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Use of M-series retention index standards in the identification of trichothecenes by electron impact mass spectrometry

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ABSTRACT

A method for the reliable identification of a series of trichothecenes as their trifluoroacetate esters in porridge flake samples is described. The esters were separated by gas chromatography and identified from their retention times relative to *n*-alkylbis(trifluoromethyl)phosphine sulphide (M-series) retention index standards and their electron impact mass spectra. The relative retention times offer an independent identification method by which the reliability of the identification can be improved. The mass spectra and the relative retention times were obtained from the same gas chromatographic–mass spectrometric run. Detection limits were of the order to 0.005–0.05 mg/kg. All the flake samples including oats (ten of fourteen samples studied) contained deoxynivalenol (0.01–0.2 mg/kg) and one oat flake sample contained HT-2 toxin (0.008 mg/kg). The other trichothecenes monitored were not found.

INTRODUCTION

Trichothecenes are a significant contamination problem in foods and feeds[1–5]. They are naturally produced by a variety of fungi, which can be formed rapidly in grain and feed during harvest, transport and storage. Because of the extreme toxicity[3,6] and natural occurrence of trichothecenes, several identification methods have been developed. Gas chromatography (GC) is often used[7–11], but the specificity is limited[8,11]. Tandem mass spectrometry (MS–MS) seems to be the most specific and sensitive method for trichothecenes in complex matrices[12–20], but needs expensive and sophisticated instrumentation. GC–MS is a very common method in the routine analysis of trichothecenes[21–23], but the specificity with complex matrices may be insufficient, leading to reduced reliability of the identification. The use of retention indices in GC–MS offers, in addition to MS data, an independent identification method by which the reliability of the identification can be improved. The detection result is accepted only if the relative abundances of the monitored ions in the mass

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spectrum do not vary by more than an allowed amount and if the monitored compounds are found inside a certain allowed retention index "window".

Retention indices have been widely used in GC[24–27] but very seldom in GC–MS[28,29]. This paper describes the use of *n*-alkylbis(trifluoromethyl)phosphine sulphides (M-series) as retention index standards in the identification of some trichothecenes (Table I) in porridge flakes by GC–electron impact (EI) MS. The M-series standards were prepared for use as universal retention index standards detectable with all common detectors used in GC[24]. However, the standards are also well suited to MS.

EXPERIMENTAL

Chemicals

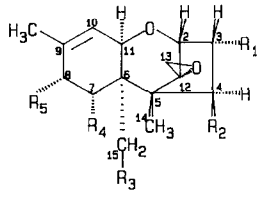
All the trichothecenes (Table I) and trifluoroacetic anhydride (TFAA) were obtained from Sigma. 4-Dimethylaminopyridine (4-DMAP) was obtained from Aldrich. The retention index standard solution ($2.5 \cdot 10^{-4}$ *M*) including even-number standards M_6–M_{20} (Table I) was obtained from HNU-Nordion.

Sample clean-up

About 100 g of porridge flakes were mixed, an aliquot (10 g) of flake was

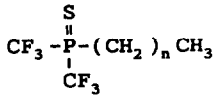
TABLE I

THE TRICHOTHECENES STUDIED



Trichothecene	R_1	R_2	R_3	R_4	R_5
T-2 toxin (T-2)	OH	OAc[a]	OAc	H	$OCOCH_2CH(CH_3)_2$
Iso-T-2 toxin (Iso-T-2)	OAc	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
HT-2 toxin (HT-2)	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
Triacetoxyscirpenol (TAS)	OAc	OAc	OAc	H	H
Diacetoxyscirpenol (DAS)	OH	OAc	OAc	H	H
Monoacetoxyscirpenol (MAS)	OH	OH	OAc	H	H
Deoxynivalenol (DON)	OH	H	OH	OH	=O

Compounds in M-series:



n = 5,7,9,11,13,15,17,19

[a] OAc = CH_3COO.

removed and 0.01 mg of Iso-T-2 was added as an internal standard. Methanol–water (95:5) (170 ml) was added and the sample was ground in a homogenizer for about 5 min. Otherwise the sample clean-up was performed according to the method described previously[17].

