Use of 1-[p-(2,3-dihydroxypropoxy)phenyl]-1-alkanones as retention index standards in the identification of trichothecenes by liquid chromatography–thermospray and dynamic fast atom bombardment mass spectrometry

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ABSTRACT

A homologous series of 1-[p-(2,3-dihydroxypropoxy)phenyl]-1-alkanones (D-standards) were used as retention index standards in the detection of trichothecenes by reversed-phase (RP) gradient elution liquid chromatography–mass spectrometry (LC–MS). Thermospray (TSP) and dynamic fast atom bombardment (dynamic FAB) were used as LC–MS interfaces. The retention indices provide independent identification of compounds, improving the reliability of the identification by LC–MS. The TSP and dynamic FAB mass spectra of the D-standards and trichothecenes are presented.

INTRODUCTION

Retention indices have been widely used in gas chromatography (GC) but sel-dom in liquid chromatography (LC). Baker and Ma [1] made the first proposal for a retention index series suitable for reversed-phase (RP) LC, studying 2-alkanones as reference standards. However, 2-alkanones have only a weak chromophore and they are of only limited use as reference compounds in UV detection. Smith [2] and Kuro-nen [3] later introduced the 1-phenyl-1-alkanones as retention index standards for RP-LC, Smith [2] in an isocratic solvent system and Kuronen [3] under gradient elution conditions. The use of retention indices in RP gradient elution LC has been applied to chemical warfare agents [3–8] and mycotoxins [9–11]. Chromatographic parameters with the greatest effect on the reliability of the gradient-programmed retention indices have been carefully studied and the long-term reproducibilities of the indices were found to be at least adequate (the average relative standard deviation was 0.3%) [8] under specified chromatographic conditions, allowing tentative identifi-cations on an interlaboratory basis [5,8].

Although the reliability of identifications can be improved by the use of retention indices, LC has some serious limitations owing to the lack of a specific and sensitive detector. Mass spectrometry, which combines high sensitivity and selectivity, has long been utilized with gas chromatography, but it is more difficult to interface a mass spectrometer to LC than GC systems. However, many interfacing techniques have been developed during the last few years and some of them, especially thermospray (TSP) and dynamic fast atom bombardment (dynamic FAB), are already routinely used in several laboratories.

We describe here the use of 1-[p-(2,3-dihydroxypropoxy)phenyl]-1-alkanones (D-standards) as retention index standards in the detection of the trichothecenes DON, MAS, DAS, TAS, HT-2 and T-2 (Table I) by RP gradient elution LC–TSP- and dynamic FAB-MS. The TSP and FAB mass spectra of the trichothecenes and D-standards are presented.

EXPERIMENTAL

Reagents

The D-standards (Table I) were synthesized according to the method presented by Kuronen [8]. All the trichothecenes (Table I) were obtained from Sigma. The D-standards and the trichothecenes were dissolved in methanol.

Equipment and conditions

The two different experimental instrumental set-ups for LC–MS measurements are summarized in Table II. The mass spectrometers were operated in low-resolution (resolution = 1000) modes. In the dynamic FAB the matrix (glycerol, 4%) in methanol was added by means of a post-column tee-connection 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). A liquid nitrogen trap was installed in the JEOL SX102 (used in FAB experiments) to increase the evacuation speed of the ion source.

RESULTS AND DISCUSSION

All the TSP mass spectra of the D-standards exhibit only a protonated molecule and the TSP mass spectra of the trichothecenes only an ammonium adduct ion, except the spectrum of DON, which exhibits peaks for both a protonated molecule and an ammonium adduct ion (Table III). The TSP mass spectra of the trichothecenes are very similar to those recorded in earlier studies [12–14]. The lack of fragmentation is due to the low exothermicity of the ionization process in the ammonium acetate-buffered TSP, owing to the high proton affinity of ammonia (858 kJ/mol) [15]. Since only one ion (ammonium adduct ion) can be used for detection, the reliability of detection of the trichothecenes is not good.

