Detection of polar and macrocyclic trichothecene mycotoxins from indoor environments

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A method is described for the qualitative and semi-quantitative simultaneous determination of both non-macrocyclic and macrocyclic trichothecene biotoxins from samples derived from indoor environments. The method includes extraction, sample pre-treatment and reversed-phase HPLC separation followed by tandem mass spectrometric identification and quantification using electrospray ionization on a quadrupole ion trap mass analyser. Aqueous methanol was used in the initial extraction and solvent partitioning and solid-phase extraction in the purification of samples. The HPLC separation was run on-line with electrospray ionization MS–MS detection. The detection limits and recoveries of the procedure varied from 1 to 1000 pg and from 31 to 92%, respectively. As the method includes few and not very labour intensive sample treatment steps, it should allow for a high throughput of samples with good prospects of automation.

Trichothecene mycotoxins are toxic fungal secondary metabolites with a characteristic tetracyclic 12,13-trichothec-9-ene ring structure. They are classified into two major groups, simple and macrocyclic, based on their chemical structures,[1,2] with the simple trichothecenes being invariably alcohols with substituents such as acetyl and isovaleryl groups (Fig. 1, Table 2). Macrocyclic trichothecenes, on the other hand, are the di- or triesters of simple trichothecenes and they have large, thermally labile, exocyclic ester bridges at positions 15 and 4 (Fig. 2, Table 4). Much of the toxic properties of the trichothecenes have been attributed to the epoxy ring at position 12.[3] In general, the most toxic of the trichothecenes belong to the macrocyclic family, which, in addition to fungal extracts and fermentation broths, can be found in *Baccharis* plants.[2,4]

Trichothecenes have been associated with human health hazards for decades.[5] The reported health implications range from nausea, vomiting, skin irritation and internal bleeding to respiratory disorders of various kinds.[6] Symptoms appear upon inhalation of trichothecene-containing aerosols or by oral or subcutaneous administration of toxin.[7] *Fusarium*, *Stachybotrys* and other trichothecene-producing moulds have most frequently been found to infect grain, with resulting losses in farm products and livestock.[1,2,8,9] On the other hand, persons handling *Stachybotrys*-contaminated fodder have suffered from cough, rhinitis, burning sensations in the mouth and nasal passages and cutaneous irritation due to contact with toxins.[10,11] In more recent years, *S. atra*, in particular, has been isolated from building materials and air samples in buildings with moisture problems with residents suffering from very similar symptoms, such as cough, irritation of eyes, skin and respiratory tract, headache and fatigue.[12–14]

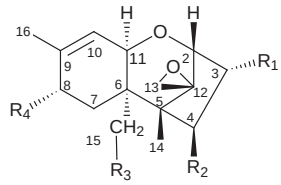


Fig. 1 Non-macrocyclic (simple) trichothecenes.

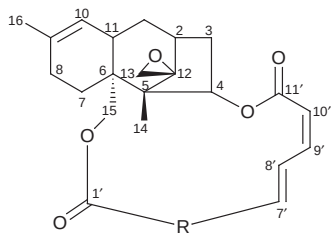


Fig. 2 Macrocyclic trichothecenes.

In addition to *S. atra*, strains of other genera known to produce trichothecenes have also frequently been reported in indoor environments.[12,15–18] However, few data are available on the toxicological implications of breathing organic dust containing toxic fungi in indoor environments and even less is known about the trichothecene concentrations in contaminated buildings. This most certainly is partially due to analytical problems, as the fungal counts in indoor air and hence the resulting toxin concentrations are low. It could also be argued, however, that dampness and fungal propagation in indoor environments is, in contrast to food poisonings, a fairly new occurrence, which only became urgent concomitantly with developments in construction techniques in the latter half of this century.

This study was undertaken with the intention of gathering relevant data about the occurrence and concentration of common fungal toxins in Finnish indoor environments and with the specific aim of developing methodology that can be used to extract and quantify a wide range of trichothecenes from samples derived from both building materials and microbial growth media. The task involved large numbers of samples with small amounts of extractable material containing minute amounts of toxin. Previously, several trichothecenes have been determined simultaneously using HPLC–MS with thermospray[19–23] and moving belt[24] introduction to the mass spectrometer. Published GC–MS methods, on the other hand, include dimethyl ether chemical ionization[25] and ammonia desorption chemical ionization mass spectrometry.[26] However, no method could be directly applied in the present investigation, as published methods either lack the necessary pre-treatment of samples, use different instrumentation or include exclusively

the simple or the macrocyclic trichothecenes. Also, ion trap data with positive electrospray ionization (ESI) have, to our knowledge, not been published elsewhere.

