Trichothecenes and Their Analysis

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ABSTRACT

The analysis of foods and feeds for the naturally occurring *Fusarium* produced trichothecenes is reviewed. Each major step (extraction, purification and detection-quantitation) is discussed. Although none of the extraction solvents has been thoroughly evaluated, aqueous methanol is the preferred system in most of the published procedures. The sample extracts are generally purified by liquid-liquid partitions followed by silica gel column and/or preparative thin layer chromatography (TLC). Both thin layer and gas liquid chromatography (GLC) are used for the detection and quantitation of the trichothecenes. The detection limit for the GLC procedures has not been determined in most of the reported methods; however, T-2 toxin, diacetoxyscirpenol, deoxynivalenol, and neosolaniol have been detected in the 25-100 ng/g range.

INTRODUCTION

The quantitative analysis of foods and feeds for trichothecenes is one of the most challenging problems of mycotoxin chemistry. Several species of fungi are known to produce trichothecenes. Many of these fungi have been implicated as the causative factors in the early reports of toxicoses in humans and animals which resulted from ingestion of moldy foods and feeds (1,2).

The basic chemical structure shared by all 43 fungal-produced trichothecenes (1,3) is the tetracyclic sesquiterpenoid system shown in Figure 1. The substituent (R) at positions 3, 4, 7, 8 and 15 may represent a hydrogen atom, a hydroxyl group, or an ester group. Most frequently, the ester group is an acetate, although esters of butenoic and isovaleric acids are also found. A series of macrocyclic diesters are also known (3). For convenience, various authors have categorized the trichothecenes according to similarity of functional groups. Ueno (1) recognized four categories; the first is represented by T-2 toxin and diacetoxyscirpenol (DAS) (Fig. 2). Hydrolysis of the ester functions of any member of this category results in the formation of the basic trichothecene ring system with one to five hydroxyl functions. This is the largest category with well over 20 members recorded in the literature. Sixteen of these metabolites are produced by various species of *Fusarium*. The second category of trichothecenes is characterized by a carbonyl function at position 8 (Fig. 3); seven of the eight naturally occurring members of this group are *Fusarium* metabolites. The third category includes the macrocyclic dilactone derivatives of verrucarol (Fig. 4); about 12 compounds of this type are known. These metabolites have been isolated from several fungal genera including *Myrothecium* (3), *Stachybotrys* (4), *Verticimonosporium* (4), and *Cylindrocarpon* (5). The fourth category has a second epoxide function at the 7-8 position. Until recently, the fourth type included only one member, crotocin (Fig. 5); it is produced by a *Trichothecium* species. Recently three additional trichothecene diepoxides have been discovered. Two are metabolites of *Cylindrocarpon* (6); the other was isolated from a vascular plant (7).

Studies of cultural conditions indicate that each fungus generally produces more than one trichothecene. The amounts of any one trichothecene produced can vary over a considerable range depending on such factors as temperature, duration of growth, substrate and strain of fungal species (1), *e.g.*, *F. tricinctum* produces primarily T-2 toxin and DAS at 8 C, while at 25 C the mold also produces HT-2 toxin (8). Most of the trichothecenes are highly toxic; the potency varies with structure (9).

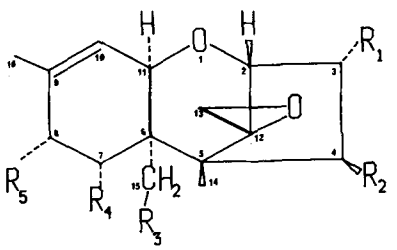
The most perplexing question surrounding the contamination of foods by trichothecenes is: which of the trichothecenes are significant contaminants of foods? With over 40 known to be produced by fungi commonly found on foods, one might expect to find a wide variety of trichothecenes as contaminants; unfortunately, the analytical methodology upon which incidence data depend is largely lacking. None of the methods in the literature (10-21) have been adequately evaluated for recovery and sensitivity. Most of these procedures were developed to detect either T-2 toxin or deoxynivalenol in a particular feed sample (10, 14, 15, 18, 19, 20).

