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Determination of mycotoxins produced by *Fusarium* isolates from banana fruits by capillary gas chromatography and high-performance liquid chromatography

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Abstract

A method of analysis for trichothecenes (nivalenol, deoxynivalenol, 3- and 15-acetyldeoxynivalenol, diacetoxyscirpenol, neosolaniol, T-2 tetraol, T-2 and HT-2 toxins), zearalenone and zearalenols, and another method for determination of fumonisin B_1 are described and applied to cultures of *Fusarium* isolated from bananas. Both methods were adapted from different techniques of extraction, clean-up and determination of these mycotoxins. The first method involves extraction with methanol–1% aqueous sodium chloride, clean-up of extracts by partition with hexane and dichloromethane, additional solid reversed-phase clean-up and analysis of two eluates by both high-performance liquid chromatography with ultraviolet detection and capillary gas chromatography. The method for fumonisin B_1 implies extraction with aqueous methanol, concentration, clean-up with water and methanol on Amberlite XAD-2 column, formation of a fluorescent 4-fluoro-7-nitrobenzofurazan derivative and analysis by high-performance liquid chromatography with fluorescence detection. Both procedures give good limits of detection and recoveries, and are considered suitable for the detection and quantification of the studied toxins in corn and rice cultures of *Fusarium* spp. isolated from banana fruits. © 1997 Elsevier Science B.V.

Keywords: *Fusarium* spp.; Fruits; Mycotoxins; Toxins; Trichothecenes; Fumonisins; Zearalenone; Zearalenols

1. Introduction

The occurrence of *Fusarium* spp. in cereal grains and other raw materials is well documented [1–5] but there are only a few published reports on the *Fusarium* mycobiota of bananas [6,7]. Mycological control in the food and feedstuffs industries is very important and mould count of feed is a useful indicator of quality [8]. However, taking into account the toxigenic potential of some fungi, the significance of both total and individual mould counts for feed quality is still not clear because only a few species appearing in these substrates are potential mycotoxins producers. The amount of mycotoxins produced can vary over a considerable range and is dependent on factors such as temperature, duration of growth period, substrate and strain of fungal species [3,9,10].

Fusarium spp. can be considered the main mycobiota in bananas [7]. Many isolates of the species found are mycotoxin producers, and, in some cases, an isolate can produce various toxic metabolites. Thus, the development of methods of analysis for multi-mycotoxins that allow for the evaluation of the toxigenic potential of *Fusarium* spp. growing in and on bananas is highly useful.

Trichothecenes are toxic metabolites responsible for mycotoxicoses in farm animals and are also

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implicated in some diseases of man [11]. Zearalenone (ZON) is an anabolic and uterotrophic metabolite with estrogenic activity often associated with hyperestrogenism and infertility in swine, poultry and cattle. The same activity is shown by the two isomers (alpha and beta) of zearalenol (α- and β-ZOL), although α-ZOL is 2–4 times more active than ZON or β-ZOL [12,13].

Methods of analysis for trichothecenes, ZON, and α- and β-ZOL in *Fusarium* spp. cultures or in cereals generally use as extracting media mixtures of methanol (MeOH) and water (sometimes added with NaCl) [4,14–18], or CH_3CN–water [5,19,20]. However, other mixtures such as ethyl acetate–CH_3CN [21] or $CHCl_3$–MeOH [22] have also been used. Fungal extracts may be purified by defatting with hexane or light petroleum followed by extraction with $CHCl_3$ [15] or CH_2Cl_2 [17,23]. Usually a further solid-phase clean up with Florisil [5,22], charcoal–alumina [20], or silica modified with C_{18} [23] or amino groups [20] has been applied. Sometimes a TLC purification step has also been carried out [14,17,24]. The cleaned up extracts can be analyzed by several techniques such as TLC (often used as screening method) [14], GC or HPLC.

