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DETECTION OF TRACE LEVELS OF TRICHOTHECENE MYCOTOXINS IN ENVIRONMENTAL RESIDUES AND FOODSTUFFS USING GAS CHROMATOGRAPHY WITH MASS SPECTROMETRIC OR ELECTRON-CAPTURE DETECTION

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SUMMARY

Methods are described for the simultaneous detection of a wide range of trichothecenes, including the most polar ones and some macrocyclics, using either gas chromatography–mass spectrometry with selected ion monitoring, or gas chromatography with electron-capture detection. Trichothecenes were extracted directly from the various matrices, or from Clin Elut columns, and cleaned up on Florisil Sep-Pak cartridges. Macrocyclics and neosolaniol were detected after hydrolysis to verrucarol and T-2 tetraol respectively. For optimum sensitivity (0.5–10 ng per sample) over the range, trichothecenes were detected, both before and after hydrolysis of ester groups, as their heptafluorobutyrate derivatives using a quadrupole mass spectrometer and negative ion chemical ionisation. The use of a magnetic sector instrument with electron-impact ionisation gave comparable sensitivity for most trichothecenes, but was less useful for the simultaneous detection of verrucarol in the presence of other trichothecenes. The methods were used to detect the presence of scirpentriol, nivalenol and 15-monoacetoxyscirpendiol in sorghum from Thailand. Trichothecenes in less complex matrices could be detected, after hydrolysis, using gas chromatography with electron-capture detection.

INTRODUCTION

In two previous papers we reported methods employing gas chromatography–mass spectrometry (GC–MS) for the detection of trichothecene mycotoxins in urine[1] and, in collaboration with Begley *et al.*[2], in blood. These methods were applicable to trichothecenes of widely differing polarities. In this present paper we report an extension of these methods to the analysis of various environmental samples and foodstuffs. The importance of being able to detect the more polar trichothecenes is demonstrated by the detection of three trichothecenes, scirpentriol, nivalenol (NIV) and 15-monoacetoxyscirpendiol (15-MAS) in sorghum samples from Thailand. Also reported is an addition to the screening procedure which allows esterified trichothecenes to be detected as their hydrolysis products, employing either

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GC–MS, or, in the case of less complex matrices, gas chromatography with electron-capture detection (GC–ECD).

The majority of GC–MS methods, reported for the detection of trichothecenes in grain samples, have been restricted to a fairly narrow range of compounds[3–10]. These methods, reviewed in our previous paper[1], have usually included deoxynivalenol (DON) and the less polar, more easily recovered trichothecenes such as T-2 toxin and diacetoxyscirpenol (DAS), but excluded their hydrolysis products, T-2 tetraol and scirpentriol. Alternatively, several methods[8–10] have been restricted to the two polar trichothecenes DON and/or NIV, which occur relatively frequently in North America and Japan respectively. Since toxins such as T-2 tetraol and scirpentriol may predominate over their esterified precursors under warm culture conditions, or after prolonged storage in the presence of moisture, it is desirable that any screening procedure should include them, and it may be that their occurrence in nature is more widespread than hitherto suspected. The screening procedures we have developed have been designed to detect a wide range of trichothecenes in a variety of matrices. Accurate quantitation has not been our goal.

Numerous GC methods have been reported for the detection of trichothecenes. In recent years sensitivity requirements have encouraged the use of heptafluorobutyrate (HFB) derivatives with GC–ECD[11–16], rather than trimethylsilyl (TMS) ethers with flame ionisation detection. The attraction of using GC–ECD is that it relieves the work load on more expensive GC–MS equipment, which may otherwise be monopolised by trace analysis for long periods of time. The major limitation to GC–ECD is that at the trace levels of interest to us, too many interfering components are present in biological matrices. This is particularly important when one is dealing with unknown and variable matrices such as environmental samples and residues. In attempting to develop a GC–ECD screening procedure employing HFB derivatives, we frequently experienced interfering components, probably lipids, around the retention times for T-2 and DAS. Scott *et al.*[3] found similar interferences at the retention times for DAS and HT-2 toxin in extracts of wheat. An alternative approach to recovering and detecting a wide range of trichothecenes, is to hydrolyse the ester functions to hydroxyls and recover only the hydrolysis products, which possess a narrower range of physicochemical properties. In conjunction with GC–ECD of HFB derivatives of trichothecenes this should also improve sensitivity in some cases, since sensitivity generally increases with the number of derivatisable hydroxy functions. During the course of this work Bata *et al.*[17] outlined a procedure, incorporating a hydrolysis step, for use in the analysis of food. However the use of TMS derivatives gave only mediocre sensitivity. As an adjunct to our GC–MS screening procedures we have developed a method wherein all trichothecenes, including the macrocyclic trichothecenes verrucarin A and roridin A, are hydrolysed to the few common hydrolysis products T-2 tetraol, scirpentriol, DON, NIV and verrucarol. Detection may then be performed using GC–ECD in the case of suitable matrices. Alternatively, for optimum sensitivity and more general applicability, GC–MS may be used to detect hydrolysis products.

