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Review

# Instrumental methods for determination of nonmacrocyclic trichothecenes in cereals, foodstuffs and cultures

Wenche Langseth*, Thomas Rundberget

*National Veterinary Institute, Department of Chemistry, P. O. Box 8156 Dep, N-0033 Oslo, Norway*

**Abstract**

An overview of the most important methods for determination of nonmacrocyclic trichothecenes (type A and B) in cereals, food/feedstuffs and solid cultures is presented. Various extraction, clean-up, and derivatization procedures, along with instrumental methods currently in use are reviewed. Possibilities and limitations of gas chromatography, high-performance liquid chromatography, and supercritical fluid chromatography in analysis of trichothecenes are discussed, including various detection techniques. Methods based on mass spectrometry are especially emphasized. The overview is based on 176 references. 

*Keywords:* Reviews; Food analysis; Sample preparation; Fusarium; Trichothecenes; Sesquiterpenoids; Mycotoxins; Toxins; Deoxynivalenol

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*Corresponding author.

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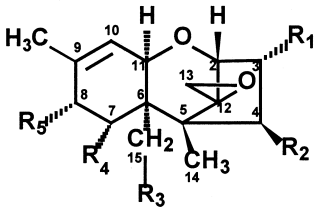
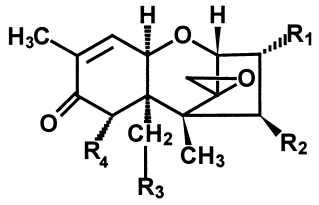
## 1. Introduction

Trichothecenes are a family of closely related sesquiterpenoids. Most of them have a double bond at position C-9,10, a 12,13-epoxide ring, and a variable number of hydroxyl and acetoxy groups (Fig. 1). This group of mycotoxins is produced by several genera of fungi; *Fusarium, Trichoderma,*




**Group A Trichothecenes**

| | MW | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|---|
| Neosolaniol | 382 | OH | OAc | OAc | H | OH |
| HT-2 Toxin | 424 | OH | OH | OAc | H | $OCOCH_2CH(CH_3)_2$ |
| T-2 Toxin | 466 | OH | OAc | OAc | H | $OCOCH_2CH(CH_3)_2$ |
| T-2 Triol | 382 | OH | OH | OH | H | $OCOCH_2CH(CH_3)_2$ |
| T-2 Tetraol | 298 | OH | OH | OH | H | OH |
| Scirpentriol | 282 | OH | OH | OH | H | H |
| 15-Monoacetoxyscirpenol (MAS) | 324 | OH | OH | OAc | H | H |
| 4,15-Diacetoxyscirpenol (DAS) | 366 | OH | OAc | OAc | H | H |




**Group B Trichothecenes**

| | MW | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|
| Deoxynivalenol (DON) | 294 | OH | H | OH | OH |
| 3-Acetyl-DON | 338 | OAc | H | OH | OH |
| 15-Acetyl-DON | 338 | OH | H | OAc | OH |
| Nivalenol | 312 | OH | OH | OH | OH |
| Fusarenon-X (4-Acetyl-nivalenol) | 354 | OH | OAc | OH | OH |

Fig. 1. Structures of type A and B trichothecenes.

*Myrothecium, Stachybotrys, Cylindrocarpon*, and *Trichothecium*, from which the trichothecene name has been derived [1,2]. The total number of trichothecenes isolated from natural sources, mainly fungal, was 170 when counted by Grove [3,4], but the number characterized continues to increase. The trichothecenes have been classified in four groups; A, B, C, and D, according to their basic structure [5,6]. Type B trichothecenes are characterized by a carbonyl group in position C-8, and include among others deoxynivalenol (DON) and nivalenol. T-2 and HT-2 toxin are the main trichothecenes belonging to type A, for which the carbonyl group is missing. Type C is characterized by a second epoxide. Type D trichothecenes are macrocyclic compounds, produced mainly by species belonging to the genera *Stachybotrys* and some higher plants [7], and constitute somewhat less than 40% of all identified trichothecenes. Neither type C nor type D trichothecenes are covered by this paper, although several of the methods may also be applied to these compounds.

Cereals contaminated by various *Fusarium* species are the main source of nonmacrocyclic trichothecenes in food- and feedstuffs. The fungi may infect grain during the growing season [8–10]. Wheat and maize seem to be especially susceptible [11]. Although the number of characterized trichothecenes is large, only a few of these have been detected so far in naturally contaminated cereals and commodities. DON is the trichothecene most frequently detected, and in the highest concentration in small grains world-wide. Up to 100% of all grain loads may under unfavourable climatic conditions be contaminated in the range 30–10 000 μg/kg, [8,12–16]. Two different risk assessments have concluded that the estimated daily intake of DON by high consumers in Europe is at the same level as the suggested amount of tolerable daily intake [17,18]. Other trichothecenes detected in naturally contaminated samples are 3-acetyl-DON, 15-acetyl-DON, nivalenol, fusarenon-X (=4-acetyl-nivalenol), T-2 toxin, HT-2 toxin, monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS), scirpentriol, and neosolaniol.

Trichothecenes are found to inhibit protein synthesis, DNA and RNA synthesis, and to have immunosuppressive and haemorrhaging effects [19–23]. Acute and subacute toxicity is characterized by feed refusal and weight loss, and increased susceptibility to infectious diseases. T-2 toxin and other type A trichothecenes are found to be up to tentimes more toxic than DON. Swine are among the most sensitive animal species, while trichothecenes are detoxified in the ruminants. Poultry appear to be relatively tolerant. The trichothecenes are rapidly distributed and metabolised after consumption by swine. There does not seem to be any accumulation of residues in the tissue or body fluid [24].

There is a clear need for sensitive and reliable methods for determination of the most common trichothecenes in cereals and cereal based food- and feedstuffs. The US Food and Drug Administration advisory levels for DON call for no more than 1 mg/kg for bran, flour, and germ for human consumption [25], while Norwegian [26] and Swedish [27] guidelines say maximum 500 and 400 μg/kg of DON, respectively, in swine feed. Methods for quality control should therefore be capable of determining trichothecenes down to 100–500 μg/kg in naturally contaminated samples. Lower detection limits, 10–50 μg/kg, are desirable for methods used in surveys, in order to establish data for risk assessments.

