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Identification of trichothecenes by frit-fast atom bombardment liquid chromatography–high-resolution mass spectrometry

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ABSTRACT

Frit-fast atom bombardment liquid chromatography–mass spectrometry (LC–frit-FAB-MS) provides a specific and reliable method for the identification of trichothecenes without derivatization. The frit-FAB spectra exhibit an abundant glycerol adduct ion, a protonated molecule and fragment ions formed by the losses of functional groups in various combinations. The use of high-resolution mass spectrometry in LC–frit-FAB-MS provides improved selectivity in the monitoring and makes the determination of the elemental compositions of the ions possible. The measured mass values with resolution 8000 were typically within 5 mmu of the calculated values. Sensitivity in the range of hundreds of picograms per microlitre was achieved with resolution 8000 using selected ion monitoring in the detection of trichothecenes.

INTRODUCTION

Trichothecenes are serious 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 their extreme toxicity [3,6] and natural occurrence, several identification methods have been developed. Trichothecenes are often detected as their derivatives by gas chromatography (GC) [7–9] and GC–mass spectrometry (MS) [5,9–12]. Trichothecenes have been identified without derivatization by desorption chemical ionization tandem mass spectrometry (DCI-MS–MS) in relative pure samples [13–18]. In spite of the high specificity of MS–MS, the separation power of the DCI probe is very poor and the total specificity of DCI-MS–MS may not suffice in the determination of trichothecenes in complex matrices.

The recently introduced thermospray (TSP) LC–MS [19] has been used in the identification of trichothecenes without derivatization [20–23]. Trichothecenes form a very abundant ammonium adduct ion with minimum fragmentation under TSP

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conditions. LC–TSP-MS provides good sensitivity by monitoring the ammonium adduct ion of trichothecenes, but the lack of fragmentation leads to a reduced reliability of the identification and makes the structure characterization very difficult. The fragmentation can be improved by using MS–MS [23], but accurate mass measurements are not possible for daughter ions using triple quadrupole mass spectrometers, which have been used in most applications of tandem MS.

The recently introduced dynamic fast atom bombardment (FAB) LC–MS often produces spectra with molecular weight information and structure-characteristic fragmentation [24–27]. The method with low-resolution MS has been applied in the analysis of many organic compounds, such as bile acids [25,26], peptides [27,28], medicines [29], amino acids [30], antibiotics [31] and oligosaccharides [32], but only a few studies have been made using the high-resolution mode [33,34]. This paper describes the use of the high-resolution mode in the identification of some trichothecenes by LC–MS using the frit-FAB interface described in detail previously [25,26].

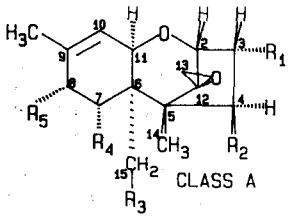
EXPERIMENTAL

The measurements were made with a Jeol JMS-SX102 high-resolution mass spectrometer with a JMA-DA6000 data system connected to an LC system by a Jeol frit-FAB interface. The ionization chamber temperature was 50°C, the xenon particle energy 4 keV, the emission current 10 mA, the accelerating voltage 8 kV and the magnetic field scanning range m/z 70–700 (3 s per scan). In order to increase the evacuation speed of the ion source, a liquid nitrogen trap was provided.

The eluent delivery was provided by two high-pressure pumps (Hewlett-Packard 1090 LC) coupled with an automated gradient controller. The pump used in the post-column addition of glycerol was a Jasco Familio-300 S. Samples were introduced into the LC system using an autoinjector with a 20-μl loop. The column used was ODS

TABLE I

THE TRICHOTHECENES STUDIED



No.	Compound	R_1	R_2	R_3	R_4	R_5
1	T-2 toxin (T-2)	OH	OAc[a]	OAc	H	$OCOCH_2CH(CH_3)_2$
2	HT-2 toxin (HT-2)	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
3	Triacetoxyscirpenol (TAS)	OAc	OAc	OAc	H	H
4	Diacetoxyscirpenol (DAS)	OH	OAc	OAc	H	H
5	Monoacetoxyscirpenol (MAS)	OH	OH	OAc	H	H
6	Deoxynivalenol (DON)	OH	H	OH	OH	=O

[a] OAc = Acetyl.