Derivatization

After clean-up the sample was evaporated to dryness with nitrogen and to the residue were added 100 μl of toluene–acetonitrile (9:1) containing 4-DMAP (2 mg/ml). Sodium hydrogencarbonate (10 mg) and 50 μl of TFAA were added and derivatization was carried out at 333 K for 20 min. After cooling, the reaction mixture was evaporated to dryness with nitrogen and 200 μl of toluene and 0.5 ml of water were added to the residue. The sample was mixed for 4 min and allowed to stand until the layers had separated. The toluene phase was transferred into a 2.5-ml vial containing anhydrous sodium sulphate. The toluene extraction was repeated twice and the final volume in the vial was adjusted to 1 ml with toluene.

Gas chromatography–mass spectrometry

All the mass spectra were obtained with an HP 5970 quadrupole mass spectrometer interfaced by direct coupling with an HP 5890 gas chromatograph. The mass spectrometer was operated in the electron impact mode (70 eV). The ion source temperature was 493 K. A 2-μl volume of the sample together with 1 μl of M-series retention index standard solution ($2.5 \cdot 10^{-4}$ *M*) were injected into the gas chromatograph using splitless (1 min) injection. The injector temperature was 533 K and the carrier gas (helium) flow-rate was about 1.5 ml/min. An HP-5 (5% phenylmethylsilicone) capillary column (25 m × 0.2 mm I.D.; 0.33 μm film thickness) was used. The interface temperature was 543 K. The temperature programme was 333 K (held for 1 min) to 473 K at 20 K/min and from 473 to 553 K at 10 K/min, the final temperature being held for 10 min.

The mass spectra were recorded by using the scan range 50–700 u. The linearity studies, determination of detection limits and the analysis of porridge flake samples were done by using multi-grouping and selected ion monitoring (Table II), in which only the most intense and characteristic ions were monitored.

TABLE II

RUN PROGRAMME FOR THE TRICHOTHECENES AND M-SERIES COMPOUNDS (*m/z* 147)

Compound	*Monitored ions*[a] *(m/z)*	*Start time (min)*
$DON(TFA)_3$	584*,259,231,147	10.00
$MAS(TFA)_2$	456*,343,329,147	13.00
DAS(TFA)	402*,359,329,147	15.00
$HT\text{-}2(TFA)_2$	472,455*454,147	16.25
TAS	348*,320,275,147	17.50
T-2(TFA)	401*,329,327,147	19.00
Iso-T-2(TFA)	460*,400,357,147	19.85

[a] Asterisks indicate ions used in quantification.

TABLE III

SELECTED ION MASS SPECTRA OF STUDIED TRICHOTHECENES

Compound	*MW*	*m/z (relative abundance, %)*
$DON(TFA)_3$	584	231(100, 259(87), 584(80)
$MAS(TFA)_2$	516	329(29), 343(21), 456(100)
DAS(TFA)	462	329(63), 359(20), 402(100)
$HT\text{-}2(TFA)_2$	616	454(50), 455(100), 472(39)
TAS	426	275(48), 320(63), 348(100)
T-2(TFA)	562	327(100), 329(52), 401(91)
Iso-T-2(TFA)	562	357(100), 400(63), 460(71)

The TFA derivatives of five barley samples containing increasing amounts (0.01, 0.03, 0.06, 0.09 and 0.2 mg/kg) of trichothecenes DON, MAS, DAS, HT-2, TAS and T-2 (Table I) and 1 mg/kg of Iso-T-2 as an internal standard were prepared for linearity studies. Each sample was analysed twice. The peak areas of the ion currents were used in the linear regression. The calibration graphs were evaluated as the ratio of the ion of *m/z* 460 of Iso-T-2 (TFA) to that of the most characteristic and if possible most abundant ion of the TFA ester (asterisks in Table II indicate the ions used in quantification). The detection limits were evaluated at a signal-to-noise ratio of 5:1.

