Analysis of Trichothecene Mycotoxins by Gas Chromatography with Electron Capture Detection†

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A sensitive gas chromatographic (GC) method for the quantitative analysis of 13 trichothecene mycotoxins in corn was developed. Derivatives sensitive to electron capture detection (ECD) were formed using heptafluorobutyric anhydride in the presence of the acylation catalyst (dimethylamino)pyridine. Optimal reaction conditions were found to consist of reacting a sample for 20 min at 60 °C in a toluene–acetonitrile (8 + 2) reaction solvent. An aqueous wash with 5% (w/v) sodium bicarbonate effectively removed excess derivatizing reagent. Chromatographic separation was achieved with a DB-1701, 15 m × 0.25 mm i.d., or SE-54, 15 m × 0.32 mm i.d., fused silica capillary column with a 0.25-μm film thickness. Both columns were used to ascertain the identity of trichothecenes coeluting with unidentified endogenous substances. The procedure was applied to samples of corn extracted with acetonitrile–water (84 + 16). The extract was pretreated with ammonium sulfate to reduce the amount of interferences and to enhance the extraction of the less polar trichothecenes prior to cleanup on a charcoal–alumina column. The fluoroacyl derivatives were prepared and the mixtures subjected to ECD-GC. Depending on the trichothecene, their minimum quantifiable limit in corn ranged from 50 to 200 μg/kg.

INTRODUCTION

The presence of mycotoxins in grains or food samples is a worldwide occurrence (Jelinek *et al.*, 1989). The trichothecenes represent a class of toxins produced as secondary metabolites by various species of *Fusarium* molds. The varied toxic properties of these compounds are the major concern in trying to control their occurrence in human foods and animal feedstuffs and in understanding the effects of chronic low-level ingestion. Over 80 different trichothecenes have been identified to date, and conditions appropriate for the production of any given secondary metabolite would produce analogous compounds via similar metabolic routes.

Trichothecenes are classified according to their chemical structures, based on a 12,13-epoxytrichothec-9-ene ring system with various functionalities. Of interest in the current study were the type A and B trichothecenes. Type A compounds are characterized by the presence of hydrogen or a hydroxyl or an ester function at R_8, while type B trichothecenes possess a carbonyl function at that position. Figure 1 shows some different combinations of hydroxyl or ester substituents at positions R_3, R_4, R_7, and R_{15} from the various members of these groups.

The correlation between the presence of certain mycotoxins and adverse physiologic responses indicates a need for specific methods of analyses of these mycotoxins in various commodities. Trichothecenes are only trace components within complex sample matrices. Most analytical methods developed to date have limited applications. For example, while enzyme-linked immunosorbent assay (ELISA) systems are ideally suited for the analysis of trace analyses in a complex matrix, chromatographic methods are better suited to screening classes of compounds. The high degree of selectivity of ELISA methods does not always lend itself to screening for a group of analogous compounds (Chu, 1990).

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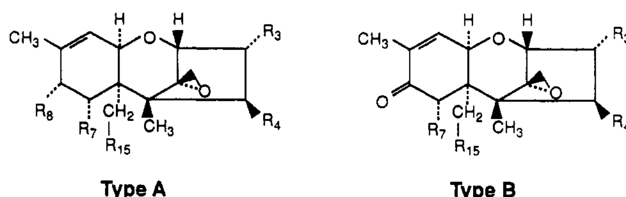

Figure 1. Structures of trichothecenes examined in this study.

