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### ► To cite this version:

Sylvia Maria Lehner, Nora Katharina Nicole Neumann, Michael Sulyok, Marc Lemmens, Rudolf Krska, et al.. Evaluation of LC - high-resolution FT - Orbitrap MS for the quantification of selected mycotoxins and the simultaneous screening of fungal metabolites in food. Food Additives and Contaminants, 2011, pp.1. 10.1080/19440049.2011.599340 . hal-00724621

**HAL Id: hal-00724621**

**<https://hal.science/hal-00724621>**

Submitted on 22 Aug 2012

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**Evaluation of LC – high-resolution FT – Orbitrap MS for the quantification of selected mycotoxins and the simultaneous screening of fungal metabolites in food**

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2011-193.R1
Manuscript Type:	Special Issue
Date Submitted by the Author:	16-Jun-2011
Complete List of Authors:	Lehner, Sylvia; University of Natural Resources and Life Sciences, Vienna, Department IFA-Tulln Neumann, Nora; University of Natural Resources and Life Sciences, Vienna, Department IFA-Tulln Sulyok, Michael; University of Natural Resources and Life Sciences, Vienna, Department IFA-Tulln Lemmens, Marc; University of Natural Resources and Life Sciences, Vienna, Department IFA-Tulln Krska, Rudolf; University of Natural Resources and Life Sciences, Vienna, Department IFA-Tulln Schuhmacher, Rainer; University of Natural Resources and Life Sciences, Vienna, Department IFA-Tulln
Methods/Techniques:	Chromatography - LC/MS, LC/MS
Additives/Contaminants:	Mycotoxins
Food Types:	
Abstract:	This study presents a liquid chromatography – high-resolution mass spectrometry based method for the quantification of 20 selected mycotoxins and the simultaneous screening for 200 fungal metabolites in food. For regulated mycotoxins, such as aflatoxins, fumonisins, ochratoxin A, zearalenone and trichothecenes, evaluation of the method performance characteristics precision, trueness, limit of detection and matrix effects have been exemplified for the matrix maize. In case of the limit of detection, an alternative evaluation approach for high resolution FT-Orbitrap data is proposed. Measurements of the signal to noise ratios obtained from “full profile mode” data led to detection limits between 8 and 160 ng g <sup>-1</sup> . Eight naturally contaminated, wheat- and maize-based matrix test materials, originating from interlaboratory comparison studies

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	were used to confirm trueness of the method for deoxynivalenol, zearalenone, fumonisin B1 and B2, HT-2 and T-2 toxin. In addition to accurate quantification of the most relevant mycotoxins, the obtained full scan chromatograms were used to investigate the potential of the FT-Orbitrap to screen simultaneously for a large number of fungal metabolites. First, a list of 200 metabolites, potentially being present in food samples has been established. Next, specific detection and identification criteria were defined, which were based on accurate mass, peak intensity and isotopologue ratio. Application of these criteria to the suspected metabolites from the list resulted in the putative identification of 13 fungal metabolites in addition to the target toxins.

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3 **Evaluation of LC – high-resolution FT – Orbitrap MS for the**  
4 **quantification of selected mycotoxins and the simultaneous screening**  
5 **of fungal metabolites in food**  
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22 A liquid chromatography – high-resolution mass spectrometry based method is  
23 reported for the quantification of 20 selected mycotoxins and the simultaneous  
24 screening for 200 fungal metabolites in food. For regulated mycotoxins, such as  
25 aflatoxins, fumonisins, ochratoxin A, zearalenone and trichothecenes, evaluation  
26 of the method performance characteristics such as precision, trueness, limit of  
27 detection and matrix effects have been exemplified for the matrix maize. In the  
28 case of the limit of detection, an alternative evaluation approach for high  
29 resolution FT-Orbitrap data is proposed. Measurements of the signal-to-noise  
30 ratios obtained from “full profile mode” data led to detection limits between 8  
31 and 160 ng g<sup>-1</sup>. Eight naturally contaminated, wheat- and maize-based matrix test  
32 materials, originating from interlaboratory comparison studies were used to  
33 confirm trueness of the method for deoxynivalenol, zearalenone, fumonisin B<sub>1</sub>  
34 and B<sub>2</sub>, HT-2 and T-2 toxin. In addition to accurate quantification of the most  
35 relevant mycotoxins, the full scan chromatograms were used to investigate the  
36 potential of the FT-Orbitrap to screen simultaneously for a large number of  
37 fungal metabolites. First, a list of 200 metabolites, potentially being present in  
38 food samples was established. Next, specific detection and identification criteria  
39 were defined, which are based on accurate mass, peak intensity and isotopologue  
40 ratio. Application of these criteria to the suspected metabolites from the list  
41 resulted in the putative identification of 13 fungal metabolites in addition to the  
42 target toxins.  
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55 **Keywords:** accurate mass, fungal metabolites, liquid chromatography, LC-MS,  
56 LOD, mycotoxins, FT-Orbitrap, high-resolution mass spectrometry  
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## Introduction

