPLC. Mean recoveries for moniliformin and zearalenone from blank samples fortified at 0.5 μg/g were 78% and 72%, respectively. Both toxins could be detected down to 0.01 μg/g, but for moniliformin, coextractive interferences in some samples and the need for peak confirmation at a second wavelength made identification difficult below 0.1 μg/g.

INTRODUCTION

Fusarium fungal species are known to infect agricultural products, particularly grains, throughout the world (Nelson et al., 1981; Booth, 1984). Several species of *Fusarium* have also been widely reported as producers of mycotoxins (Marasas et al., 1984; Chelkowski, 1989), primarily zearalenone (Mirocha et al., 1971; di Menna et al., 1987), moniliformin (Thiel et al., 1986), and the nonmacrocyclic trichothecenes (Pathre and Mirocha, 1979; Ueno, 1983a; Snyder, 1986; Grove, 1988). This latter group consists of a diverse range of structurally related compounds of which over 80 have been isolated from natural sources (Grove, 1988). Many of these, and in particular most of those that have been reported from agricultural products, derive from four basic trichothecene skeletons, namely nivalenol (NIV), deoxynivalenol (DON), scirpentriol (Sctol), and T-2 tetraol (T-2tol). While it is unlikely that trichothecenes of these four chemical families would be the only contaminants in naturally contaminated products, culture work suggests that they would form the major portion of total trichothecene contamination.

Zearalenone may be analyzed by high-performance liquid chromatography (HPLC) (Bagneris et al., 1986; Tanaka et al., 1985), as can moniliformin (Shepherd and Gilbert, 1986; Scott and Lawrence, 1987). Trichothecenes are commonly analyzed by gas chromatography (GC) after derivatization (Scott and Kanhere, 1986). Numerous other analytical methods have been published, most frequently employing thin-layer chromatography (TLC), HPLC, or GC [see Kamimura et al. (1981), Romer (1986), and reviews by Scott (1982), Gilbert (1984), and Ueno (1983b)]. Kamimura et al. (1981) described a multitoxin method for several *Fusarium* toxins including seven common trichothecenes. Indeed, most published trichothecene procedures describe sensitive methods for a limited range of toxins (Tanaka et al., 1985; Romer, 1986; Lauren and Greenhalgh, 1987; Scott et al., 1989).

Since attempts to analyze products for all trichothecenes individually would be extremely difficult and time-consuming, there would be some advantage in converting all trichothecenes to their basic chemical skeletons and analyzing for these. In this way the total trichothecene content could be more readily determined. Methods have been published that screen for trichothecene content of the four main families (NIV, DON, Sctol, T-2tol) after hydrolysis of any toxins present to the basic alcohol skeletons (Rood et al., 1988a,b; Kroll et al., 1988). These methods offer great potential for simplified analysis and for more fully measuring the trichothecene content of products. However, the method of Kroll et al. (1988) involves a 24-h hydrolysis step which we have found to give low recoveries, and we, and others (Scott, 1990), have also found that the methods described by Rood et al. (1988a,b) can give variable results. The method described here overcomes these problems.

This paper describes a simple and reproducible method for combined analysis of all trichothecenes of the NIV, DON, Sctol, and T-2tol types. An addition to the method allows a separate aliquot of extract to be worked up to yield fractions for analysis of zearalenone and moniliformin.

MATERIALS AND METHODS

Standard Materials. NIV was purchased from Romer Labs, Inc. (Washington, MO). T-2tol was purchased from Sigma Chemical Co. (St. Louis, MO). DON, 3-acetyldeoxynivalenol (3-ADON), and T-2 toxin (T-2) were provided by R. Greenhalgh and J. D. Miller, Plant Research Centre, Agriculture Canada (Ottawa, Canada). Preparation of 4,15-diacetylnivalenol (DANIV), Sctol, and 4,15-diacetoxyscirpenol (DAS) has been described (Grove and Mortimer, 1969). Zearalenone was provided by C. J. Mirocha, University of Minnesota (St. Paul, MN). Moniliformin was provided by M. J. Shepherd, MAFF (Norwich, U.K.). All standards were stored in sealed vials at −10 °C.

