Journal of Chromatography, 562 (1991) 555–562
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5532

Identification of trichothecenes by thermospray, plasmaspray and dynamic fast-atom bombardment liquid chromatography–mass spectrometry

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ABSTRACT

Thermospray, plasmaspray and dynamic fast-atom bombardment liquid chromatography–mass spectrometry are compared for the identification of six trichothecenes. Thermospray spectra of the trichothecenes exhibit only a very abundant ammonium adduct ion. Plasmaspray, which provides a more energetic ionization process than thermospray, produces some fragment ions in addition to an abundant ammonium adduct ion. The spectra obtained by dynamic fast-atom bombardment exhibit a protonated molecule, a glycerol adduct ion and numerous fragment ions formed by the losses of functional groups as neutrals in various combinations. Thermospray and plasmaspray are suitable only for monitoring of the trichothecenes, whereas dynamic fast-atom bombardment is suitable for monitoring and for structure characterization.

INTRODUCTION

Trichothecenes are significant contaminants in foods and feeds [1–4]. Because of their extreme toxicity [3] and natural occurrence, several identification methods have been developed. Trichothecenes are most often detected as their different types of derivative by gas chromatography (GC) [5,6] or gas chromatography–mass spectrometry (GC–MS) [4,7,8]. The time-consuming derivatization step is not needed in the identification of trichothecenes by liquid chromatography (LC) [9,10], but the specificity and sensitivity of conventional LC detectors are limited. A mass spectrometer provides a nearly ideal detector, but its coupling to LC is more difficult than to GC.

However, several interfacing techniques have been developed [11,12]. One of the most commonly used interfaces is thermospray (TSP) [13], which produces with trichothecenes a very abundant ammonium adduct ion with minimal fragmentation [14,15]. TSP LC–MS is suitable for the monitoring of trichothecenes, but the lack of fragmentation makes the structure characterization difficult.

The recently introduced interfacing techniques of plasmaspray (PSP) [16,17] and dynamic fast-atom bombardment (FAB) [18,19] provide more energetic ionization processes than TSP and also often more fragmentation. In PSP a differ-

0378-4347/91/$03.50

ential voltage is applied between the thermospray vaporizer and the ion-source block to create a d.c. plasma of sample and solvent ions [16]. In dynamic FAB an eluent, containing 1–5% glycerol, is bombarded continuously by neutral atoms with high kinetic energy [19]. The bombardment results in sputtering of molecules and formation of positive and negative ions. Both methods have been applied to the analysis of several compounds [12,16,17]. This study compares the spectra of six trichothecenes (DON, MAS, DAS, TAS, HT-2, and T-2) recorded by TSP, PSP and dynamic FAB LC–MS.

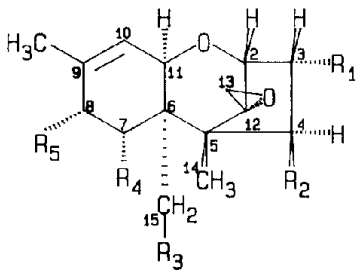
EXPERIMENTAL

Reagents

All the trichothecenes (Table I) were obtained from Sigma (St. Louis MO, U.S.A.) and dissolved in methanol.

TABLE I

THE SIX TRICHOTHECENES STUDIED



Compound	R_1	R_2	R_3	R_4	R_5
T-2 Toxin (T-2)	OH	OAc	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

Equipment and conditions

The three different experimental instrumental set-ups in LC–MS measurements are summarized in Table II. The mass spectrometers were operated in low-resolution (1000 R) mode. In dynamic FAB the matrix (glycerol) in methanol was added by means of a post-column at a flow-rate of 250 μl/min. The eluent was split (1:200) before the mass spectrometer by a Jeol pneumatic splitter. Xenon was used in the bombardment (particle energy 5 keV).