Experimental

Chemicals

All solvents were of either HPLC or analytical-reagent grade. Water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Diacetoxyscirpenol (DSA) and T-2 toxin, manufactured by Sigma (St. Louis, MO, USA) were provided by Dr. Paula Vanninen of VERIFIN (Finnish Institute for Verification of the Chemical Weapons Convention, Helsinki, Finland). Verrucarin A (VERA), roridin A (RDRA) and satratoxin G and H (SATG, SATH) were provided by Professor Eeva-Liisa Hintikka of the National Veterinary and Food Research Institute (Helsinki, Finland). All other mycotoxins were purchased from Sigma. The alkaloid reserpine (Sigma) was used as an internal standard.

The following solid-phase extraction (SPE) cartridges, with sequentially differing polarity, were used in the trials: Bond Elut C_{18} (Analytichem International, Harbor City, CA, USA), Isolute C_{18} (non-end-capped), Isolute MF C_{18} (non-end-capped), Isolute C_8 (end-capped), Isolute C_8 (non-end-capped) and Isolute ENV$^+$ (International Sorbent Technology, Hengoed, Mid-Glamorgan, UK).

Sample composition

The method was applied to building materials, ranging from paper-covered gypsum board to carpeting and wooden material, to fungal isolates grown on solid agar plates and to dust collected either by suction through air filters or by dusting of surfaces for fallen dust particles.

Sample preparation

Samples were stored at −20 °C prior to analysis. Material to be extracted was initially ground by hand, using appropriate tools or, when feasible, by mortar and pestle. The samples were weighed prior to application of the extraction solvent. The extraction solvent was applied directly to agar plates and the surface scraped to remove aerial mycelia and spores.

Extraction and purification

Sample preparations were carried out under dim light when possible. Samples and extracts were stored at 5 °C when not in use. If the samples had to be stored for longer periods, they were cooled to −20 °C. All samples were saturated with nitrogen between preparation steps, to minimize oxidative decomposition. Samples were extracted twice with 95% methanol (PA, Merck, Darmstadt, Germany) on a rotary shaker at 165 rpm (IKAKS 501, IKA-Labortechnik, Staufen, Germany). The initial extraction was continued for 2 h, after which the slurry was centrifuged (5 min, 1500*g*, ambient temperature) and the supernatant was retained. The pellet was re-extracted for 30 min, the slurry was centrifuged and the supernatants were combined.

The combined supernatants were partitioned twice against half their volume of hexane prior to evaporation of methanol in a rotary evaporator (Heidolph 51111, Heidolph-Elektro, Kelheim, Germany), saturated with N_2, at 35 °C. The volume of aqueous samples after evaporation of methanol was about 2 ml. The defatted, concentrated samples were next transferred to solid-phase extraction (SPE) cartridges solvated with methanol (1 ml per 100 mg of sorbent) and pre-equilibrated with water containing 0.5% methanol (1 ml per 100 mg of sorbent). A vacuum manifold (Lida Manufacturing, Kenosha, WI, USA) with an operating pressure of 3 in Hg was used to standardize the operating conditions in SPE. The cartridges were washed with one volume of 0.5% methanol, containing 10 mM ammonium acetate buffer (PA, Merck), to remove polar impurities, before extraction of the analytes with pure methanol through a filter of pore size 0.45 μm (Spartan 13, Schleicher & Schüll, Dassel, Germany).

The methanol extract was evaporated to dryness under N_2 at 35 °C. The residues was next transferred to 200 μl HPLC vials with 200 μl of methanol [the recovery of internal standard was poor using the HPLC elution solvent (20% methanol in 10 mM ammonium acetate buffer), hence pure methanol was used at this stage].

HPLC conditions

The analytes were introduced to the MS detector by injecting 10 μl of sample through an HPLC system consisting of an Alliance 2690 separation module (Waters, Milford, MA, USA) connected to a LiChrocart 250-3 Purospher RP18 column (Merck, Darmstadt, Germany) on-line with a Lichrocart 4-4 Purospher RP18e precolum (Merck), both operated at 30 °C (Model 7981 column oven, Jones Chromatography, Hengoed, Mid-Glamorgan, UK). A methanol–aqueous buffer (10 mM ammonium acetate) solvent system was used; 20 μM sodium acetate (PA, Merck) was added to the solvents for enhancement of cationization in ESI-MS. An initial methanol concentration of 20% was held for 4 min, after which it was raised linearly to 70%, the final concentration being held for 18 min. The flow rate was 400 μl min^{-1}. Between samples, 10 μl of pure methanol were injected and the column was first eluted for 4 min with 90% methanol, then the methanol concentration was lowered to 20% in 1 min followed by conditioning for 4 min with this solvent composition. This was done to minimize cross-contamination of samples.