Chemicals and Reagents. Solvents were all of either AR or HPLC grade. Ethyl acetate and toluene were redistilled before

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use. Water was distilled in glass and then passed through a Millipore Milli-Q water purifier. Trifluoroacetic anhydride (TFAA) was purchased from Pierce Chemical Co. (Rockford, IL) and stored sealed at 4 °C. Tetrabutylammonium dihydrogen phosphate, 1 M solution in water (TBAHP), was purchased from Aldrich Chemical Co. (Milwaukee, WI). Alumina was of neutral grade 1, purchased from Woelm (Eschwege, Germany), and used as received. Carbon was Darco G-60 from J. T. Baker Chemical Co. (Phillipsburg, NJ) and used as received. Cation-exchange resin was Dowex 50W-X16 from Bio-Rad Laboratories (Richmond, CA). The resin was washed before use as described by Lauren and Greenhalgh (1987). Extract-Clean C_{18} columns (500 mg/2.8 mL) were purchased from Alltech Associates, Inc. (Deerfield, IL). The sodium hydroxide hydrolysis solution was freshly prepared at least monthly.

Apparatus. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-6A gradient system fitted with autosampler, column oven, variable-wavelength UV detector, an RF-535 fluorescence detector, and a C-R3A data processor. Gas chromatography with electron capture detection (GC/ECD) was performed on a Varian 3700 fitted with a megabore on-column injector adaptor, a Shimadzu AOC-7 autosampler, and a Shimadzu C-R1A data processor.

Extraction. The sample was finely ground and mixed well. A subsample (20 g) was mixed with acetonitrile–methanol–water (80:5:15, 100 mL) and shaken for 2 h. The contents were allowed to settle, and then aliquots were removed for cleanup. Alternatively, the supernatant may be removed and stored at −20 °C for up to 4 weeks before use. A 10-mL aliquot (2 g equiv) was removed for trichothecene analysis and a 25-mL aliquot (5 g equiv) for zearalenone and moniliformin analysis.

Cleanup. *Trichothecenes.* The procedure was adapted from the method described earlier for NIV and DON (Lauren and Greenhalgh, 1987). A glass column (10 mm i.d. × 250 mm) tapered at one end was plugged with glass wool and dry-packed with alumina–carbon (20 + 1) mixture (1 g) followed by prewashed cation-exchange resin (2 g). A 10-mL aliquot of extract was applied to the column and allowed to drain under gravity. The first 2 mL of eluate was discarded and the rest (ca. 6 mL) collected and mixed to ensure homogeneity. A 5-mL aliquot (equivalent to 1 g of grain) of this solution was evaporated to dryness under vacuum at ≤58 °C, and the residue was treated with 0.2 N sodium hydroxide in methanol–water (90:10, 150 μL). This mixture was mixed by vortex and then allowed to stand at room temperature (ca. 20 °C) for 60 min. After this time, 0.25 N hydrochloric acid in methanol–water (95:5, 150 μL) was added and the solution mixed by vortex.

To this solution was added methanol–acetonitrile (3:1, 2 mL), and the resultant solution mixed and applied to a minicolumn made from a Pasteur pipet (145 mm) plugged with lightly compressed glass wool and dry-packed with Celite (30–50 mg) followed by carbon (120 mg) (Lauren and Greenhalgh, 1987). [This minicolumn must be washed with methanol–acetonitrile (3:1, 2 mL) immediately before use.] The column was allowed to drain by gravity and the eluate collected. The hydrolysis flask was rinsed with a further two batches of methanol–acetonitrile (3:1, 2 mL), and each was applied in turn to the minicolumn once the preceding rinse had completely drained. The total 6-mL eluate was collected in a 15-mL tube and mixed. This solution was evaporated to dryness (N_2, 45 °C) and was ready for analysis by either HPLC or GC/ECD, or split to yield fractions for analysis by both methods. The dried residue was stable for at least 2 weeks at 4 °C in sealed tubes.

Zearalenone and Moniliformin. The procedure was adapted from the method for moniliformin by Scott and Lawrence (1987). A 25-mL aliquot of extract was washed with hexane (25 mL) in a stoppered tube, and then the hexane was removed and discarded. An aliquot (20 mL) of the washed extract was evaporated to dryness at 45 °C under vacuum and then dissolved in methanol (0.5 mL). An Extract-Clean C_{18} column was preconditioned with methanol (2 mL) and water (2 mL) at about 3 drops/s under gentle vacuum, ensuring the column was taken to damp-dry by suction (ca. 5–10 s) after each addition. The suction was removed, and a sample collection flask was then placed under the cleanup column. A 100-μL aliquot of the methanol solution (equivalent to 0.8 g of grain) was then applied to the preconditioned column, which was again dried by suction (ca. 5 s). One fraction was eluted with water (2 mL) under gentle vacuum. This fraction was then evaporated to dryness under vacuum at 45 °C and the residue dissolved in the HPLC mobile phase for moniliformin (0.8 mL). This solution was passed through a small alumina column (150 mg in a Pasteur pipet) and the eluate collected and used immediately for analysis of moniliformin or stored at −10 °C until analysis.