compound	R_3[a]	R_4	R_7	R_8	R_{15}
type A					
verrucarol (VER)	H	OH	H	H	OH
scirpentriol (SCT)	OH	OH	H	H	OH
diacetoxyscirpenol (DAS)	OH	OAc[b]	H	H	OAc
T-2 tetrol (T24)	OH	OH	H	OH	OH
HT-2 toxin (HT2)	OH	OH	H	O-ival[c]	OAc
T-2 toxin (T-2)	OH	OAc	H	O-ival	OAc
iso-T-2 toxin (iT2)	OAc	OH	H	O-ival	OAc
type B					
nivalenol (NIV)	OH	OH	OH	=O	OH
fusarenon-X (F-X)	OH	OAc	OH	=O	OH
deoxynivalenol (DON)	OH	H	OH	=O	OH
3-acetyl-DON (3AD)	OAc	H	OH	=O	OH
15-acetyl-DON (FAD)	OH	H	OH	=O	OAc
3,15-diacetyl-DON (DAD)	OAc	H	OH	=O	OAc

[a] The subscripts denote the position of attachment on the corresponding trichothecene nucleus. [b] Ac, $COCH_3$ (acetyl). [c] ival, $COCH_2CH(CH_3)_2$ (isovaleryl).

Analyses of trichothecenes by thin-layer chromatography (TLC) typically lack either selectivity (Eppley *et al.*, 1986; Trucksess *et al.*, 1987) or sensitivity (Shannon *et al.*, 1985), requiring different separation systems and spray reagents to visualize and to characterize the type A and B compounds (Romer, 1986; Siame and Lovelace, 1989). However, increased sensitivity can be obtained with increased sample cleanup by multistep cleanup procedures (Trucksess *et al.*, 1987) or by using two-dimensional TLC (Siame and Lovelace, 1989).

Liquid chromatographic (LC) analyses are complicated by the absence of a chromophore in the type A trichothecenes. Diode array detection in combination with retention index monitoring allows for the detection and partial characterization of these compounds (Kuronen,

1989), but derivatization is usually required to allow for adequate detection of type A trichothecenes (Bayliss *et al.*, 1988; Maycock and Utley, 1985). Sensitive methods of analysis using UV detection (Lauren and Greenhalgh, 1987; Visconti and Bottalico, 1983), electrochemical detection (Sylvia *et al.*, 1986; Childress *et al.*, 1990), or fluorescence detection following postcolumn derivatization (Sano *et al.*, 1987) have been limited to the type B trichothecenes because of their specificity. Cleanup procedures can still be time-consuming for methods that offer analyte selectivity or specificity with suitable sensitivity (Sano *et al.*, 1987).

Gas chromatography (GC), while also requiring extensive cleanup of samples, offers high-resolution separation using capillary columns, selectivity and sensitivity of certain methods of detection, and applicability to most trichothecene mycotoxins. The versatility of GC can facilitate the development of methods for the routine analysis of samples containing several trichothecenes. Derivatization is typically required to obtain suitable volatility and good chromatographic results. Trimethylsilylation with either flame ionization detection (FID) or electron capture detection (ECD) have been applied successfully to several trichothecenes (Kuroda *et al.*, 1979; Chu, 1990; Möller and Gustavsson, 1992), as have fluoroacyl derivatives with ECD (Romer *et al.*, 1978; Cohen and Lapointe, 1984; Scott and Kanhere, 1986; Scott *et al.*, 1989). The use of ECD provides considerable sensitivity to the analytical method.

This paper outlines the development of an ECD-GC method to screen for 13 trichothecenes in corn, detected as their heptafluorobutyryl derivatives, which could also be used as a quantitative tool. Reproducible derivatization and chromatographic resolution of the major trichothecenes found typically in grains or animal feedstuffs were obtained.

MATERIALS AND METHODS

Chemicals and Reagents. The mycotoxin standards nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3AD), and 15-acetyldeoxynivalenol (FAD) were provided by J. D. Miller, and 3,15-diacetyldeoxynivalenol (DAD) and fusarenon-X (F-X) were graciously donated by M. E. Savard, both from the Plant Research Centre, Agriculture Canada, Ottawa, ON. Verucarrol (VER), scirpenetriol (SCT), T-2 toxin (T-2), T-2 tetrol (T24), HT-2 toxin (HT2), and iso-T-2 toxin (iT2) were purchased from Sigma Chemical Co., St. Louis, MO, as were heptafluorobutyric anhydride (HFBA) and 4-(*N*,*N*-dimethylamino)-pyridine (DMAP). Solvents (acetonitrile, ethyl acetate, toluene) were all of ACS grade, distilled in glass. Water was distilled and then passed through a Sybron/Barnstead water purifier. The sodium bicarbonate solution was of ACS grade, 5% (w/v) in deionized distilled water.