EXPERIMENTAL

Materials

DON was purchased from Myco-Lab (Chesterfield, MO, U.S.A.). 15-Acetyl-DON (15-AcDON) was a gift from Agriculture Canada (Ottawa, Canada). Other trichothecenes were isolated and purified at the Chemical Defence Establishment. Fisons Distol grade solvents and HPLC grade water were used. Florisil Sep-Pak and C_{18} Sep-Pak cartridges were purchased from Waters Assoc. (Northwich, U.K.). Clin Elut columns (1005) were made by Analytichem International (Harbor City, CA, U.S.A.). Heptafluorobutyrylimidazole (HFBI) was purchased from Lancaster Synthesis (Morecambe, U.K.) and redistilled before use. Samples of grain, vegetation, pollen and honey were obtained from various locations in the U.K. and South East Asia.

Extraction and clean-up

GC–MS analysis of unhydrolysed toxins. A sample of sorghum (or other grain) (5 g) was ground with a pestle and mortar. An aliquot (0.2–0.5 g) was weighed into a 3-ml vial and extracted by tumbling for 1 h with 90% methanol (3 ml). After centrifuging, the extract was transferred to a 15-ml screw-capped vial, and the extraction procedure repeated on the residue using a further 2 ml of 90% methanol and tumbling for 15 min. The combined extracts were defatted by tumbling for 10 min with light petroleum (b.p. 60–80°C) (3 × 5 ml). The methanolic fraction was concentrated to dryness in a 50-ml round-bottomed flask using a rotary evaporator. The residue was transfered to a Florisil Sep-Pak cartridge (pre-rinsed with chloroform–methanol, 7:3) in dichloromethane (2 × 1 ml). The flask was washed with chloroform–methanol (7:3, 5 ml) and the washings eluted through the Florisil cartridge. The eluate was collected in a 15-ml centrifuge tube and concentrated to dryness under a stream of nitrogen.

Samples of pollen, leaves, rice, maize and mung beans were extracted and cleaned up similarly.

Honey (0.5 g) was dissolved in water (4 ml) and loaded onto a 5-ml Clin-Elut tube. The tube was then eluted with ethyl acetate–methanol, 95:5 (4 × 8 ml), the combined extracts were dried with sodium sulphate (2 g), and then concentrated to dryness in a 50-ml round-bottomed flask on a rotary evaporator. This residue was then cleaned up on a Florisil cartridge as described above.

GC–MS and GC–ECD analysis after hydrolysis. Pollen, honey or other environmental sample was initially extracted and cleaned up as described above, and the cleaned up residue dissolved in methanol (1 ml). Half was then transferred to a 1-ml vial, concentrated to dryness under nitrogen and stored in a freezer for future GC–MS analysis of unhydrolysed toxins, if required. To the remaining half was added 2% (w/v) sodium hydroxide in methanol (0.5 ml), and the solution allowed to stand overnight at ambient temperature. Excess base was neutralised by shaking with acidic Amberlite IR-120 ion-exchange resin (0.3 g). The resin was removed by filtration through glass wool and washed with methanol (3 × 2 ml). The combined filtrates were concentrated to dryness on a rotary evaporator, the residue taken up in water (2 ml), and transferred to a preconditioned reversed-phase C_{18} Sep-Pak cartridge[1]. The flask was rinsed sequentially with water (2 ml) and methanol–water (40:60, 2

ml), and each passed in turn through the cartridge. The aqueous eluate was discarded. The 40:60 eluate was concentrated to dryness on a rotary evaporator, the residue transferred in methanol to a 1-ml vial for derivatisation, and the methanol removed under a stream of nitrogen.

As a refinement to the GC–ECD method, 7-hydroxyDAS (7-OH DAS) was added to the sample before extraction. The product of hydrolysis of 7-OH DAS, 3,4,7,15-tetraol, could then be used as an internal standard for measuring relative retention times, and at the same time acted as a check of the recovery procedure (but not as an internal standard for quantitation).

Glassware blanks were taken as controls before the analysis of unknown samples.

Derivatisation

Residues were heated with HFBI (20 μl) in toluene–acetonitrile (95:5, 200 μl) for 2 h at 60°C. After cooling, the solution was washed by vortexing for 1 min with 5% sodium bicarbonate solution (500 μl), and then with water (2 × 500 μl). Aliquots (0.5 μl) were injected. Derivatised samples could be stored at −20°C for 24–48 h without deterioration. For GC–MS analysis, 7-OH DAS was added prior to derivatisation as internal standard as described previously[1].

GS–MS analysis

Two methods were employed, as used for the analysis of urine[1].

(i) A VG7070EQ mass spectrometer was coupled to an 11/250 data system, and equipped with a Dani 3800 gas chromatograph. The gas chromatograph was fitted with a BP1 25 m × 0.2 mm I.D. fused-silica column, film thickness 0.25 μm (SGE U.K.), leading directly into the ion source. GC–MS conditions, using electron-impact (EI) ionisation and selected ion monitoring, were the same as those reported previously for the detection of trichothecenes in urine[1]. T-2, HT-2, T-2 tetraol, DAS, MAS, scirpentriol, DON and NIV were detected monitoring the ions as described previously. In addition, fusarenon-X and 15-AcDON were detected monitoring the ions: fusarenon-X *m/z* 942 and 923 (retention time 9 min 36 s); 15-AcDON *m/z* 730 and 688 (retention time 10 min 18 s).