ESI-CID-MS–MS

Mass spectral analysis was performed on a Finnigan (Sunnyvale, CA, USA) LCQ instrument fitted with an ESI probe. The operating conditions were optimized in the working flow range using T2-toxin, RDRA and T2-tetraol and were as follows. The ESI probe was operated in the positive ion mode and set at a voltage of 1.10 kV. Pressurized nitrogen (6.9 bar) was used as auxiliary and sheath gas with a flow rate of 5 (arbitrary units) and 75 (arbitrary units), respectively. Helium was used for collision induced dissociation (CID) at a pressure of 2.75 bar. The capillary temperature was set to 260 °C and the capillary voltage to 46.0 V with a tube lens offset of 55 V. The system includes two octapole ion guides with an interoctapole lens in between. The first octapole dc offset potential was −3.24 V and the second −6.5 V, with the interoctapole lens voltage set at −16.0 V and the octapole rf amplitude at 400. The electron multiplier voltage was set to −800 V. For CID experiments, the relative collision intensity in the ion trap varied from 12.6 (verrucarol) to 25.0 (SATH and RDRA). The maximum injection time was 200.03 ms and the total microscans were set to 3. It should be pointed out, however, that these operating conditions are optimum, or nearly optimum, for the specific instrument used in this study only and that optimization should always be performed for other similar instruments.

Method yield, linear range and error limits

The error limits of the method were measured by performing a double-sided Student's *t*-test with 95% confidence interval on the deviation from the mean of 12 standards, of three different toxin concentrations, with the same concentration of internal standard (see Table 6). The standards were subjected to the same treatment as the samples. The overall recoveries of the toxins were expressed as the mean recovery of the four strongest standards. The term recovery in this paper means the amount of

substance obtained in the last quantification step in relation to the amount of substance added to the material before extraction, and is expressed as a percentage.

Results and discussion

Sample pre-treatment

Non-end-capped C_8 was found to be the best suited solid-phase sorbent for the whole range of toxins. The most polar toxins did not adhere properly to non-end-capped materials or to the less polar C_{18} materials (results not shown). The most polar of the materials was ENV$^+$, which gave a slightly better yield for all compounds. This carrier was discarded, however, owing to leakage of the carrier material with the resulting expected blockage of HPLC columns.

Chromatographic separation of toxins

With the given conditions, the 10 toxins and the internal standard eluted between 5.5 and 19.5 min. Some of the toxins co-eluted (Fig. 3), but this is of minor importance, since the MS–MS detection allowed the separation of co-eluting peaks while at the same time minimizing the influence of the background (Fig. 4). Judging from the comparison of the solid-phase sorbents in the sample pre-treatment procedure, however, a non-end-capped C_{18} or C_8 column might yield a better separation of the compounds than the end-capped C_{18} column used here.

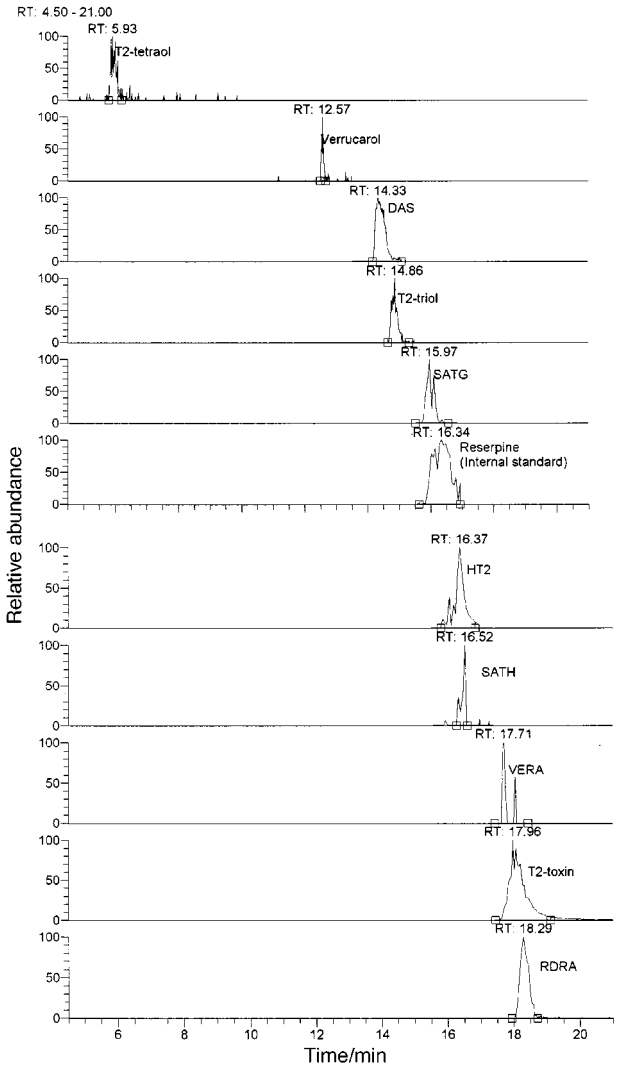


Fig. 3 HPLC–MS of a sample containing 10 toxin standards at concentrations between 20 and 1 ng μl^{-1}.