A second fraction was eluted from the C_{18} cleanup column under gentle vacuum with methanol–water (80:20, 2 mL). This fraction was evaporated to dryness (N_2, 45 °C) and the residue dissolved in methanol–water (50:50, 0.8 mL). This solution was used for zearalenone analysis.

Trichothecene Analysis. *NIV and DON by HPLC.* The cleaned-up residue was dissolved in an appropriate volume of methanol–water (5:95) to give the equivalent of 1 g of grain/mL of solution. (Use 1 mL if the total dried residue is used for HPLC analysis.) The HPLC column was a Chrompak CP Spher C_8 (4.6 mm i.d. × 250 mm), and the mobile phase was methanol–water (15:85) at 1 mL/min. The column and precolumn in-line filter were held at 35 °C. The detector was set at 222 nm with an attenuation of 0.01 AUFS. Quantitation of toxins was relative to a mixed external standard containing NIV and DON at 0.9 and 1.0 μg/mL, respectively, in methanol–water (5:95). Typical injections were of 100 μL, and under these conditions the standard solution showed 50% full-scale deflection (fsd) at 422 s for NIV and 36% fsd at 773 s for DON. Estimations were possible at about 0.03 μg/g grain for both toxins. An alternative detection wavelength of 245 nm was useful for peak confirmation and in the few instances when coextractives interfered with analysis. Responses for NIV and DON at 245 nm were about half those at 222 nm.

GC/ECD Analysis for NIV, DON, Sctol, and T-2tol. The residue for GC analysis was disssolved in ethyl acetate (200 μL) with vortexing in a 15-mL screw-top culture tube. TFAA (100 μL) was added and the solution mixed by vortex, the tube was stoppered, and the mixture was heated at 75–80 °C. After 1 h, the tube was removed from heat, and the mixture was evaporated to dryness under a *gentle* stream of nitrogen (40 °C, 10 min). (Note: A too vigorous nitrogen flow, or use of a short tube, can lead to loss of the volatile trichothecene derivatives, especially the NIV derivative). The residue was dissolved in an appropriate volume of toluene for analysis. These samples were stable for at least 24 h in screw-top vials at room temperature. Quantitation was by comparison with a range of mixed external standards prepared with the samples. The analytical column was a DB-5 (0.536 mm i.d. × 15 m, 1.5-μm film) held at 175 °C with a helium gas flow of 4 mL/min. Nitrogen makeup gas flow was 50 mL/min. An injection size of 1 μL was used. Injector temperature was 220 °C. Typical retention times for the TFAA derivatives were 372, 499, 575, and 627 s for NIV, Sctol, DON, and T-2tol, respectively. Estimations were possible to 0.02 μg/g of grain for the four toxins.

Zearalenone Analysis by HPLC. The HPLC column was a Zorbax ODS (4.6 mm i.d. × 240 mm) with a mobile phase of methanol–1% aqueous acetic acid (62:38) at 1 mL/min and a column temperature of 35 °C. The detector was set at 280 (excitation) and 460 nm (emission). Injection volume was 40 μL, and the retention time of zearalenone was 880 s. Quantitation was relative to an external standard of 1.0 μg/mL in methanol–water (50:50) and was possible at or below 0.01 μg/g of grain.

Moniliformin Analysis by HPLC. The analytical column was a Zorbax ODS (4.6 mm i.d. × 240 mm) with a mobile phase of 0.01 TBAHP in acetonitrile–water (16:84) at 1 mL/min with a column temperature of 26 °C. The detector was set at 229 nm (or alternatively 260 nm) with an attenuation of 0.01 AUFS. Injection volume was 40 μL, and the retention time of moniliformin was 1014 s. Quantitation was made relative to an external standard of 0.23 μg/mL in the mobile phase. All standard solutions were stored at −10 °C between uses and were allowed to return to ambient temperature before use. Estimations on standards were possible to 0.01 and 0.03 μg/mL at 229 and 260 nm, respectively, but coextractive peaks in many samples made identification difficult below 0.1 μg/mL (equivalent to 0.1 μg/g of grain). Confirmation by estimation at both wavelengths was essential.