Mycotoxins are fungal secondary metabolites, produced by various mold species. By definition, they are toxic to vertebrates and other animal groups in low concentrations (Bennet and Klich, 2003). Mycotoxins can enter the food and feed chain by fungal infection of crop plants, either on the field or during storage. The use of contaminated raw cereals or processed commodities therefore constitute a threat to animal and human health. For this reason regulations or guidelines exist in approximately 100 countries (van Egmond et al., 2007).

The need for monitoring a huge number of regulated compounds in different matrices led to the development of multi-target methods for the simultaneous detection of several analytes in a single method (e.g. Sulyok et al., 2006; Mol et al., 2008). A wide range of physicochemical properties of the analytes makes simultaneous and adequate sample clean-up and complete HPLC separation for all target compounds impossible. Therefore, highly sensitive, selective and robust MS instruments that allow the injection of crude sample extracts with minimum or no clean-up are necessary. Triple-quadrupole (QqQ) instruments fulfill these requirements when operated in selected reaction monitoring (SRM) mode and are therefore well suited for quantitative target analysis. They show a high sensitivity and a wide linear range, but they also show limitations inherent to their targeted acquisition mode: the number of analytes is limited in one method, time-consuming optimization of acquisition parameters is needed for each compound (e.g. dwell time, collision energy, acquisition time window) in order to achieve maximum sensitivity, and retrospective data analysis is not possible. Operated in full scan mode, QqQ instruments produce unit resolution spectra and show low sensitivity, limiting their capabilities for screening applications.