RESULTS AND DISCUSSION

The TFA esters of the trichothecenes were identified on the basis of the selected ion mass spectra and relative retention times. Selected ion monitoring was used to increase the sensitivity. The ions of the TFA esters monitored were chosen so that the chemical noise from the matrix would be at a minimum and the maximum signal-to-noise ratio would be achieved. Ions with high mass values often meet these requirements. The selected ion mass spectra are presented in Table III. The mass spectra of the TFA esters and their partial fragmentation pathways are presented elsewhere[23].

Selective ion monitoring together with retention time information is in most instances sufficient for a reliable identification. However, the retention times are very dependent on the GC conditions, *e.g.*, on the condition of the column, the stability of the temperature programme and the carrier gas flow-rate. More reliable results can be obtained by using relative retention times, which are much less dependent on the conditions than the actual retention times. The retention index series used in this study (M-series) (Table I) was prepared mainly for GC detectors[24], but is also well suited to GC–EI-MS analysis. All compounds in the M-series produced two characteristic and common ions, *viz.*, *m/z* 147 ($C_2H_3SPF_3$) and *m/z* 229 ($C_4H_4SPF_6$), which can be selected for monitoring together with characteristic ions of the analyte. The elemental compositions of the ions of *m/z* 147 and 229 were confirmed by high-resolution mass spectrometry (resolution, $R = 10\ 000$). Fig. 1 presents an example of the identification of the trichothecenes as their TFA esters in a standard solution and in an oat flake sample.

The relative retention times (*RRT*) of the TFA esters were calculated with the equation

$$RRT = 100M_n + 100(M_{n+i} - M_n) \cdot \frac{T_x - T_n}{T_{n+i} - T_n} \quad (1)$$

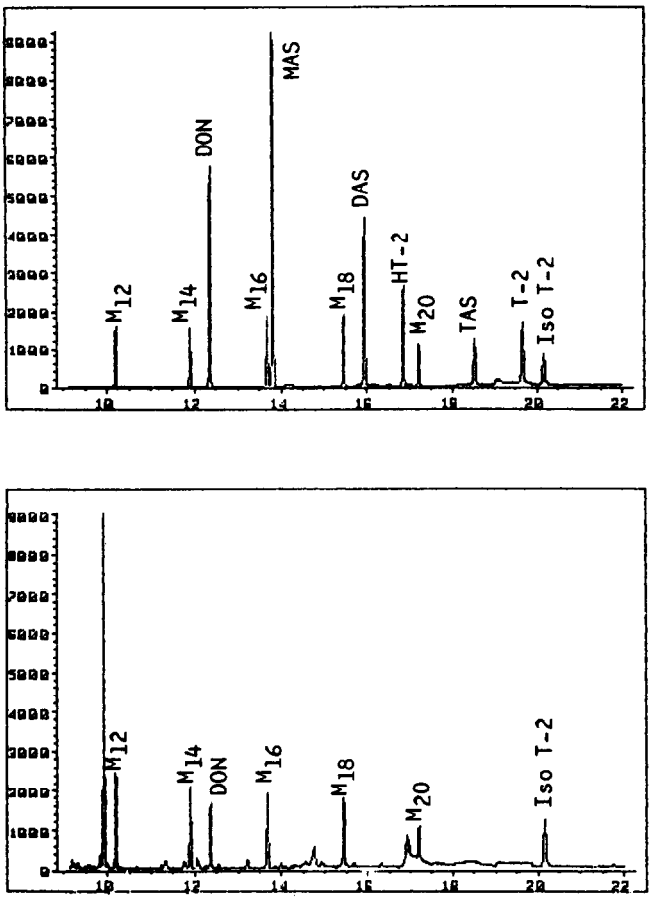


Fig. 1. Selected ion current chromatograms recorded (a) from a TFA-derivatized standard sample and (b) from a TFA-derivatized oat flake sample. x-Axis and y-axis represent retention time (in minutes) and intensity, respectively. The monitored ions are presented in Table II.