(ii) A Finnigan 4600 GC–MS system was fitted with a BP5 12 m × 0.22 mm I.D. fused-silica column, film thickness 0.25 μm (SGE U.K.). GC–MS conditions used for screening purposes, employing negative ion chemical ionisation (NICI) and selected ion monitoring, were as described previously[1], with the dial reading for the source temperature set at 60°C. Fragmentation is minimal at this temperature giving optimum sensitivity. Additional trichothecenes verrucarol, fusarenon-X, 15-AcDON and T-2 triol were detected monitoring the ions: verrucarol *m/z* 638 and 658 (retention time 8 min 33 s); fusarenon-X *m/z* 942 (retention time 9 min 10 s); 15-AcDON *m/z* 730 and 710 (retention time 10 min 20 s); and T-2 triol *m/z* 970 and 950 (retention time 12 min 20 s). For confirmation of structure, the source temperature was raised to 80°C inducing a small amount of fragmentation to allow additional ions to be monitored. Confirmation of the identification of scirpentriol, nivalenol and 15-MAS was made monitoring additional ions: scirpentriol *m/z* 851, 637, 375 and 347; NIV 1097, 1095, 863 and 670; 15-MAS 483 and 347.

GC–ECD analysis

A Pye 304 gas chromatograph was employed, equipped with a ^{63}Ni electron-capture detector and connected to a Perkin-Elmer Sigma 15 data station. The gas chromatograph was fitted with an SGE Unijector operated in split mode, and a 25 × 0.22 mm I.D. fused-silica BP1 column (SGE). Conditions were as follows: injector temperature 260°C; detector temperature 275°C; oven held at 165°C for 5 min, programmed from 165–185°C at 5°C min, held at 185°C for 5 min, programmed from 185 to 250°C at 4°C/min, and finally held at 250°C for 5 min. The carrier gas was hydrogen at 16 p.s.i.; the make-up gas was nitrogen at 30 ml/min; split-flow 10 ml/min; septum purge 3 ml/min. Trichothecenes were detected as the hydrolysis products T-2 tetraol (T-2, HT-2, T-2 triol, T-2 tetraol and neosolaniol), scirpentriol (DAS, 15-MAS, scirpentriol), DON (15-AcDON and DON), NIV (fusarenon-X and NIV), verrucarol (verrucarin A, roridin A and verrucarol), 3,4,7,15-tetraol (7-hydroxy DAS). (Other trichothecenes, not examined, would also be hydrolysed to these hydrolysis products). Retention times, absolute and relative to 3,4,7,15-tetraol are as shown in Table I.

RESULTS AND DISCUSSION

Clean up procedures

The clean up procedures using Florisil Sep-Pak cartridges gave good recoveries over the range of trichothecenes. Recoveries from sorghum spiked at 1 ppm (200 ng in 200 mg) are shown in Table II. Nivalenol was the worst case reflecting both the high polarity of this toxin and the variability in its derivatisation under the derivatising conditions employed[1]. Kamimura *et al.*[18] previously advocated the use of Florisil to clean up extracts of grain. Florisil rather than silica cartridges gave improved clean up for GC–ECD. It was necessary to use a highly polar solvent (chloroform–methanol, 7:3) to afford good recoveries of the most polar trichothecenes nivalenol and T-2 tetraol. Although accurate quantitation was not our main objective, reproducibility in sorghum samples was reasonably good (Table II). Recoveries from honey, after extraction from Clin Elut columns and clean up as above, were in the range 30–90%, but were not optimised or assessed for quantitative purposes.

Recoveries after additional hydrolysis and clean up on C_{18} Sep-Pak cartridges were lower (Table II), but were still acceptable over the range. The method was

TABLE I

RETENTION TIMES OF TRICHOTHECENE HYDROLYSIS PRODUCTS

Trichothecene	*Retention time* (min)*	*Relative retention time*
3,4,7,15-Tetraol	9.95	1
NIV	10.23	1.028
Scirpentriol	12.17	1.223
DON	12.76	1.282
Verrucarol	13.40	1.347
T-2 tetraol	13.71	1.378

* BP1 column.

TABLE II

RECOVERIES OF TRICHOTHECENES

Trichothecene	*Recovery (%)*	
	*Sorghum**	*Pollen***
T-2	83–100	60–85
HT-2	81–93	
Neosolaniol		67–69
DAS	93–100	65–75
15-MAS	60–100	
T-2 triol	74–91	
T-2 tetraol	74–91	
Scirpentriol	56–83	
NIV	41–55	50–54
DON	53–78	44–52
15-AcDON	89–100	
Verrucarol	62–80	
Verrucarin A		30–42

* Determined by GC–MS (NICI), three determinations.
** After hydrolysis, determined by GC–MS (NICI), two determinations.

primarily developed for the recovery of T-2 tetraol, scirpentriol, DON and NIV, and the lower recovery of verrucarol can be improved if required by increasing the proportion of methanol in eluting the C_{18} cartridge.