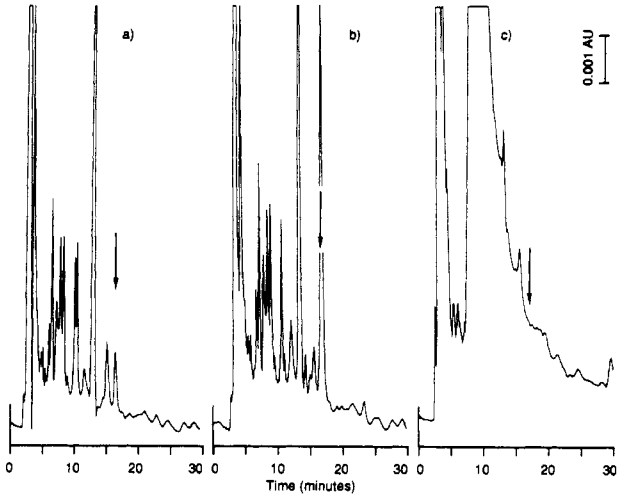

Figure 1. Maize and wheat samples analyzed for moniliformin by HPLC. Conditions as in text, with detection wavelength 229 nm. (a) Maize extract showing 0.04 mg/kg; (b) extract of maize used in (a) spiked at 0.5 mg/kg, shows 0.34 mg/kg; (c) wheat extract, moniliformin not detectable at a 0.01 mg/kg limit. Arrow indicates retention time of moniliformin.

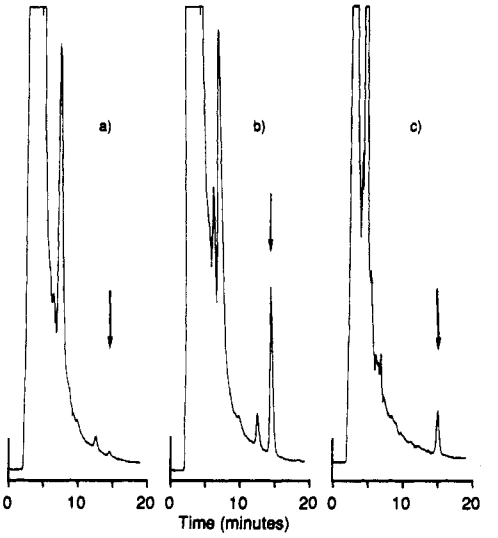

Figure 2. Maize and wheat samples analyzed for zearalenone by HPLC. Conditions as in text. (a) Maize extract showing 0.01 mg/kg; (b) maize extract showing 0.46 mg/kg; (c) wheat extract showing 0.13 mg/kg. Arrow indicates retention time of zearalenone. Minor peaks in (a) and (b) are not α- or β-zearalenol.

RESULTS AND DISCUSSION

The method described for analysis of zearalenone and moniliformin is based on the moniliformin method of Scott and Lawrence (1987). Conditions are modified to improve results for moniliformin, and an extra fraction is taken for zearalenone analysis.

The volume of methanol extract solution added to the solid-phase cleanup column was set at 100 μL since it was found that interferences for moniliformin analysis increased significantly when greater than 100 μL was used or when the column was not dried by suction after this addition. Figure 1 shows the analysis of maize and wheat samples for moniliformin by the described method. Wheat samples show more background interference than maize, but both give generally satisfactory conditions for analysis. Recovery for replicate spiked blank samples fortified with 0.5 μg/g of standard material was 78%, range 60–92%.

Coextractive interferences in many samples made identification of moniliformin difficult below 0.1 μg/g. A number of samples showed suspected moniliformin at 229-nm detection, but this was not confirmed at the alternative wavelength of 260 nm. Attempts to overcome these problems by using different mobile phases, analytical columns, and column temperatures were not successful. Therefore, suspected positives for moniliformin must be confirmed at two wavelengths and by peak shape. Column temperature control is essential to obtain consistent retention times. The method gives results similar to those obtained with the method of Shepherd and Gilbert (1986) but is more rapid, and also yields a fraction suitable for zearalenone analysis.

Results for zearalenone analysis are shown in Figure 2. Sample chromatograms are shown for both maize and wheat samples and show that interference was negligible at the detection limit of 0.01 μg/g. The mean recovery for fortified samples (0.25 and 0.5 μg/g) taken over several different analytical runs was 72%, range 60–82%. When this method is used for analysis of zearalenone alone, each Extract-Clean C_{18} column may be reused up to four times provided it is reconditioned between each sample. There is no noticeable deterioration in either recovery of zearalenone or removal of coextractives.

The method for trichothecenes gives an estimate of the total content by converting any trichothecenes present to their parent alcohols. It has been verified for the four main families, namely NIV, DON, Sctol, and T-2tol. If the hydrolysis step is omitted, the method is also suitable as a cleanup for individual trichothecene analysis.