Apparatus. Gas chromatography (GC) was performed on a Hewlett-Packard Model 5890 equipped with a split/splitless injector operating in the splitless mode, a HP Model 7671 autosampler, a ^{63}Ni electron capture detector, and a HP Model 3396 integrator. GC separation was acheived with a 15 m × 0.32 mm i.d. SE-54 or a 15 m × 0.25 mm i.d. DB-1701 fused silica capillary column with a 0.25-μm film thickness.

Extraction. Samples were ground to pass through a 0.5-mm mesh. To 50 g of ground sample in a 500-mL Erlenmeyer flask was added 250 mL of acetonitrile–water (84 + 16). Flasks were shaken for 2.5 h on a wrist-action shaker set at medium speed. The contents were allowed to settle for 15–30 min. Portions of extracts were stored refrigerated in glass scintillation vials for up to 24 h before cleanup.

Sample Pretreatment. To 7 mL of extract (equivalent to 1.4 g of sample; 0.2 g/mL) in 16 × 125 mm screw-cap tubes was added approximately 1.4 g of anhydrous ammonium sulfate. The tubes were capped tightly and vortexed for 10–15 min at high speed. The phases were then separated by centrifugation at 1000*g* for 2 min, and the upper phase was retained for cleanup.

Cleanup. Sample cleanup was performed using charcoal–alumina columns prepared as described by Trenholm *et al.* (1985). Briefly, a 10-mL disposable glass pipet with the upper restriction removed was fitted with a silanized glass-wool plug in the tip. The column was then packed sequentially with 0.75 g of Darco G-60 activated charcoal, 200–400 mesh, and 0.7 g of neutral alumina, 70–230 mesh, with tapping while vacuum was applied to the pipet tip. A glass-wool plug was placed on top of the alumina.

Immediately before use, each column was washed with 15 mL of acetonitrile–water (84 + 16), and the solvent level was brought down to the level of the glass-wool plug. A 5-mL aliquot of the upper phase (obtained following ammonium sulfate treatment of the extract; equivalent to 1.0 g of sample) was applied to the top of a column and run in until the sample reached the glass-wool plug. Eluent was collected in 16 × 125 mm test tubes. Two more 5-mL aliquots of acetonitrile–water (84 + 16) were collected in the first tube. Two additional fractions, each containing 15 mL of eluent, were then collected. The three fractions were evaporated to 1–2 mL, transferred to 13 × 100 mm screw-cap tubes, and evaporated to dryness, either separately or after pooling.

Derivatization. To the dried eluate in a 13 × 100 mm screw-cap test tube was added 100 μL of catalyst solution [DMAP, 2 mg/mL in toluene/acetonitrile (8/2)]. The tube was vortexed for 3 s, and 50 μL of HFBA was added. The tube was vortexed for another 3 s and placed in a heating block at 60–65 °C for 20 min. The reaction mixture was allowed to cool to room temperature for 3–5 min. Excess derivatizing agent was destroyed by adding 1 mL of an aqueous 5% (w/v) sodium bicarbonate solution and vortexing for 15 s. The sample was diluted with an additional 400 μL of toluene, vortexed for another 15 s, and centrifuged for 2 min at 1000*g* to separate the layers. Because of its miscibility, the acetonitrile portion (20 μL) of the catalyst solution would now separate out with the aqueous layer. The upper organic layer (480 μL of toluene) was transferred to an autosampler vial, which was then sealed with a crimp-top cap for GC analysis.