GC–MS analysis

Both methods gave sensitivity comparable to that reported in the analysis of urine samples. Detection limits based on signal-to-noise ratios greater than or equal to 3 for the screening ions, were in the range 0.5–10 ng per sample except where large amounts of chemical background was present, *e.g.* in some vegetation samples. The detection of T-2 tetraol and scirpentriol spiked into honey (500 mg) at levels of 50 ppb* (25 ng each), using the magnetic sector mass spectrometer and EI ionisation, is shown in Fig. 1. Good signal-to-noise ratios were observed. A disadvantage in using heptafluorobutyrate derivatives is a poor sensitivity for detecting neosolaniol, which appears to be unstable in the GC–MS system. Its presence can however be indicated with very good sensitivity (< 1 ng per sample) after hydrolysis to T-2 tetraol. Fig. 2 shows the detection of neosolaniol (as T-2 tetraol) spiked into honey at 50 ppb (25 ng), again using the magnetic sector instrument and EI ionisation. A complication was experienced in the analysis of verrucarol using the magnetic sector instrument. The retention time for verrucarol-$(HFB)_2$ is very close to that for T-2 tetraol-$(HFB)_4$; however the ions used to monitor verrucarol are more than 200 mass units below those used for T-2 tetraol, and the magnetic sector instrument cannot be calibrated with the large change in accelerating voltage required. The quadrupole instrument does not have this problem, and is therefore preferred when simultaneous detection of verrucarol and T-2 tetraol as their HFB derivatives is required. This is

* Throughout the article the American billion (10^9) is meant.

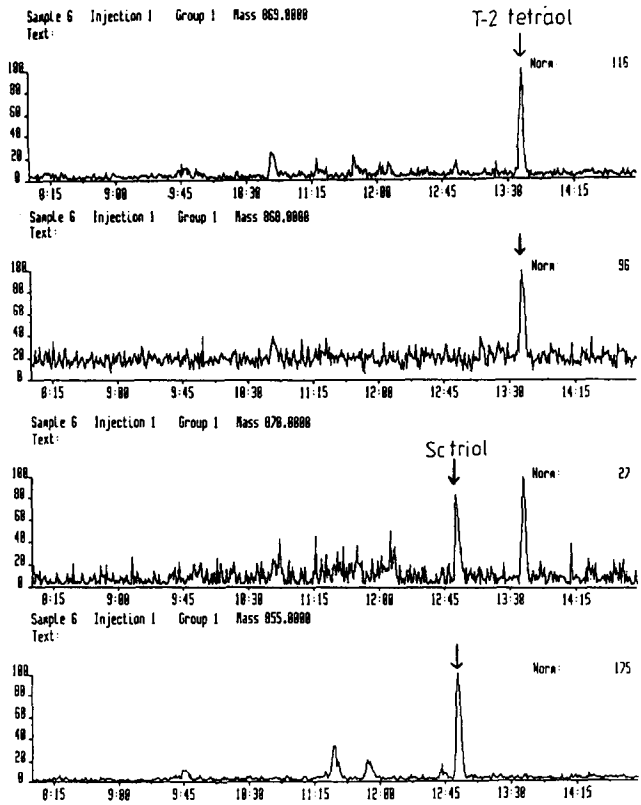

Fig. 1. Selected ion current profiles (EI ionisation) demonstrating the detection of T-2 tetraol and scirpentriol (25 ng each, 50 ppb) in spiked honey (500 mg).

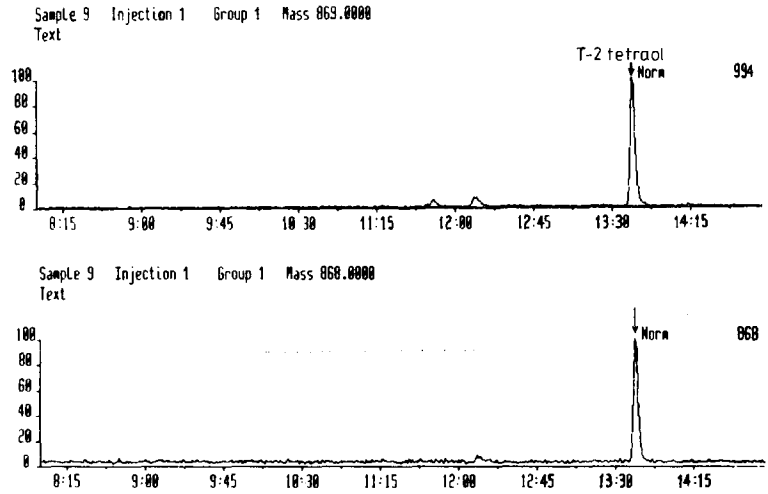

Fig. 2. Selected ion current profiles (EI ionisation) demonstrating the detection of neosolaniol (25 ng, 50 ppb), after hydrolysis to T-2 tetraol, in spiked honey (500 mg).