Table I. Recoveries of Trichothecene Alcohols[a] and Esters[b] Spiked[c] into Wheat and Maize Extracts before Cleanup

substrate	spike	recovery,[d] % NIV	DON	Sctol	T-2tol
wheat	alcohols	73 ± 9	69 ± 4	78 ± 9	82 ± 4
	esters	90 ± 3	55 ± 2	60 ± 4	70 ± 6
maize	alcohols	71 ± 2	70 ± 8	82 ± 4	83 ± 6
	esters	103 ± 6	57 ± 2	62 ± 12	62 ± 7

[a] NIV, DON, Sctol, T-2tol. [b] DANIV, 3-ADON, DAS, T-2. [c] 1 μg of each toxin added to 10 mL of extract solution (equivalent to 2 g of substrate) before cleanup. [d] Mean recovery ± standard error, calculated on a molar basis; n = 3 for wheat, n = 5 for maize.

The method was adapted from a method for NIV and DON described earlier by Lauren and Greenhalgh (1987). That method was shown to give good recoveries (83–94%), at least comparable to other accepted methods. The new extractant of acetonitrile–methanol–water (80:5:15) in place of acetonitrile–water (85:15) and the new carbon minicolumn solvent of methanol–acetonitrile (3:1) in place of methanol have been found to give higher results for naturally contaminated samples. The third major change was the introduction of a hydrolysis step.

Recoveries for the hydrolysis method have been determined by using both parent alcohols and representive ester derivatives of each alcohol added to both wheat and maize extracts. The results are presented in Table I. The recovery varies for each structural type and also between components spiked as alcohols or esters, but generally the results are in the same range reported by Rood et al. (1988a,b). As stated by those authors, the hydrolysis conditions chosen are a trade-off between the relative rates of ester hydrolysis and degradation of the different trichothecene skeletons and the interaction of these factors with the sample matrix. Different values are obtained in the absence of the sample matrix; therefore, reactions with pure standards, while useful to show trends, were not helpful in defining the final hydrolysis conditions.

When we chose our hydrolysis conditions, among the first examined were those decribed by Rood et al. (1988a,b).

Table II. Recovery of NIV[a] and DON[a] after Hydrolysis Treatments

test[b]	hydrolyzed	neutralized	recovery,[c] % NIV	DON
1	no	no	97	99
2	yes	HOAc	9	8
2	yes	HCl	88	76
3	yes	HOAc	71	64
3	yes	HCl	92	92

[a] 250 ng of each toxin in 25 μL of MeOH added to clean test tubes for each test. [b] Tests as follows: 1, evaporate solvent and leave dry overnight at room temperature; 2, hydrolyze and neutralize as in method but with acids as shown, evaporate, and leave dry overnight at room temperature; 3, hydrolyze and neutralize as in test 2, pass through carbon–Celite minicolumn as in method, leave in solution overnight at room temperature, evaporate. [c] All residues dissolved in 0.5 mL of methanol–water (5:95) for analysis by HPLC.

While those conditions gave satisfactory results for samples processed through to analysis within the same day, considerable variability resulted for samples when analysis was delayed for 1 day or more after hydrolysis. This effect was examined, and the predominant influence was the use of acetic acid (HOAc) as the neutralizing agent after hydrolysis, in particular if the hydrolysis solution was evaporated before storage. It was found that using hydrochloric acid (HCl) as the neutralizing agent overcame the problem. This is illustrated for NIV and DON in Table II. Such instability after the hydrolysis step was alluded to by Rood et al. (1988a), although no explanation was given. The presently described method was found to give extracts that were stable for up to 14 days when stored dry in stoppered tubes at or below 4 °C. Storage for more than 1 or 2 days at ambient temperature is not advised, however, since this can lead to low estimates. If this is unavoidable, the extracts should be left in solution.

The particular brand of carbon used in the cleanup, in particular in the carbon–Celite minicolumn (i.e., the second column), was found to be critical. Eight different forms of carbon were tested, but most produced low recoveries or poor cleanup. Only Darco G-60 from J. T. Baker Chemical Co. gave a satisfactory cleanup and recovery.

Derivatization of trichothecenes for GC analysis has been extensively studied, and various reasons have been proposed for poor or variable results (Gilbert et al., 1985; Kientz and Verweij, 1986, 1987; Rizzo et al., 1986). Interlaboratory differences also seem a major contributing factor. In our hands derivatization with TFAA in the presence of ethyl acetate cosolvent was found to be both simple and reliable, in addition to producing a moderately stable derivative solution. Addition of dimethylaminopyridine to the reaction gave a small increase in derivatization yield but greatly increased the rate of degradation of NIV and T-2tol derivatives. The use of solid sodium bicarbonate (Kientz and Verweij, 1986) had no beneficial action and requires more steps in workup. It was noticed that not all brands of TFAA yielded satisfactory results. Some yielded no product unless treated with solid sodium carbonate directly before use. Some new batches of the preferred brand also required this treatment for optimum results. This effect could account for the preference of Kientz and Verweij (1986) for TFAA derivatization in the presence of solid sodium bicarbonate.