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3 New generations of high-resolution mass spectrometers, such as time-of-flight  
4 (TOF), fourier-transformation-ion-cyclotron resonance (FT-ICR) and FT-Orbitrap  
5 instruments are promising alternatives for the simultaneous analysis of multiple  
6 compounds. Their particular strength lies in their high mass resolving power and high  
7 mass accuracy. While TOF instruments show lower sensitivity and dynamic range than  
8 QqQ instruments in SRM mode, FT-ICR instruments have been rarely used due to their  
9 high costs, their slow scan time (1-3 seconds) and their elaborate mode of operation.  
10 FT-Orbitrap instruments offer a better dynamic range and a sensitivity close to that of  
11 many QqQ instruments (Krauss et al., 2010).  
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23 High-resolution mass spectra show high selectivity when generating extracted  
24 ion chromatograms (EICs) of the exact mass of the respective compound out of full scan  
25 data with a narrow relative mass extraction window (typically  $\pm 5$  ppm). Hence, no pre-  
26 selection of compounds and time-consuming set-up of acquisition parameters is  
27 necessary. Retrospective analysis of full scan data makes it possible to assess virtually  
28 all compounds present in a sample. This makes liquid chromatography – high-resolution  
29 – mass spectrometry (LC-HR-MS) on FT-Orbitrap instruments particularly interesting  
30 for screening purposes (Krauss et al., 2010).  
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41 Guidelines for screening approaches for the monitoring of regulated substances  
42 exist (e.g. 2002/657/EC, SANCO/10684/2009). However, such qualitative screening  
43 methods usually aim to avoid only false-negative results since the outcome needs  
44 verification by an appropriate confirmatory method (for which standards are needed).  
45 Nielen et al. (2007) and Blokland et al. (2008) made suggestions for implementing HR-  
46 MS measurements for confirmatory analysis in 2002/657/EC.  
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53 According to Krauss et al. (2010), screening approaches on LC-HR-MS  
54 instruments can be separated into non-target screenings (in the search of unknowns) and  
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3 suspects screenings (where full scan data can be examined for a positive list of  
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5 compounds of interest). Both of these approaches aim to generate a list of highly likely  
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7 substances present in a sample without the availability of reference standards (which is  
8  
9 in contrast to the target analysis). To the best of the authors knowledge, no detailed  
10  
11 guidelines or requirements for non-target or suspects screening applications using LC-  
12  
13 HR-MS exist. Besides accurate mass (due to the high resolving power and mass  
14  
15 accuracy) with which (de-)protonated molecules or adducts can be searched for, criteria  
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17 such as the relative isotope abundance (RIA) of naturally occurring isotopic ions ( $^{13}\text{C}$ )  
18  
19 can be included for increasing the confidence in the presence of suspects (conformance  
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21 with theoretical isotopic pattern). Xu et al. (2010) evaluated the accuracy with which  
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23 isotope patterns can be determined on FT-Orbitrap instruments. For obtaining structural  
24  
25 information, it is possible to include data dependant MS/MS (e.g. of the most intense  
26  
27 ion of a full scan) to facilitate retrospective analysis as well as confirmation of the  
28  
29 detected substances.  
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34 LC-HR-MS using FT-Orbitrap instruments in full scan mode has been  
35  
36 successfully used for the (semi-)quantitative determination of e.g. small molecules in  
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38 biological samples (Zhang et al., 2009), veterinary drugs in food matrices (Kaufmann et  
39  
40 al., 2010), hormone and veterinary drug residue analysis (van der Heeft et al., 2009),  
41  
42 residue analysis in food and feed (Kellmann et al., 2009), mycotoxin analysis in maize,  
43  
44 wheat (Herebian et al., 2009) and barley (Zachariasova et al., 2010a) and mycotoxin  
45  
46 analysis in beer (Zachariasova et al., 2010b). However, certain methodical aspects when  
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48 dealing with HR-MS data remain unanswered or are to be further discussed in the  
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50 scientific community.  
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54 One of these aspects is how to estimate the limit of detection (LOD) for HR-MS  
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56 data. In literature, values for the LOD are often given as the concentration level at a  
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3 signal to noise ratio (S/N) of 3 (e.g. Herebian et al., 2009). This method for the  
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5 estimation of LODs is frequently used, regardless of the fact, that with narrow relative  
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7 mass extraction windows (e.g.  $\pm 5$  ppm) the corresponding extracted ion chromatograms  
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9 (EICs) usually do not show any noise, due to the high mass resolving power of these  
10  
11 instruments. Owing to this fact, other approaches have been suggested, such as the  
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13 lowest calibration level (LCL, Zachariasova et al., 2010b) which is “the lowest  
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15 concentrations of matrix-matched standards which it was possible to repeatedly  
16  
17 determine during a longer time period” and alternative approaches for the calculation of  
18  
19 detection limits (Kaufmann, 2009). While the LCL is defined as the lowest analyte  
20  
21 concentration of a calibration (SANCO/10684/2009) but has no definition regarding its  
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23 use as performance characteristic (LOD) of an analytical method, calculation  
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25 approaches are prone to errors caused by model assumptions. So far no standardised  
26  
27 procedure for the determination of the LOD in HR-MS has been established and is  
28  
29 accepted throughout the scientific community. In our work we present the successful  
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31 application of an alternative approach for the estimation of LOD values which is based  
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33 on “full profile mode” LC-MS chromatograms that can be used in case of FT-Orbitrap  
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35 instruments.  
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41 Our main focus was to explore the capabilities of an LTQ Orbitrap XL system  
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43 for the establishment of an LC-HR-MS method for the quantitative analysis of the most  
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45 relevant mycotoxins in food samples in combination with a first explorative approach  
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47 for the simultaneous screening of a large number of fungal metabolites potentially being  
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49 present in food. The extraction procedure and LC method is based on previous work of  
50  
51 our group in this field (Sulyok et al., 2006). After generating fit for purpose calibration  
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53 functions, we evaluated the limits of detection in pure solvents and in presence of the  
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55 matrix maize (including critical assessment regarding European regulations) and matrix  
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3 effects for maize. Precision and trueness were assessed from spiking experiments as  
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5 well as the measurement of matrix test materials. A preliminary approach for exploring  
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7 the general applicability of HR-MS using an LTQ Orbitrap XL for a suspects screening  
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9 for 200 fungal secondary metabolites in food samples was conducted. Therefore,  
10  
11 specific detection and identification criteria were defined and applied to the HR-full  
12  
13 scan chromatograms.  
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## 15 16 17 18 **Materials and methods**

