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Determination of macrocyclic trichothecenes in mouldy indoor materials by LC-MS/MS

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Abstract

Stachybotrys occurring in mouldy indoor environments is associated with the so called "sick building syndrome" in humans or cases of idiopathic pulmonary hemorrhages. Samples of mouldy materials from indoor environments (n=15) were analysed for the occurrence of this fungus and its secondary metabolites by a sensitive LC-MS/MS method. In four samples, *Stachybotrys* and macrocyclic trichothecenes have been detected. Maximum values for Satratoxin G and H in wallpaper were determined with 9.7 µg/cm² and 12.0 µg/cm², respectively.

Keywords: macrocyclic trichothecenes, *Stachybotrys,* Satratoxin

Introduction

Macrocyclic trichothecenes produced by *Stachybotrys* species are highly toxic compounds associated with the so called "sick building syndrome" in humans. As several case control studies have shown, the occurrence of *Stachybotrys* in indoor environments could be related to headaches, fatigue, nausea, vomiting, hemorrhages, depression, sleep disturbances, anxiety, vertigo, and amnesia. Also cases of pulmonary hemosiderosis in infants have been related to *Stachybotrys* growth (1, 2). In 1938, this mould was first identified as etiologic agent of stachybotryotoxicosis, a severe disease of horses, which has been caused by ingestion of mouldy straw. Later, macrocyclic trichothecenes were supposed to be the reason of this disease (3). Because of its high cellulolytic potential, *S. chartarum*, which is mainly responsible for toxin production, also grows on humid cellulose-containing materials like wallpaper or gypsum board, *e.g.* after water damages (4). In this study a sensitive LC-MS/MS method has been developed to determine Satratoxins and other macrocyclic trichothecenes in these matrices.

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Materials and Methods

Materials

15 samples were taken from mouldy indoor materials like wallpaper (n=3), wall (n=10) or gypsum board (n=2). Standard substances of Roridin A and Verrucarin A were purchased from Sigma (Deisenhofen, Germany). Standards of Satratoxin G and H were kindly provided by Prof. Gareis (BfEL Kulmbach, Germany). Acetonitrile and methanol (HPLC-grade) were purchased from J.T. Baker (Griesheim, Germany). Water was purified by a milli-Q water purification system (Millipore GmbH, Schwalbach, Germany).

Mycological examination

For mould differentiation, smears of the mouldy area were plated on Sabouraud agar and incubated overnight at 37 °C. After an incubation time of one week at room temperature, micro-morphological characterisation was carried out by tape preparations and staining with lactophenol blue solution at 1000-fold magnification.

Sample preparation and recovery experiments

About 15 cm² wallpaper, wall or gypsum board was reduced to small pieces and extracted with 10 ml acetonitrile/water 84/16 (v/v) by shaking for 1 h using a horizontal shaker. The extract was centrifuged for 5 min at 5000 rpm. For analysis, an aliquot was diluted 1:5 with water and filtered through a 0.45 µm PTFE syringe filter (Supelco, Deisenhofen, Germany). For

testing the recovery, extracts of non-contaminated wallpaper were spiked with Satratoxin G and H at a level of 1 µg/cm² (n=3).

LC-MS/MS parameters

The LC-MS/MS system consisted of a HPLC apparatus Series 200 (Perkin Elmer, Rodgau-Jügesheim, Germany) with two pumps, a degasser, an autosampler and a column oven as well as an API 3200 triple quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany). As analytical column a Gemini 150x2 mm, 5 µm (Phenomenex, Aschaffenburg, Germany) was used. The oven temperature was set at 30 °C. The binary linear gradient consisted of eluent A (deionized water +5 mM ammonium formate) and eluent B (methanol+5 mM ammonium formate) with a flow rate of 400 µl/min: 0 min 10% B, 26 min 100% B, 30 min 100% B, 35 min 10% B.

Table 1. MRM-transitions, declustering potentials (DP) and collision energies (CE) for 8 macrocyclic trichothecenes

	Precursor Ion $[M+NH_4]^+$		Fragment Ions				Retention time [min]
Analyte	Q1	DP [V]	Q3 (1)	CE [eV]	Q3 (2)	CE [eV]	
Roridin A	550.4	26	249.1	23	133.0	41	17.75
Roridin E *	532.4	31	231.1	21	249.1	25	19.90
Roridin L-2 *	548.3	31	249.1	21	231.1	21	15.38
Satratoxin F *	560.3	41	249.1	21	231.1	21	17.01
Satratoxin G	562.3	21	249.1	19	231.1	21	15.82
Satratoxin H	546.3	21	245.2	25	231.1	25	16.39
Verrucarin A	520.2	31	249.1	23	231.1	27	17.37
Verrucarin J *	502.4	30	231.1	25	249.1	25	19.58

* Putative mass transitions as no standard substances available

Table 2. Identified fungi from mouldy indoor materials

	Alternaria spp.	*Aspergillus spp.*	*Chaetomium spp.*	*Cladosporium spp.*	*Gliocladium spp.*	*Memnoniella spp.*	*Mucor spp.*	*Penicillium spp.*	*Stachybotrys spp.*	*Ulocladium spp.*
Sample										
Gypsum board 1		x						x	x	
Gypsum board 2		x						x		
Wall 1		x				x		x		
Wall 2								x		
Wall 3		x			x		x			
Wall 4				x				x		
Wall 5		x						x		x
Wall 6				x				x		
Wall 7							x	x		
Wall 8										
Wall 9										x
Wall 10		x								
Wallpaper 1	x		x					x	x	
Wallpaper 2								x	x	
Wallpaper 3		x							x	

The MS/MS experiments were carried out in positive electrospray ionisation mode with an ion spray voltage of 4000 V and a source temperature of 300 °C. The nebulizer gas flow was set at 50 psi and the heating gas at 30 psi. As there were no standards available for Roridin E, Roridin L-2, Satratoxin F and Verrucarin J, probable mass transitions were assumed for these toxins. All substances showed best sensitivity as adduct-ions of ammonium $[M+NH_4]^+$. The MRM-transitions, declustering potentials and collision energies used as well as the retention times of the toxins are shown in Table 1.