The dynamic FAB mass spectra of trichothecenes exhibit an abundant protonated molecule, a glycerol adduct ion and fragment ions formed by the losses of functional groups as neutral species in various combinations (Table IV). The spectra have been discussed in more detail in earlier studies [12,16].

The dynamic FAB mass spectra of the D-standards exhibit an abundant pro-

TABLE I

D-STANDARDS (D_1–D_6) AND TRICHOTHECENES STUDIED

D-Standards[a]
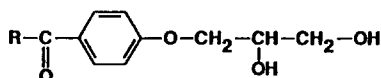
R—C—⬡—O—CH$_2$—CH—CH$_2$—OH
‖ |
O   OH

Trichothecenes[b]
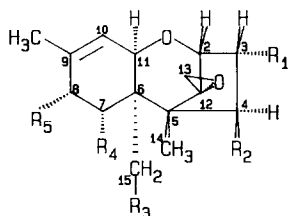
	R$_1$	R$_2$	R$_3$	R$_4$	R$_5$
T-2 toxin (T-2)	OH	OAc	OAc	H	OCOCH$_2$CH(CH$_3$)$_2$
HT-2 toxin (HT-2)	OH	OH	OAc	H	OCOCH$_2$CH(CH$_3$)$_2$
Triacetoxyscirpenol (TAS)	OAc	OAc	OAc	H	H
Diacetoxyscirpenol (DAS)	OH	OAc	OAc	H	H
Monoacetoxyscirpenol (MAS)	OH	OH	OAc	H	H
Deoxynivalenol (DON)	OH	H	OH	OH	=O

[a] $R = CH_3(CH_2)_{n-1}$; $n = 1$–6.
[b] OAc = Acetyl.

TABLE II

DESCRIPTIONS OF SYSTEMS

Component/parameter	LC–TSP-MS	LC–dynamic FAB-MS
Liquid chromatograph	LKB 2249	HP 1090 LC
Column	Spherisorb ODS-18, 3 μm,	ODS-18, 3 μm,
	15 cm × 4.6 mm I.D.	15 cm × 4.6 mm I.D.
	(Phase Separations, Queensferry, U.K.)	(Nomura Kagaku, Tokyo, Japan)
Column temperature	Ambient	Ambient
Mobile phase A	H$_2$O + 0.1 M CH$_3$ COONH$_4$	H$_2$O
Mobile phase B	CH$_3$OH + 0.1 M CH$_3$ COONH$_4$	CH$_3$ OH
Elution programme	B; 30–80% for 0–15 min	B; 30–80% for 0–15 min
LC flow-rate	1 ml/min	1 ml/min
Injection volume	20 μl	20 μl
Splitting ratio	–	1:200
Flow-rate to MS	1 ml/min	5 μl/min
Mass spectrometer	VG TRIBRID	JEOL JMS-SX102
Interface	VG Plasmaspray	JEOL Frit Fab
Ion source temperature	220°C	50°C
Vaporizer temperature	200°C	–

TABLE III

TSP MASS SPECTRA OF D-STANDARDS AND TRICHOTHECENES

Compound	m/z (relative intensity, %)		
	$[M + H]^+$	$[M + NH_4]^+$	Other ions
D_1	211 (100)	–	–
D_2	225 (100)	–	–
D_3	239 (100)	–	–
D_4	253 (100)	–	–
D_5	267 (100)	–	–
D_6	281 (100)	–	–
T-2	–	484 (100)	–
HT-2	–	442 (100)	–
TAS	–	426 (100)	–
DAS	–	384 (100)	–
MAS	–	342 (100)	–
DON	297 (46)	314 (100)	–

tonated molecule and a fragment ion $[RCOC_6H_4OH]^+$, which is formed by the loss of $CH_2CH(OH)CH_2OH$ after protonation of the ether oxygen. The more extensive fragmentation in FAB than in TSP indicates a more energetic ionization process with FAB. The formation of abundant fragment ions in FAB allows reliable detection of the trichothecenes by selected ion monitoring. However, the energetic ionization process in FAB may lead to decreased selectivity in the detection of trichothecenes in