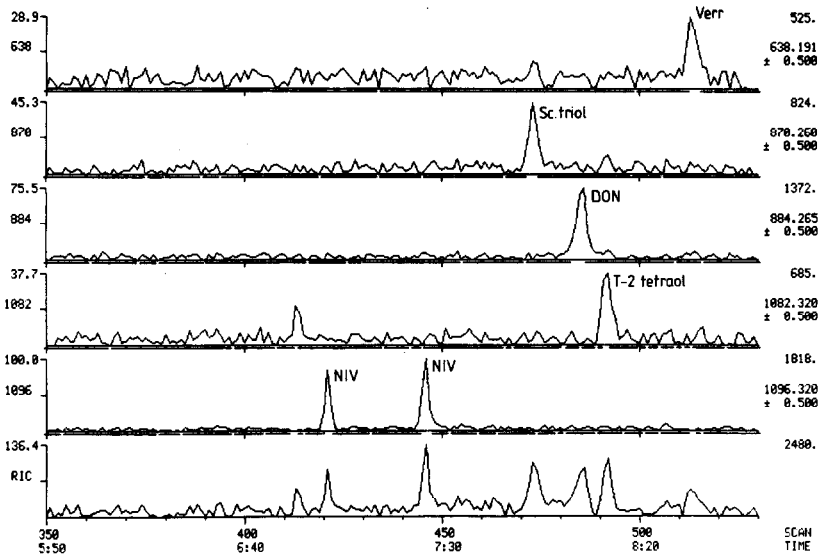

Fig. 3. Selected ion current profiles (NICI) demonstrating the detection of verrucarin A (as verrucarol), DAS (as scirpentriol), DON, neosolaniol (as T-2 tetraol) and NIV (5 ng each, 0.5 ppm) in spiked pollen (500 mg).

demonstrated in Fig. 3 which shows the simultaneous detection, after hydrolysis, of verrucarin A, neosolaniol, DAS, DON and NIV (5 ng of each), in a 10-mg aliquot of pollen spiked at 0.5 ppm.

The application of the methods to real life samples is demonstrated in Figs. 4–9. The detection of scirpentriol in a sample of sorghum, using the magnetic sector instrument and EI ionisation, is shown in Fig. 4. The sample was collected from the Experimental Agricultural station of Kasetsart University in Thailand, and was known to be old and mouldy. Scirpentriol was detected and confirmed by monitoring six ions, which gave signal-to-noise ratios greater than 3 and which were shown to be in ratios similar to those of a standard. Scirpentriol was quantitated as 20 ppb (4 ng) in the aliquot (200 mg) analysed, making no allowance for recovery. Positive

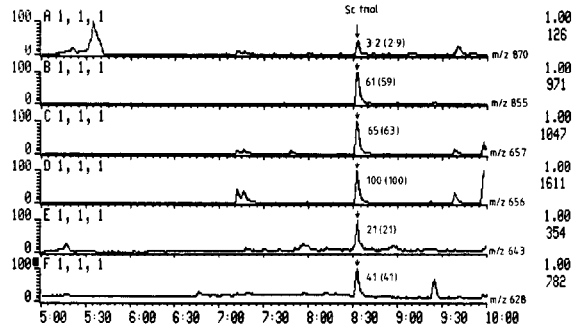

Fig. 4. Selected ion current profiles (EI ionisation) confirming the detection of scirpentriol in naturally contaminated sorghum. Ion ratios are shown, with those of a standard in parentheses.

responses were also obtained for the screening ions at the retention times for NIV and 15-MAS. However low signal-to-noise ratios, due to interference from chemical background, prevented confirmation by monitoring additional fragment ions. A glassware blank taken through the analytical procedure and analysed immediately before the sample, which we regard as mandatory for trace analysis of this nature, was negative. A different aliquot of the sample was analysed using the quadrupole instrument and NICI. The reconstructed ion current profile, indicating the presence of scirpentriol, NIV and a smaller amount of 15-MAS, is shown in Fig. 5. Scirpentriol and nivalenol were confirmed monitoring six ions. Fig. 6 shows selected ion current profiles confirming the identification of NIV. As discussed previously for the analysis of urine, either of the two derivatives formed by nivalenol can be used for confirmation; in the case of sorghum extracts the derivative with the shorter retention time is the more useful. It was quantitated as *ca.* 70 ppb (35 ng) in the 500 mg aliquot analysed, making no allowance for recovery. 15-MAS was confirmed monitoring three ions (Fig. 7) and was quantitated as 19 ppb (9.5 ng). Scirpentriol was quantitated as 41 ppb. Trace amounts of a compound with the same retention time as DON were also detected at a level equivalent to 2 ppb (Fig. 8). However the identification could not be confirmed, because although ion ratios were similar to those in a standard, the signal-to-noise ratios for three of the ions were less than the minimum ratio required of 3:1. Likewise suspected traces of DAS (*ca.* 2 ppb) could not be confirmed. Levels of scirpentriol in three separate aliquots of the sample varied between 15 and 41 ppb, NIV 10–70 ppb and 15-MAS 11–33 ppb, making no allowance for recovery. This variation reflects inhomogeneity in the small sample aliquots analysed. (For accurate quantitation of grain samples a rigorous sampling procedure is required.) A second sample of sorghum, taken from the same location as the first but less visibly mouldy, was also found to contain scirpentriol at levels determined in separate ali-