The first methods developed for determination of trichothecenes in grain were based on thin-layer chromatography (TLC). The trichothecenes were visualised by various reagents, of which aluminium chloride was most commonly employed for type B trichothecenes. These methods have been reviewed by Shotwell [1], Snyder [28] and Pohland et al. [29], and will not be further dealt with in this paper. Later, gas chromatographic (GC) methods were established based on electron-capture (ECD) or mass spectrometric (MS) detection, and are the methods most widely employed today. High-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) are other techniques used for determination of trichothecenes. Different immunochemical methods, especially enzyme-linked immunosorbent assay systems, have been established for determination of DON, T-2 toxin and other trichothecenes. These methods are, however, not based on chromatographic principles, and are therefore not covered by the present paper, but the field has been reviewed elsewhere [30–32]. A detailed review of GC methods described up to the early

nineties is given by Scott [33]. Other reviews of mycotoxin analysis, where trichothecenes are included, are the ones by Crosby [34], Cole [35], Chu [31,36], Gilbert [37], Betina [38], Scott [39–41] and Trucksess [25].

The aim of the present paper is to give an overview of existing methods used for determination of nonmacrocyclic trichothecenes (type A and B) in cereals, feed and foodstuffs, and to some extent, fungi cultures. No attempts have been made to cover all papers published, where methods for trichothecene analysis are included, but rather to summarize the most common methods currently in use, focusing on various approaches to trichothecene analysis and problems associated with them.

## 2. Trichothecene analysis

### *2.1. Sampling*

Mycotoxins are unevenly distributed in grain and naturally contaminated samples. One spikelet may contain up to 20 mg/g of DON while many kernels are completely free of toxins [8]. Less than 0.1% of the kernels may be infected. The chaff is normally from two to twenty times more contaminated than the kernel [42]. To reduce the variance, large sample sizes are required. Each sample should be based on a series of small portions taken from different locations throughout the lot, preferably by an automatic sampling system. In general, the sample size depends on several factors; lot size, the individual particle or unit size, the concentration level, the homogeneity of the lot, and the manner of sample collection. General guidelines on sampling for mycotoxins have been published [43–46]. Special procedures for aflatoxins, another important group of mycotoxins, have been worked out by FAO [47], and is under preparation by CODEX [48] and CEN [49] for control purposes. The same principles can be applied to sampling for trichothecene analysis. The sample should be thoroughly blended and subdivided to a minimum 1 kg sample, before the entire subsample is milled finely to pass through a 1 mm sieve. It is important that the ground sample is thoroughly mixed to achieve complete homogenisation before the amount required for extraction is taken out [46].

### *2.2. Extraction*

Various combinations of solvents have been used for extraction of grain, foodstuffs and other solid materials. To obtain a representative sample, extraction of 20 to 50 g of sample is generally recommended. Quantities down to 10 g have been used [50], but such low quantities require great care in securing a homogenous sample. In most procedures, 5 ml extraction solvent is applied per g of sample, but larger volumes have also been used [51]. Extraction of straw feed requires larger volumes of solvent than grain and feed, because of more voluminous sample matrix.

In the original procedure published by Scott et al. [52], the sample was extracted with methanol–water in the ratio (1:1, v/v), a solvent mixture which has been adopted by several other workers [53,54]. In a later version the ratio was changed to 7:3, methanol–water [55,56], but also the ratio 9:1 has been used [50,57]. Acetonitrile–water, in somewhat variable ratios, has been applied by a number of laboratories. Romer et al. [58] introduced extraction with acetonitrile–water (84:16), followed by purification on a charcoal–alumina column (see Section 2.3). This solvent mixture is probably the extraction medium most extensively used for trichothecene analysis today [59–63]. Small modifications have been published, including addition of small amounts of methanol [64] and basic cupric carbonate [59] to the solvent mixture. Other solvents applied are ethyl acetate–acetonitrile–water (20:5:1) [65] and acetone acidified with sulfuric acid [66].

Trenholm et al. [61] demonstrated that acetonitrile–water (84:16) resulted in less coextraction of interfering contaminants than methanol–water (1:1). Tanaka et al. [51] found that the highest recovery of nivalenol and DON was obtained with acetonitrile–water (3:1), followed by acetonitrile–water (3:2) and methanol–water (3:1). Extraction with acetonitrile–water (9:1) resulted in a 20% lower yield. Möller and Gustavsson [65] found that extraction with ethyl acetate–acetonitrile–water resulted in a cleaner extract than solvent mixtures of either acetonitrile or methanol and water. In a Nordic intercomparison study, lower recovery was obtained by the laboratories that used methanol–water than those that used acetonitrile–water or ethyl acetate–acetonitrile [67].

The choice of extraction medium is partly related to the selected clean-up system. Acetonitrile–water is preferred when charcoal–alumina or MycoSep columns are applied, because the crude extract can be transferred directly to the column.

Both high speed blenders and shakers have been applied for the extraction of grain and foodstuffs. Extraction time periods down to 3 min with high speed blenders [59] and 30 min with shakers [51] have been recommended. Trenholm et al. [61] demonstrated, however, that the extraction of DON from spiked samples is very much faster than from naturally contaminated samples. While 3 min blending was enough for a spiked sample, 16 min was required for naturally contaminated samples with acetonitrile–water (84:16). Up to 120 min was required with a wrist-action shaker. We experienced that maximum recovery was obtained after 30 min shaking for a naturally contaminated sample in our laboratory, 60 min being the prescribed time, ensuring complete extraction of all types of sample matrixes [63]. The extraction efficiency depends very much on the speed of the blender or shaker, and on the shape and size of the extraction flask. The required extraction time should therefore be tested in each laboratory. Use of a high-speed blender results in faster extraction per sample, but large sample series can conveniently be extracted simultaneously with a shaker, with less effort per sample.

In addition to traditional liquid extraction of solid samples, supercritical fluid extraction has been carried out for the determination of trichothecenes in grain and feed. A polar modifier is required to obtain sufficient recovery (>85%) [68,69]. Huopalahti et al. [68] used carbon dioxide modified with 5% methanol, at 550 atm pressure, and 60°C. With HPLC–MS in selected ion monitoring (SIM) mode, trichothecenes were detected down to 250 μg/kg in cereal extracts without further purification steps.

### *2.3. Clean-up*

Trichothecenes vary considerable in polarity, from nivalenol to T-2 toxin. Thus nivalenol has four hydroxyl groups whereas T-2 toxin contains an isovaleryl group and only one hydroxyl group. No single published method is optimal for determination of all trichothecenes simultaneously. A compromise has to be made. Separate clean-up steps may be applied for different groups of trichothecenes, but such approaches are time-consuming. More often the same purification procedure is used for all trichothecenes, which generally results in reduced recovery for at least one of the toxins. The extent of clean-up required depends on the aim of the analysis, the toxin concentration, the detection system, the matrix, and whether only type B or all trichothecenes are determined. Less purification is required when screening for high concentrations of DON, or fungal cultures are being analysed. Interferences from other compounds are less problematic in capillary GC analysis because of higher separation efficiency of capillary GC columns compared to normal HPLC columns. Use of a selective detection method, especially MS, reduces the need for extensive clean-up of the extract. In contrast to type A, type B trichothecenes contain a conjugated carbonyl group sensitive to ECD. A relatively selective detection is obtained when the toxins are determined as their trimethylsilyl (TMS) derivatives.