The type B trichothecenes nivalenol (NIV), deoxynivalenol (DON), 3- and 15-acetyldeoxynivalenol (3- and 15-AcDON) have chromophoric groups that show an UV absorption maximum at about 225 nm, and can be analyzed by HPLC–UV [15,19,22]. They can be also determined as trimethylsilyl (TMS) or trifluoroacetyl (TFA) derivatives with GC with flame ionization detection (GC–FID) or electron-capture detection, respectively [25]. The type A trichothecenes diacetoxyscirpenol (DAS), neosolaniol (NEO), T-2 toxin (T-2), HT-2 toxin (HT-2) and T-2 tetraol (T-2 TOL) absorb light at low wavelengths (ca. 200 nm), so they are generally analyzed by GC of their TMS or TFA derivatives [4,16,24]. The two isomers of ZOL can be separated by HPLC–UV [23] and by GC [23,25,26]. ZON has been analyzed by HPLC with fluorometric [17,27,28] or UV detection [20,22,29] and by GC [16,25,26]. GC–MS is used as a confirmation technique for TMS or TFA derivatives of these toxins [5,26].

Fumonisins are mycotoxins produced by *F. moniliforme* and *F. proliferatum* whose interest has increased greatly in recent years as they have short-term toxic effects in cancer initiation/promotion in rat liver bioassays [30,31] and cause animal disorders. Fumonisin B_1 (FB_1) is the most well-known of these toxins. Extraction of fumonisins in cultures or cereals is carried out with MeOH–water [30,32–36] or CH_3CN–water [37–39]. The extracts are cleaned up by chromatography on strong anion-exchangers such as XAD-2 [32] or SAX [35–37,40], although C_{18} columns have also been used [38,39,41]. These toxins can be derivatized with fluorogenic reagents such as *o*-phthaldialdehyde [33], 4-fluoro-7-nitrobenzofurazan [40], 9-fluorenilmethyl chloroformate [37], fluorescamine [42] or naphthalene-2,3-dicarboxaldehyde–potassium cyanide [38,40,43]. Then they can be separated by HPLC and detected with a fluorimeter. TLC procedures for inexpensive analysis are also available [32]. Fumonisins can be confirmed by HPLC–MS [44,45].

The aim of this work was to design a suitable methodology for the identification and determination of trichothecenes, ZON, α- and β-ZOL, and FB_1 in cultures of *Fusarium* spp. isolated from bananas [7]. The adaptation and modifications of different techniques of extraction, clean-up and determination of these mycotoxins has given rise to a method of analysis for FB_1 and another for the remaining toxins.

2. Experimental

2.1. Reagents and standards

All the solvents and reagents used were analytical or HPLC grade. Standards of mycotoxins NIV, DON, 3-AcDON, 15-AcDON, NEO, DAS, T-2, HT-2, T-2 TOL, ZON, α-ZOL and β-ZOL, the resin Amberlite XAD-2 and the reagent 4-fluoro-7-nitrobenzofurazan (NBD-F) were purchased from Sigma (St. Louis, MO, USA). A standard of FB_1 was obtained from the Department of Food Science and Technology, CSIR (Republic of South Africa). Tri-Sil TBT, a mixture of N-trimethylsilylimidazole–N,O-bis(trimethylsilyl)acetamide – trimethylchlorosilane (3:3:2), was a product from Pierce (Rockford, IL, USA). The following solutions were used as mobile phases for the analysis of FB_1 by HPLC: (A)

MeOH–NaH_2PO_4 buffer 0.05 *M*, pH 4.4 (1:1) and (B) CH_3CN–water (4:1).

2.2. Production of mycotoxins

Five isolates of *Fusarium* belonging to *F. graminearum, F. acuminatum, F. equiseti, F. moniliforme and F. proliferatum* were used for mycotoxin production. These mycotoxin-producing isolates had been previously selected by TLC screening, following the procedure of Bottalico et al. [14], from our collection of fungi isolated from banana fruits [7]. The isolates were used to inoculate 500 ml Erlenmeyer flasks containing 100 g of rice or corn with a moisture content of 40%, which were autoclaved at 115°C for 30 min. The Erlenmeyer flasks were incubated at 25°C for 3 weeks. Then cultures were dried at 50°C for 48 h and ground to a powder with a mill.