As noted under Materials and Methods, care in evaporation of excess derivatization agent is necessary if losses of the volatile derivatives are to be avoided. In our hands the more variable results were obtained with NIV and DON, with Sctol and T-2tol being more consistent both within and between runs. It is therefore our practice to analyze for NIV and DON by HPLC and for Sctol and T-2tol by GC/ECD, which also serves as a qualitative confirmation for NIV and DON.

Table III. Reproducibility of Trichothecene Method

sample	trichothecene content, μg/g NIV	SD	DON	SD
maize 47[a]	1.04	0.1	3.06	0.26
maize 47[b]	1.13	0.08	3.70	0.24
wheat 6[b]	0.24	0.05	1.16	0.13

[a] Five replicates analyzed as a single batch. [b] Seven samples analyzed over several sample batches.

Table IV. Mean NIV and DON Content of Maize Samples from Three Regions Using Trichothecene Analysis Method with and without the Hydrolysis Step

region	harvest	no. of samples	trichothecene content, μg/g −hydrolysis NIV	DON	+hydrolysis NIV	DON
1	1987	9	0.36	0.65	0.52	0.78
	1988	15	0.39	0.29	0.50	0.28
	1989	15	0.15	0.25	0.21	0.29
2	1987	6	0.55	0.47	0.74	0.56
	1988	9	0.67	0.21	0.85	0.18
	1989	4	0.60	0.38	0.75	0.42
3	1987	13	0.11	0.09	0.20	0.10
	1988	12	0.51	1.61	0.74	2.06
	1989	9	0.31	0.03	0.43	0.04

The trichothecene method described has been used to analyze over 300 grain samples including maize, wheat, barley, oats, and sorghum. (It is also suitable for many feed samples but is not generally usable for poultry feed samples which show considerable coextractive interference.) The reproducibility of the method was illustrated by analyzing a reference maize and wheat sample several times either in one sample batch or as a single determination over several sample batches. The results are given in Table III. In each case the standard deviation is about 10% of the estimated values. It is our practice to analyze each batch of samples without undue delays and to include a reference sample in each batch to check batch-to-batch consistency.

The analysis of 91 maize samples from three different harvest seasons showed the value of the method for determining the full trichothecene content compared with analysis of individual toxins. These maize samples were analyzed by the method both with and without the hydrolysis step. NIV and DON occurred frequently, but few of the samples had Sctol or T-2tol and then at low levels. Table IV summarizes the results for NIV and DON in all maize samples expressed as seasonal means for samples from each of three growing regions. In regions 1 and 2 almost all samples contained both NIV and DON, and the estimated mean after hydrolysis increased by up to 45% for NIV and by up to 20% for DON. Similar increases after hydrolysis were noted in samples from region 3, although in this case only about half of the samples contained NIV or DON. These results indicate the common occurrence of significant proportions of trichothecenes as esterified forms in the naturally contaminated maize samples.

Sample HPLC chromatograms of maize samples both with and without hydrolysis are shown in Figure 3 and illustrate the increase in NIV and DON content after hydrolysis. Figure 4 shows the GC/ECD chromatograms of similar samples after hydrolysis.

The method described is useful for the analysis of the major *Fusarium* mycotoxins. The individual parts of the method give recoveries and detection limits comparable

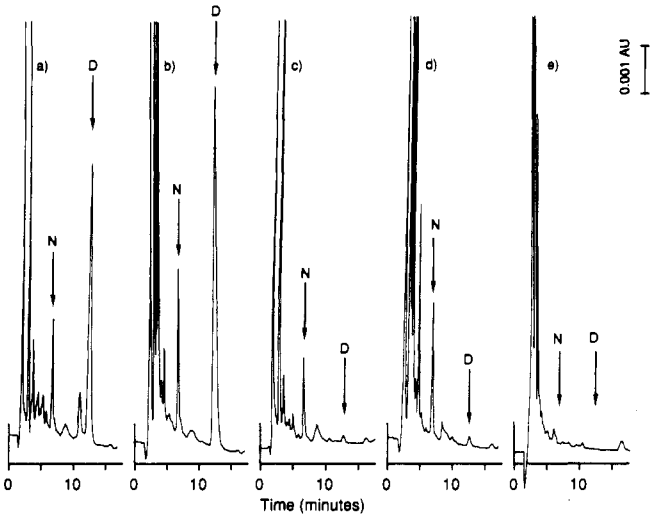

Figure 3. HPLC chromatograms of maize samples analyzed for NIV (N) and DON (D) after cleanup with and without hydrolysis. Conditions as in text except for injection volume (a)–(d) of 50 μL. Detection wavelength was 222 nm. (a) Maize 47 extract without hydrolysis showing NIV (0.79 mg/kg) and DON (2.97 mg/kg); (b) maize 47 extract with hydrolysis showing NIV (1.15 mg/kg) and DON (3.52 mg/kg); (c) maize extract without hydrolysis showing NIV (0.56 mg/kg) and DON (0.07 mg/kg); (d) maize extract as in (c) but with hydrolysis showing NIV (0.92 mg/kg) and DON (0.10 mg/kg); (e) maize extract with hydrolysis showing no NIV or DON.