Many scientists have experienced great difficulties and frustration when trying to establish published procedures for trichothecene analysis in their own laboratory. Interferences, low recovery, or double peaks are often observed for one or more of the trichothecenes. Adsorption to glassware may constitute a significant problem in trichothecene analysis, and differences in the glassware may account for some of these difficulties. Although silanization is often recommended to reduce adsorption, most procedures do not describe special treatment of the glassware. However, it has been applied also to trichothecene analysis [70]. Trenholm et al. [61] chose to heat all glassware for 2 h in a muffle furnace at 500°C before use. Several authors have pointed out that the solvent applied for dissolving residues after evaporation to dryness is critical. Pure acetone, ethyl acetate or other medium polar solvents do not completely dissolve nivalenol and DON. These compounds tend to adsorb to glass and require a more polar solvent like methanol in the mixture [60,62]. Tanaka et al. [51] found the best recovery was obtained with pure methanol combined with sonication.

A large number of combinations have been described for the purification of crude extracts. In the

paper of Seidel et al. [50] an overview of different combinations of purification steps described in the literature is given. Most procedures are roughly based on one of the four methods described below. Romer and coworkers [58,62] described the use of a charcoal–alumina column, and later the MycoSep column [71] as the basic clean-up step. Scott et al. [52], recommended extraction into ethyl acetate and purification on silica as the main clean-up steps. A fourth alternative is defatting with hexane followed by purification on Florisil, as described by Tanaka et al. [51]. Application of immunoaffinity columns for purification prior to instrumental methods has been a great success for several mycotoxins [31]. High specificity and speed may be obtained, but commercial columns are not available for most trichothecenes, and the columns are generally only applicable to one toxin. In a study by Scott and Trucksess [72] low recovery was obtained for DON with a commercial column (Vicam).

In most of the published procedures, the concentration of each individual trichothecene is determined. Stahr et al. [73] described, however, a method where the esterified trichothecenes are hydrolyzed to their corresponding parent alcohols by alkaline hydrolysis. By this method most trichothecenes are converted to four compounds, making it easier to detect low concentrations of trichothecenes present in naturally contaminated samples. However, care should be taken to avoid degradation of DON and nivalenol. This method was later been adopted by several others [57,64,74,75].

### *2.3.1. Charcoal–alumina column*

The most extensively applied column for purification of crude extracts is the charcoal–alumina column, first described by Romer and coworkers [58,62]. The ratio between charcoal and alumina may vary somewhat, 0.5:0.7 (w/w), probably being the most common combination [60]. The charcoal should be of quality Darco G-60. Diatomaceous earth, e.g., Celite is often included in the packing material. Trucksess et al. [60] used 1.5 g packing material per column, but various column dimensions have later been reported, from 3 mm [63] to 20 mm I.D. [62] and 20 to 50 mm in length. Various vacuum systems, to which one or more columns can be fitted, have been described. The system may also be partly automated. Each column is used for several samples, with acetonitrile washing between each injection [63]. Crude acetonitrile–water (84:16) extracts of the sample, 0.25 to 20 ml depending on the size of the column, may be added directly onto the stationary phase. The trichothecenes are eluted by further addition of acetonitrile–water (84:16), while fat and other nonpolar components are retained. A relatively large elution volume is required to obtain complete elution of the less polar trichothecenes, about 25 ml for 1.5 g packing material. Special arrangements are therefore needed for vacuum suction of the solvent through the column. However, the 84:16 mixture is relatively easy to evaporate to dryness since it represents an azeotropic composition of acetonitrile–water. Type B trichothecenes have been determined without any further purification steps by TLC [60,62] and HPLC [59], or as their TMS derivatives by GC–ECD [63].

### *2.3.2. MycoSep column*

MycoSep 225, which is a modified charcoal–alumina column, was introduced by Romer Labs [71]. The stationary phase consists of various adsorbents, including charcoal, Celite, and ion-exchange resin. The acetonitrile–water (84:16) extract is purified by slowly pushing the flange end of the column into the tube containing the extract until the required amount, 2–3 ml, have passed through the column. The extract can be transferred directly to the reaction vial for evaporation and subsequent derivatization. More than 90% recovery was obtained for DON in a survey of barley and malt by Hastings and Stenroos [76]. Weingaertner et al. [77] validated the method for five type B trichothecenes. For DON, 3- and 15-acetyl-DON, and fusarenon-X the mean recovery was 95–97%, while the recovery of the more polar nivalenol was 66%. In our laboratory, we have applied MycoSep to five type A trichothecenes, too, and obtained more than 80% recovery for all trichothecenes studied, except nivalenol, for which it was on average 67%.

In a comparison study by Weingaertner et al. [77] chromatograms of similar quality, but better precision, were obtained with the MycoSep column. The method of comparison, which was already established in the analysis laboratory, was similar to that described by Scott et al. [55] with several clean-up

steps. We experienced that application of the MycoSep column resulted in less interferences than with a procedure consisting of two different purification steps, one on the charcoal–alumina–Celite column and one on a silica cartridge. In an interlaboratory comparison study for the determination of DON in wheat, good accuracy was achieved by the participants that used the MycoSep columns [78].

*2.3.3. Silica column*

In the procedure by Scott and coworkers [52,55] aqueous ammonium sulphate is added to the methanol–water extract before further purification. The trichothecenes are extracted into ethyl acetate from the methanol–water sample extract by liquid–liquid extraction prior to further clean-up on silica. In a later version, this step was replaced by application of an extraction column (Clin-Elut) [55], a method also adopted by Sydenham and Thiel [54]. In the original procedure by Scott et al. [52] the trichothecenes were eluted from the silica material with dichloromethane–methanol (95:5). Later dichloromethane–methanol (90:10) [55], diethyl ether [54] and acetone [79,80] have been applied as eluent. While no further purification was described by Scott and coworkers [52,55], others have chosen to include an additional step.