2.3. Procedures

For the analysis of trichothecenes, ZON and ZOL, ground cultures (20 g) were extracted with MeOH–1% aqueous NaCl (60:40) (2×50 ml) using an Ultraturrax homogenizer (Ika-Werk, Germany). The hydroalcoholic extracts were filtered through Whatman No. 1 paper, defatted with *n*-hexane (2×25 ml) and extracted with dichloromethane (3×25 ml). The combined CH_2Cl_2 extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residues were dissolved with 2 ml of MeOH–water (40:60) and filtered through Sep-Pak C_{18} reversed-phase cartridges (Waters Associates, Milford, MA, USA) previously conditioned. Cartridges were eluted sequentially with 4 ml of MeOH–water (40:60) and with 4 ml of MeOH. Two fractions (1 and 2) were collected accordingly, which were evaporated to dryness under N_2 and redissolved with 0.5 ml of MeOH. Fraction 1 was used for analysis of the more polar type B trichothecenes, and fraction 2 for analysis of type A trichothecenes, ZON, and α- and β-ZOL.

Trichothecenes were detected and quantified in fraction 1 by HPLC with the UV detector set at 225 nm. The separation was performed in a Spherisorb ODS-2 reversed-phase column. The mobile phase was MeOH–water (35:65) at a flow of 1.0 ml/min. ZON, α- and β-ZOL were analyzed in fraction 2 by HPLC–UV at 236 nm with the same column, but the mobile phase was in this case MeOH–water (65:35) at 1.0 ml/min. Identified toxins were confirmed by stopping the flow and recording the UV spectrum when the suspected compound reached the detector cell.

An aliquot of fraction 2 was evaporated to dryness under N_2 with slight warming. The residue was treated with 50 μl of Tri-Sil TBT for 1 h at 45°C and was analyzed by GC–FID. Usually 3–4 μl of the solution were injected into the gas chromatograph. The mycotoxins were determined by the external standard procedure. Analysis by GC was also used as a technique for confirming the trichothecene mycotoxins found in fraction 1 by HPLC.

FB_1 was extracted by a modification of the procedure of Vesonder et al. [32]. Briefly, 20 g of ground culture were extracted with 2×100 ml of MeOH–water (65:35). The extracts were filtered, combined and concentrated at 50°C in a rotary evaporator connected to a water pump. The concentrated liquid was brought to 100 ml and a 20 ml aliquot was loaded on a column (25×1 cm) packed with 10 g of Amberlite XAD-2. The column was eluted first with 100 ml of water and then with 100 ml of MeOH. The aqueous eluate was rejected and the methanolic eluate containing FB_1 was collected and brought to 100 ml with MeOH. If the sample was supposed to contain high levels of FB_1, a 0.25 ml volume (equivalent to 0.01 g sample) was evaporated to dryness under N_2 at 40°C and derivatized according to the method of Scott and Lawrence [40] with modifications. The residue was dissolved with 100 ml of 0.04 *M* sodium borate buffer, pH 9.3. Then 50 μl of a fresh solution of 50 m*M* of NBD-F in ethanol was added. The solution was heated at 60°C for 1 min and cooled with ice for 1 min. The mix was diluted quickly with 100 μl of mobile phase A and injected, as soon as possible, into the liquid chromatograph provided with the same Spherisorb ODS-2 column used for trichothecenes. The column was eluted at 1 ml/min with mobile phases A and B, as follows: 5 min with A; 0.5 min with gradient 100% A to 50% A +50% B; 10 min with 50% A +50% B. The fluorescence detector was operated at 460 nm (excitation wavelength) and 500 nm (emission wavelength). In the case of low levels of FB_1,

the MeOH eluate had to be concentrated by rotary evaporation at 10 ml before the volume was taken. Peak heights were compared with those of FB_1 standards of appropriate concentrations. The NBD-F derivative of FB_1 is unstable so it was analyzed immediately or kept in a freezer (−18°C) for up to 2 days [40].

2.4. Apparatus

A HP 1050 liquid chromatograph provided with a variable-wavelength UV–Vis detector and a programmable fluorescence detector HP 1046 A (Hewlett-Packard, Palo Alto, CA, USA) was used for the HPLC separations. The sample loop was 20 μl. A reversed-phase column Spherisorb ODS-2 (250×4 mm I.D., 5 μm d_p) (Tracer Analitica, Barcelona, Spain) protected with a guard precolumn packed with the same phase was used throughout. Chromatograms were recorded and integrated by a HP 3396A integrator.