Detection

Positive ion mode ESI was clearly more effective than the negative ion mode in ionization of the trichothecenes. Partially this was due to the strong tendency of these compounds to form sodiated adducts in ESI. Protonated molecules could not be detected at all, or were present in very low abundance, regardless of whether sodium acetate was added to the HPLC buffer or not. Sodium acetate at a level of 20 μM was added to the buffer to enhance further the cationization, as suggested by Voyksner[27] and Schneider *et al.*,[28] among others. In this way also, the proportion of molecules cationized by ammonium decreased dramatically (below 1%, compared with the sodiated molecules). Similarly to Krishnamurthy *et al.*[19] and Voyksner *et al.*,[23] ammonium acetate buffer was included in the eluent, but at a lower concentration of 10 mM, in order not to impair the electrospray ionization.[27] Buffering clearly reduced peak broadening compared with a non-buffered eluent.

Methanol was the preferred modifier even though for chromatographic purposes acetonitrile would have been better with the column used. This was due to the fact that a water content of higher than 20%, which would have been necessary with an acetonitrile buffer, impaired the electrospray ionization.

The use of ion source collision-induced dissociation (SCID) at a level of 10% clearly increased the abundance of the sodiated molecular ions relative to the abundance of the sodiated dimers and trimers (Table 1). With all toxins except RDRA, this increase was reflected as an increase in the height of the sodiated molecular ion chromatographic peak and hence as an improved detection limit. Even with RDRA, the area of the chromatographic peak was slightly improved when SCID was used (Fig. 5).

At a SCID level higher than 10, however, the abundance of even the sodiated molecular ions started to decrease (results not shown).

ESI tandem mass spectra

The simple trichothecenes included in this study were mono- to tetraols with no ester-linked substituent groups or with acetyl groups at positions 15 and/or 4 and/or with an isovaleryl group at position 8 (Table 2). Contrary to the dimethyl ether chemical ionization mass spectra reported by Burrows,[25] the most predominant fragments in the ESI tandem mass spectra of the simple trichothecenes were due to the loss of isovaleric acid (*i*-$C_4H_9CO_2H$) and acetic acid (CH_3CO_2H) when these substituents were present in the parent molecule (Tables 2 and 3). This is as expected, since it has long been known that the most abundant ion in the EI mass spectra of trimethylsilyl ether derivatives of T-2 and HT-2, among others, is due to isovaleric acid.[29]

Interestingly, with T-2 tetraol and verrucarol, both of which lacked the ester-linked substituents, the ion of *m*/*z* 231 was among the predominant fragments, as it was in the spectra of all of the macrocyclic trichothecenes (Tables 4 and 5). As the parent ion in all cases was the sodiated molecule, ruling out the protonated basic tetracyclic 12,13-trichothec-9-ene unit, this fragment is very difficult to account for. Other major fragments in the spectra of the macrocyclic trichothecenes, all of which yielded similar neutral losses, were $[M + Na - 18]^+$ (loss of water) $[M + Na - 28]^+$, $[M + Na - 230]^+$, $[M + Na - 248]^+$ ($[M + Na - 230 - water]^+$) and $[M + Na - 276]^+$ ($[M + Na - 230 - ethanol]^+$) (Table 5).

A significant new finding was the dominant presence of the product ion $[M + Na - 230]^+$ with accompanying neutral losses of water and ethanol in all the spectra of all macrocyclic trichothecenes. Even though it would be difficult to attempt to distinguish between classes of such structurally diverse compounds as the trichothecenes on the basis of the presence of any one or more types of adduct ions, the presence of all of the