The other methods (11-13, 16, 17, 21) were developed for the detection of either DAS, T-2 toxin, fusarenon-X or some combination of the three. These trichothecenes have received the greatest attention in method development because of the ready availability of reference standards. Mirocha et al. (16) included a procedure for the analysis of deoxynivalenol along with the procedure for DAS and T-2 toxin.

Smalley and Strong (22) suggested an approach to the analysis of the trichothecenes which involved hydrolysis to the corresponding parent alcohols. As many as 12 different alcohol products are possible. Since many of the trichothecenes would be expected to yield the same alcohol product, the analysis will indicate only total trichothecene content.

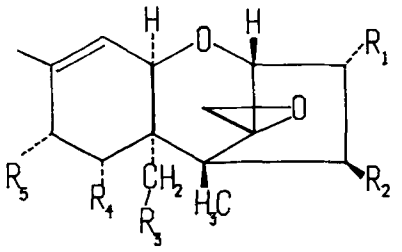
Another more appealing approach would be to initially limit method development to those trichothecenes suspected of occurring most frequently either through analytical reports or indirectly from reports of frequent occurrence of a fungus known to produce particular trichothecenes. According to the latter approach, the trichothecenes produced by various species and strains of the *Fusarium* genus should be given highest priority.

The *Fusarium* have been frequently implicated in mycotoxicoses (1). The literature contains information on 23 trichothecenes produced by the *Fusarium* under laboratory conditions (1,3). However, the laboratory data indicate that only 7 of these trichothecenes are produced in significant yields by various strains of the *Fusarium*. These metabolites are T-2 toxin, HT-2 toxin, DAS, neosolaniol, fusarenon-X, nivalenol, and deoxynivalenol (Figs. 3, 4). Generally only one or two of these metabolites will be produced in significant yield at any one time, with the others occurring in relatively low yield (8,15,16). These *Fusarium* metabolites are members of the first two categories of Ueno (1), as previously mentioned.



12,13-EPOXYTRICHOTHEC-9-ENE

FIG. 1.



	R_1	R_2	R_3	R_4	R_5
DIACETOXYSCIRPENOL	OH	OAc	OAc	H	H
T-2 TOXIN	OH	OAc	OAc	H	$OC(O)CH_2CH(CH_3)_2$
HT-2 TOXIN	OH	OH	OAc	H	"
NEOSOLANIOL	OH	OAc	OAc	H	OH

FIG. 2.

This review will discuss the analytical procedures which have been developed for detection and quantitation of the trichothecenes produced by the *Fusarium*. The analytical methods for any group of compounds usually consists of three distinct steps, once a representative sample is available: extraction, purification, and detection-quantitation. Each of these steps will be reviewed.

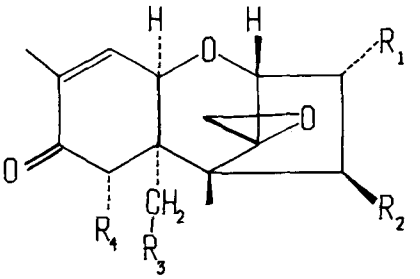
Extraction Procedures

The most commonly used extraction solvents for the trichothecenes are chloroform, ethyl acetate, methanol, aqueous methanol, and acetonitrile; none has been thoroughly evaluated for recovery of all of the trichothecenes from a sample. Pathre and Mirocha (23) have divided the trichothecenes into two groups based on solubility and, presumably, extractability. Group A included most of the compounds with ester and hydroxyl functions (e.g., T-2 toxin, neosolaniol, DAS). Group B compounds have multiple hydroxyl functions and no ester groups; scirpentriol, nivalenol and deoxynivalenol are examples. Group A trichothecenes are most soluble in aprotic solvents such as chloroform, ethyl acetate, diethyl ether and acetone, while group B compounds are most soluble in polar solvents such as water, alcohols, and acetonitrile. Some of the group B compounds can also be extracted by group A solvents; Pathre and Mirocha (23) report a high recovery of scirpentriol using ethyl acetate. Methods for the simultaneous extraction of trichothecenes representative of both groups use aqueous alcohol. Pathre and Mirocha (23) use a procedure which requires two extractions (Tables I and II); the group A toxins with ethyl acetate, followed by group B with aqueous methanol. The published recovery studies are summarized in Table I.