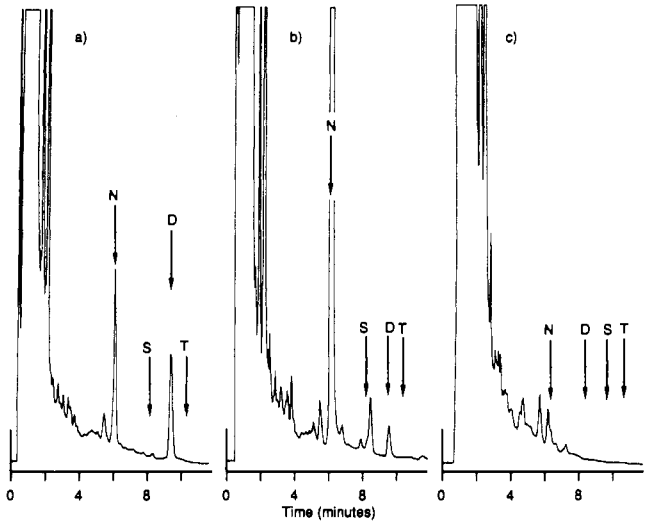

Figure 4. GC/ECD chromatograms of maize samples analyzed for NIV (N), DON (D), Sctol (S), and T-2tol (T) after cleanup with hydrolysis. Conditions as in text. Sample concentration was 0.125 g equiv/mL. (a) Extract showing NIV (0.30 mg/kg) and DON (0.42 mg/kg) with no Sctol or T-2tol; (b) extract showing NIV (4.40 mg/kg) and DON (0.12 mg/kg) with no Sctol or T-2tol; (c) extract showing no trichothecenes.

to those reported in existing individual methods for moniliformin, zearalenone, or trichothecenes. The trichothecene method may be employed with hydrolysis to give information on the full content of the four main structural types or without hydrolysis to give information on individual trichothecenes. Stability problems with some existing methods have been overcome.

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LITERATURE CITED

Bagneris, R. W.; Gaul, J. A.; Ware, G. M. Liquid chromatographic determination of zearalenone and zearalenol in animal feeds and grains, using fluorescence detection. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 894–898.

Booth, C. The *Fusarium* problem: historical, economic and taxonomic aspects. In *The Applied Mycology of Fusarium*; Moss, M. O., Smith, J. E., Eds.; Cambridge University Press: Cambridge, U.K., 1984; pp 1–13.

Chelkowski, J., Ed. *Fusarium. Mycotoxins, Taxonomy and Pathogenicity*; Elsevier Science Publishers: Amsterdam, 1989.

di Menna, M. E.; Lauren, D. R.; Poole, P. R.; Mortimer, P. H.; Hill, R. A.; Agnew, M. P. Zearalenone in New Zealand pasture herbage and the mycotoxin-producing potential of *Fusarium* species from pasture. *N. Z. J. Agric. Res.* **1987**, *30*, 499–504.

Gilbert, J. The detection and analysis of *Fusarium* mycotoxins. In *The Applied Mycology of Fusarium*; Moss, M. O., Smith, J. E., Eds.; Cambridge University Press: Cambridge, U.K., 1984; pp 175–193.

Gilbert, J.; Startin, J. R.; Crews, C. Optimisation of conditions for the trimethylsilylation of trichothecene mycotoxins. *J. Chromatogr.* **1985**, *319*, 376–381.

Grove, J. F. Non-macrocyclic trichothecenes. *Nat. Prod. Rep.* **1988**, *5*, 187–209.

Grove, J. F.; Mortimer, P. H. The cytotoxicity of some transformation products of diacetoxyscirpenol. *Biochem. Pharmacol.* **1969**, *18*, 1473–1478.

Kamimura, H.; Nishijima, M.; Yasuda, K.; Saito, K.; Ibe, A.; Nagayama, T.; Ushiyama, H.; Naoi, Y. Simultaneous detection of several *Fusarium* mycotoxins in cereals, grains, and foodstuffs. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1067–1073.

Kientz, C. E.; Verweij, A. Trimethylsilylation and trifluoroacetylation of a number of trichothecenes followed by gas chromatographic analysis on fused-silica capillary columns. *J. Chromatogr.* **1986**, *355*, 229–240.