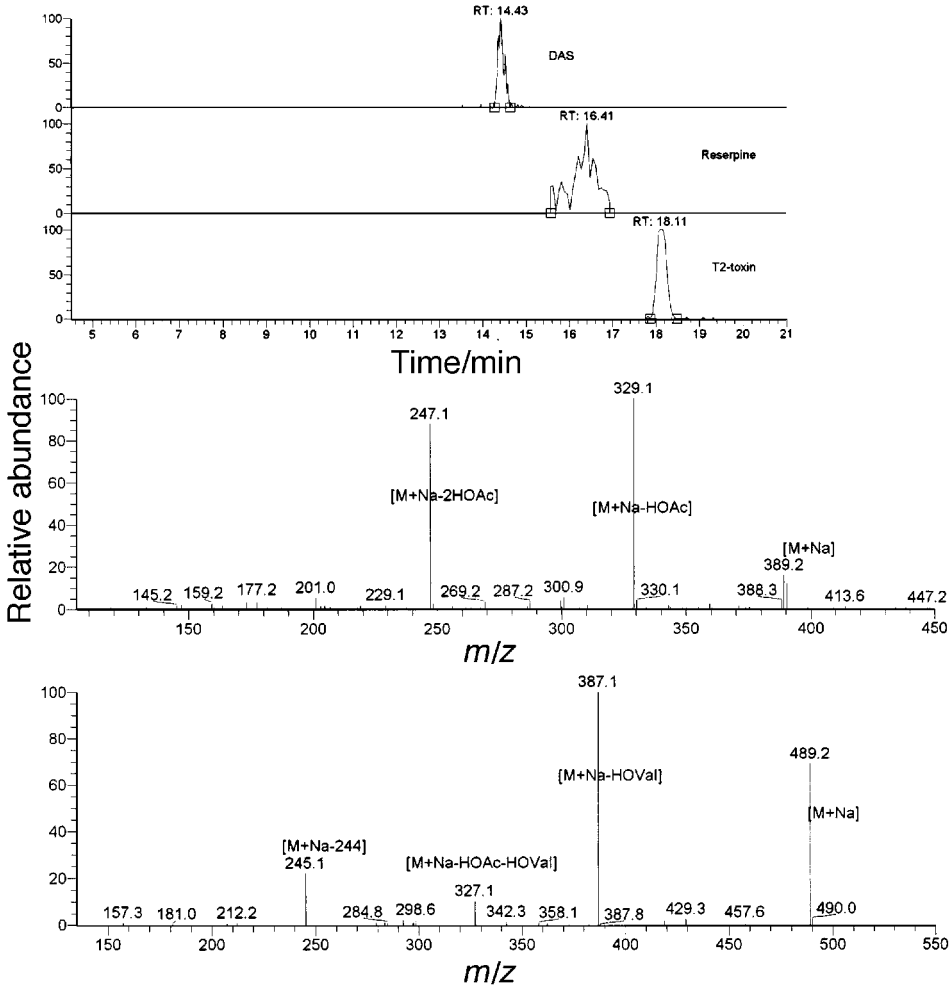


Fig. 4 HPLC–MS of a native sample from a water-damaged day-care centre containing DAS and T2-toxin, together with respective tandem mass spectra. The sample had been extracted from paper-covered gypsum board contaminated with a toxin-producing mould, identified as *Stachybotrys* spp., by the City of Vantaa Environment Centre, Finland.

Table 1 Effect of ion source collision-induced dissociation (SCID) on the relative abundance of sodiated molecular ions, $[M+Na]^+$, sodiated dimers, $[2M+Na]^+$, and sodiated trimers, $[3M+Na]^+$, and on the height of the sodiated molecular ion chromatographic peaks

			Relative abundance (%)			
SCID (%)	Toxin	Concentration/ ng μl^{-1}	$[M+Na]^+$	$[2M+Na]^+$	$[3M+Na]^+$	Peak height‡
0	T2-tetraol*	20	69	100	14,5	216878
10			100	87	5	240905
0	Verrucarol*	20	100	29	—	226472
10			100	27	—	250751
0	DAS*	12.5	100	13	—	8977203
10			100	8	—	12009129
0	T2-triol*	20	100	92	5	2704228
10			100	67	1	2883992
0	SATG†	2.94	36	100	4	1462147
10			27	100	1	1929086
0	HT2*	20	100	16	—	4124367
10			100	6	—	4771604
0	SATH†	2.94	23	100	3	852797
10			20	100	—	1228475
0	VERA†	1.67	100	41	—	2809694
10			100	20	—	2573022
0	T2-toxin*	12.5	100	8	—	22611897
10			100	4	—	25834475
0	RDRA†	2.5	100	14	—	5662756
10			100	6	—	5605601

* Acquired by direct injection. † Subjected to HPLC separation owing to low concentration of standards. ‡ Height of integrated chromatographic peak.