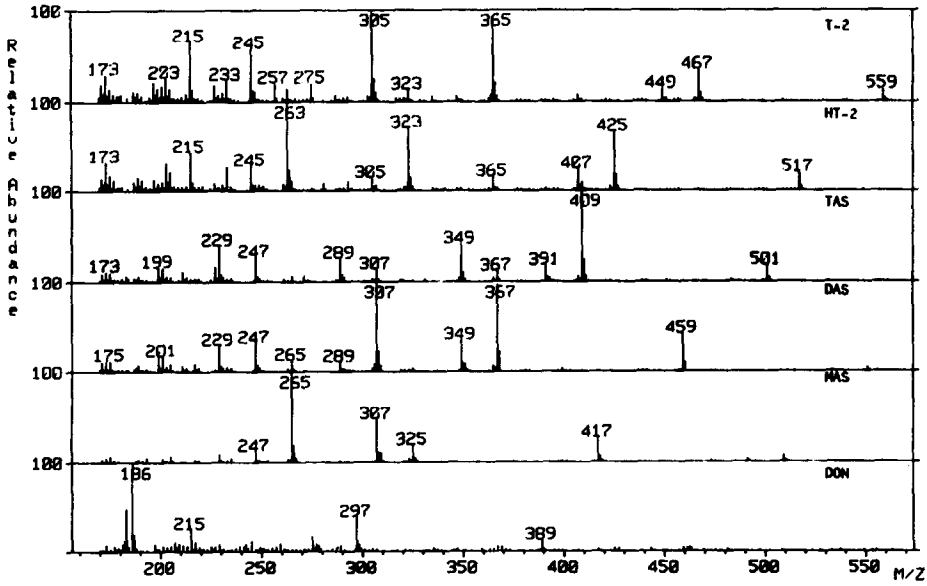


Fig. 1. Frit-FAB-MS spectra of the trichothecenes studied. Amount of trichothecenes introduced on-column, 2 μg (100 ng/μl; 20-μl injection).

(15 cm × 4.6 mm I.D.) (Nomura Kagaku). The trichothecenes were separated by means of a gradient at a flow-rate of 1.0 ml/min. Mobile phase A was water and B was methanol, with a gradient from 30% to 80% B from 0 to 15 min. Glycerol (4%) in methanol was added by means of a post-column at a flow-rate of 250 μl/min. Polyethylene glycol (0.02%) in the methanol solution including glycerol was used as a calibrant in the high-resolution measurements. The effluent from the column was split by a pneumatic splitter and the flow into the frit was 5 μl/min.

All the trichothecenes (Table I) were obtained from Sigma and dissolved in methanol. Glycerol was purchased from Waeko and polyethylene glycol (PEG) from Kanto.

RESULTS AND DISCUSSION

All the frit-FAB spectra of the trichothecenes (Fig. 1) exhibit an abundant glycerol adduct ion $[M+H+92]^+$ and a protonated molecule $[M+H]^+$ from which molecular weights can be reliably determined. Fragment ions are formed by the losses of functional groups as neutral species (water, acetic acid, formaldehyde and isovaleric acid) in various combinations (Table II). The most abundant fragment ions are formed by the loss of isovaleric acid and/or by successive losses of units of acetic acid. The loss of water in the case of TAS, which does not include any hydroxy group, probably occurs from the acetoxy group. This suggests the possibility that the ions $[M+H-H_2O]^+$ are partly formed by the loss of water from the acetoxy or isovaleroyloxy groups in the other trichothecenes.