### 19 20 21 *Chemicals and reagents*

22  
23 Methanol (MeOH, LiChrosolv, LC gradient grade) and glacial acetic acid (HAc) were  
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25 purchased from Merck (Darmstadt, Germany); acetonitrile (ACN, HiPerSolv  
26  
27 Chromanorm, HPLC gradient grade) was purchased from VWR (Vienna, Austria);  
28  
29 formic acid (FA, MS grade) was obtained from Sigma-Aldrich (Vienna, Austria). Water  
30  
31 was purified successively by reverse osmosis and an ELGA Purelab Ultra-AN-MK2  
32  
33 system (Veolia Water, Vienna, Austria). Mycotoxin standards were purchased from  
34  
35 different sources and were dissolved in ACN if not stated otherwise. Stock solutions of  
36  
37 3-acetyldeoxynivalenol (3ADON), aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, M<sub>1</sub> (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>,  
38  
39 AFG<sub>2</sub>, AFM<sub>1</sub>), deoxynivalenol (DON), fumonisins B<sub>1</sub> and B<sub>2</sub> (FB<sub>1</sub>, FB<sub>2</sub>, in ACN:water  
40  
41 1:1, v/v), HT-2 toxin (HT-2), ochratoxin A (OTA), T-2 toxin (T-2) and zearalenone  
42  
43 (ZON) were obtained from Biopure Referenzsubstanzen GmbH (Tulln, Austria).  
44  
45 Alternariol, mycophenolic acid (MPA) and tentoxin were purchased from Sigma-  
46  
47 Aldrich (Vienna, Austria). Roquefortine C (RFC) was purchased from Iris Biotech  
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49 GmbH (Marktredwitz, Germany).  $\alpha$ -Ergocryptine was obtained from Dr. Miroslav  
50  
51 Flieger (Academy of Sciences of the Czech Republic, Prague). A stock solution of  
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53 enniatin B and B<sub>1</sub> (EnnB, EnnB<sub>1</sub>) was provided as a gift by Dr. Marika Jestoi (National  
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3 Veterinary and Food Research Institute, Finland). All standards were stored at -20 °C,  
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5 except for FB<sub>1</sub> and FB<sub>2</sub>, which were stored at 4°C.  
6

7 For external calibration, a multi-analyte stock solution of the 20 mycotoxins  
8  
9 with concentrations ranging from 100 – 5351 ng g<sup>-1</sup> was prepared freshly prior to  
10  
11 analysis. The stock solutions were diluted with ACN:water 1:1 (v/v) 1:10 to 1:1000  
12  
13 over various levels, ranging from 1 – 535 ng g<sup>-1</sup>. Since samples are diluted by a factor of  
14  
15 eight during sample preparation (extraction and dilution of raw extract) this corresponds  
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17 to a concentration range of 8 – 4281 ng g<sup>-1</sup> in food samples.  
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### 20 21 22 ***Sample preparation*** 23

24 Sample extraction and further preparation was based on a recently described procedure  
25  
26 that allows efficient extraction of fungal metabolites in food samples from various  
27  
28 matrices (Sulyok et al., 2006). 20 ml extraction solvent (ACN:water:HAc, 79:20:1  
29  
30 (v/v/v)) were added to 5 g of ground sample. The sample was extracted for 90 min at  
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32 170 rpm using a GFL 3017 rotary shaker (GFL, Burgwedel, Germany) and then left for  
33  
34 5 min to allow sedimentation of the solids. An aliquot of 350 µl of the supernatant was  
35  
36 diluted with the same volume of a mixture consisting of ACN:water:HAc, 20:79:1  
37  
38 (v/v/v) and homogenized using a vortex mixer (Janke+Kunkel IKA Labortechnik VF2,  
39  
40 Müller-Scherr, Vienna, Austria). 5 µl of the diluted extract were injected into the LC-  
41  
42 MS system. The final concentration of sample equivalent in the extract was  
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44 0.125 g ml<sup>-1</sup>, corresponding to 0.625 mg of sample injected into the LC-MS system.  
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### 50 51 ***LC-MS analysis*** 52

53 The chromatographic separation of the analytes was carried out using an HPLC system  
54  
55 (Accela, Thermo Fisher Scientific, San Jose, CA, USA) equipped with a reversed-phase  
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57 Gemini C<sub>18</sub> analytical column, 150 x 2.0 mm i.d., 5 µm particle size, equipped with a  
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3 C<sub>18</sub> 4 x 2 mm i.d. security cartridge (all from Phenomenex, Torrance, CA, USA). The  
4  
5 column temperature was maintained at 20 °C. Eluent A was water, eluent B was MeOH,  
6  
7 both containing 0.1 % FA. The chromatographic method held the initial mobile phase  
8  
9 composition (90 % A) constant for 2 min, followed by a linear gradient to 100 % B in  
10  
11 12 min. This final condition was held for 4 min, followed by 5 min column  
12  
13 re-equilibration at 90 % A. The flow rate was 350 µl min<sup>-1</sup>.  
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15