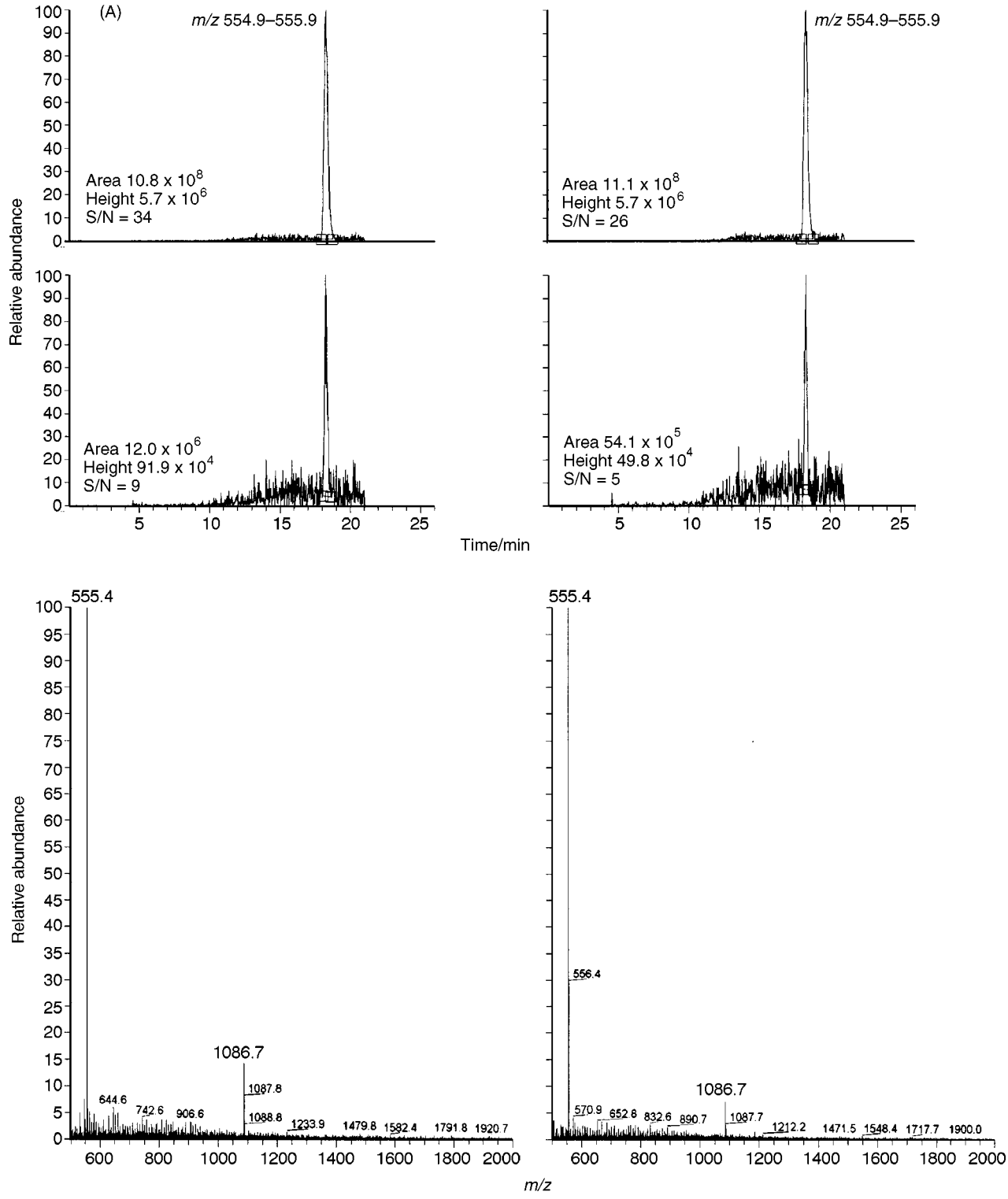


Fig. 5 Selected ion chromatograms and mass spectra of an RDRA standard, showing the effect of SCID on the abundance of the sodiated molecular ion (555.4 u) relative to the sodiated dimer (1086.7 u). A, SCID not applied; and B, SCID 10%.

Table 2 Molecular masses (M_r) and structures of the simple trichothecenes (R_1–R_4 as in Fig. 1)

Compound	M_r	R_1	R_2	R_3	R_4
T-2 tetraol	298.8	OH	OH	OH	OH
Verrucarol	266.3	H	OH	OH	H
Diacetoxyscirpenol (DAS)	366.4	OH	OAc*	OAc	H
T-2 triol	382.5	OH	OH	OH	OVal†
HT-2	424.5	OH	OH	OAc	OVal
T-2 toxin	466.5	OH	OAc	OAc	OVal

* OAc = $OCOCH_3$. † OVal = $OCOCH_2CH(CH_3)_2$.

mentioned product ions in the ESI tandem mass spectra of an unknown compound with characteristic toxic attributes would point towards the presence of a macrocyclic trichothecene.