Ion series of *m/z* 367, 307 and 247 in the spectrum of TAS are possibly formed by the losses of 0, 1 or 2 units of acetic acid combined with the loss of ketene or by the

TABLE II

ION SPECIES OF THE TRICHOTHECENES STUDIED

Compound	Ion species (m/z)[a]
T-2	559 = $[M+H+92]^+$, 467 = $[M+H]^+$, 449 = $[M+H-18]^+$, 407 = $[M+H-60]^+$, 365 = $[M+H-102]^+$, 323 = $[M+H-102-42]^+$ or $[M+H-60-84]^+$, 305 = $[M+H-102-60]^+$, 275 = $[M+H-102-60-80]^+$, 263 = $[M+H-102-60-42]^+$ o $[M+H-60-60-84]^+$, 257 = $[M+H-102-60-30-18]^+$, 245 = $[M+H-102-60-60]^+$ 233 = $[M+H-102-60-30-42]^+$ or $[M+H-60-60-30-84]^+$, 227 = $[M+H-102-60-60-18]^+$, 215 = $[M+H-102-60-60-80]^+$
HT-2	517 = $[M+H+92]^+$, 425 = $[M+H]^+$, 407 = $[M+H-18]^+$, 365 = $[M+H-60]^+$, 323 = $[M+H-102]^+$, 305 = $[M+H-102-18]^+$, 293 = $[M+H-102-30]^+$, 281 = $[M+H-102-42]^+$ or $[M+H-60-84]^+$, 263 = $[M+H-102-60]^+$, 245 = $[M+H-102-60-18]^+$, 233 = $[M+H-102-60-30]^+$, 215 = $[M+H-102-60-30-18]^+$
TAS	501 = $[M+H+92]^+$, 409 = $[M+H]^+$, 391 = $[M+H-18]^+$, 367 = $[M+H-42]^+$, 349 = $[M+H-60]^+$, 307 = $[M+H-60-42]^+$, 289 = $[M+H-60-60]^+$, 247 = $[M+H-60-60-42]^+$, 229 = $[M+H-60-60-60]^+$
DAS	459 = $[M+H+92]^+$, 367 = $[M+H]^+$, 349 = $[M+H-18]^+$, 307 = $[M+H-60]^+$, 289 = $[M+H-60-18]^+$, 265 = $[M+H-60-42]^+$, 247 = $[M+H-60-60]^+$, 229 = $[M+H-60-60-18]^+$
MAS	417 = $[M+H+92]^+$, 325 = $[M+H]^+$, 307 = $[M+H-18]^+$, 265 = $[M+H-60]^+$, 247 = $[M+H-60-18]^+$, 229 = $[M+H-60-18-18]^+$
DON	389 = $[M+H+92]^+$, 297 = $[M+H]$

[a] 18 = H_2O; 30 = CH_2O; 42 = CH_2CO; 60 = CH_3COOH; 84 = $(CH_3)_2CHCHCO$; 102 = $(CH_3)_2CHCH$; 92 = $CH_2OHCHOHCH_2OH$.

substitution reaction with water. In an earlier study, the respective ions were shown to be formed by the losses of functional groups with the loss of ketene in the collision-activated dissociation (CAD) of ammonium adduct ions [13]. This suggests the possibility that the ion series of m/z 367, 307 and 247 in the frit-FAB spectrum of TAS and the respective ions in the spectra of the other trichothecenes are partly formed via a similar pathway.

The fragmentation in the frit-FAB spectra is higher than in the TSP spectra obtained in previous studies [20–22], but is very similar to the CAD spectra of ammonium adduct ions [13] and to the isobutane desorption chemical ionization (DCI) [16,35] spectra of the trichothecenes reported earlier. It might be only in this instance that the amount of energy transferred into the internal energy of the molecule is nearly the same in DCI as in frit-FAB. However, the conclusion cannot be generalized, as the exothermicity of the proton transfer reaction determines the amount of fragmentation in isotubtane CI, but in addition to exothermicity many other factors affect the fragmentation in the frit-FAB mode.

Fig. 2 shows mass chromatograms of the studied trichothecenes recorded by LC–frit-FAB-MS with whole mass range scanning and resolution 1000 from the standard sample (100 μg/ml). All the studied trichothecenes separated well with relatively symmetrical and narrow peaks (peak width 0.5–1 min) under the chosen LC conditions, although post-column addition of the glycerol causes peak broadening.