TABLE II

SYSTEM DESCRIPTIONS

TSP HPLC–MS	PSP HPLC–MS	Dynamic Fab HPLC–MS	
Chromatograph	Perkin Elmer, Series 2 (Ueberlingen, F.R.G.)	LKB-2249 (Bromma, Sweden)	HP 1090 LC, (Palo Alto, CA, U.S.A.)
Column	LiChrosorb RP-18, 5 μm, 15 cm × 4.6 mm I.D. (Merck, Darmstadt, F.R.G.)	Spherisorb ODS-2, 3 μm, 10 cm × 4.6 mm I.D. (Phase Separations, Queensferry, U.K.)	ODS-18, 3 μm 15 cm × 4.6 mm (Nomura Kagaku, Tokyo, Japan)
Mobile phase A	H_2O + 0.1 *M* NH_4CH_3COO	H_2O + 0.1 *M* NH_4CH_3COO	H_2O
Mobile phase B	CH_3OH + 0.1 *M* NH_4CH_3COO	CH_3OH + 0.1 *M* NH_4CH_3COO	CH_3OH
Run programme	65% B isocratic	B; 20–70% for 0–20 min	B; 30–80% for 0–15 min
HPLC flow-rate	1 ml/min	0.8 ml/min	1 ml/min
Injection volume	20 μl	20 μl	20 μl
Split ratio	–	–	1/200
Flow-rate to MS	1 ml/min	0.8 ml/min	5 μl/min
Mass spectrometer	Finnigan MAT 90 (Bremen, F.R.G.)	VG ZAB-E (Manchester, U.K.)	Jeol JMS-SX102 (Tokyo, Japan)
Interface	Finnigan MAT thermospray	VG plasmaspray	Jeol frit FAB
Ion-source temperature	220°C	250°C	50°C
Vaporizer temperature	200°C	250°C	–

RESULTS AND DISCUSSION

All the TSP spectra of the trichothecenes exhibit a very abundant ammonium adduct ion without fragmentation (Table III). The spectra are very similar to ammonia desorption chemical ionization (DCI) spectra recorded in an earlier study [20]. This is because the ionization process in ammonium acetate-buffered TSP is analogous to that in ammonia CI. The proton affinities of the trichothecenes [21,22] are similar to the proton affinity of ammonia (859 kJ/mol) [23], leading to low exothermicity of the ion–molecule reactions between trichothecenes and ammonium ions and thus to the formation of an abundant ammonium adduct ion with minimal fragmentation. Ammonium acetate-buffered TSP provides good selectivity in the identification of the trichothecenes, since compounds with a proton affinity of less than 787 kJ/mol cannot be ionized efficiently and they are transparent in the analysis [24]. Good sensitivity (0.1–1 ng/μl) is achieved in the single-ion (ammonium adduct ion) monitoring of the pure trichothecenes, but the lack of fragmentation decreases the reliability of the identification and makes structure characterization very difficult.

TABLE III

THERMOSPRAY SPECTRA OF THE SIX TRICHOTHECENES STUDIED

Compound	$[M+H]^+$	$[M+NH_4]^+$
T-2	– –	484 (100)
HT-2	– –	442 (100)
TAS	– –	426 (100)
DAS	– –	384 (100)
MAS	– -	342 (100)
DON	297 (46)	314 (100)

TABLE IV

PLASMASPRAY SPECTRA OF THE TRICHOTHECENES STUDIED

Compound	$[M+H]^+$	$[M+NH_4]^+$	Other ions
T-2	– –	484 (100)	365 (2), 335 (4), 305 (5), 275 (3), 257 (2), 245 (3), 215 (3)
HT-2	425 (100)	442 (42)	323 (6), 293 (4), 275 (3), 263 (14), 245 (7), 215 (8)
DAS	367 (1)	384 (100)	307 (4)
MAS	325 (3)	342 (100)	307 (17), 282 (20), 265 (33), 247 (3)
DON	297 (89)	314 (100)	284 (5), 249 (8)