16 The HPLC system was coupled to an LTQ Orbitrap XL (Thermo Fisher  
17  
18 Scientific, San Jose, CA, USA) equipped with an electrospray ionization (ESI) interface  
19  
20 which was operated in positive ionization mode using the following settings:  
21  
22 electrospray voltage: 4 kV, sheath gas: 40 arbitrary units, auxiliary gas: 5 arbitrary units,  
23  
24 capillary temperature 350°C. All other source parameters were automatically tuned for a  
25  
26 maximum MS signal intensity of reserpine (Sigma Aldrich (Vienna, Austria)) solution  
27  
28 (10 mg L<sup>-1</sup>). To this end, 10 µl min<sup>-1</sup> of reserpine solution (dissolved in ACN:water =  
29  
30 8:2 (v/v)) were infused via syringe pump into mobile phase (Eluent A:B, 1:1) of a flow  
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32 rate of 350 µl min<sup>-1</sup>.  
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36 For the FT-Orbitrap, the automatic gain control was set to a target value of  
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38 5\*10<sup>5</sup> and a maximum injection time of 500 ms was chosen. The mass spectrometer was  
39  
40 used with a resolving power setting of 60,000 FWHM (at *m/z* 400) and a scan range of  
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42 *m/z* 100-1000. Data was generated using Xcalibur 2.1.0 (Thermo Fisher Scientific, San  
43  
44 Jose, CA, USA).  
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46

47 Mass calibration of the LTQ Orbitrap XL system was done using MSCAL5  
48  
49 ProteoMass™ LTQ/FT-Hybrid ESI Pos. Mode CalMix (Sigma Aldrich, Vienna,  
50  
51 Austria) at an interval of maximum one week. Mass calibration was checked prior to  
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53 measurements with common background ions (Keller et al., 2008). In case of a relative  
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55 mass deviation ≥ 3 ppm, mass calibration was carried out using Thermo TunePlus  
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3 software (Version 2.5.5. SP1, Thermo Fisher Scientific, San Jose, CA, USA). With  
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5 external calibration, 95 % of the data showed less than 3 ppm relative mass deviation at  
6  
7 the peak apex (most intense scan of chromatographic peak; data not shown). Less  
8  
9 intense scans at the beginning and end of a chromatographic peak occasionally showed  
10  
11 slightly higher mass deviations. Hence, for generating extracted ion chromatograms  
12  
13 (EICs) a relative mass extraction window size of exact mass  $\pm$  5 ppm was applied  
14  
15 during data analysis, resulting in reasonable chromatographic peak shapes.  
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### 20 ***Data analysis***

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22 Data processing was done using Xcalibur 2.1.0 QualBrowser, QuanBrowser (using  
23  
24 Genesis peak detection algorithm, relative mass extraction window:  $\pm$  5 ppm), and the  
25  
26 validation software Validata, a Microsoft Excel macro developed by Wegscheider et al.  
27  
28 (1999). For all analytes the protonated molecules (exceptions: HT-2 and T-2 toxins:  
29  
30 ammonium adducts) were used for data evaluation.  
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### 35 ***Estimation of method performance characteristics***

#### 36 ***Spiking experiments***

37  
38 For the evaluation of matrix-induced signal suppression/enhancement (SSE), the  
39  
40 method precision and the determination of the limit of detection in presence of matrix  
41  
42 ( $LOD_{matrix}$ ), maize was used as model matrix. Non-contaminated maize was extracted  
43  
44 and diluted as described under sample preparation. Subsequently, to 900  $\mu$ l of diluted  
45  
46 blank maize extract, 100  $\mu$ l of the multi-analyte stock solution was added to obtain the  
47  
48 highest spiking level. Further dilution with diluted blank maize extract was carried out  
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50 to achieve nine defined concentration levels of the toxins (covering the same range as  
51  
52 for the external calibration) without diluting the matrix more than 10 %. Blank maize  
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3 was extracted, diluted and spiked at three different days for obtaining three independent  
4 data sets. These were used for estimation of matrix effects and of the overall precision  
5 of the method.  
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#### 9 10 11 *Determination of the limit of detection*