GC analyses were performed in a Hewlett-Packard HP 5890 Series II gas chromatograph provided with FID and split/splitless injector. HP 3365 ChemStation software was used for data processing. Separation was carried out on a SPB-5 (30 m×0.25 mm I.D., 0.25 μm d_f) fused-silica capillary column (Supelco, Bellefonte, PA, USA) attached to the injector by a deactivated fused-silica retention gap (400×0.25 mm I.D.). Injector and detector temperatures were 275°C and 300°C, respectively. The oven temperature was programmed from 150°C (held for 1 min) to 280°C (held for 10 min) at 8°C/min. He at a flow-rate of 1 ml/min was the carrier gas. Split injections at split ratio 1/20 were made.

3. Results and discussion

3.1. Trichothecenes, zearalenone and zearalenol

The separation obtained by HPLC with a standard mixture of type B trichothecenes NIV, DON, 3-AcDON and 15-AcDON in the conditions described above is shown in Fig. 1. The two last toxins could not be resolved either by changing the composition (over a range compatible with analysis) or the flow of the mobile phase. Enrichment of the mobile phase in MeOH lead to a decrease in all retention times, which speeded the analysis but made the integration of NIV difficult. The retention times of these mycotoxins and their reproducibility, after ten consecutive injections, are listed in Table 1.

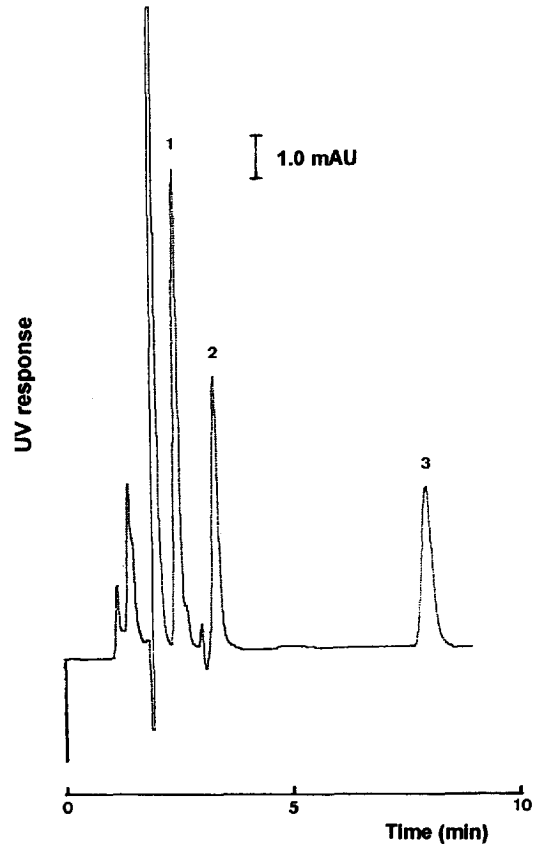

Fig. 1. Separation of a standard mixture of NIV, DON, 3-AcDON and 15-AcDON by HPLC with UV detection at 225 nm. Conditions: Spherisorb ODS-2 (250×40 mm, 5 μm d_p) reversed-phase column; mobile phase: methanol–water (35:65) at 1 ml/min. Peaks: 1, NIV; 2, DON; 3, 3-AcDON and 15-AcDON.

The four type B trichothecenes were separated by GC of the TMS derivatives obtained with Tri-Sil TBT (Fig. 2). This technique is suitable for confirmation purposes, and preferable for the identification and quantification of 3-AcDON and 15-AcDON, as they were resolved. A small peak due to the chromatographic system appeared at about 17.5 min but it did not match any peak from the toxins studied. HPLC was chosen as the initial technique to analyse for these metabolites owing to its higher

Table 1
Retention times of trichothecenes, zearalenone, zearalenols and fumonisin B_1 by HPLC and/or GC[a]

Mycotoxin	HPLC			GC	
	t_R (min)	S.D.	Mobile phase	t_R (min)	S.D.
Nivalenol	2.49	0.018	A	16.78	0.05
Deoxynivalenol	3.34	0.017	A	16.13	0.04
3-Acetyldeoxynivalenol	7.85	0.06	A	16.35	0.04
15-Acetyldeoxynivalenol	7.96	0.06	A	16.52	0.05
Diacetoxyscirpenol	–	–	B	16.40	0.04
T-2 tetraol	–	–	B	16.83	0.05
Neosolaniol	–	–	B	18.01	0.05
T-2 toxin	–	–	B	21.09	0.06
HT-2 toxin	–	–	B	21.85	0.06
β-Zearalenol	5.87	0.04	B	23.03	0.10
α-Zearalenol	8.32	0.07	B	22.81	0.09
Zearalenone	9.27	0.09	B	22.11	0.09
Fumonisin B_1	9.49	0.03	C, D	–	–