Chromatography. A Hewlett-Packard Model 7671 autosampler was used to inject 2 μL of the toluene layer (equivalent to 4.17 mg of sample) into a gas chromatograph with an injector temperature of 250 °C and a detector temperature of 300 °C. Separation was achieved with a temperature program consisting of 1 min at 80 °C, then increase 80–140 °C at 30 °C/min, followed by 140–280 °C at 5 °C/min. The same chromatographic conditions were used for both the SE-54 and DB-1701 columns.

Recovery from Grain Samples. Clean ground corn obtained from the Greenbelt Farm, Agriculture Canada, Ottawa, ON, was extracted as described above. The corn was spiked with a mixture of trichothecenes in extraction solvent at levels of 100, 250, or 1000 μg/kg. This gave a final concentration of 0.02, 0.05, or 0.2 μg/mL following extraction. Each milliliter of extract was equivalent to 0.2 g of grain. The spiked samples were pretreated, cleaned up, and derivatized as described above.

Calibration Curve. For subsequent analysis of suspect contaminated grain samples, preparation of a standard

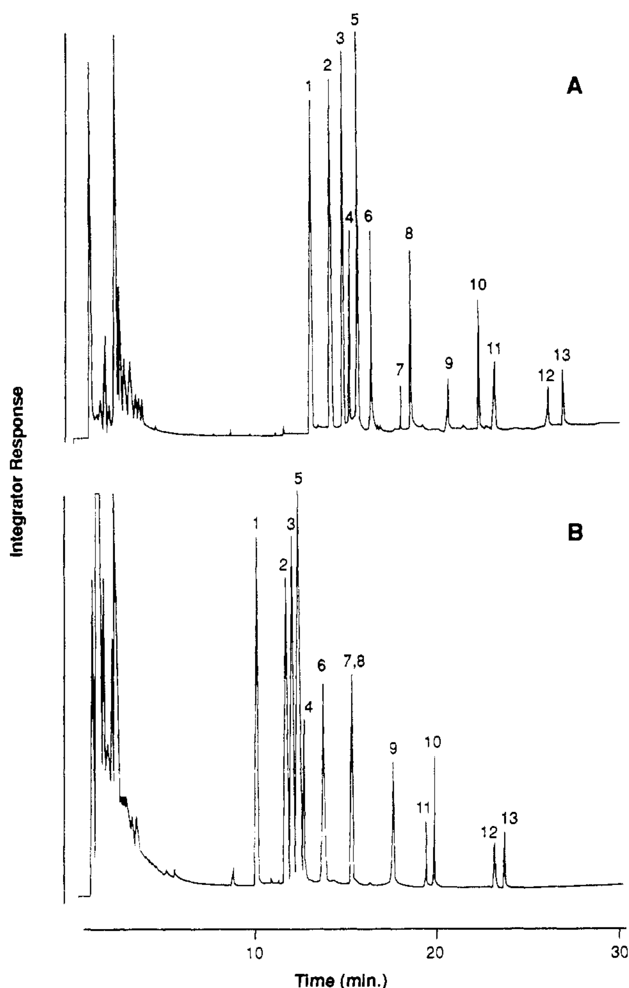

Figure 2. Chromatographic separation of HFB–trichothecenes on columns (A) DB-1701 and (B) SE-54. Peaks: 1, NIV; 2, SCT; 3, DON; 4, VER; 5, T24; 6, F-X; 7, FAD; 8, 3AD; 9, DAS; 10, HT2; 11, DAD; 12, T-2; 13, iT2.

curve for comparison would be carried out as above with the recovery study. Toxin concentrations used to construct the curve would depend on level of natural contamination envisioned.