In a comparison of methods Tanaka et al. [51] obtained low recovery for nivalenol with the described method [52]. The toxin was found not to be extractable with ethyl acetate. The traditional silica column has been replaced by modern solid-phase extraction cartridges in several described methods [53,54]. When large volume columns are interchanged with modern silica or Florisil solid-phase extraction cartridges, the polar solvent required for solvatisation of the sample prior to clean-up may cause problems. In order to retain the toxins on small volume columns, it is important that the toxins are added to the stationary phase in a relatively apolar solvent. The dissolved residue may be diluted with a apolar solvent prior to the transference to the column, but high quantities are normally required. Often, the toxins are added to the stationary phase in a polar solvent, and eluted directly from the column without any washing step in between, making the purification step less effective [50,65,75,80]. Modern solid-phase extraction columns are generally delivered prepacked in plastic cartridges. It is well known that plastic softeners, especially phthalates, may be coeluted and cause considerable interferences in the GC determination of toxins. The problem can be reduced or eliminated by using glass or well washed plastic reservoirs. However, several choose to pack their own columns, using glass columns, which is time-consuming and inconveniences the use of silica and Florisil as stationary phase for clean-up.

*2.3.4. Florisil column*

In the method described by Tanaka and coworkers [51,81] the crude acetonitrile–water (3:1) extract is defatted with hexane, and the extract further purified on a column packed with Florisil (magnesium silicate), a material first applied for trichothecenes analysis by Kamimura et al. [82]. The stationary phase is washed with hexane, and DON and nivalenol eluted with chloroform–methanol (9:1). Quantities down to 10 μg/kg can be detected without further purification steps. For lower concentrations, an additional clean-up step on a silica Sep-pak cartridge is recommended. In the original procedure, extract equivalent to 10 g of sample is purified. The traditional Florisil column was later been replaced by small particle solid-phase extraction cartridges [50,65]. The eluent has been substituted with chloroform–methanol (7:3) without any washing step in between [50]. Kamimura et al. [82] and Seidel et al. [50] found that Florisil was more successful in removing matrix interferences than silica.

*2.3.5. Combinations*

When analysing for both type A and B trichothecenes, an extra clean-up step is often recommended in addition to purification on the charcoal–alumina column. Some of these clean-up steps are, however, very simple. Croteau et al. [83] combined the charcoal–alumina column with the addition of ammonia sulphate to the crude extract, which separates the acetonitrile–water phase into two phases. The result was less interferences with the early-eluting trichothecenes, nivalenol and DON, from polar compounds in the GC analysis. Furlong and Soares [57] introduced an extra extraction step with chloroform prior to purification on the charcoal–alumina column. Others have included purification

on one or two additional stationary phases in their procedure, like Rood et al. [75], who used both Florisil and silica columns, the last one after alkaline hydrolysis of the acylated trichothecenes. The Florisil column was eluted with ethyl acetate–methanol (3:1) and the silica column with dichloromethane–acetone (1:4). Lauren and Greenhalgh [84] introduced a column consisting of alumina–charcoal (20:1) with a cation-exchange resin in front for determination of nivalenol and DON by HPLC. Additional purification on charcoal was recommended for nivalenol. This clean-up method was later been expanded to also cover other trichothecenes [64].

In the procedure for determination of type A trichothecenes by Cohen and Lapointe [53], a purification step on a cyano cartridge was added to the method described by Scott et al. [52]. DAS and T-2 toxin were eluted with chloroform–hexane (1:1), while HT-2 toxin was eluted in another fraction with chloroform–ethanol (98:2). Rajakylä et al. [85] chose to use an amino column instead of the cyano cartridge.

Seidel et al. [50] replaced the silica column in the procedure of Tanaka et al. [51] with a $C_{18}$ solid-phase extraction cartridge. The more polar trichothecenes were eluted by methanol–water (1:1), while HT-2 and T-2 toxin were eluted by methanol–water (9:1), followed by extraction into dichloromethane. Takino and Yamaguchi [66] chose first to apply a $C_{18}$ cartridge, then derivatize with a TMS reagent, followed by purification on a Florisil column. Stahr et al. [73] used a $C_{18}$ cartridge after the extraction step with ethyl acetate described by Scott et al. [52]. Sano et al. [86] replaced the silica column with a cyano cartridge. The trichothecenes were eluted with methanol–water (1:4). Application of an anion-exchange resin, Amberlite XAD-4, prior to the Florisil column has been described by Kamimura et al. [82].

Many of the described methods are tedious and time-consuming, containing several purification steps. The chance of sample loss increases with the number of clean-up steps included. Especially steps involving evaporation to dryness followed by dissolution are critical, and may cause significant losses. The problems described for silica columns are also applicable to other stationary phases. To obtain sufficient recovery of the more polar trichothecenes, nivalenol and DON, the toxins generally have to be added to the column in a polar organic solvent. When the purification is carried out on small volume columns, the toxins generally have to be eluted directly from the column without any washing step in between.

### *2.4. Derivatization for GC analysis*

Almost all GC methods for determination of trichothecenes in food and cereals currently in use are based on derivatization of the hydroxyl groups forming TMS, trifluoroacetyl (TFA), pentafluoropropionyl (PFP) or heptafluorobutyryl (HFB) derivatives. Acetylation with acetic anhydride has also been carried out [73]. The choice of derivatization reagent depends on the type of trichothecene to be determined and the method of detection. The conjugated carbonyl group in type B trichothecenes makes them sensitive to ECD, while enhanced sensitivity of type A trichothecenes is obtained when reacted with a fluoroacyl reagent.

#### *2.4.1. Trimethylsilylation*

Silylation of trichothecenes was first described by Ikediobi et al. [87]. All hydroxyl groups are derivatized to form TMS ethers. Various reagent mixtures have been applied. Type A trichothecenes readily react with bis(trimethylsilyl)acetamide (BSA) alone [88,89], while two peaks often arise for type B trichothecenes with certain reagent mixtures, because of incomplete derivatization [90–92]. Gilbert et al. [92], who studied the optimum conditions for the formation of TMS derivatives, postulated that the double peaks arise from difficulties in derivatization of the C-7 hydroxyl group, because of hydrogen bonding to the adjacent carbonyl group. Tanaka et al. [90] showed that trimethylsilylimidazole (TMSI) is a necessary ingredient for complete silylation of type B trichothecenes. In our laboratory we have experienced that double peaks for type B trichothecenes may appear with pure TMSI under certain circumstances, especially for 3-acetyl-DON. A mixture of TMSI and trimethylchlorosilane (TMCS) and a solvent has been applied by a number of workers [82,90,93–95]. Today, commercial mixtures are more widely employed, either bis(trimethylsilyl)trifluoroacetamide–TMSI–TMCS, (3:3:2) (Regisil

323) [92], or more commonly, BSA–TMSI–TMCS, (3:3:2) (Tri-Sil, Sylon BTZ) [55,65,96–99]. The derivatization mixture, 50–100 μl, may be added without solvent [51,63,65,74,79]. Various reaction conditions have been applied, from 10 min at room temperature [55] to overnight at 60°C [100], 10–30 min at 30–80°C being recommended [63,65,74,77]. It is important to remove all residues of water prior to derivatization, which may be obtained by addition of benzene to the residue to form an azeotrope [63]. In the procedures first published, excess reagent was not removed, or removed by nitrogen evaporation. Rizzo et al. [93] showed, however, that a much cleaner chromatogram was obtained if the reaction mixture was washed with an aqueous solution after dilution with hexane, a method which has been adopted by most others. The water treatment not only removes excess reagent, which may cause damage to the GC column, it also effectively removes coloured pigments and other impurities present in the sample. Another advantage is higher stability of the TMS derivatives. We have found that the TMS derivatives can be stored for at least 10 days at −18°C with proper washing and removal of residual water by drying with anhydrous sodium sulphate.