[a] Results are mean values and standard deviations from ten injections of standard solutions.
Mobile phases: A, Methanol–water (35:65); B, methanol–water (65:35); C, methanol–0.05 *M* NaH_2PO_4 buffer, pH 4.4 (50:50); D, acetonitrile–water (80:20).
–: Not detected (type A trichothecenes) or not applicable (fumonisin B_1).
HPLC and GC conditions: see Section 2.

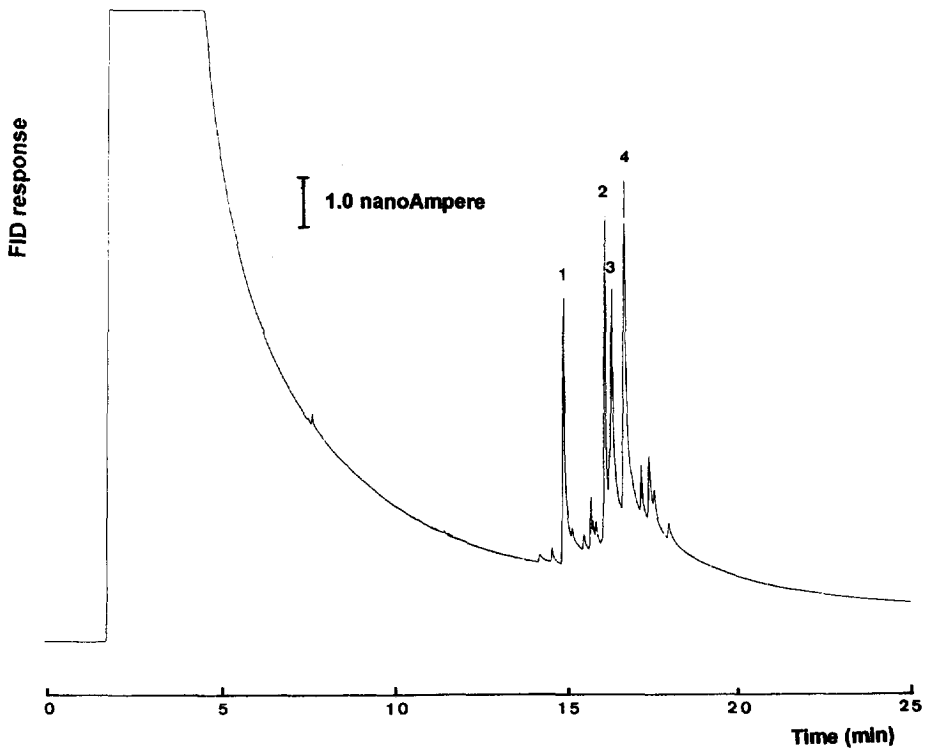

Fig. 2. Separation of a standard mixture of NIV, DON, 3AcDON and 15AcDON by GC–FID of their TMS derivatives. Conditions: SPB-5 (30 m×0.25 mm I.D., 0.25 μm film thickness) fused-silica capillary column. Column temperature: programmed from 150°C (held 1 min) at 8°C/min to 280°C (held 10 min). Helium head pressure 130 kPa. Split ratio 1:20. Peaks: 1, NIV; 2, DON; 3, 3-AcDON and 4, 15-AcDON.

speed (lack of evaporation and derivatization steps, lower retention times) and better resolution (except for 3- and 15-AcDON).

Both α- and β-ZOL and ZON, less polar than type B trichothecenes, are well separated in a reasonable time by HPLC with the mobile phase MeOH–water (65:35) (Fig. 3 Table 1). The resolution between ZON and α-ZOL is acceptable and both toxins may be analyzed without interference. Moreover, type A trichothecenes, which may be present in fraction 2, do not interfere at 236 nm. Separation of ZON and ZOL by GC as TMS derivatives appeared to be more problematic. Their retention times were higher than those of TMS derivatives of trichothecenes (Table 1) and, although ZON, α-ZOL and β-ZOL were resolved, sensitivity was low, peaks tailed severely and the response was not reproducible. Derivatization with Tri-Sil TBT and GC separation of ZON and ZOL can be used for confirming these toxins at relatively high levels. Problems may be due to residual amounts of trimethylsilylimidazole or related degradation products at the beginning of the column [25].