TABLE IV

DYNAMIC FAB MASS SPECTRA OF D-STANDARDS AND TRICHOTHECENES

| Compound	m/z (relative intensity, %)		
	$[M + H]^+$	$[M + H + 92]^+$	Other ions
D_1	211 (100)	–	137 (11)
D_2	225 (100)	–	151 (9)
D_3	239 (100)	–	165 (11)
D_4	253 (100)	–	179 (13)
D_5	267 (100)	–	193 (12)
D_6	281 (100)	–	207 (11)
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)
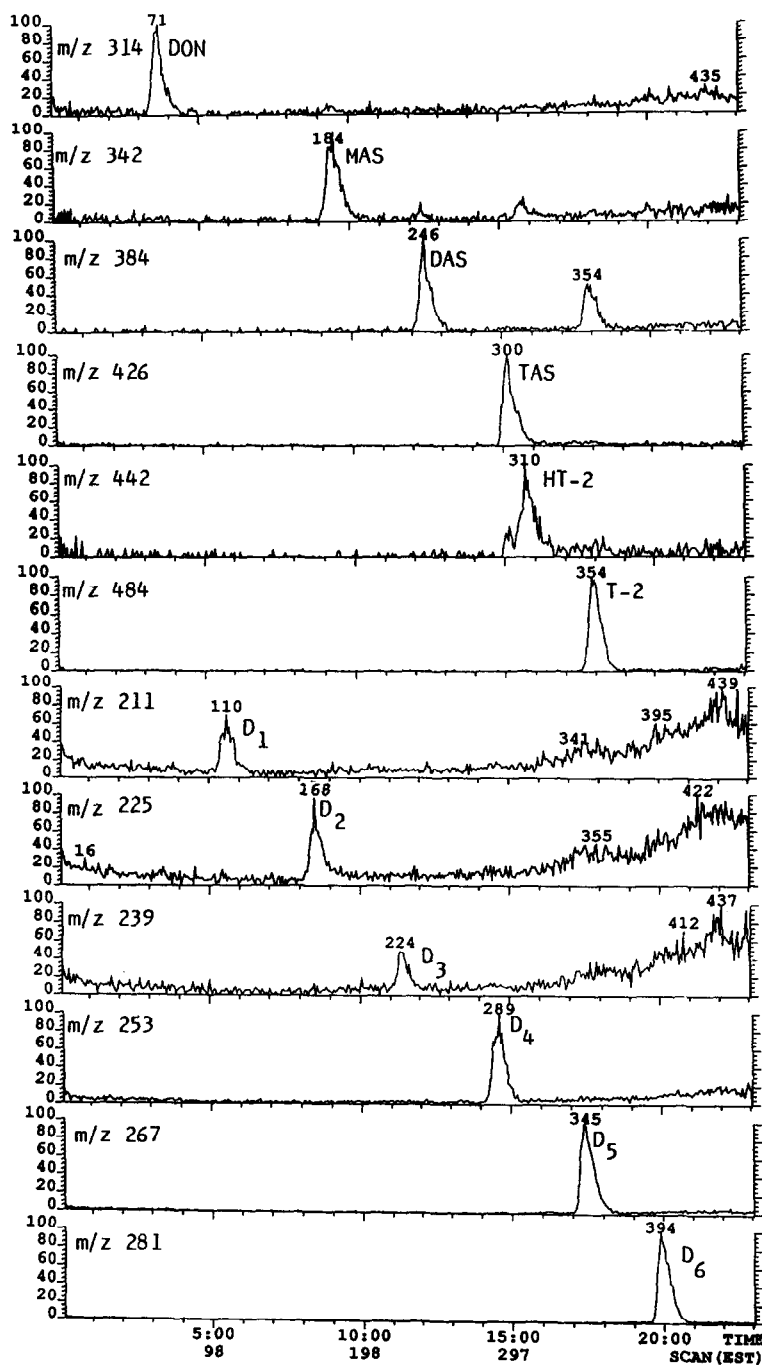
Fig.1. Mass chromatograms of ammonium adduct ions of the trichothecenes and protonated molecules of the D-standards recorded by LC–TSP-MS. The