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13 For estimation of the limit of detection (LOD) two different approaches were applied.  
14 One common approach, according to Harris (2006), is to assume that the standard  
15 deviation of the signal of an analyte in a sample at a concentration level close to its  
16 detection limit is similar to the standard deviation of the analyte signal in a blank. For  
17 estimation of the LOD according to this approach, linear calibration curves were  
18 generated at low concentrations for which linearity of the detector response was  
19 assumed. The slopes ( $m$ ) of linear regression lines and the standard deviations ( $s$ ) of ten  
20 replicate measurements were used for the estimations of the LOD according to Equation  
21 (1).  
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$$33 \quad LOD = \frac{3s}{m} \quad (1)$$

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37 As alternative approach, the concentration at which a signal to noise ratio (S/N) of 3 is  
38 achieved when acquiring “full profile mode” data, was determined.  
39

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41 From results obtained for low concentrations of standard solutions in pure  
42 solvents, the instrument detection limit ( $LOD_{\text{solvent}}$ ) was estimated. The LOD in  
43 presence of the matrix maize ( $LOD_{\text{matrix}}$ ) was estimated by measuring spiked, diluted  
44 extracts of blank maize. LOD values obtained were multiplied by a factor of eight in  
45 order to obtain LODs in  $\text{ng g}^{-1}$  sample.  
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#### 52 53 54 *Evaluation of trueness*

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56 For evaluating the trueness of the method, several well defined matrix test materials  
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3 originating from interlaboratory comparison studies were used. Wheat flour originated  
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5 from Biopure Referenzsubstanzen GmbH (Tulln, Austria), two quality control test  
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7 materials (maize #1 and #2) originated from FAPAS (FERA, York, UK), and other  
8  
9 proficiency testing materials (maize #3, breakfast cereal, wheat draff, grinded wheat and  
10  
11 animal feed) originated from Bipea (Gennevilliers, France). Table 1 gives the assigned  
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13 values ( $X$ ) and expanded uncertainty  $U_X$  (confidence level 95 %) of the test materials.  
14  
15 When available, the uncertainty  $u_x$  (confidence level 68 %) of the test materials has  
16  
17 been directly taken for obtaining  $U_X$ . Otherwise, it has been calculated from the robust  
18  
19 standard deviation  $s_x$  of the results of the participating laboratories, according to  
20  
21 equation (2), where  $n$  is the number of the participating laboratories of the  
22  
23 interlaboratory comparison studies.  
24  
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26

$$U_X = 2 * u_x = 2 * \frac{s_x}{\sqrt{n}} \quad (2)$$

### 27 28 29 30 31 ***Suspects screening***

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33 In order to exploit the possibilities HR-MS can offer for the screening of microbial  
34  
35 metabolites suspected to be present in the test materials, criteria were defined. These  
36  
37 criteria were applied to full scan data obtained after analysis of the test materials.  
38

39  
40 First, a positive list of 208 fungal metabolites was constructed including  
41  
42 molecular formula, CAS number (if available) and exact masses of ions likely to be  
43  
44 present in HR-mass spectra (see Supplement Table 1). Eight of those have a molecular  
45  
46 mass of  $< 100$  or  $> 1000 \text{ g mol}^{-1}$ , so they cannot be covered with the method applied.  
47  
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49  
50 Full scan LC-MS chromatograms were examined for peaks indicating the  
51  
52 presence of these “suspected” metabolites using the following criteria (automated  
53  
54 detection):  
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- 56  
57 (1) Presence of at least two ion species ( $[M+H]^+$  and/or  $[M+NH_4]^+$  and/or  $[M+Na]^+$ )  
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3 with a maximum relative mass deviation of  $\pm 3$  ppm of the exact mass of the  
4  
5 metabolites from the above mentioned positive list. This mass deviation was  
6  
7 chosen due to the accuracy that was determined prior to measurements (see  
8  
9 materials and methods section). Depending on the mass accuracy achieved, this  
10  
11 parameter needs to be adjusted.  
12