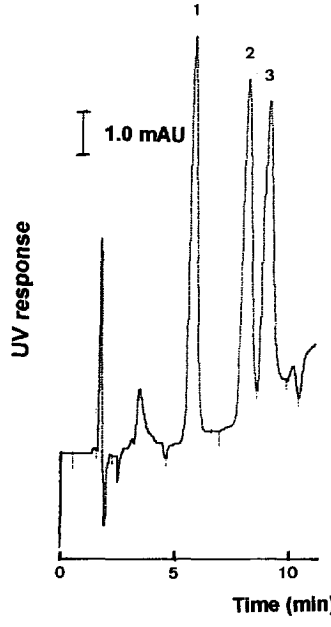

Fig. 3. Separation of a standard mixture of α-ZOL, β-ZOL and ZON by HPLC with UV detection at 236 nm. Conditions: as in Fig. 1, but the mobile phase was methanol–water (65:35). Peaks: 1, β-ZOL; 2, α-ZOL; 3, ZON.

The separation of the TMS derivatives of type A trichothecenes by GC is shown in Fig. 4. DAS, T-2 TOL and NEO are well separated from each other and from both T-2 and HT-2. However, these two last toxins have nearly the same retention times and overlapped severely. A more efficient column is needed to separate these two compounds. Table 1 lists the retention times of the TMS derivatives of the trichothecenes, ZON and ZOL. There are no problems of interference, as probably overlapping trichothecenes are collected in different fractions by the reversed-phase clean-up.

Recovery of the procedure was tested by addition of the analytes (dissolved in MeOH) to ground corn previously analyzed and where toxins were undetectable. The spiking levels were 2, 10 and 20 μg/g. In fraction 1 the mean recoveries of NIV, DON and 3-AcDON by HPLC were 69, 90 and 86%, respectively. In fraction 2 the mean recoveries of α-ZOL, β-ZOL and ZON, also by HPLC, were 87, 89 and 94%, respectively; and for DAS, T-2 TOL, NEO and T-2 by GC, were 90, 92, 86 and 84%, respectively. These values can be considered good (except for NIV) and fall within the normal limits for these toxins.

The limits of detection (LD) of the complete method (signal-to-noise 3:1) by HPLC–UV, were 2, 3, 5, 5, 9 and 9 ng/g for ZOL (α and β), ZON, NIV, DON, 3- and 15-AcDON, respectively. In the case of type A trichothecenes determined by GC–FID the LD of the method for DAS, T-2 TOL, NEO and T-2 were 18, 18, 20 and 23 ng/g, respectively. LD of type B trichothecenes were similar to those found for type A when they were separated by GC (17–20 ng/g). So HPLC–UV was more sensitive than GC–FID for type B trichothecenes. Relative standard deviations of responses (heights) from five replicate injections of standard solutions of 10–20 μg/ml by HPLC were <5% for NIV, DON, 3-AcDON, 15-AcDON, ZON and ZOL.

3.2. *Fumonisin B_1*

Fig. 5 shows the peak produced by the NBD-F derivative of standard FB_1 (40 ng). In this case LD (signal-to-noise 3:1) was 0.13 μg/g (about 1 ng