scan range was 200–600 u. The amount introduced on-column was 200 ng for trichothecenes and 100 ng for D-standards (5 ng/μl, 20-μl injections). For conditions, see Table II. Time scale in min.
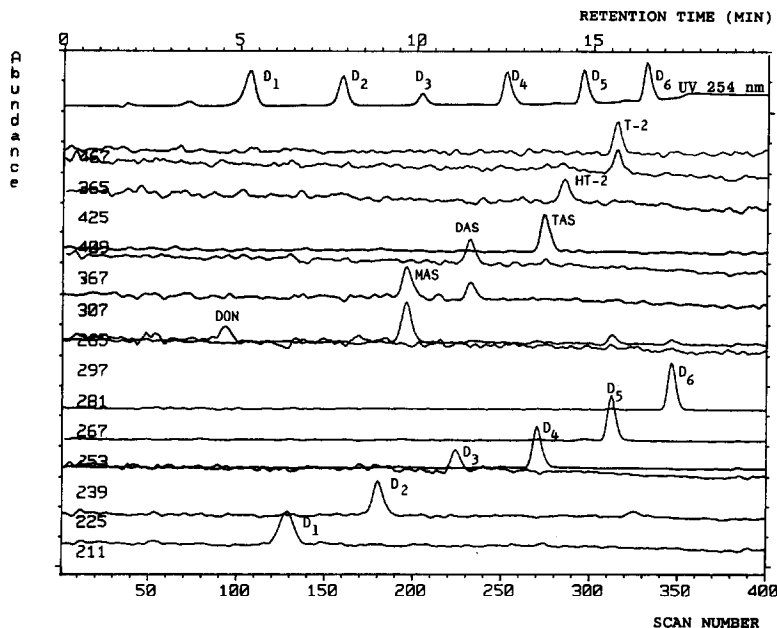
Fig. 2. Mass chromatograms of protonated molecules of the trichothecenes and D-standards recorded by LC–dynamic FAB-MS. The scan range was 200–600 u. The amount introduced on-column was 1 μg for trichothecenes and 1 μg for D-standards (50 ng/μl, 20-μl injections). For conditions, see Table II.