Aqueous methanol was used as the extractant in four of the six recovery studies. The ratio of methanol to water used for extraction varies from 1 to 9 for fusarenon-X in feed (11), to a ratio of 9 to 1 (19) for added T-2 toxin, fusarenon-X and deoxynivalenol in corn. Other procedures use a methanol/water mixture of 50:50 (12,17,20). The addition of 1-10% sodium chloride to the water is used in some of the procedures (11,12), presumably to prevent emulsion formation.

Pathre and Mirocha (23) have compared the recoveries of four trichothecenes from corn and mixed feed using ethyl acetate and acetonitrile, although the details of these experiments are not given. The ethyl acetate extraction yielded somewhat higher recoveries; however, it also required a more extensive purification procedure. The implication is that for a screening method, acetonitrile might be the extraction solvent of choice, whereas for quantitative recoveries ethyl acetate would be preferred.

Most of the remaining procedures in the literature were developed for application to the assay of naturally contaminated samples; recovery studies, in general, were not



	R_1	R_2	R_3	R_4
NIVALENOL	OH	OH	OH	OH
FUSARENON-X	OH	OAc	OH	OH
DEOXYNIVALENOL	OH	H	OH	OH

FIG. 3.

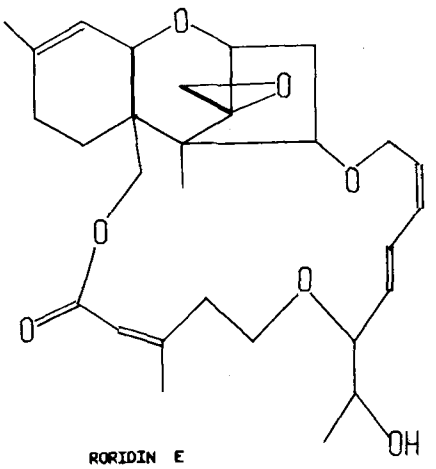


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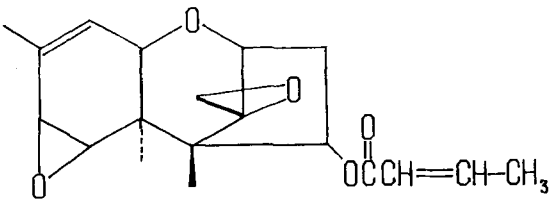
FIG. 4.



CROTOCIN

FIG. 5.

done (Table II). The extraction solvent of choice for most of the methods is aqueous methanol (11,12,15,18,20). Both chloroform and ethyl acetate have been successfully used for T-2 toxin analysis of naturally contaminated samples (10,14,16).

Purification Procedures

The adequate purification of a sample extract for detection and quantitation is probably the most refractory step in determination of trichothecenes. Most of the published methods use traditional cleanup procedures, such as liquid-liquid partition, silica gel column chromatography, and preparative thin layer chromatography (TLC). Usually two or more purification steps are required for detection of the trichothecenes. Table III summarizes the procedures that have been used to purify aqueous methanol extracts of various grain and feed samples. The aqueous methanol

TABLE I

Recovery of Added Trichothecenes from Natural Substrates

Extraction solvents (v/v)	Substrate(s)	Trichothecenes	Amount added (ug/g)	Recovery (%)	Method	Ref.
$MeOH/H_2O$ (3:1)	Corn	Deoxynivalenol	10	85	GLC	(18)
		Fusarenon-X	10	75		
		T-2 Toxin	10	71		
$MeOH/H_2O$ (10:90)	Feed	Fusarenon-X	40	86		
				65[a]	GLC	(11)
$MeOH/H_2O$ (1:1)	Corn and mixed feed	T-2 Toxin	1	91-105	GLC	(17)
		DAS	0.2	89-112		
$MeOH/H_2O$ (55:45)	Cereal grains	DAS	0.05-0.3	89	TLC	(12)
		Fusarenon-X	0.25-1.25	100		
	Powdered rice	DAS	0.05-0.03	83	TLC	
		Fusarenon-X	0.25-1.25	86		
Ethyl Acetate	Corn and mixed feed	T-2 Toxin		87		(23)[b]
		DAS		99		
		Monoacetoxyscirpenol		97		
		Scirpentriol		115		
Acetonitrile	Corn and mixed feed	T-2 Toxin		80		(23)[b]
		DAS		98		
		Monoacetoxyscirpenol		93		
		Scirpentriol		83		

[a]Recovery from spiked sample after 2 days.