Kientz, C. E.; Verweij, A. Decomposition of trifluoroacetyl derivatives of some trichothecenes on fused-silica capillary columns using gas chromatographic analysis. *J. Chromatogr.* **1987**, *407*, 340–342.

Kroll, J.; Giersch, Ch.; Guth, S. *Nahrung* **1988**, *32*, 75–77.

Lauren, D. R.; Greenhalgh, R. Simultaneous analysis of nivalenol and deoxynivalenol in cereals by liquid chromatography. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 479–483.

Marasas, W. F. O.; Nelson, P. E.; Toussoun, T. A. *Toxigenic Fusarium Species. Identity and Mycotoxicology*; The Pennsylvania State University Press: University Park, PA, 1984.

Mirocha, C. J.; Christensen, C. M.; Nelson, G. H. F-2 (zearalenone) estrogenic mycotoxin from *Fusarium*. In *Microbial Toxins*; Kadis, S., Ciegler, A., Ajl, S. J., Eds.; Academic Press: New York, 1971; Vol. 7, pp 107–138.

Nelson, P. E., Toussoun, T. A., Cook, R. J., Eds. *Fusarium: Diseases, Biology, and Taxonomy*; The Pennsylvania State University Press: University Park, PA, 1981; pp 3–221.

Pathre, S. V.; Mirocha, C. J. Trichothecenes: Natural occurrence and potential hazard. *J. Am. Oil Chem. Soc.* **1979**, *56*, 820–823.

Rizzo, A. F.; Saari, L.; Lindfors, E. Derivatization of trichothecenes and water treatment of their trimethylsilyl ethers in an anhydrous apolar solvent. *J. Chromatogr.* **1986**, *368*, 381–386.

Romer, T. R. Use of small charcoal/alumina cleanup columns in determination of trichothecene mycotoxins in foods and feeds. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 699–703.

Rood, H. D., Jr.; Buck, W. B.; Swanson, S. P. Diagnostic screening method for the determination of trichothecene exposure in animals. *J. Agric. Food Chem.* **1988a**, *36*, 74–79.

Rood, H. D., Jr.; Buck, W. B.; Swanson, S. P. Gas chromatographic screening method for T-2 toxin, diacetoxyscirpenol, deoxynivalenol, and related trichothecenes in feeds. *J. Assoc. Off. Anal. Chem.* **1988b**, *71*, 493–498.

Scott, P. M. Assessment of quantitative methods for determination of trichothecenes in grains and grain products. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 876–883.

Scott, P. M. General referee reports: Mycotoxins. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 98–105.

Scott, P. M.; Kanhere, S. R. Comparison of column phases for separation of derivatized trichothecenes by capillary gas chromatography. *J. Chromatogr.* **1986**, *368*, 374–380.

Scott, P. M.; Lawrence, G. A. Liquid chromatographic determination and stability of the *Fusarium* mycotoxin moniliformin in cereal grains. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 850–853.

Scott, P. M.; Lombaert, G. A.; Pellaers, P.; Bacler, S.; Kanhere, S. R.; Sun, W. F.; Lau, P.-Y.; Weber, D. Application of capillary gas chromatography to a survey of wheat for five trichothecenes. *Food Addit. Contam.* **1989**, *6*, 489–500.

Shepherd, M. J.; Gilbert, J. Method for the analysis in maize of the *Fusarium* mycotoxin moniliformin employing ion-pairing extraction and high-performance liquid chromatography. *J. Chromatogr.* **1986**, *358*, 415–422.

Snyder, A. P. Qualitative, quantitative and technological aspects of the trichothecene mycotoxins. *J. Food Prot.* **1986**, *49*, 544–569.

Tanaka, T.; Hasegawa, A.; Matsuki, Y.; Lee, U.-S.; Ueno, Y. Rapid and sensitive determination of zearalenone in cereals by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr.* **1985**, *328*, 271–278.

Thiel, P. G.; Gelderblom, W. C. A.; Marasas, W. F. O.; Nelson, P. E.; Wilson, T. M. Natural occurrence of moniliformin and fusarin C in corn screenings known to be hepatocarcinogenic in rats. *J. Agric. Food Chem.* **1986**, *34*, 773–775.

Ueno, Y., Ed. *Trichothecenes. Chemical, Biological and Toxicological Aspects*; Elsevier Science Publishers: Amsterdam, 1983a.

Ueno, Y., Ed. Analysis. In *Trichothecenes. Chemical, Biological and Toxicological Aspects*; Elsevier Science Publishers: Amsterdam, 1983b; pp 113–133.

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