Reproducibility, detection limits and recoveries of toxin analyses

The detection limits of the compounds varied from 1 to 1000 pg, with the more polar toxins having a higher detection limit than the less polar, macrocyclic toxins (Table 6). This is explained by differences in molecular mass and polarity, leading to varying yields in ESI and also in the impulse of the electron multiplier. Furthermore, differences in the chromatographic behaviour of

Table 3 Parent ions (sodiated molecular ions, depicted as M_{Na}) and fragment ions in the positive-ion ESI-CID tandem mass spectra of the simple trichothecenes

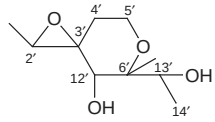
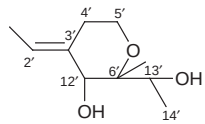
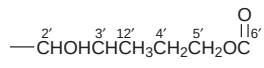
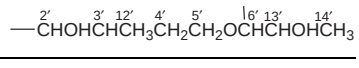
		Relative abundance (%) and *m/z*								
Compound	RCI* (%)	$[M_{Na}]^+$	$[M_{Na}-H_2O]^+$	$[M_{Na}-HOAc]^+$	$[M_{Na}-HOVal]^+$	$[M_{Na}-2HOAc]^+$	$[M_{Na}$-HOAc−HOVal$]^+$	$[M_{Na}-2HOAc-HOVal)]^+$	$[M_{Na}-30]^+$	Other major fragments
T−2 tetraol	15.8	21.8 (*m/z* 321.1)	27.7 (*m/z* 303.1)	—	—	—	—	—	100 (*m/z* 291.1)	68.6 (*m/z* 261.0 = $[M_{Na}-60]$) 92.2 (*m/z* 263.1 = $[M_{Na}-58]$) 13.5 (*m/z* 231.0 = $[M_{Na}-90]$)
Verrucarol	14.6	100 (*m/z* 289.1)	18.6 (*m/z* 271..0)	—	—	—	—	—	25.2 (*m/z* 258.9)	61.5 (*m/z* 245.1 = $[M_{Na}-44]$) 7.9 (*m/z* 231.4 = $[M_{Na}-57.7]$)
DAS	19.0	23.6 (*m/z* 389.2)	—	100 (*m/z* 329.1)	—	65.8 (*m/z* 247.1)	—	—	—	9.8 (*m/z* 269.1 = $[M_{Na}-120.1]$) 5.7 (*m/z* 287.2 = $[M_{Na}-102.0]$) 5.6 (*m/z* 201.2 = $[M_{Na}-188.0]$)
T−2 triol	19.0	24.2 (*m/z* 405.3)	—	—	100 (*m/z* 303.1)	—	—	—	4.3 (*m/z* 375.1)	43.7 (*m/z* 334.4 = $[M_{Na}-70.7]$)
HT−2	17.7	12.4 (*m/z* 447.2)	—	9.5 (*m/z* 387.1)	100 (*m/z* 345.1)	—	9.6 (*m/z* 285.1)	—	—	—
T−2 toxin	19.7	12.9 (*m/z* 489.1)	—	3.1 (*m/z* 429.2)	100 (*m/z* 387.1)	—	16.5 (*m/z* 327.3)	2.7 (*m/z* 267.3)	—	21.5 (*m/z* 245.3 = $[M_{Na}-243.8]$)

* Relative collision intensity in ion trap (the ion source was operated at a level of 10% collision−induced dissociation).

these compounds affected the detection limits, with broader peaks yielding lower detection limits.

For semi-quantitative purposes, calibration curves with acceptable correlation between concentration and impulse could be constructed for all compounds but, owing to the nature of the ion trap, caution should be exercised when analysing samples outside the concentration range of the calibration curves.

Table 4 Molecular masses (M_r) and structures of the macrocyclic trichothecenes (R as in Fig. 2)

Compound	M_r	R
Satratoxin G (SATG)	544.2	
Satratoxin H (SATH)	528.2	
Verrucarin A (VERA)	502.2	
Roridin A (RDRA)	532.6	

Conclusion

Quadrupole ion trap tandem mass spectrometry with positive electrospray ionization is well suited for the detection and quantification of trichothecene mycotoxins, separated by reversed phase HPLC. ESI is a very soft ionization method well suited for the present study, with the benefits including low flow rates and hence good detection limits due to lesser dilution of analyte fronts. The accuracy of quantitative analysis is, however, limited by the characteristics of the ion trap. More precise measurements would also require labelled internal standards of all toxins, which at present are not commercially available.

The sample pre-treatment and the HPLC separation were made difficult by the wide polarity range of the analyte compounds. Particularly the most polar compound, T2-tetraol, was sensitive to the polarity of the solid-phase sorbent used. All compounds could, however, be purified and determined simultaneously, with only a few, not very labour- and time-consuming, treatment steps.