[b]Details of experimental conditions were not reported.

TABLE II

Analysis of Samples Naturally Contaminated with Trichothecenes

Extraction solvent (v/v)	Sample	Trichothecenes	Method	Amount Found, mg/kg	Ref.
$CHCl_3$ (Soxhlet)	Corn	T-2 Toxin	TLC, GLC	2	(10)
Ethyl Acetate	Barley	T-2 Toxin	TLC	25	(14)
$MeOH/H_2O$ (60:40)	Corn	Deoxynivalenol	GLC-MS	8	(15)
$MeOH/H_2O$ (9:1)	Corn	Deoxynivalenol	TLC	7.5	(19)
Ethyl Acetate	Mixed feed	T-2 Toxin	GLC-MS	0.076	(16)
		DAS	GLC-MS	0.38	
		DAS	GLC-MS	0.5	
$MeOH/H_2O$ (6:4)	Mixed feed	Deoxynivalenol	GLC-MS	0.05	(16)
				1.0	
				1.0	
	Corn	Deoxynivalenol	GLC-MS	0.1	
				1.0	
				1.8	
$MeOH/H_2O$ (1:1)	Barley	Deoxynivalenol	GLC	5-7	(20)
$MeOH/H_2O$ (3:1)	Corn	Deoxynivalenol	GLC	7.2	(18)

extracts either are extracted directly with hexane, followed by transfer of the trichothecenes into chloroform, or are evaporated to dryness before cleanup. Both Vesonder et al. (15) and Mirocha et al. (16) concentrate the extraction solvent under vacuum and then add acetone to precipitate extraneous materials. The concentrated material is then further purified by preparative thin layer chromatography or column chromatography. Forsyth et al. (18), Ishii et al. (19), and Yoshizawa et al. (20) concentrate the aqueous methanol extract, defat the extract with hexane, and then transfer into either chloroform or ethyl acetate. The chloroform or ethyl acetate solution is evaporated and purified further by silica gel column chromatography and/or preparative thin layer chromatography. The remaining procedures (11-13,17) include the addition of a salt solution either during the extraction of the sample or at the liquid-liquid partition stage. Sodium chloride is used in all but one of the procedures; Romer et al. (17) adds a 30% aqueous ammonium sulfate solution to the aqueous methanol extract before liquid-liquid partition. Twelve of the thirteen procedures use silica gel in a purification step involving either preparative thin layer or column chromatography. Seven of these procedures use chloroform/methanol as the elution solvent. The ratio of methanol to chloroform ranged from 10:90 for deoxynivalenol elution to 3:97 for elution of T-2 toxin and DAS. Naoi et al. (13) use a mixture of silica gel and Florisil in the analysis of wheat powder for T-2 toxin and DAS. Forsyth et al. (18) resort to two preparative thin layer chromatography steps in the purification of deoxynivalenol from ground corn. The deoxynivalenol purification procedure used by Vesonder et al. (15) requires a silica gel column followed by

TABLE III

Methods Used for Purification of Aqueous Methanol Extracts for Trichothecene Analyses