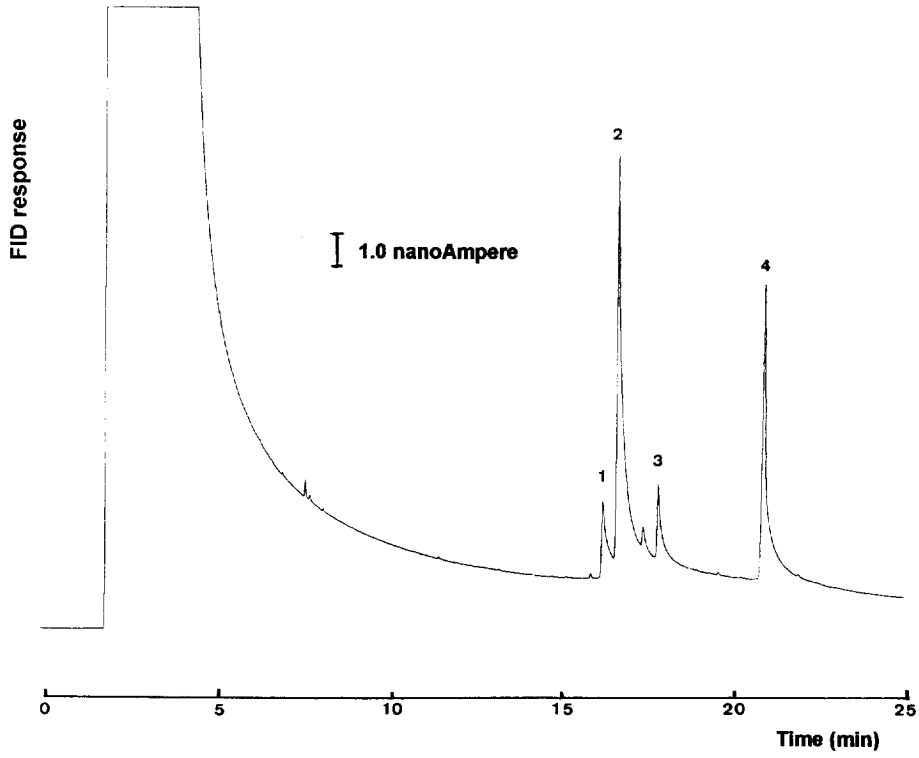

Fig. 4. Separation of a standard mixture of DAS, T-2 TOL, NEO, T-2 and HT-2 by GC of their TMS derivatives. Conditions: as in Fig. 2. Peaks: 1, DAS; 2, T-2 TOL; 3, NEO; 4, T-2 and HT-2.

injected), which agrees with reported values [40]. Lower values can be obtained if the aliquot taken for evaporation and derivatization is >0.25 ml or the eluate becomes more concentrated. Recovery assays in corn with undetectable levels of this mycotoxin at 0.5, 2.5 and 5 μg/g spiking levels, gave 78, 83 and 89% recoveries (mean 83%). The low stability of the derivative is reported to be a problem [40] but no significant decrease in response was found along seven consecutive injections (130 min) when the solution was stored in refrigerator between injections.

3.3. Analysis for toxins in Fusarium cultures

Table 2 lists the mycotoxins found in corn and rice cultures from five isolates of *Fusarium* previously obtained from banana fruits and selected by TLC as high mycotoxin-producers. The methodology described was applied to identify/confirm the mycotoxins studied here and to quantify them in the cultures. The listed levels are the mean of five replicates. The toxins found were DON, 15-AcDON, α-ZOL, ZON, T-2 TOL, NEO and FB_1. T-2 TOL and NEO were determined by GC; the remaining toxins were detected and determined by HPLC with GC confirmation (except for FB_1). Corn was always a better substrate than rice for mycotoxin production. *F. graminearum* yielded DON, 3-AcDON and ZON. *F. acuminatum* produced only T-2 TOL and NEO whereas ZON and α-ZOL were the only mycotoxins found in *F. equiseti* cultures. Finally, no trichothecenes, ZON or ZOL were detected in the cultures of *F. moniliforme* and *F. proliferatum* isolates. Instead, they showed very high levels of FB_1 (2180–2760 μg/g) so no concentration of methanol eluate was necessary for NBD-F derivatization. This is the first time that these methods of analysis are applied