- 13
- 14 (2) For the most intense ion species a minimum intensity of 10,000 counts was  
15  
16 required.  
17
- 18 (3) Of the most intense ion species (I) the peak corresponding to the first  $^{13}\text{C}$   
19  
20 isotopologue had to be present (maximum relative mass deviation  $\pm 5$  ppm).  
21  
22 This mass tolerance was chosen to account for the low intensities of the  
23  
24 isotopologue ion species, which can lead to slightly higher mass deviations. The  
25  
26 ratio of the measured intensity of the I+1 ion to the calculated (theoretically  
27  
28 expected) intensity  $[\text{Int}(\text{I}+1)_{\text{meas}}]/[\text{Int}(\text{I}+1)_{\text{calc}}]$  had to be 0.65-1.05. This tolerance  
29  
30 was chosen in order to take the accuracy with which relative isotope abundances  
31  
32 can be determined on FT-Orbitrap instruments (Xu et al., 2010) into account.  
33  
34
- 35 (4) Criteria (1) – (3) had to be fulfilled in at least 5 scans within a period of 25  
36  
37 seconds to be considered “putatively identified”.  
38  
39  
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41 For the automated data evaluation of these criteria for full scan FT-Orbitrap data  
42  
43 a python script was implemented (Neumann et al., not published). Finally, EICs of  
44  
45 putatively detected suspects were evaluated manually to ensure reasonable  
46  
47 chromatographic peak shapes.  
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## Results and discussion

### *Chromatographic separation of the analytes*

In Figure 1 a typical chromatogram for the separation and detection of 20 selected mycotoxins is shown. For all analytes the protonated molecules (exceptions: HT-2 and T-2: ammonium adducts) were used for generating EICs. No ammonia was added to the eluents, but is most likely present in the solvents or from previous experiments (Berger et al., 1999) or formed in the source by redox processes in the electrospray (Nielsen and Smeesgaard, 2003). Typical chromatographic peak widths of 15 – 25 seconds lead to approximately 15 – 25 spectra across one peak. The most polar metabolites (DON, 3ADON) show broader peaks. This is caused by the injection of 5  $\mu\text{l}$  of the diluted sample extract (containing 50 % ACN in water) into an HPLC-flow (mobile phase consisting of 10 % ACN in water) of 350  $\mu\text{l min}^{-1}$ .

### *Generation of calibration curves*

For generating external standard calibration curves, nine concentration levels were measured, ten times each (exception: Fumonisin: five to ten times each). Using linear calibration over the whole concentration range, highly negative intercepts (y axis) were observed. Additionally, sensitivity (slopes) increased with increasing analyte concentration. Therefore, quadratic fitted curves were used to describe the detector response as a function of toxin concentrations. This is in agreement with the findings of Kellmann et al. (2009) who made similar observations when using FT-Orbitrap in full scan mode for the determination of residues and contaminants in honey and animal feed. Also, signal intensities showed higher absolute standard deviations at high analyte concentrations (heteroscedasticity). Kaufmann et al. (2010), who developed a method for the quantification of veterinary drugs in different food matrices, made similar



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3 observations and also used quadratic regression. Table 2 gives the calibration ranges  
4  
5 and precision estimates obtained for standards in pure solvents.  
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### 8 9 *Determination of the limit of detection*

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11 The limit of detection (LOD) is usually defined as the lowest analyte concentration at  
12  
13 which the analyte signal can be reliably differentiated from the blank signal. It is a  
14  
15 parameter to prevent the detection of false positives.  
16  
17

18  
19 Applying the approach according to Harris (2006) lead to LODs at which often  
20  
21 only a few (0-3) data points could be found across the chromatographic peaks of the  
22  
23 corresponding EICs. Therefore, this approach resulted in estimated LODs that were too  
24  
25 optimistic and cannot be used for the reliable estimation of the detection limits (data not  
26  
27 shown).  
28

29  
30 Hence, we suggest an alternative approach for the estimation of LOD when  
31  
32 using FT-Orbitrap in full scan mode. The most straightforward way to estimate the LOD  
33  
34 is to determine the concentration at which a signal-to-noise ratio (S/N) of 3 is achieved.  
35  
36 When working with high-resolution mass spectrometers, such as TOF, FT-ICR or FT-  
37  
38 Orbitrap instruments, usually no noise is observed, owing to the high mass resolving  
39  
40 power of these instruments. EICs generated with relative mass extraction window sizes  
41  
42 of  $\pm 5$  ppm are highly selective for the molecule of interest. Only in exceptional cases  
43  
44 background signals with similar  $m/z$  ratios cause the presence of detectable noise in  
45  
46 EICs generated out of full scan data.  
47  
48