RESULTS AND DISCUSSION

Chromatographic separation of the 13 trichothecenes tested is shown in Figure 2. The SE-54 column, a relatively nonpolar phase, and the DB-1701 column, a phase of intermediate polarity, were chosen for their ability to resolve several HFB–trichothecenes within a 30-min period. The inability of the SE-54 column to discriminate between the 3-acetyl- and 15-acetyl isomers of acetyl-DON did not pose a serious problem. The DB-1701 column could be used to ascertain the identity of an acetyl-DON isomer observed on the SE-54 column. Also, the two isomers seldom occur simultaneously. The geographic distribution of the strains that produce them do not appreciably overlap. The 3-acetyl isomer is typically produced by *Fusarium* strains found in Asia and Europe, while 15-acetyl-DON is most frequently found in North America (Jelinek *et al.*, 1989). Scott and Kanhere (1986) also examined several GC column phases for their abilities to separate HFB and TMS derivatives of trichothecenes, and they similarly found that the SE-54 and DB-1701 columns complement each other with respect to individual separation of combinations of trichothecenes. Furthermore, due to the effective resolution afforded by utilizing both columns separately (compounds not resolved on one system may be resolved on the other), this procedure is amenable for the inclusion of other trichothecenes for quantitation.

Table 1. Limits of Quantitation and Relative Sensitivities of Trichothecenes Used in This Study

compound	MQL[a] (μg/kg)	relative sensitivity[b]
nivalenol (NIV)	125	13.7
scirpentriol (SCT)	100	13.3
deoxynivalenol (DON)	50	15.8
verucarrol (VER)	100	7.5
T-2 tetrol (T24)	100	13.6
fusarenon-X (F-X)	150	7.5
15-acetyl-DON (FAD)	200	1.4
3-acetyl-DON (3AD)	50	6.1
diacetoxyscirpenol (DAS)	100	1.9
HT-2 toxin (HT2)	50	3.3
3,15-diacetyl-DON (DAD)	200	1.8
T-2 toxin (T-2)	50	1.0
iso-T-2 toxin (iT2)	50	1.5

[a] Estimated minimum quantifiable limit in corn utilizing the extraction and cleanup procedure detailed in this study. [b] Integrator response of HFB-derivatized standards on a weight-for-weight basis (prederivatized compounds) relative to T-2 toxin.

Heptafluoroacetylation procedures have been used previously with a catalyst (Kanhere and Scott, 1990; Yagen *et al.*, 1985; Kostiainen and Rizzo, 1988) and without (Rothberg *et al.*, 1983; Lauren and Agnew, 1991; Black *et al.*, 1987; Cohen and Lapoint, 1984) for standards (Mirocha *et al.*, 1986; Krishnamurthy *et al.*, 1986; Visconti *et al.*, 1987; Hewetson and Mirocha, 1987) and as a screening procedure for parent alcohols following sample hydrolysis (Black *et al.*, 1987; Krishnamurthy *et al.*, 1987). Heptafluorobutyrylation offers the greatest sensitivity to electron capture detection without sacrificing too much volatility (Poole and Zlatkis, 1980), although the high molecular weights of some HFB derivatives make them less suitable for low-resolution mass spectrometry (Wreford and Shaw, 1987). The HFB derivatization also provides an increase in derivative stability over trifluoroacetylation (Kientz and Verweij, 1986).

In the current study overall sensitivity for detection of individual trichothecene standards depended on number and position of hydroxyl functional groups available for derivatization (Table 1). As expected, the more hydroxyl groups, the greater the sensitivity, although absence of the R_{15} hydroxy appeared to decrease the response (i.e., 3AD vs FAD, VER vs HT2). The fluoroacyl derivatives offer similar sensitivity for type A and B trichothecenes (based on OH groups) when using ECD (Romer *et al.*, 1978; Cohen and Lapointe, 1984; Scott and Kanhere, 1986; Scott *et al.*, 1989), whereas, in comparison, the trimethylsilyl (TMS) derivatives of type B trichothecenes have greater ECD sensitivity than those of type A (Kuroda *et al.*, 1979; Möller and Gustavsson, 1992). Furthermore, fluoroacyl derivatives are more stable to heat and less sensitive to moisture than TMS derivatives (Blau and King, 1977).