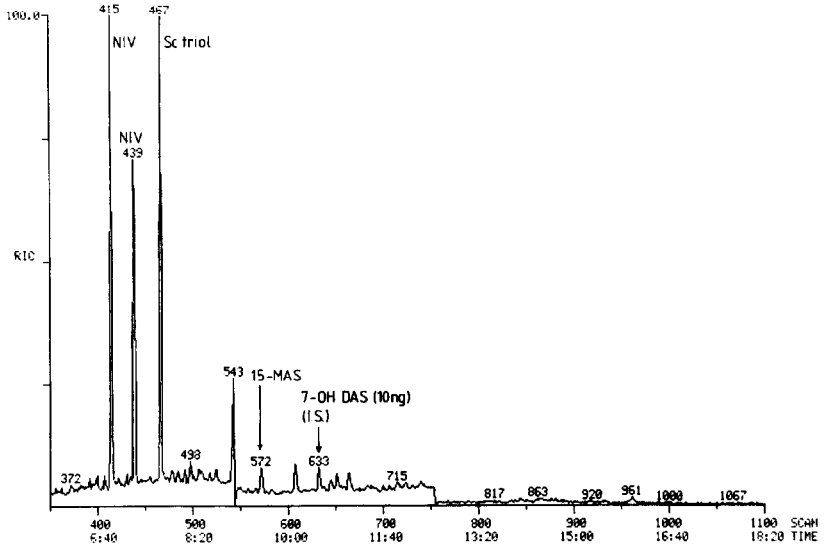

Fig. 5. Reconstructed ion current (NICI) showing the detection of NIV (as both HFB derivatives), scirpentriol and 15-MAS in naturally contaminated sorghum.

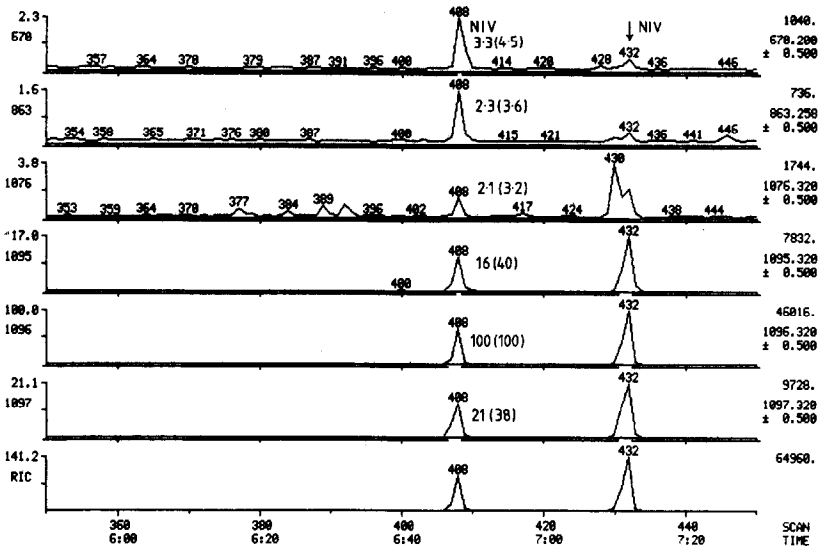

Fig. 6. Selected ion current profiles and reconstructed ion current, (NICI) confirming the detection of NIV in naturally contaminated sorghum. Ion ratios are shown, with those of a standard in parentheses.

quots as 3–27 ppb. Confirmation of the presence of scirpentriol in this sample, monitoring six ions and using NICI is shown in Fig. 9. Unconfirmable traces of NIV and 15-MAS were also detected. The appropriate glassware blanks were negative.

GC–ECD analysis

The use of GC–ECD for analysis at trace levels is much more limited with

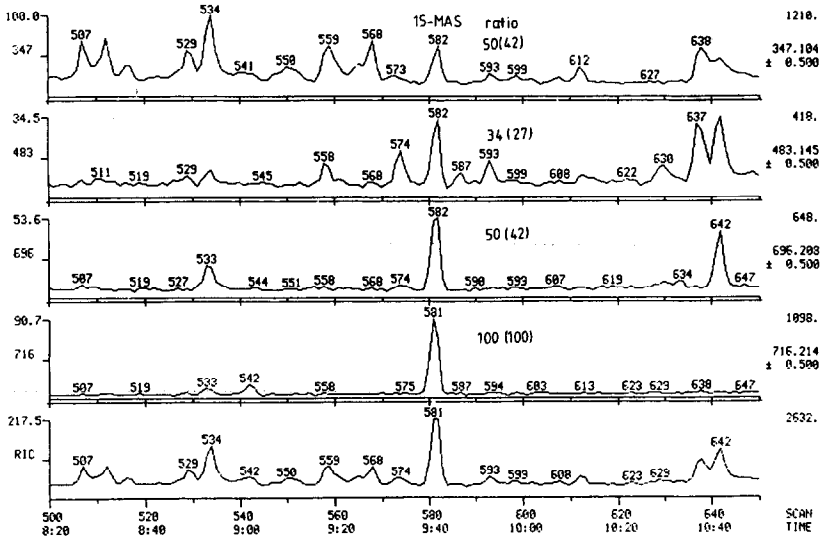

Fig. 7. Selected ion current profiles and reconstructed ion current (NICI) confirming the detection of 15-MAS in naturally contaminated sorghum. Ion ratios are shown, with those of a standard in parentheses.