*2.4.2. Fluoroacylation*

Formation of fluoroacyl derivatives, TFA, PFP or HFB derivatives, are commonly applied in order to increase the sensitivity, particularly of type A trichothecenes, to ECD. The reaction is carried out with either the imidazole or the anhydride. The more hydroxyl groups present, the greater the sensitivity. Heptafluorobutyrylation of trichothecenes was first described by Romer et al. [101] and Milama and Lelièvre [102], and was later adopted by Scott et al. [52] and others [50,53,54,83,103]. Type A trichothecenes react readily with heptafluorobutyrylimidazole (HFBI) at room temperature, while DON and other type B trichothecenes require higher temperatures. Scott et al. [52] added HFBI, dissolved in toluene–acetonitrile (95:5), and heated for 1 h at 60°C, a method which has been adopted by several others [53,54]. Higher temperatures have been employed [103], but adverse effects have been observed, in the same way as with longer reaction times [104]. Formation of HFB derivatives is also carried out using the anhydride (HFBA), with 4-dimethylaminopyridine (DMAP) as catalyst, dissolved in an organic solvent [50,83,105]. Croteau et al. [83] studied various derivatization conditions with DMAP, and found that the best results were obtained with toluene–acetonitrile (80:20) as derivatization solvent. Faster derivatization of type B trichothecenes is achieved with HFBA/DMAP compared to HFBI. Complete reaction was accomplished within 10 min at 60°C, 20 min being recommended for routine analysis. A disadvantage of HFB and PFP reagents, is that the neosolaniol derivative is unstable, and often decomposes during the GC–MS analysis [106,107].

After derivatization, excess reaction reagent should be removed. Kanhere and Scott [108] described a method where reaction byproducts were removed by adsorption on a solid-phase consisting of DMAP on a polystyrene matrix, subsequently removed by centrifugation. The method has been validated by Seidel et al. [50]. More commonly, the reaction mixture is washed with an aqueous phase in the same way as for TMS reagents. Initial washing with 1 ml 5% (w/v) aqueous sodium bicarbonate followed by water seems to give the best result [109,110]. The sample is often diluted with hexane or toluene before GC analysis [83], a step which should be done prior to washing with the aqueous phase, to improve the reproducibility [110]. It has been claimed that fluoroacyl derivatives are more stable to heat and less sensitive to moisture than TMS derivatives [111]. Our experience is that with fluoroacyl derivatives, the washing step is more critical.

Derivatization with PFP reagents is carried out in very much the same way as for HFB reagents, using either pentafluoropropionylimidazole [112], or the anhydride (PFPA) in the presence of a catalyst like DMAP [75], triethylamine [106], or imidazole [110].

The TFA derivatives are generally prepared by heating with trifluoroacetic anhydride, which can be evaporated off prior to GC analysis [64,113,114]. Excess reagent may also be removed by washing with aqueous solution in the same way as for the HFB and PFP reagents [75,115]. The reaction is normally carried out in the presence of a catalyst; DMAP, pyridine or sodium carbonate or bicarbonate [116,117]. Trifluoroacetylimidazole [106,118] or N-

methylbis(trifluoroacetamide) [118–120] may also be employed. Application of these three reagents have been studied by Kientz and Verweij [118], and various problems have been noted on the use of TFA reagents. The derivatives seem to be less stable than the HFB and PFP derivatives, both during storage and the GC run [121]. It is essential to remove completely residual water by means of dry sodium sulphate.

### *2.5. GC analysis without derivatization*

Trichothecenes are the only mycotoxins for which GC analysis is the method most widely applied. Normally the hydroxyl group(s) of trichothecenes are derivatized in order to attain the volatility and sensitivity required for trace analysis. However, several scientists have omitted the derivatization step [122–125]. Reasonable peak shape and sensitivity may be obtained for the less polar trichothecenes like DAS, HT-2 and T-2 toxin, while significant adsorption of DON and nivalenol to the column is often registered. Application of flame ionization detection (FID) has been described [124,125], but MS detection is generally employed [125–127]. A detection limit of 50 pg for DON has been obtained with oxygen negative ion chemical ionization (NICI) in SIM mode [128]. However, GC analysis of underivatized trichothecenes is first of all found to be useful in characterization of fungal culture extracts, and identification of unknown trichothecenes, as described by Miller and coworkers [129,130]. The analysis may be carried out on a relatively short DB-5 capillary column with on-column injection and retention gap. Electron impact ionization (EI) spectra of the underivatized compounds can be obtained, making it easier to identify the metabolites without being in possession of the pure compounds. The mass spectra of underivatized trichothecenes and other secondary metabolites of Fusarium are collected in different handbooks [131–133].

### *2.6. GC analysis after derivatization*

Although the trichothecenes may be injected directly into the GC without derivatization, most methods currently in use are based on derivatization of the hydroxyl groups in order to increase the volatility and sensitivity. Most of the original methods for determination of trichothecenes by GC were established with packed GC columns [51,52,62]. Today almost all laboratories use capillary columns, which are essential for multiple trichothecene determination in food and cereals, yielding higher separation efficiency and sensitivity. Scott and Kanhere [134] have compared different stationary phases for capillary GC analysis. Chromatograms and retention times are shown for the TMS and HFB derivatives of eight of the most common trichothecenes with different columns. Either 100% dimethyl siloxane (e.g. DB-1, SE-30), 5% phenyl (e.g. DB-5, SE-54), or 7% phenyl 7% cyanopropyl (e.g. DB-1701) dimethyl siloxane are most frequently employed for both TMS, PFP and HFB derivatives. GC–ECD analysis is often carried out on a DB-5 column with either a DB-1701 or a DB-1 column as verification column.