Extraction solvents (v/v)	Preliminary	Liquid/liquid partition	Liquid chromatography		Trichothecene	Ref.
			Solid phase	Eluant (v/v)		
110 ml MeOH	Add 90 ml H_2O (16% NaCl)	Hexane 3x $CHCl_3$	10 g Silica gel 10 g Florisil Column	Hexane, Ether, MeOH/$CHCl_3$ (3:97)	T-2 Toxin DAS	(13)
MeOH/H_2O (10:90) 10% NaCl		Hexane $CHCl_3$			Fusarenon-X	(11)
MeOH/H_2O (60:40)	Pre-extd. with BuOH, concn., Add EtOH, concn., MeOH/Acetone, concn.		(1) Silica gel Column (2) Sephadex LH-20	$CHCl_3$/MeOH (1:1-1:3) Acetone	Deoxynivalenol	(15)
MeOH/H_2O (60:40)	Concn., Acetone pptn.		PTLC Silica gel	$CHCl_3$/MeOH/H_2O (90:10:1)	Deoxynivalenol	(16)
MeOH/H_2O (55:45) 1% NaCl		Hexane 3x $CHCl_3$	Silica gel Column 1x20 cm	$CHCl_3$/MeOH (97:3)	Fusarenon-X DAS	(12)
MeOH/H_2O (50:50)		Concn. Hexane Ethyl Acetate	PTLC Silica gel	(1) Ethyl Acetate/toluene (3:1) (2) $CHCl_3$/Acetone (3:2)	Deoxynivalenol	(20)
MeOH/H_2O (50:50)	Add 30% aq. $(NH_4)_2SO_4$	$CHCl_3$	2 g Silica gel Column	Hexane, Benzene Acetone/Benzene (5:95), Et_2O	T-2 Toxin DAS	(17)
MeOH/H_2O (3:1)	Concn.	Hexane, 3x $CHCl_3$	PTLC Silica gel	(1) Ethyl Acetate/ Toluene (3:1) (2) $CHCl_3$:Acetone (3:2)	Deoxynivalenol	(18)
MeOH/H_2O (9:1)	Concn.	Hexane, 3x $CHCl_3$ MeOH, Acetone solns.	(1) Silica gel column (2) PTLC silica gel	Hexane/Acetone (1:1) $CHCl_3$/MeOH(3:1)	Deoxynivalenol	(19)

purification on a Sephadex LH-20 column.

The cleanup procedures (10,14,16) for both the chloroform and ethyl acetate extracts use a liquid-liquid partition step followed by either thin layer or column chromatography (Table IV). Hsu et al. (10) uses a liquid-liquid partition followed by both a column and a preparative thin layer chromatography step in the analysis of corn for T-2 toxin. The ethyl acetate extracts (14,17) are concentrated, and the residue is then partitioned between aqueous methanol and chloroform/ethyl acetate or acetonitrile and petroleum ether. In either case the extract is further purified on silica gel, in one instance by column chromatography with toluene/ethyl acetate (3:1) as the eluant and in the other case by preparative thin layer chromatography with chloroform/methanol (98:2) as the eluant. Pathre and Mirocha (23) note that ethyl acetate is an efficient solvent for the extraction of group A trichothecenes, but is not selective, giving an extract that requires extensive cleanup.

Roberts and Patterson (24) use a novel purification procedure for the detection of twelve mycotoxins, including T-2 toxin and DAS, in animal feeds. An acetonitrile solution of the sample extract is dialyzed against water/acetone (70:30). The aqueous acetone is then extracted with chloroform and the mycotoxins are detected by TLC.

Detection and Quantitation

Methods (1,3,23) for the detection of the trichothecenes are numerous. Most of these are bioassay procedures; consequently, they are not specific and only roughly quantitative. Some of the biological procedures are extremely sensitive for groups of trichothecenes (25), and these are useful for screening and additional confirmation of suspected trichothecene contamination. This review, however, will consider only chemical assay procedures.

As previously noted, the trichothecenes all have the 12,13-epoxy function which might be used for development of a selective method. Unfortunately, a specific and sensitive detection procedure for the trichothecene epoxide function has not been found. One enzymatic assay procedure for the epoxide group has been suggested (26). Glutathione-S-epoxide-transferase was used, and an analysis for the unreacted glutathione in the reaction mixture gave an indirect measure of epoxide concentration. Submicrogram concentrations could be measured, but no data were presented on the detection of trichothecenes in naturally contaminated samples.

Polarography has been used to detect trichothecin in liquid cultures (27). The compound was detected at a concentration of 220 ng/ml with an error of not more than 3%. Recent advances in high pressure liquid chromatography (HPLC) using polarographic detectors may offer a direct method for detecting and quantitating trichothecenes.