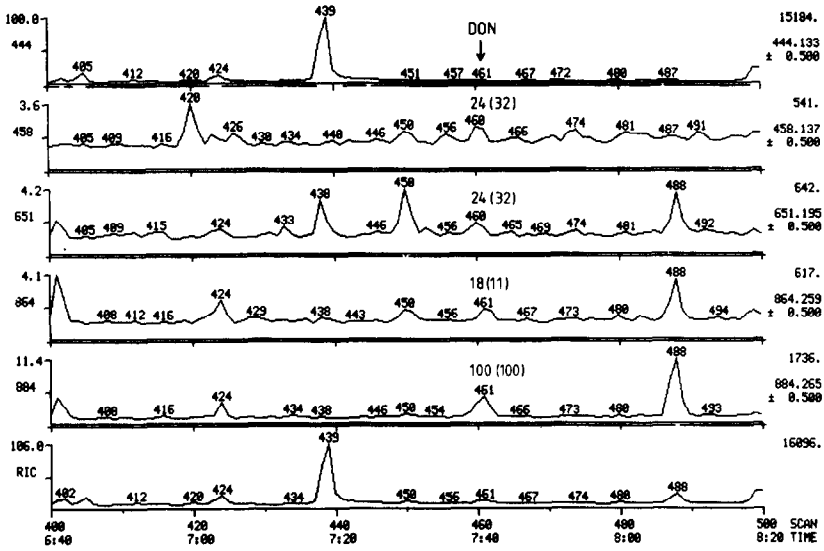

Fig. 8. Selected ion current profiles and reconstructed ion current (NICI) indicating possible, but unconfirmable, trace levels of DON in naturally contaminated sorghum. Ion ratios are shown, with those of a standard in parentheses.

regard to the sample matrix. In our hands, for detection of levels less than *ca.* 50 ng per sample, its use has been restricted to the analysis of certain powder samples and hard surfaces, which we investigated for trichothecene contamination. The use of 7-OH DAS (detected as 3,4,7,15-tetraol) as internal standard gave relative retention

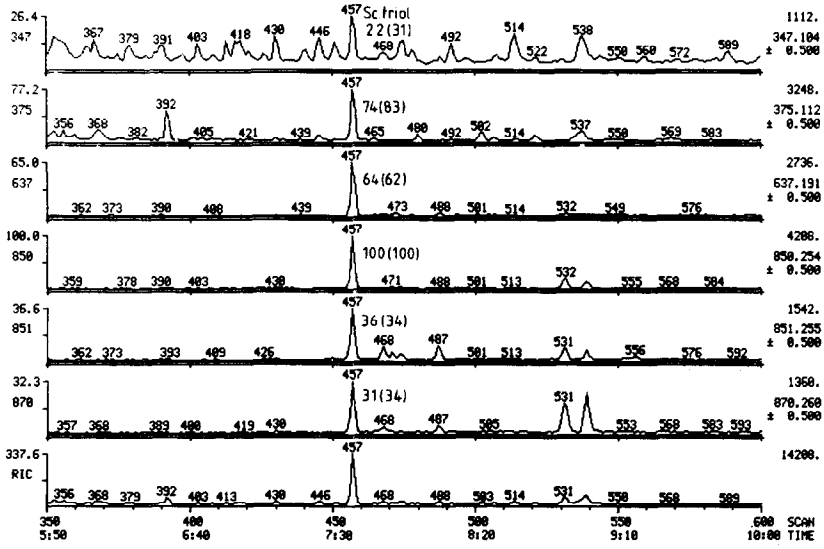

Fig. 9. Selected ion current profiles and reconstructed ion current (NICI) confirming the detection of scirpentriol in naturally contaminated sorghum. Ion ratios are shown, with those of a standard in parentheses.

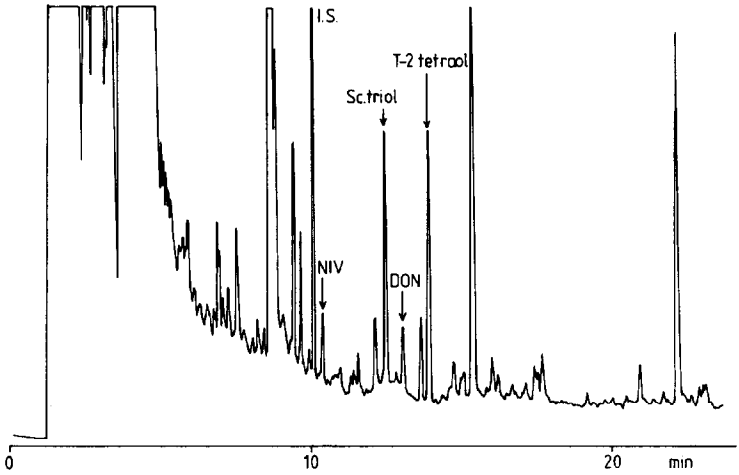

Fig. 10. GC–ECD chromatogram demonstrating the detection of T-2 (as T-2 tetraol), DAS (as scirpentriol), DON and NIV (50 ng each, 5 ppm) in spiked pollen. 7-OH DAS (detected as 3,4,7,15-tetraol) was added as internal standard.