complex matrices, where the number of interfering ions from background compounds is increased.

The reliability of the detection of the trichothecenes can be greatly improved by the use of retention indices, which offer an independent identification parameter, in addition to the TSP or dynamic FAB mass spectra, from the same LC run. Figs. 1 and 2 illustrate the detection of trichothecenes by LC–TSP- and dynamic FAB-MS where the D-standards are used as retention index standards. The mass chromatograms show that the trichothecenes and the D-standards are eluted with sufficiently symmetrical and narrow peaks under the chosen LC–MS conditions. The noisier peaks in the TSP than in the dynamic FAB mass chromatograms are due to background noise rather than instability of the LC or TSP conditions, as the sample used in the TSP measurements was of lower concentration than that used in the dynamic FAB measurements (5 ng/μl vs. 50 ng/μl per trichothecene, 20-μl injections). The post-column addition of glycerol in LC–FAB-MS has no significant effect on the retention times, but causes peak broadening.

Fig. 3 presents plots of the absolute retention time against number of carbon atoms in the alkyl group of the D-standards recorded by LC–TSP- and dynamic FAB-MS. Under the chosen LC conditions, the curves are non-linear. This non-linearity shows up more clearly when more than six D-standard components are used [8]. In the non-linear situation, the retention indices can be calculated by the polygonal technique [17] or by the more accurate [8] cubic spline interpolation method [18].
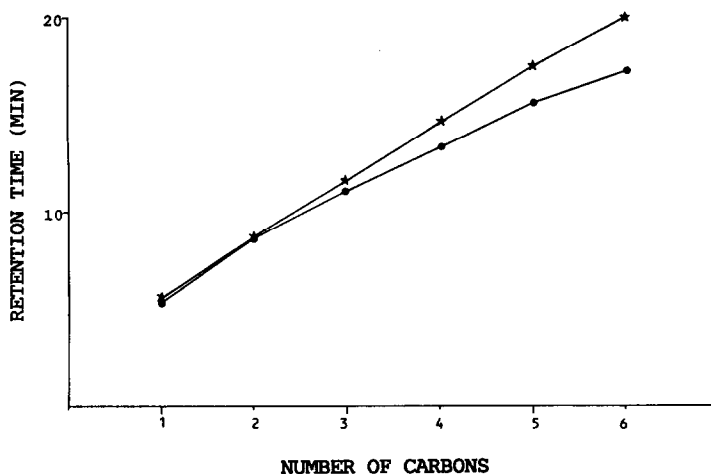
Fig. 3. Plot of retention times of the D-standards against number of carbons in the alkyl chain under linear gradient conditions recorded by (*) LC–TSP-MS and (●) LC–dynamic FAB-MS. For conditions, see Table II.

Table V reports the retention indices of the trichothecenes calculated by the cubic spline interpolation method. DON eluted before the first standard and its retention indices were calculated by extrapolation. The variations in the retention indices obtained with the TSP and dynamic FAB methods are due to the different instrumentation, the use of the buffer solution (ammonium acetate) in TSP and the different columns used (Table II). It has been shown earlier that the retention indexes with C_{18} columns vary with the manufacturer [8]. Slight asymmetry and noise in the LC peaks obtained using TSP due to low concentration (5 ng/μl) may lead to reduced repeatability of the retention indices. The concentrations of the retention standards therefore need to be sufficiently high. In our experiments, the concentration of the D-

TABLE V

RETENTION INDICES (RI_D) OF THE TRICHOTHECENES RECORDED BY LC–TSP-MS AND LC–DYNAMIC FAB-MS CALCULATED BY THE CUBIC SPLINE METHOD

The LC and MS conditions are presented in Table II.

Compound	RI_D	
	TSP	Dynamic FAB
T-2	517.8	508.2
HT-2	435.2	433.4
TAS	418.0	408.8
DAS	334.7	317.8
MAS	229.1	235.7
DON[a]	34.2	40.4

[a] The value of RI_D was calculated by extrapolation, as DON elutes before the first retention index standard.

standards should be tens of nanograms per microlitre for reliable detection of the standards with whole mass range scanning, and a few nanograms with selected ion monitoring (SIM).

The problem with SIM is that several ions must be selected, as trichothecenes and D-standards do not form common ions with either TSP or dynamic FAB, leading to reduced sensitivity. The number of simultaneously monitored ions can be reduced by using a multi-grouping technique in which the monitored mass values are programmed to change during the SIM run. However, this technique also makes the runs more difficult.

CONCLUSIONS

A homologous series of 1-[p-(2,3-dihydroxypropoxy)phenyl]-1-alkanones (D-standards) used as retention index standards in RP gradient elution LC–MS where TSP or dynamic FAB provides the interface improves the reliability of identifications made by LC–MS. The D-standards are efficiently ionized by both the TSP and dynamic FAB techniques. The retention indices provide a second level of identification for the compounds of interest, in addition to the MS data, from the same LC run. In general, retention indices can provide a useful means of indentifying peaks of interest in analytical work where the primary analysis is carried out by LC with UV detection and further information is sought by LC–MS.

ACKNOWLEDGEMENTS

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