TABLE V

DYNAMIC FAST-ATOM BOMBARDMENT SPECTRA OF THE SIX TRICHOTHECENES STUDIED

Compound	$[M+H]^+$	$[M+H+92]^+$	Other ions
T-2	467 (33)	559 (12)	449 (12), 407 (5), 365 (96), 323 (10) 305 (100), 275 (18), 263 (15), 257 (18) 245 (59), 233 (21), 215 (65), 203 (22) 197 (19)
HT-2	425 (67)	517 (19)	407 (21), 365 (15), 323 (69), 305 (12), 263 (100), 245 (25), 233 (26), 215 (42), 203 (28)
TAS	409 (100)	501 (20)	391 (17), 367 (13), 349 (37), 307 (15), 289 (23), 247 (26), 229 (35), 201 (13), 199 (14)
DAS	367 (97)	459 (39)	349 (34), 307 (100), 289 (9), 265 (10), 247 (30), 229 (28), 201 (13) 199 (12)
MAS	325 (17)	417 (25)	307 (46), 265 (100), 247 (10), 229 (8)
DON	297 (100)	389 (32)	215 (68)

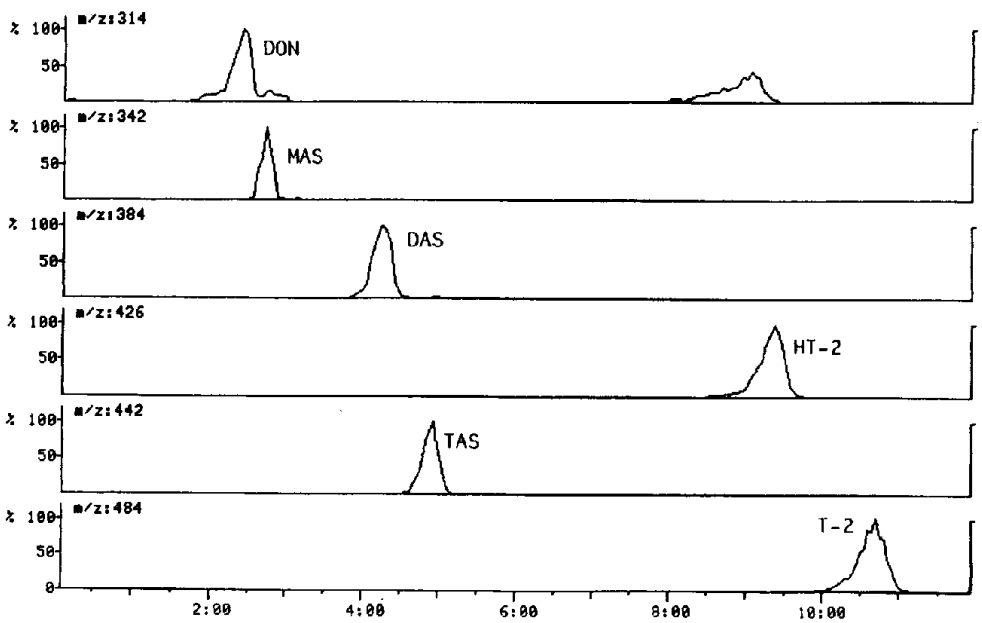


Fig. 1. Mass chromatograms of ammonium adduct ions of the pure trichothecenes (10 ng/μl; 20-μl injection) recorded by TSP LC–MS (isocratic).