A modification of the Poltorak colorimetric test for α,β-unsaturated ketones has been used to assay for trichothecin in culture extracts (28). A brown color develops when a benzene solution of trichothecin is treated with sulfuric acid; this mixture gives a blue color (measured at 600 mm) when added to methanol. The minimum detectable level was reported to be 200 μg/ml. Another colorimetric method (29) uses chromotropic acid for the measurement of pure fusarenon-X and T-2 toxin at the 50-1000 ng/ml concentration. The absorbance was measured at 583 nm in a microcell. Neosolaniol, nivalenol, tetraacetylnivalenol, and HT toxin gave a positive color reaction in this test.

Thin layer chromatography and gas liquid chromatography have so far been the detection and quantitation

TABLE IV

Methods Used for Purification of Extracts for Trichothecene Analysis

Extraction solvents (v/v)	Preliminary	Liquid/liquid partition	Liquid chromatography		Trichothecene	Ref.
			Solid phase	Eluant (v/v)		
$CHCl_3$	Concn., Add MeOH:H_2O (4:1)	(1) Skellysolve B (2) Add H_2O to 1:1 (3) $CHCl_3$/EtOAc (1:1)	(1) Silica gel column 2.2x64cm	Toluene/EtOAc (3:1)		(10)
			(2) PTLC Silica gel	THF/Benzene (15:85)	T-2 Toxin	
$CHCl_3$	Add hexane		10 g Silica gel Column	Hexane, Benzene, Acetone/Benzene (5/95), Et_2O MeOH/$CHCl_3$ (3:97)	T-2 Toxin	(21)
Ethyl Acetate	Concn., add MeOH:H_2O	(1) Hexane (2) Add H_2O to 1:1, adjust pH-9 (3) $CHCl_3$/EtOAc (1:1)	15g Silica gel Column	Toluene/EtOAc (3:1)	T-2 Toxin	(14)
Ethyl Acetate	Concn., add acetonitrile	(1) Pet. Ether (2) Ferric gel, filter (3) Add dimeth-oxypropane	PTLC Silica gel	$CHCl_3$/MeOH (98:2)	T-2 Toxin DAS	(16)

TABLE V

Conditions for Gas Liquid Chromatography of Trichothecenes

Column	Temp. (°C)	Trichothecenes	Derivatization reagent	Detector	Ref.
3% SE-30	210, 238 160-250	13 Compounds	BSA[a]	Flame Ionization	(34)
(1) 3% SE-30 (2) 3% QF-1	160-240 145-210	T-2 Toxin	BSA	Flame Ionization	(10)
3% OV-1	150-250	Deoxynivalenol	BSTFA[b]	(1) Flame Ionization (2) Mass Spectrometer	(15)
3% OV-1	150-280	Deoxynivalenol	"TMS Reagent"	Flame Ionization	(18)
3% SE-30	200	T-2 Toxin DAS	HFBI[c]	Electron Capture	(17)
3% OV-1	150-275	T-2 Toxin DAS	TMCS[d]-BSA, MBTFA[e]	(1) Flame Ionization (2) Mass Spectrometer	(16)

[a]BSA = Bis(trimethylsilyl)acetamide.
[b]BSTFA = Bis(trimethylsilyl)fluoroacetamide.
[c]HFBI = Heptafluorobutyrylimidazole.
[d]TMCS = Trimethylchlorosilane.
[e]MBTFA = N-methyl-bis(trifluoroacetyl)amine.

methods of choice for trichothecenes (23). Thin layer chromatography (TLC) has been the most frequently used method of analysis because of the greater specificity resulting from a combination of multiple solvent development systems and spray reagents. The major drawback has been the relatively high detection limit compared to gas liquid chromatography (GLC). A large number of TLC solvent systems have been reported for separating various trichothecenes from each other and from extraneous sample extract components (23,29,30). Many of these are combinations of chloroform and an alcohol (23,29,30), with methanol generally preferred. Combinations of benzene/acetone, ethyl acetate/hexane, ethyl acetate/toluene and benzene/tetrahydrofuran have all been successfully used for the separation of trichothecenes. Depending on the silica gel used and the environmental conditions, the proportions within each combination are varied to achieve the desired migration and separation.