where M_n and M_{n+i} are carbon numbers in the alkyl chain of the compounds eluted one on each side of the monitored trichothecene, T_x is the retention time of the monitored trichothecene and T_n and T_{n+i} are the retention times of the compounds of M_n and M_{n+i}. The relative retention times for the compounds eluted after the last M-series compound (M_{20}) (TAS, T-2 and Iso-T-2) are obtained by using calculated values for M_{22} and M_{24} [$T_{22} = T_{20} + (T_{20} - T_{18})$; $T_{24} = T_{20} + 2(T_{20} - T_{18})$].

The relative retention times of the TFA esters were measured using pure compounds and are presented together with standard deviations ($n = 6$) in Table IV. The relative retention times are more accurate for compounds eluted between the standards than for those eluted after the last standard, M_{20}. Standard M_{24} is not commercially available. However, accurate enough results are obtained for TAS, T-2 and Iso-T-2 with the calculated retention times of M_{22} and M_{24}. The relative retention times were measured six times and the measurements were made every second week, but no systematic changes in the results were observed.

TABLE IV

RELATIVE RETENTION TIMES (RRT), DETECTION LIMITS AND LINEAR REGRESSION OF THE QUANTIFICATION

The concentrations of the studied trichothecenes in the spiked barley samples were 0.01, 0.03, 0.06, 0.09 and 0.2 mg/kg; Iso-T-2 was used as an internal standard (1 mg/kg).

Compound	*RRT* (±*S.D.*) (*n* = 6)	r^a	*Slope*	*Intercept* (*mg/kg*)	*Detection limit* (*pg*)
$DON(TFA)_3$	1451.89 ± 0.12	0.9926	2.0342	0.00377	10
$MAS(TFA)_2$	1614.61 ± 0.18	0.9963	0.5102	−0.00009	5
DAS(TFA)	1855.94 ± 0.27	0.9991	0.8357	0.00383	15
$HT\text{-}2(TFA)_2$	1958.03 ± 0.16	0.9985	1.5168	0.00502	10
TAS	2152.99 ± 0.86	0.9997	3.0450	0.00213	40
T-2(TFA)	2283.67 ± 1.26	0.9991	2.0282	0.00438	30
Iso-T-2(TFA)	2341.25 ± 1.63				

[a] Correlation coefficient

The identification of a trichothecene was accepted if the relative abundance of the monitored ion of the TFA ester did not vary more than ±10% and the relative retention time by more than ±2 units. If the relative retention time of the analyte peak was within the acceptable values but the relative abundance of the monitored ion showed intergerence by the matrix, the ion was changed to one not subject to interference and the run was repeated. In all instances where the relative retention times varied by more than 2 units the relative abundances of the monitored ions also varied by more than ±10%.

The studied trichothecenes were quantified by using Iso-T-2, which does not occur naturally, as an internal standard. The correlation coefficients showed good linearity between concentration levels of 0.02 and 0.2 mg/mg. The detection limits were between 0.005 and 0.05 mg/kg with the porridge flake samples and between 5 and 40 pg with standards (Table IV).

Fourteen porridge flake samples were analysed by the method described above. Eight of the samples were prepared from oats, three from a mixture of four corns (oats, rye, barley and wheat), two from rye, one from barley and one from wheat. DON was found at levels 0.01 and 0.2 mg/kg in all of the samples containing oats. One rye sample contained 0.005 mg/kg of DON and one oat sample contained 0.008 mg/kg of HT-2.

CONCLUSIONS

The use of relative retention times in GC–MS offers, in addition to the MS data, an independent identification method, by which the reliability of the identification can be improved. The MS and relative retention time data are obtained from the same GC run. In an analytical problem in which unknown peaks detected by GC detectors are to be identified by GC–MS, it might be difficult to find the unknown peak by GC–MS owing to the response differences. In this event the use of relative retention times helps to establish the peak of interest.

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