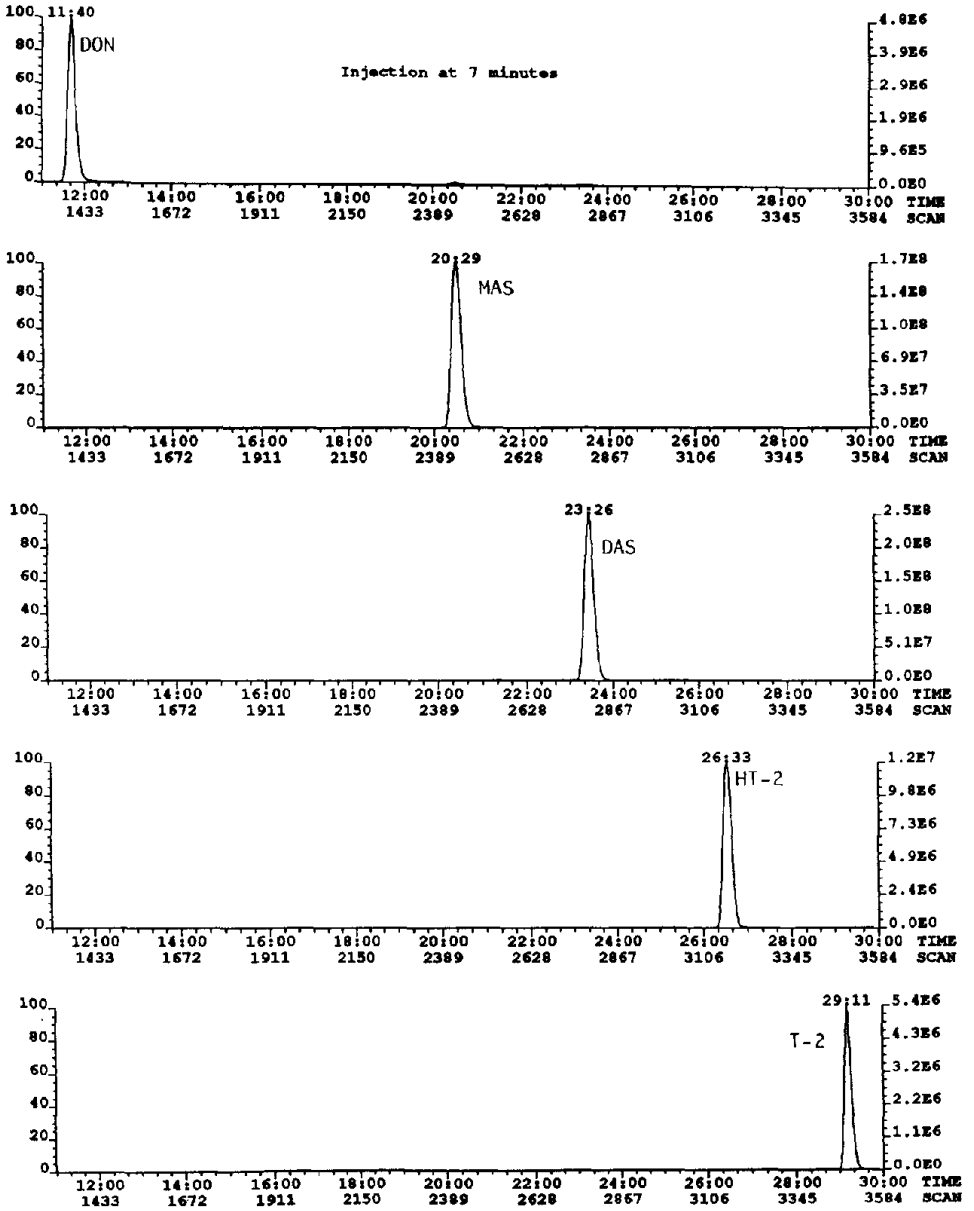


Fig. 2. Mass chromatograms of ammonium adduct ions of the trichothecenes (10 ng/μl; 20-μl injection) recorded by PSP LC–MS (gradient).

The recently introduced plasmaspray (PSP) LC–MS method provides a more energetic ionization process than TSP, owing to a differential voltage applied between the thermospray vaporizer and the ion-source block to create a d.c. plasma of sample and solvent ions (H_3O^+ and $CH_3OH_2^+$). The lower proton

affinities of the solvent ions than that of ammonia result in a more exothermic ionization of the sample [23]. All the PSP spectra of the trichothecenes exhibit an abundant ammonium adduct ion (Table IV). The trichothecenes with two or more hydroxy groups in the molecule (DON, MAS and HT-2) produce more abundant fragment ions than those with one hydroxy group (DAS and T-2). DON, MAS, and HT-2 can reliably be identified by multiple-ion monitoring, whereas DAS and T-2 must be identified by single-ion monitoring, which decreases the reliability of the identification.