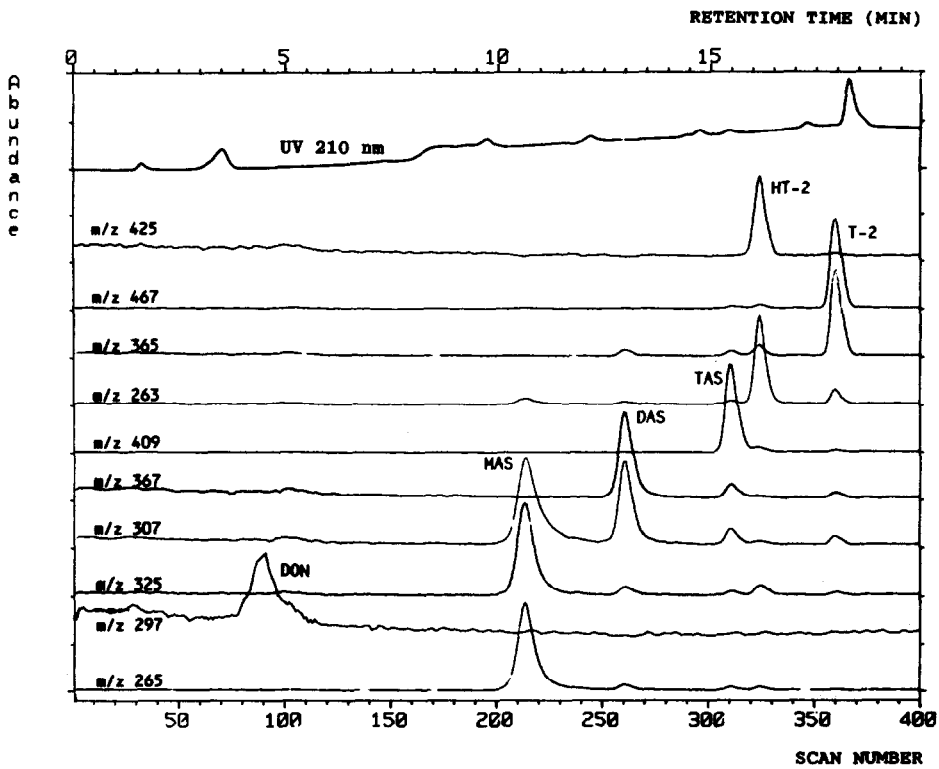


Fig. 2. Mass chromatograms of the trichothecenes studied, recorded by LC–frit-FAB-MS with resolution 1000 from the standard sample. Amount of trichothecenes introduced on-column, 2 μg (100 ng/μl; 20-μl injection). The scan range was from *m/z* 70 to 700.

The specificity of the method can be improved by using high-resolution MS. Fig. 3 shows mass chromatograms of the protonated molecules of the studied trichothecenes recorded with resolution 8000 from the standard sample (10 μg/ml) using selected ion monitoring and a multi-grouping technique: the monitored mass values *m/z* 297.134 (DON), 325.165 (MAS), 367.176 (DAS) and 371.228 (PEG; lock mass) were changed after 14.5 min to *m/z* 409.186 (TAS), 425.216 (HT-2), 467.228 (T-2) and 459.281 (PEG; lock mass). The mass window used in the lock mass monitoring was 200 mmu. Stable lock ion current profiles show good stability of the LC–frit-FAB-high-resolution MS. The detection limits with resolution 8000 using selected ion monitoring were about 5 μg/ml for DON and MAS and slightly less than 1 μg/ml for the other trichothecenes.

The use of high resolution in LC–frit-FAB-MS also allows the determination of elemental compositions of the ions from the components represented in the LC peaks. Table III presents accurate mass measurements of the protonated molecules and some fragment ions of the studied trichothecenes with resolution 8000. The measurements were made with column injection from the standard samples including trichothecenes (100 μg/ml). The measured mass values are typically within 5 mmu of the calculated values. The results are accurate enough for a reliable determination of the elemental compositions of the ions.