Interestingly, there were similarities not only in the CID spectra of the simple trichothecenes but also in the spectra of the macrocyclic trichothecenes. A significant new finding was the

Table 5 Parent ions (sodiated molecular ions, depicted as M_{Na}) and fragment ions in the positive-ion ESI CID-MS/MS spectra of the macrocyclic trichothecenes

		Relative abundance (%) and *m/z*									
Compound	RCI* (%)	$[M_{Na}]^+$	$[M_{Na}-H_2O]^+$	$[M_{Na}-28]^+$	$[M_{Na}-44]^+$	$[M_{Na}-74]^+$	$[M_{Na}-230]^+$	$[M_{Na}-248]^+$	$[M_{Na}-276]^+$	$[M_{Na}-304]^+$	*m/z* 231.1
SATG	24.0	0.8 (*m/z* 567.3)	0.8 (*m/z* 549.2)	2.3 (*m/z* 538.9)	28.3 (*m/z* 523.2)	100 (*m/z* 493.2)	4.9 (*m/z* 337.0)	1.8 (*m/z* 319.0)	2.2 (*m/z* 293.1)	22.1 (*m/z* 263.1)	26.7
SATH	25.0	2.1 (*m/z* 551.6)	16.5 (*m/z* 533.3)	66.1 (*m/z* 523.1)	29.8 (*m/z* 507.2)	—	29.8 (*m/z* 321.1)	100 (*m/z* 303.0)	6.4 (*m/z* 275.0)	2.0 (*m/z* 247.1)	2.3
VERA	20.8	27.8 (*m/z* 525.2)	6.2 (*m/z* 507.2)	44.4 (*m/z* 497.1)	—	—	100 (*m/z* 295.1)	2.9 (*m/z* 277.0)	36.2 (*m/z* 249.0)	—	23.4
RDRA	25.0	0.6 (*m/z* 555.3)	0.6 (*m/z* 537.2)	100 (*m/z* 527.2)	6.9 (*m/z* 511.2)	—	62.8 (*m/z* 325.1)	0.7 (*m/z* 307.1)	33.1 (*m/z* 279.1)	—	27.2

* Relative collision intensity in ion trap (the ion source was operated at a level of 10% collision-induced dissociation).

Table 6 Linear regression analysis data (95% confidence interval), reproducibilities, detection limits and recoveries for the quantification of the toxins. Reserpine (71.25 ng ml^{-1}) was used as an internal standard. The number of data points in toxin measurements was 12

Compound	Parent ion	Ions in MRM*	Concentration determined/ng ml^{-1}	Regression line†	Pearson correlation coefficient	Standard error for the *y*-estimate	Detection limit range/pg	Recovery‡ (%)
T2–tetraol	321.1	260.5–263.5; 291.1	5–500	$y = 53.85x + 5.20$	0.9785	13.96	1000	31
Verrucarol	289.1	245.1; 259.1; 274.5–276.5	5–500	$y = 114.1x + 4.8$	0.9634	18.14	1000	46
DAS	389.2	247.3; 329.1	25–500	$y = 1.396x - 4.224$	0.92077	10.89	100	52
T2–triol	405.3	303.1; 333.9	25–500	$y = 109.8x + 19.8$	0.8718	20.82	100	63
SATG	567.3	492.0–493.5; 523.2	0.62–7.35	$y = 0.3818x + 0.2823$	0.9589	0.2612	10	88
HT2	447.2	285.1; 345.1	25–500	$y = 2.338x + 4.085$	0.9110	17.53	10	76
SATH	551.5	468.1; 523.1	0.62–7.35	$y = 0.4884x + 0.0127$	0.9792	0.1865	10	39
VERA	525.2	295.1; 494.0–498.0	0.19–1.88	$y = 1.090x + 0.0497$	0.8933	0.07040	1	47
T2–toxin	489.1	245.1; 327.1; 387.1	25–500	$y = 0.1502x - 7.4770$	0.8498	22.40	1	82
RDRA	555.3	279.1; 325.1; 527.2	0.19–1.88	$y = 0.01526x + 0.06368$	0.9661	0.04045	1	92
Reserpine	609.3	609.3; 397.3; 448.4	14.25	—	—	—	0.1	76

* Multiple reaction monitoring. † y = concentration of HPLC vials in ng μl^{-1}; x = area of toxin peak/area of reserpine peak. ‡ Mean of the recovery of the four strongest standards.

dominant proportion of the product ion $[M - 230]^+$ with accompanying neutral losses of water and ethanol in all the spectra of all macrocyclic trichothecenes, the presence of which in the ESI tandem mass spectra of an unknown compound with toxic attributes would suggest a macrocyclic trichothecene. If sufficient amounts of toxins are available, this can be verified through multiple MS experiments.

With the present methodology we shall, in the near future, conduct a wide microbiological and toxicological investigation of contaminated buildings.

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