In dynamic FAB LC–MS the eluent was bombarded continuously by xenon atoms. In the bombardment the trichothecenes produced an abundant protonated molecule, a glycerol adduct ion and several abundant fragment ions formed by loss of functional groups as neutral species in various combinations (Table V). The ionic species were described in a previous report [25]. The greater fragmentation obtained with FAB than with TSP and PSP indicates that the ionization process with FAB is more energetic than with TSP and PSP. The formation of abundant fragment ions by FAB allows reliable identification of the trichothecenes by multiple-ion monitoring and makes the structure characterization easier than with TSP and PSP. The detection limits of the pure trichothecenes (1–10 ng/μl following 20-μl injection), with dynamic FAB are only one order of magnitude worse than with TSP, although the eluent was split with the

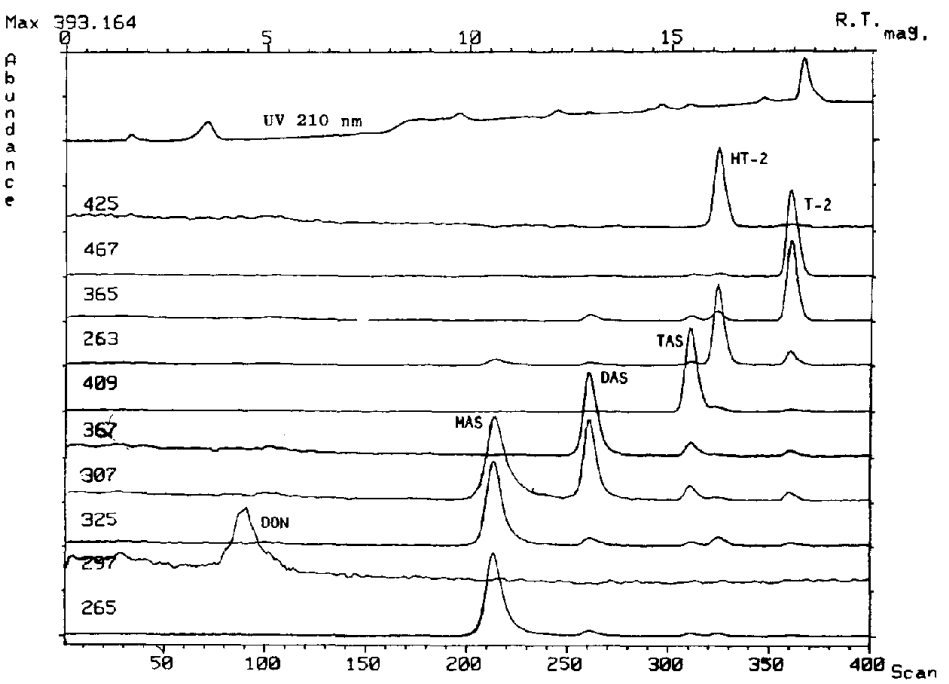


Fig. 3. Mass chromatograms of protonated molecules and some fragment ions of the trichothecenes (10 ng/μl; 20-μl injection) recorded by dynamic FAB LC–MS (gradient).

ratio of 1:200 in the dynamic FAB. This suggests the possibility that the ionization process with FAB is more efficient than with TSP.

Fig. 1–3 show the mass chromatograms of the trichothecenes obtained by TSP, PSP and dynamic FAB LC–MS with multiple-ion monitoring. The mass chromatograms show that the trichothecenes were eluted with adequately symmetric and narrow peaks under the chosen gradient LC–MS conditions (PSP and dynamic FAB). All the trichothecenes can be detected under the chosen isocratic conditions (TSP), although the peaks of T-2 and HT-2 became wider. The wider peaks in dynamic FAB than in PSP are due to the post-column addition of glycerol.

ACKNOWLEDGEMENTS

Dr. Ernst Schröder from Finnigan MAT, Dr. Nick Ordsmith and Dr. Chris Harbach from VG Analytical, and Dr. Matsuura from Jeol Ltd. are acknowledged for the operating of the instruments. Financial support from Magnus Ehrnrooth and Oskar Öflund foundations is acknowledged.

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