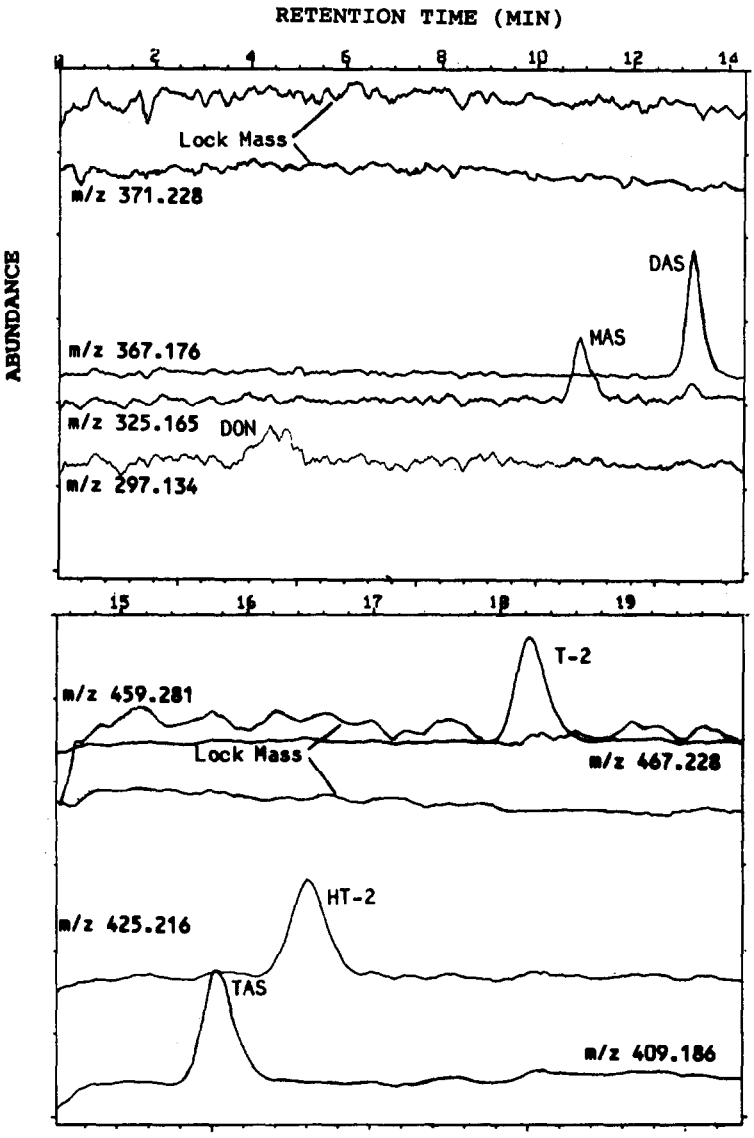


Fig. 3. Selected ion current profiles of the protonated molecules of the thichothecenes studied, recorded by LC–frit-FAB-MS with resolution 8000 using selected ion monitoring and the multi-grouping technique from the standard solution. Amount of trichothecenes introduced on-column, 200 ng (10 ng/μl; 20-μl injection).

CONCLUSIONS

LC–frit-FAB-MS is well suited to the identification of trichothecenes at concentration levels higher than hundreds of picograms per microlitre. The selectivity of the detection can be increased by using high-resolution mass spectrometry, which

TABLE III

ELEMENTAL COMPOSITIONS AND ACCURATE MASSES OF SOME IONS OF THE TRICHOTHECENES STUDIED, OBTAINED BY FRIT–FAB LC–MS

The resolution was 8000 and the scan range was from *m/z* 150 to 550. Trichothecenes were separated with an ODS C_{18} column and the standard sample concentration was 100 μg/ml.

Compound	Measured mass	mmu	Elemental composition
T-2	467.2140	−14.2	$C_{24}H_{35}O_9$
T-2	365.1581	− 2.0	$C_{19}H_{25}O_7$
T-2	305.1449	6.0	$C_{17}H_{21}O_5$
HT-2	425.2260	8.5	$C_{22}H_{33}O_8$
TAS	409.1823	− 4.0	$C_{21}H_{29}O_8$
DAS	367.1758	0.1	$C_{19}H_{27}O_7$
DAS	349.1707	5.7	$C_{19}H_{25}O_6$
DAS	307.1516	− 3.0	$C_{17}H_{23}O_5$
MAS	325.1607	− 4.4	$C_{17}H_{25}O_6$
MAS	307.1584	3.8	$C_{17}H_{22}O_5$
MAS	265.1486	4.6	$C_{15}H_{21}O_4$
DON	297.1412	7.4	$C_{15}H_{21}O_6$

also allows accurate mass measurements of the components represented in the LC peaks.

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