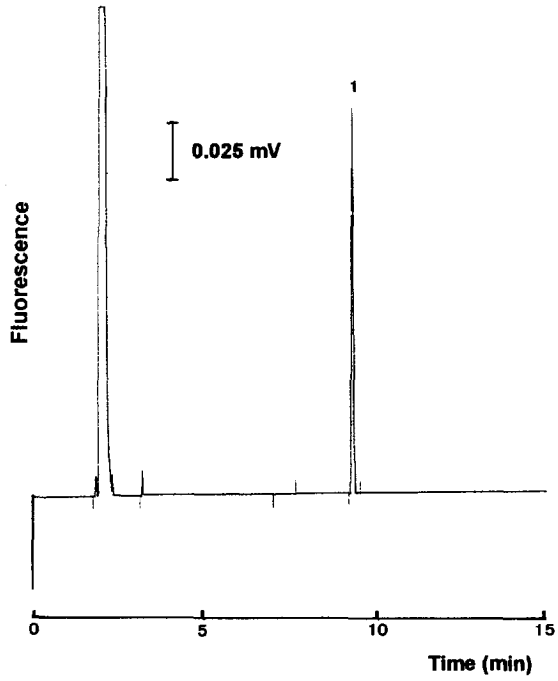

Fig. 5. Liquid chromatogram of standard FB_1 (1) as NBD-F derivative with fluorescence detection. Conditions: column, as in Fig. 1; mobile phases: (A) MeOH–NaH_2PO_4 buffer 0.05 *M*, pH 4.4 (50:50) and (B) CH_3CN–water (80:20); programme: 5 min with A; 0.5 min with gradient 100% A to 50% A +50% B; 10 min with 50% A +50% B; flow-rate: 1 ml/min; excitation wavelength: 460 nm, emission wavelength: 500 nm.

to corn and rice cultures of *Fusarium* spp. isolated from banana fruits. As these species can yield various mycotoxins at any one time, these methods for extraction/detection of multitoxins are considered very suitable for screening and establishing the potential toxigenicity of isolates.

4. Conclusion

One method for the multidetection of trichothecenes, ZON and ZOL by HPLC–UV and capillary GC–FID and another for the determination of FB_1 by HPLC with fluorescence detection were devised by adapting several other procedures. The former had good recoveries in general (69–94%) and acceptable limits of detection, low for HPLC determination of ZON and ZOL (2–3 ng/g) and somewhat higher for type B trichothecenes. The limits of detection for trichothecenes by GC were about 20 ng/g. T-2 and HT-2 were not resolved by our GC system and a more efficient one is needed. 3-AcDON and 15-AcDON were not resolved by HPLC, but they were by GC. The method for FB_1 provided acceptable recoveries (mean 83%) but the limit of detection was relatively high (0.13 μg/g). Both methods were suitable for analysis of the studied mycotoxins (except for T-2 and HT-2) in corn and rice cultures of *Fusarium* spp. isolated from banana fruits. Five species showed a high toxigenic potential which was always somewhat higher in corn cultures. *F. moniliforme* and *F. proliferatum* yielded very high FB_1 levels but did not produce the other toxins. The remaining species produced relatively high

Table 2
Determination of mycotoxins (μg/g dry matter) in corn and rice cultures of *Fusarium* species isolated from banana fruits[a]

Mycotoxins[b]	*F. graminearum*		*F. acuminatum*		*F. equiseti*		*F. moniliforme*		*F. proliferatum*	
	Corn	Rice	Corn	Rice	Corn	Rice	Corn	Rice	Corn	Rice
DON	30	24	–	–	–	–	–	–	–	–
3-AcDON	10	7	–	–	–	–	–	–	–	–
T2 TOL	–	–	15	12	–	–	–	–	–	–
NEO	–	–	210	181	–	–	–	–	–	–
ZON	520	488	–	–	45	40	–	–	–	–
a-ZOL	–	–	–	–	7	5	–	–	–	–
FB_1	–	–	–	–	–	–	2370	2180	2760	2630

[a] Concentrations (mean values of five replicates) were determined by HPLC except for type A trichothecenes (GC).
[b] DON, deoxynivalenol; 3-AcDON, 3-acetyldeoxynivalenol; T-2 TOL, T-2 tetraol; NEO, neosolaniol; ZON, zearalenone; α-ZOL, α-zearalenol; FB_1, fumonisin B_1.
Nivalenol, 15-acetyldeoxynivalenol, diacetoxyscirpenol, T-2 toxin, HT-2 toxin and β-zearalenol were not detected.
–: Not detected.

levels of DON, ZON and NEO and lower levels of 3-AcDON, T-2 TOL and α-ZOL, but no FB_1.

5. Mycotoxin abbreviations

3-AcDON	3-Acetyldeoxynivalenol
15-AcDON	15-Acetyldeoxynivalenol
DAS	Diacetoxyscirpenol
DON	Deoxynivalenol
FB_1	Fumonisin B_1
HT-2	HT-2 toxin
NEO	Neosolaniol
NIV	Nivalenol
T-2	T-2 toxin
T-2 TOL	T-2 tetraol toxin
α-ZOL	α-zearalenol
β-ZOL	β-zearalenol
ZON	Zearalenone

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