Results and Discussion

Mould characterisation

Stachybotrys and its toxins could be detected on three wallpaper samples and one gypsum board sample. Other moulds like *Aspergillus* and *Penicillium* were also detected frequently (Table 2). From one wall sample no mould growth could be observed.

LC-MS/MS analysis

Spiking experiments with Satratoxin G and H at a level of 1 µg/cm² (n=3) resulted in recovery rates of 92.9±0.2% (mean±RSD) for Satratoxin G and 107.4±2.1% for Satratoxin H.

All *Stachybotrys* positive samples (n=4) also contained macrocyclic trichothecenes. In the other samples none of the toxins analysed could be identified. From two wallpaper samples different pieces have been examined. The analysis gave varying results (Table 3). This indicates an unequal distribution of the toxins in the mouldy material. Satratoxin G and H showed maximum levels of 9.7 µg/cm² and 12.0 µg/cm², respectively. Furthermore, substances with defined retention times and fragmentation patterns corresponding to those supposed for Roridin E, Roridin L-2, Satratoxin F and Verrucarin J could be identified (Figure 1). A co-occurrence of these toxins with Satratoxin G and H has been described formerly by Andersen *et al.* (5). However, a definite identification by the given methods was not possible due to lacking standard substances. Roridin A and Verrucarin A were not detected.

It can be concluded that the occurrence of Satratoxins in such high amounts implicates a severe contamination of indoor environments. However, these toxins were determined in indoor materials and not in indoor air or dust. Thus, a direct exposure of humans to these toxins is not yet proven. More information about the possible health risk will be gained in a further study, examining dust and/or air from the locations where the samples have been drawn.

Table 3. Results of LC-MS/MS analysis for 8 macrocyclic trichothecenes (ng/cm²)

	Roridin A	Roridin E*	Roridin L-2*	Satratoxin F*	Satratoxin G	Satratoxin H	Verrucarin A	Verrucarin J*
Sample								
Gypsum board 1	-	-	-	-	18	22	-	-
Wallpaper 1a**	-	-	-	-	27	20	-	-
Wallpaper 1b**	-	+	-	-	740	740	-	-
Wallpaper 2a**	-	+++	-	-	1560	3260	-	-
Wallpaper 2b**	-	++	++	+	9650	12000	-	++
Wallpaper 3	-	+++	++	++	260	360	-	++

* Quantitation not possible lacking standard substances ** a/b: Different spots of one sampled area

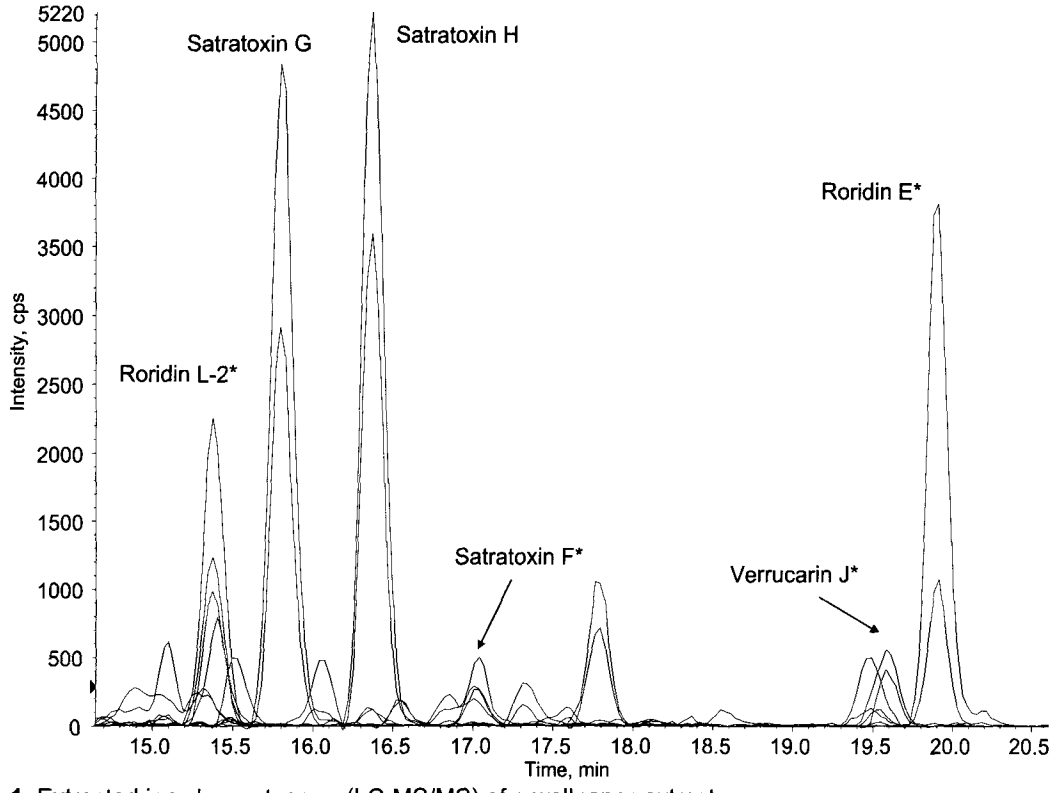

Figure 1. Extracted ion chromatogram (LC-MS/MS) of a wallpaper-extract

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