GC analysis of trichothecenes is generally carried out with a split/splitless injector in splitless mode. Use of electric pressure control of the injector have made it possible to inject large quantities of sample solution into the GC, resulting in higher detector response [66]. A temperature gradient may be run from 60 or 80°C to 250 or 270°C [56,63,83,135], but a gradient starting at 160°C or higher has also been described [50,54,64,75]. Our experience is that starting the gradient at 170°C is possible with one injector in our laboratory, while double peaks are observed for the type B trichothecenes with another one. But even when injected at 120°C the peaks become somewhat broader, and the response of neosolaniol is significantly diminished. Lower responses are often obtained for pure standards compared to spiked samples because of adsorption to the injector glassliner and column. Proper silanization of the glassliner has been proven to be essential to avoid adsorption.

The first report on GC analysis of trichothecenes was by Ikediobi et al. [87], who formed TMS derivatives and detected them by FID. Application of FID has been described by others [57,136], but ECD, first described by Kuroda et al. [137], is much more sensitive and selective for determination of trichothecenes. The conjugated carbonyl group in type B trichothecenes makes them sensitive to ECD, but even the TMS derivatives of type A trichothecenes

can be detected [82,137,134,138]. However, much higher sensitivity is obtained with a fluorinated acylation reagent, the more hydroxyl groups the greater the sensitivity, and the higher the number of fluorine atoms, the greater the detection response. Croteau et al. [83] found that the optimum detector temperature for HFB derivatives was 300°C, which is the temperature most commonly in use, independent of derivatization method. The response is also very much affected by the amount of make-up gas, generally nitrogen, used for the ECD. Our experience is that the day to day variation is reduced with a somewhat higher flow-rate than the optimum.

GC–Fourier transform infrared spectroscopy is a relatively new technique [139], which has been applied to Fusarium toxins, including trichothecenes. The technique has been applied to both underivatized compounds [140] and TMS derivatives [141]. Several characteristic absorption bands were observed. The method is especially applicable to analysis of fungi cultures, because additional structural information on unknown secondary metabolites present in a culture may be obtained. The relatively high quantities required for detection makes it less suitable for analysis of naturally contaminated samples.

*2.6.1. GC analysis with MS detection*

As early as 1981 Scott et al. [52] described the use of GC with MS detection for the determination of DON. MS is the preferred technique for reliable determination of small quantities of trichothecenes, especially type A [142]. In the first methods described, MS was employed for confirmation of results obtained with other detection techniques, especially GC–ECD [51–53,79,107,143]. Gilbert et al. [99] were the first to employ MS for detection of trichothecenes in a survey of grain samples, while Mirocha et al. [144] analysed for trichothecenes by GC–MS in various samples implicated in the biological warfare in Southeast Asia. But only after the introduction of relatively easily operated benchtop GC–MS instruments with autosamplers in the early eighties, did GC–MS methods become applicable to routine analysis. Vesonder and Rohwedder [145] have reviewed application of GC–MS to mycotoxin analysis until 1986.

Both TMS [55,79,100,141] and HFB [52,55,103,107], and also PFP [75,106,110,112] and TFA derivatives [113,116,117] have been widely employed for GC–MS determination of trichothecenes. Full scan MS spectrum in EI mode at 70 eV is generally obtained with quantities down to 0.1–1 ng for both TMS and fluoroacyl derivatives. The most important mass peaks with full scan EI conditions of the HFB derivatives of 14 trichothecenes are given by Krishnamurthy et al. [103]. All spectra were recorded in the mass range of $m/z$ 200–950. The high molecular mass of some of the trichothecenes is, however, a disadvantage when employing HFB reagents. The HFB derivative of nivalenol has a molecular mass of 1096. Alternatively the PFP derivatives may be applied [75,106,110,112]. Similar fragmentation patterns are obtained, but the ions have a mass units of 50 less per PFP group present in the ion. The mass spectra of TFA and TMS derivatives of selected trichothecenes are given by Mirocha and coworkers [146,147], along with the major fragmentation pathways. A problem with derivatized trichothecenes in general is that only the MS spectra of the most common trichothecenes are published, although special libraries have been established [148]. The provided EI spectra only give limited structural information about the compounds, making it difficult to recognise unknown trichothecenes from their MS spectra, which strongly depend on number and localisation of derivatized groups. However, some of the peaks are typical for certain groups of trichothecenes, and fluoroacyl derivatives of type B trichothecenes can normally be recognised by the $m/z$ 298 and 321 (HFB derivatives) or $m/z$ 244 and 271 (PFP derivatives) peaks. Mirocha (personal communication) found that it is easier to recognise the trichothecenes from MS spectra of their TMS derivatives than their TFA derivatives. However, the intensity of the molecular ion is higher for fluoroacyl than TMS derivatives. The molecular ion constituted from $<1\%$ to 24% of the base peak for TFA derivatives. The molecular ion was not observed for any of the derivatives of T-2 toxin and related trichothecenes [146].

For quantitative determination and detection of small quantities of trichothecenes, the MS has to be run in SIM mode. More intense peaks are observed in the high mass region for the fluoroacyl derivatives [146], resulting in more selective and sensitive

detection than for TMS derivatives. The following masses have been recorded for HFB derivatives [56]: *m/z* 1096 and 883 for nivalenol, 884 for DON, 656 for 15-MAS, 474 and 459 for DAS, 732, 672 and 655 for HT-2 and 501 for T-2 toxin. We have experienced that almost the same ions can be employed for PFP derivatives, taking into account the difference in molecular mass between HFB and PFP derivatives; *m/z* 896 for nivalenol, 734 for DON, 556 for 15-MAS, 572 and 555 for HT-2. We found that two or preferable three ions were needed for reliable detection of T-2 toxin, 451 and 468, and preferable also 407. For DAS *m/z* 379 and 452 are recorded. Black et. al. [149] recorded six ions for each toxin and found that confirmation of identity should in general be achieved with at least three ions. Better sensitivity and reproducibility is normally obtained in quantitative work when the MS is tuned with lower resolution, and increased peak intensity, and if possible specifically enhancing the higher mass ions. Quantities down to 10 pg can be detected for HFB and PFP derivatives with most instruments, making it possible to detect about 10 μg/kg or less in grain samples. Most MS spectra are recorded at 70 eV, but lower voltage has also been applied in order to increased abundance of the molecular ion [135].

Mirocha et al. [146] found that positive chemical ionization (PCI) spectra with methane as reagent gas are more useful in qualitative identification of the trichothecenes than the EI spectra. Fewer fragments, but more distinct peaks for the purpose of identification are obtained with PCI. The PCI spectra of TMS and TFA derivatives of the most common trichothecenes are given along with their fragmentation pathways [146]. A library, containing more than 250 entries of both EI and PCI spectra of TMS, HFB, PFP and TFA derivatives of trichothecenes has been compiled [148]. The most abundant peaks of the HFB derivatives are given by Krishnamurthy et al. [103]. The $[M+1]^+$ peak constituted from 6 to 99% of the base peak for all the common trichothecenes, with a source temperature at 100°C. Chemical ionization has also been carried out using ammonia [125,150], methane–ammonia (1:1) [113] and dimethyl ether [151,152] as reaction gas.