Sensitivity was also dependent on instrumentation. Poole and Zlatkis (1980) reported that two major mechanisms of the ionization processes in electron capture detectors have opposing dependencies on temperature. During the current study the operating temperature of the detector was varied in 25 °C increments between 250 and 350 °C, and it was observed that the ECD temperature which produced the optimum response for each of the HFB–trichothecenes examined was found to be 300 °C (data not shown).

Hydroxyl groups at certain positions on the trichothecene nucleus require more rigorous conditions for acyl-

ation. The R_7 hydroxyl group of DON or NIV, for example, has been shown to be the hydroxyl function on the trichothecene nucleus most resistant toward acylation due to steric hindrance (Kanhere and Scott, 1990; Grove, 1988). Hydrogen bonding of the 7-α-hydroxyl with the R_8 carbonyl provides a further shielding influence. Some trichothecenes, such as T-2 and DAS, without the R_7 hydroxyl group, can be derivatized in 15 s with HFBI at room temperature (Romer *et al.*, 1978), while DON requires 60–120 min at 60 °C (Black *et al.*, 1987; Scott *et al.*, 1986; Cohen and Lapointe, 1984; Ware *et al.*, 1984) or 15 min at 110 °C (Krishnamurthy *et al.*, 1986). High temperatures or long reaction times may have adverse effects on derivatization; side reactions may result in the formation of byproducts which would reduce the apparent derivatization efficiency and increase potential interferences.

The use of a nucleophilic catalyst such as trimethylamine or 4-(*N*,*N*-dimethylamino)pyridine with an anhydride can increase the rate of acylation considerably and can facilitate the acylation of sterically hindered functionalities. For example, DON is completely derivatized by HFBA/DMAP in less than 20 min at 60 °C (Ware *et al.*, 1984). An extractive wash step which follows derivatization aids in removing possible interferences due to the presence of catalyst and of excess reagent. The use of an insoluble polymer-bound form of DMAP could obviate the need for a basic wash; excess anhydride could be removed by careful evaporation. However, when Kanhere and Scott (1990) used polystyrene-bound 4-(*N*-benzyl-*N*-methylamino)-pyridine, they found incompletely derivatized forms of DON and NIV when these were reacted under conditions where DMAP yielded fully derivatized products.

Complete derivatization of the trichothecenes tested was accomplished within 10 min at 60–65 °C for the conditions outlined under Materials and Methods. Reaction times of up to 60 min showed no improvement in the response of any of the trichothecenes examined. Consequently, a reaction time of 20 min was chosen to ensure complete derivatization of the trichothecenes. Furthermore, it was noted that this time when compared to a shorter reaction period also reduced the number of interfering signals, possibly by driving impurities to complete derivatization as well, precluding more peaks from being formed due to only partial derivatization.

The efficiency of the derivatization procedure was evaluated with different volumes of catalyst solution and of derivatizing reagent. A greater reaction volume allowed for better miscibility of the HFBA with the catalyst solution at room temperature. When lower volumes of catalyst solution were used, the HFBA still became miscible when placed in the 60 °C heating block. Thus, a lower reaction volume effectively created a more concentrated derivatizing environment. Despite the caution that side reactions are possible in concentrated solutions of DMAP and anhydride (Hofle *et al.*, 1978), no interferences were observed when 50 μL of HFBA was added to 100 μL of DMAP solution rather than to 1 mL of DMAP solution as used in the method of Ware *et al.* (1986).