Detection of the trichothecenes on TLC plates involves measurement of quenching of a fluorescent background or use of a chromogenic spray. Sulfuric acid sprays have been commonly used to visualize the trichothecenes on TLC plates. Ueno et al. (31) observed that those trichothecenes with an α,β-enone system (B-type) give a nonfluorescent, brown color when sprayed with sulfuric acid, while those compounds without such a system (A-type) give a blue fluorescent color under longwave ultraviolet light. The detection limit on TLC plates was observed to be 0.1 to 0.2 μg for the A-type and several micrograms for the B-type. (The A- and B-types of Ueno (31), based on the presence of the α,β-enone system, and the A and B groups of Pathre and Mirocha (23), based on solubility, should not be confused.) One other spray reagent, p-anisaldehyde (30), has also been extensively used to detect the trichothecenes. This reagent also causes the Ueno A-type trichothecenes to fluoresce blue and colors the B-type a nonfluorescent yellow. Bamburg (32) used Ehrlich's spray reagent (p-dimethylaminobenzaldehyde in hydrochloric acid) to detect DAS in the presence of T-2 toxin. This reagent gives a pink color with DAS, whereas T-2 toxin does not react. Tatsuno et al. (33) found that nivalenol gives a pink-violet color when sprayed with α-naphthol in sulfuric acid. The limit of detection for trichothecenes sprayed with these last two reagents has not been reported.

Gas liquid chromatography (GLC) eliminates much of this variability and has a lower detection limit than TLC for most compounds that can be made volatile. Several methods (9,10,14-17,19,34,35) have been developed using GLC for the detection and quantitation of the trichothecenes (Table V). Because of the presence of one or more hydroxyl functions, derivatives are formed before GLC analysis. The trimethylsilyl ether derivatives have been investigated by Ikediobi et al. (34) for several trichothecene combinations with 3% SE-30 as the liquid phase. The trimethylsilyl ethers of the trichothecenes have been formed by using several different reagents; bis-(trimethylsilyl)-acetamide has been used most frequently. However Tanaka et al. (35) and Pathre and Mirocha (23) report that nivalenol and similar trichothecenes in solubility group B do not react completely with this reagent. Tanaka et al. (35) use a combination of N-trimethylsilylimidazole and trimethylchlorosilane in pyridine for the nivalenol derivatization, while Mirocha et al. (16) use a mixture of bis(trimethylsilyl)acetamide, trimethylchlorosilane and N-trimethylsilylimidazole for derivatization of T-2 toxin and DAS. Romer et al. (17), using the reagent heptafluorobutyrylimidazole, form the heptafluorobutyryl ethers of T-2 toxin and DAS. The fluoro derivatives are then detected by electron capture GLC. The detection limit is lower with electron capture detectors for the halogenated derivatives than with the flame ionization detector for the silyl ethers.

Nearly all of the methods that have been developed for naturally occurring trichothecenes have been for T-2 toxin and deoxynivalenol (Table V). These methods used either a methylsilicone (SE-30, OV-1) or a methyltrifluoropropylsilicone (OF-1) as the liquid phase. Tanaka et al. (35) and Pathre and Mirocha (23) recommend the use of a methylphenylsilicone (OV-17) column. Pathre and Mirocha (23) achieved baseline separation of the trimethylsilyl ethers of deoxynivalenol, monoacetoxyscirpenol, DAS, neosolaniol, T-2 toxin and HT-2 toxin with 3% OV-17 as the liquid phase. Ikediobi et al. (34) found that QF-1 was useful for the separation of verrucarol, crotocol and trichothecolone; these could not be separated on SE-30 columns.

Only a few of the methods described in the literature report a limit of detection for any of the trichothecenes, and these few are for pure trichothecenes. Romer et al. (17) list a detection limit for T-2 toxin in feed of 100 ng/g and for DAS in feed of 25 ng/g. Vesonder et al. (15) and Mirocha et al. (16) have used combined GLC-mass spectrometry to detect and confirm the trichothecenes. Vesonder et al. (15) also used this technique in the analysis of corn for vomitoxin (deoxynivalenol). Mirocha et al. (16) have used selected ion monitoring mass spectrometry to detect the trichothecenes.

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