The most soft ionization of trichothecenes is achieved by negative ion chemical ionization (NICI). Although traditionally classified as chemical ionization, NICI is actually not a true chemical ionization for trichothecenes [146]. The method is applicable to compounds that can undergo electron capture, like halogen compounds, including fluoroacyl derivatives of trichothecenes. The sensitivity is directly proportional to the number of electron attracting groups in the molecule. In most instances $[M]^-$ or $[M\text{-}HF]^-$ are the most abundant ions with methane as reagent gas [103,106,153]. The ions are very much similar for PFP and HFB derivatives, when corrected for difference in molecular masses of the HFB and PFP groups [106]. NICI has also been carried out using hydroxide and chloride ion attachment conditions [126,154].

NICI has been applied to the determination of trace levels of trichothecenes [75,103,106,107,112,149,153,155]. Enhanced sensitivity has been obtained with this technique. In SIM mode, with methane as reaction gas, detection down to femtogram quantities has been obtained [103,106]. The detection limits are similar or somewhat lower for HFB compared to PFP derivatives. The ion source temperature has been found to have a dramatic effect on the NICI spectra [112,153]. The abundance of the $[M]^-$ ion decreased dramatically with increasing temperature. Optimum sensitivity and selectivity were obtained with a source temperature of 60°C and high source pressure (0.5–1 Torr; 1 Torr=133.322 Pa). Elevated electron energies have been applied by several scientists; 150 eV [112,153] and 200 eV [148]. However, one should be aware of that the conditions described for chemical ionization are not applicable to all instruments, especially not benchtop quadrupole instruments, although claimed to be able to run both chemical ionization and NICI. For many instruments the electron voltage cannot be increased beyond 70 eV. Temperatures below 100–150°C may not be obtainable. We have experienced that the temperature increases to above 100°C during the run, regardless of setting temperature, due to heat generated by the filament, with a Fisons Trio 1000 quadrupole instrument. At 150°C all type B trichothecenes exhibited a significant $[M]^-$ ion, while a peak of very low intensity was observed for T-2 toxin and some other type A trichothecenes. Broader peaks were another result of source temperatures below 200°C.

In several studies tandem MS (MS–MS) has been applied to trichothecene analysis [100,151,153,156]. This technique is especially applicable as an aid to structural elucidation of unknown metabolites [37]. By utilizing its high specificity, direct analysis of crude sample extracts has been possible. DON has been detected directly at levels down to 0.1 mg/kg in solvent extracts of cereals without prior clean-up steps [156,157].

### 2.7. Internal standard for GC analysis

Addition of an internal standard is generally recommended for quantitative GC analysis, especially with MS detection. Various internal standards have been used for determination of trichothecenes. However, none of the commercially available standards meet all the requirements set to an internal standard. Several of the standards in use function only as GC standards, for example heptachlor [52], 5α-cholestane [158], methoxychlor [159], and Mirex, a highly chlorinated pesticide [77]. They are not derivatized by any of the reagents employed. Seidel et al. [50] recommended the use of nandrolone for derivatization with HFBI. This compound contains one hydroxyl group and forms a derivative which elutes between HT-2 and T-2 toxin. However, the TMS ether of nandrolone was claimed to have poor ECD sensitivity in spite of the conjugated carbonyl group. Chloramphenicol was found to be a better internal standard of TMS derivatives; yielding higher sensitivity and being effective in the control of the derivatization step. In our laboratory, we found that α-chloralose meets most of the requirements for an internal standard for TMS and PFP derivatives, and presumably also HFB derivatives. The compound which contains a glucofuranonse group is quite polar, and can therefore be used also to control the clean-up step when the charcoal–alumina–Celite or the MycoSep columns are employed. Krishnamurthy et al. [103] have investigated the potential of using synthetically modified trichothecenes, deoxyverrucarol and 16-hydroxyverrucarol, as internal standards. These compounds are true internal standards, having the same characteristics as the analysed trichothecenes. The disadvantage is that none of these compounds are commercially available, and it is essential that they are free of residual trichothecenes from initial preparation. Deuterated T-2 toxin has also been employed for MS determination. The compound is commercially available.

### 2.8. HPLC

Several HPLC procedures have been published for the determination of DON and a few other selected trichothecenes in food and cereals [59,84,160–163]. Nivalenol and DON have been detected without derivatization down to 15 μg/kg and 50 μg/kg, respectively, with UV detection at 222 nm in cereal samples [84]. Separation was achieved on a RP-18 column with methanol–water (14:86) as mobile phase. For type A trichothecenes, HPLC with UV detection is only applicable to cultures with high toxin concentrations [164]. Considerably improved selectivity and a tenfold increase in sensitivity was obtained for DON with electrochemical detection in reductive mode [165]. Successful separation was achieved within 5 min on a $C_{18}$ column with methanol–40 m*M* borate buffer (35:65) as mobile phase. Although not described, this method is probably applicable to other type B trichothecenes, since the response is obtained from reduction of the carbonyl group.

Several workers have described determination of trichothecenes by HPLC after derivatization [86,166,167]. The trichothecenes have been derivatized with *p*-nitrobenzoyl chloride in the presence of 4-DMAP in acetonitrile prior to HPLC separation, resulting in a derivative detectable at 254 nm. A disadvantage is that a large peak from the reagent partly interferes with the trichothecenes. A post-column derivatization system is described by Sano et al. [86] where the trichothecenes are detected by fluorescence spectrophotometer at 370 nm (Ex.) and 460 nm (Em.). An aqueous sodium hydroxide solution (0.15 *M*) is added to the eluate from the HPLC column followed by a mixture of methyl acetoacetate (30 m*M*) and ammonium acetate (2 *M*). The detection limit was 20 μg/kg for nivalenol and DON after purification of maize extracts on two different clean-up columns. Although claimed to be simple, the method requires two extra HPLC pumps, several reaction coils, and an oil bath at elevated temperature to ensure proper mixing of the solutions.