times reproducible within 1%. It also served as a check of the clean up procedure. At present, 7-OH DAS and its hydrolysis product are not known to occur naturally in the environment. Fig. 10 shows the detection of 5 ppm (50 ng of each) of T-2, DAS (as their hydrolysis products), DON and NIV in spiked pollen (10 mg). Fig. 11

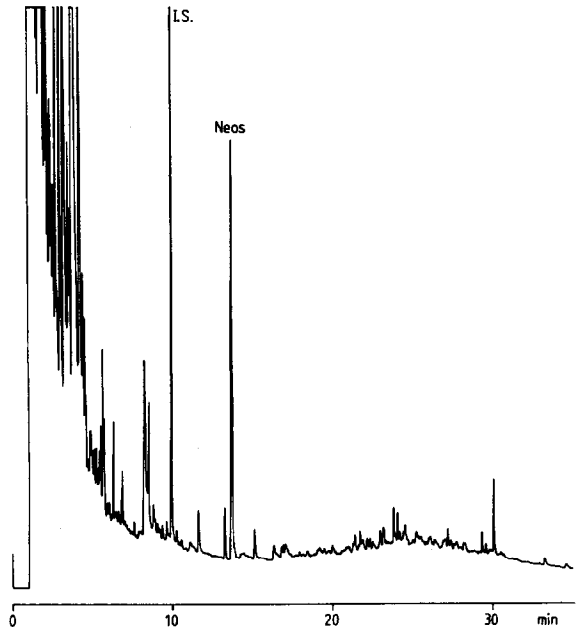

Fig. 11. GC–ECD chromatogram demonstrating the detection of neosolaniol (100 ng, 10 ppm), after hydrolysis to T-2 tetraol, in spiked pollen (10 mg).

shows the detection of neosolaniol (10 ppm, 100 ng) in a sample of spiked pollen. Limits of detection in pollen samples, based on minimum signal-to-noise ratios of 3:1, were *ca.* 5 ng per 10 mg sample (5 ppm) for T-2, DAS and neosolaniol, and *ca.* 20 ng per sample for NIV, DON and verrucarin A. Detection limits for the other trichothecenes fell between these limits. Because of the increased chances of interferences, GC–ECD in trace analysis of this nature should be limited to use as a screening procedure. A positive analysis must be confirmed by the more specific molecular characteristics monitored by GC–MS.

CONCLUSIONS

The screening procedures described herein allow a wide range of trichothecenes to be detected in a variety of matrices, down to levels of 0.5–10 ng per sample, but depending of course on the sample size and chemical background present. Detection limits with confirmation of structure, monitoring at least three ions, depend on the level of chemical background present in a particular matrix. In sorghum, levels down to 10 ppb could be confirmed. Optimum sensitivity over the range was obtained using a quadrupole mass spectrometer and NICI. When used to analyse samples both before and after hydrolysis, this method allowed a very wide range of trichothecenes to be detected, including some macrocyclics which are less amenable to sensitive GC–MS analysis. Specific structural information on the original toxin is of course lost on hydrolysis, but in our procedure half the original extract is saved for analysis for non-hydrolysed toxins. The use of a magnetic sector instrument and EI ionisation gave comparable sensitivity for most toxins, though chemical background was in some instances higher than when using NICI, *e.g.* the detection of 15-MAS in sorghum. The simultaneous detection of verrucarol and T-2 tetraol as their HFB derivatives is best performed on a quadrupole instrument. GC–ECD can be used as a screening procedure for less complex environmental samples but is less sensitive than GC–MS, particularly for DON, NIV and verrucarin A. The procedures described are not intended to be rigorously quantitative, and would need further refinement if such was desired.

The detection of naturally occurring scirpentriol, NIV and 15-MAS illustrates the desirability of screening for a wide range of trichothecenes in environmental and other samples. It is, to our knowledge, the first unequivocal demonstration of the natural occurrence of trichothecenes in food crops from this region of South East Asia, and the first example of the natural occurrence of scirpentriol and 15-MAS. Greenhalgh *et al.*[19] have previously demonstrated low level trichothecene production in the laboratory by a strain of *F. equisetti,* isolated from a leaf sample collected in Thailand. Curiously, the toxins produced by this strain, DAS, 15-MAS and fusarenon X, are closely related to those we detected in sorghum from Thailand. The sorghum samples, though deliberately collected because of their mouldy appearance, were two of only five grain samples (sorghum, maize, rice and mung beans) analysed for trichothecenes. It may therefore be that low-level contamination of grain with trichothecenes is widespread in South East Asia, and that under the conditions prevailing, the hydrolysed toxins such as scirpentriol predominate.

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