HFBA is not miscible with toluene or with acetonitrile, however, a mixture of toluene/acetonitrile in approximately 3:1 ratio allows for up to 20% (v/v) of HFBA to be present before phase separation occurs at room temperature. The catalyst solution composition was varied to include ratios of 95:5, 80:20, 50:50, and 0:100 toluene/acetonitrile. Reaction conditions were kept constant, i.e., 20 min at 60–65 °C. The 80:20 and 50:50 ratios showed increased peak areas for five of the trichothecenes examined (NIV, DON, 3AD, DAS, T-2) (Figure 3). Other solvent

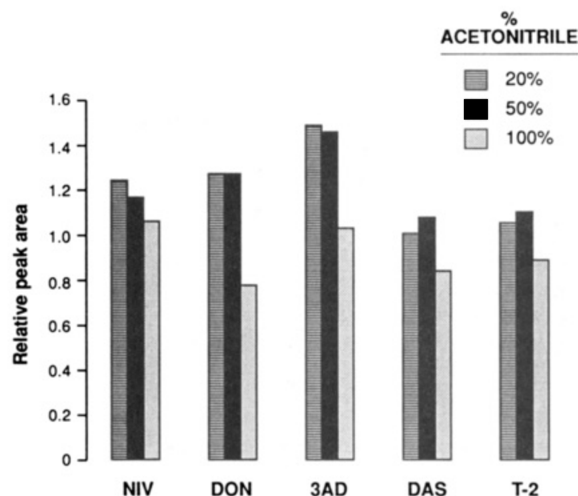

Figure 3. Effect of derivatization solvent composition on peak areas of five trichothecenes relative to 5% acetonitrile in toluene.

compositions produced more secondary peaks which may have been due to components at varying levels of derivatization.

The parent alcohols (VER, SCT, NIV, DON, T24) were fully derivatized without signs of hydrolysis or transesterification of the various esters of these polyols. The derivatized compounds were stable for at least 24 h at room temperature or 1 week in the freezer. With NIV, elevated derivatization temperatures or longer derivatization times produced variable responses, with evidence of decomposition.

A more polar solvent can increase the rate of formation of charged reactive intermediates from uncharged reactants by better solvating the charged species. Increasing the proportion of acetonitrile in the derivatization solvent increases the rate of formation of the ion pair produced by DMAP and HFBA. However, acylation with DMAP proceeds best in nonpolar solvents (Hofle *et al.*, 1978). The better response achieved with a derivatization solvent of 20% acetonitrile in toluene (Figure 3) rather than the 5% acetonitrile in toluene used by Ware *et al.* (1986) represents a compromise in the rate of formation of the catalytically active species and the rate of derivatization. It appears to be more than a question of miscibility as proposed by Kanhere and Scott (1990), since in the 60 °C reaction conditions there is only one phase.

Grain samples were extracted with acetonitrile–water (84 + 16) as a compromise to overall recoveries and to minimize the coextraction of interfering contaminants (Trenholm *et al.*, 1985) and facilitate removal of the solvent through a novel saturation effect. The 84 + 16 mixture represents the azeotropic composition for a mixture of acetonitrile/water. When the extract was then treated with ammonium sulfate, the acetonitrile portion separated from the aqueous portion which had become saturated with ammonium sulfate. The less polar trichothecenes, such as 3AD, T-2, and HT2, displayed a salting-out phenomenon where they remained effectively with the upper acetonitrile layer. Consequently, recoveries would increase marginally when the extraction solvent contained a greater proportion of water, for example, acetonitrile–water (75 + 25) (unpublished data). This effect, however, was not observed for the more highly water-soluble compounds such as DON and NIV, which preferred the aqueous layer. As a result, the phase separation resulted in slightly decreased recoveries in the acetonitrile, and accordingly, the loss increased with an ammonium sulfate treatment that used 75% acetonitrile extraction solvent