HPLC analysis of trichothecenes, including mac-

rocyclic trichothecenes, are most commonly described with MS detection (LC–MS), either with thermospray [85,168–171] or fast-atom-bombardment (FAB) interface [170,172]. Moving belt [173] and direct inlet [143] interfaces have also been used. Separation may be achieved with a gradient on a 150 or 250 mm×4.6 mm I.D. RP-18 column, the mobile phase consisting of either methanol or acetonitrile and an aqueous phase. For thermospray and atmospheric pressure ionization sources a buffer is used, normally 0.1 *M* ammonium acetate, while 4% glycerol in methanol is added postcolumn with FAB. With thermospray an ammonium adduct is achieved for most trichothecenes in positive ion mode. Additionally, the $[M+H]^+$ peak is observed for DON and some other trichothecenes. In negative mode the $[M+CH_3COO]^-$ ion is generally recorded, but the $[M\text{-}H]^-$ or $[M]^-$ are sometimes achieved for trichothecenes [169]. FAB spectra of the trichothecenes exhibit an abundant glycerol adduct ion $[M+H+92]^+$ in addition to the protonated molecule $[M+H]^+$ [170,172]. Several fragment ions are generally also observed. A more detailed description of the MS conditions used for trichothecenes with FAB, thermospray, and moving belt interfaces is not given here, since the use of these interfaces have been taken over by other ones; electrospray, and especially atmospheric pressure chemical ionization for mycotoxins. Most papers published up to now are on method development. The application of LC–MS for determination of trichothecenes in real samples is very limited [85]. The quantities determined have been relatively high, although Rajakylä et al. [85] reported detection of T-2 toxin down to 75 pg. With modern equipment, also designed for routine analysis, detection down to the same level as with GC–MS is probably possible. A disadvantage of LC–MS in general is that limited fragmentation is obtained, giving scanty structural information, or possibilities for confirmation. However, with MS–MS instruments or ion trap instruments, for which sequential fragmentation of ions can be obtained ($MS^n$), more information for structural elucidation of unknown compounds is obtainable. Work is going on with this techniques for the analysis of trichothecenes.

### *2.9. SFC*

SFC is a relatively new technique. Separation of several trichothecenes have been demonstrated on 10 or 15 m×50 μm I.D. capillary columns, with supercritical carbon dioxide at 100°C as mobile phase [150,154,174]. High selectivity and sensitivity was obtained by SFC–MS, applying both PCI and NICI. The method can be applied to both type A and B trichothecenes, and macrocyclic trichothecenes. In addition to capillary GC column Young and Games [175,176] applied packed HPLC columns, combined with both UV detection and moving belt MS. Methanol (10%) was used as modifier to carbon dioxide. With chemical ionization, the SFC–MS spectra are very much similar to the GC–MS spectra (see Section 2.6.1), while EI spectra with direct inlet interface actually are a result of charge exchange ionization, due to the carbon dioxide present. The spectra are, however, similar to the EI spectra for most compounds, which seems to be true also for the trichothecenes [150]. In all these studies SFC was only applied to pure toxins or culture material. However, the potential of performing SFC on trichothecene analysis has been demonstrated. Detection limits in the same range as with GC–MS seem to be obtainable [150].

## 3. Conclusion

### *3.1. Extraction*

Acetonitrile–water, in the ratio 84:16, seems to be the solvent mixture most suitable for extraction of real samples, as described by Romer et al. [58], especially combined with the MycoSep or the charcoal–alumina column. The ratio 3:1, as described by Tanaka et al. [51], may also be employed. Satisfactory recovery can also be obtained with ethyl acetate–acetonitrile–water (20:5:1), as described by Möller and Gustavsson [65]. However, this method is less frequently employed, and is therefore less validated.

### *3.2. Sample clean-up*

The MycoSep multifunctional clean-up column is a very simple and fast purification method, giving chromatograms comparable to methods consisting of several clean-up steps. Only one evaporation step, prior to derivatization, is required [71,77]. Although

only 65–70% recovery is obtained for nivalenol, this seems to be the situation for most of the described procedures that cover all trichothecenes. As long as the recovery is reproducible, it is correctable. The charcoal–alumina column is cheaper in use than the MycoSep column, and still the clean-up procedure is relatively effective and simple compared to many of the other described methods. Several samples can be run simultaneously, and the procedure can be more or less automated [63]. For determination of low quantities of type A trichothecenes by GC–ECD, an additional clean-up step is required. Use of traditional silica or Florisil columns demand large elution volumes, which limit the number of samples conveniently purified simultaneously. Special problems are connected with application of small volume cartridges, because of limited solubility of the more polar trichothecenes in less polar organic solvents. Several evaporation and dissolution steps are often included in these procedures, critical steps which may cause significant losses. These circumstances make the use of silica, Florisil and other solid-phase extraction columns less convenient.

### *3.3. Choice of derivatization reagent for GC analysis*

With ECD a TMS reagent, preferably a mixture of BSA, TMCS and TMSI, should be used for determination of type B trichothecenes. All compounds with a hydroxyl group in the sample are derivatized, but the reagent itself is not sensitive to ECD. A relatively selective detection is therefore obtained. Fluoroacylation reagents are, however, preferable for type A trichothecenes, because of enhanced sensitivity. In view of the lower stability of TFA derivatives compared to HFB and PFP derivatives, HFB or PFP reagents should be used. The derivatization procedure of choice seems to be of less importance, but the procedure using HFBA or PFPA with DMAP is probably the one most extensively studied [83]. For determination of trichothecenes by GC–MS in naturally contaminated samples, derivatization with HFB or PFP reagents is preferable to derivatization with TMS reagents, because of more abundant peaks in the high mass region. The availability of MS instrument determines the choice of reagent. For instruments with a mass range up to 800 or 1000, the formation of PFP derivatives is preferable.

### *3.4. Choice of instrumental method*

The method of choice depends on the required detection level, the matrix, and instrumentation available. HPLC can be employed for high concentrations of type B trichothecenes, but with traditional LC detectors more sophisticated and complicated arrangements are required for determination of low quantities and type A trichothecenes. However, modern LC–MS instruments enable detection of low quantities of all types of trichothecenes in real samples. SFC is a technique suitable for compounds of low volatility and high polarity, like trichothecenes. However, although introduced in the early eighties, the technique is still not very suitable for routine analysis. Still few laboratories are in the possession of the equipment. GC analysis is the method of choice for most laboratories. Type B trichothecenes can routinely be determined in grain and mixed feed samples as TMS derivatives with ECD. MS detection is however essential for reliable detection of small quantities of type A trichothecenes [142]. If GC–MS is not available, GC–ECD may be employed after derivatization with a fluoroacylation reagent (HFB or PFP). However, it is important that verification is carried out on another GC column, preferably also with another derivatization reagent. GC analysis with MS detection (SIM), run in EI mode, is probably the method that can be established with least effort today, with reliable results for both type A and B trichothecenes. Up to a tenfold increase in sensitivity can be obtained, if needed, with NICI if the correct instrumentation is available. Both EI, PCI and NICI spectra may be useful for structural elucidation.

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