Table 2. Percentage Recoveries (±SD)[a] of Various Trichothecenes from Corn Spiked at 100, 250, or 1000 μg/kg

compound	100 μg/kg	250 μg/kg	1000 μg/kg
nivalenol (NIV)	ND[b]	60 ± 4.0	77 ± 5.0
scirpentriol (SCT)	56 ± 2.2	–[c]	87 ± 2.2
deoxynivalenol (DON)	73 ± 1.4	108 ± 6.5	81 ± 6.4
verucarrol (VER)	87 ± 2.0	–	102 ± 4.2
T-2 tetrol (T24)	70 ± 3.5	–	83 ± 3.9
fusarenon-X (F-X)	28 ± 3.8	47 ± 2.3	60 ± 3.4
15-acetyl-DON (FAD)	ND	65 ± 5.3	79 ± 2.5
3-acetyl-DON (3AD)	35 ± 4.2	69 ± 2.6	80 ± 3.9
diacetoxyscirpenol (DAS)	52 ± 3.8	100 ± 3.3	96 ± 3.8
HT-2 toxin (HT2)	121 ± 6.8	95 ± 4.5	99 ± 2.7
3,15-diacetyl-DON (DAD)	ND	25 ± 5.0	43 ± 3.8
T-2 toxin (T-2)	81 ± 3.0	83 ± 2.9	79 ± 3.9
iso-T-2 toxin (iT2)	71 ± 2.7	–	80 ± 2.1

[a] $N = 3$, run in duplicate. [b] Not detected. [c] No data at this concentration.

compared to 84%, due to the greater proportion of water (unpublished data).

Table 2 shows the recoveries obtained when clean corn was spiked with the trichothecenes at levels of 100, 250, or 1000 μg/kg. Overall, relatively high recoveries were obtained for most of the trichothecenes when spiked at the higher concentrations, with the exception of DAD and F-X. At lower concentrations, somewhat decreased recoveries were typically observed due to the adsorption characteristics of the charcoal–alumina cleanup columns and also due to the greater degree of interferences which needed to be removed, particularly for the early-eluting compounds (see Figure 2). Estimates of the minimun quantifiable limits (MQL) in corn for the 13 trichothecenes are provided in Table 1.

The ECD-GC method was found to be linear ($r^2 > 0.99$) over the range 40–800 pg administered on-column for the 13 standards tested when the analysis was performed within 24 h of derivatization; the final samples contained 20–400 ng/mL of each trichothecene. The greatest sensitivity was achieved with DON, where as little as 4 pg on-column produced a response suitable for quantitation, that is, a signal-to-noise ratio greater than 3. Final quantitation of DON in grain samples and feedstuffs, however, did not allow for the detection of DON below 100 pg on-column, corresponding to approximately 50 μg/kg.

It was observed that treatment of sample extracts with ammonium sulfate prior to cleanup significantly improved the sample chromatography compared to no pretreatment. This procedure reduced many of the polar interferences in grain samples which carried over through the charcoal–alumina cleanup and interfered with the earlier-eluting trichothecenes such as DON and NIV. The later-eluting compounds tend to be less susceptible to endogenous interferences and can be quantitated at approximately the same level as DON despite the differences in sensitivity noted in Table 1. This sample pretreatment procedure, while effective with GC, did not offer any further beneficial effect on the LC analysis for DON routinely used in our laboratory (Trenholm *et al.*, 1985). Attempts to improve the LC detection of NIV, SCT, and T24 using this extraction/cleanup procedure were also not successful. Overall, though, GC analysis of toxins in corn gave good results. Recoveries and detection limits were comparable to or marginally better than those of other HFBI ECD-GC methods screening fewer trichothecenes (Scott *et al.*, 1989; Cohen and Lapointe, 1984; Lauren and Agnew, 1991; Sydenham and Thiel, 1987). Furthermore, detection of type B trichothecenes was at least similar to that reported in a recent publication quantitating 13 trichothecenes by EC detection of the TMS derivatives, whereas detection limits for type A compounds in the current study appeared to be marginally better (Möller and Gustavsson, 1992).

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