49  
50 Usually, when recording FT-Orbitrap data, most of the noise is removed  
51  
52 automatically by the instrument software in the so-called "reduced profile mode". This  
53  
54 is achieved in the following way: the system determines noise during booting of the  
55  
56 instrument and subtracts all mass peaks below a certain threshold automatically during  
57  
58 the acquisition of data. This reduces the data file size of a typical LC-MS run in full  
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3 scan mode to approximately 130 MB per file for the above described method. When  
4  
5 generating FT-Orbitrap data files in “full profile mode” no signals are automatically  
6  
7 removed. Therefore, only when acquiring data in “full profile mode”, noise can be  
8  
9 observed in the corresponding data files when generating EICs with narrow mass  
10  
11 extraction windows. By generating EICs of data that was recorded in “full profile  
12  
13 mode”, the toxin concentrations corresponding to a S/N of 3 was determined for the  
14  
15 estimation of LODs. It shall be noted however that acquiring “full profile mode “ data  
16  
17 results in very a large file size (ca. 3.2 GB per run for the method in use) and therefore  
18  
19 cannot be used for routine measurements. Concentration values for the LODs obtained  
20  
21 by this procedure were a factor three to four higher compared to the values achieved  
22  
23 according to Harris (2006) and are given in Table 2 (LOD<sub>solvent</sub>) and Table 3 (LOD<sub>matrix</sub>).  
24  
25 This approach seems to give a more reliable estimation of the LOD for high-resolution  
26  
27 data, resulting in reasonable peak shapes with approximately 8-10 data points across a  
28  
29 peak. An example EIC of tentoxin (Delaforge et al., 1997) in solution without matrix,  
30  
31 recorded in “reduced profile mode” and “full profile mode” (concentration at S/N=3) is  
32  
33 given in Figure 2. Applying this approach to spiked maize extracts, the limits of the  
34  
35 European Regulations could be achieved for FB<sub>1</sub>, FB<sub>2</sub> and DON. For ZON the  
36  
37 regulations were achieved, but with the following exceptions: ZON for “Processed  
38  
39 cereal-based food for infants and young children” (20 ng g<sup>-1</sup>), for “Bread, pastries and  
40  
41 biscuits” (50 ng/g) and “Cereal snacks and breakfast cereals” (50 ng g<sup>-1</sup>). Maximum  
42  
43 residue levels for aflatoxins (0.025 - 15 ng g<sup>-1</sup>) and for OTA (0.5 - 10 ng g<sup>-1</sup>) however  
44  
45 are at concentrations which cannot be measured with the presented method. For some  
46  
47 analytes (e.g. DON, 3ADON, ZON) measurements in negative ionization mode and/or  
48  
49 addition of acetate to the eluents (adduct formation) might be beneficial for achieving  
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51 lower LODs.  
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3 ***Performance characteristics obtained from matrix matched calibration***  
4 ***(maize)***  
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6  
7 Maize is a matrix known to be associated with pronounced SSE effects (Sulyok et al.,  
8  
9 2006). For this reason it was used for assessing matrix effects. Application of the  
10  
11 proposed method to other matrices requires detailed investigations for the matrix in use.  
12  
13 Recovery functions were constructed for extracted and diluted blank maize that was  
14  
15 spiked with the analytes at several concentration levels. For this, the spiking  
16  
17 concentrations of the maize extracts were put on the x axis; experimentally derived  
18  
19 concentrations (external calibration with standards in pure solvents) were put on the y  
20  
21 axis. The resulting calibration functions showed linearity (tested by Mandel Test  
22  
23 (Validata, Wegscheider et al., 1999). RSDs of the recovery functions were used as  
24  
25 estimates for the precision of the method. The slope of the recovery function can be  
26  
27 used as estimate for matrix induced signal suppression/enhancement (SSE).  
28  
29

30  
31  
32 RSDs of the recovery functions was found to be around 10 % and generally  
33  
34 lower than 20 %. The SSE generally showed values of 77 – 124 % indicating little  
35  
36 influence of matrix components on ionization efficiencies. AFB<sub>1</sub> and AFB<sub>2</sub> show matrix  
37  
38 effects of 55 and 69 % SSE, respectively. Sulyok et al. (2006) also found strong matrix  
39  
40 effects, namely 18 and 48 % SSE for AFB<sub>1</sub> and AFB<sub>2</sub>, respectively. Roquefortine C  
41  
42 shows uncommonly large matrix effects (39 % SSE) together with low precision (60 %  
43  
44 RSD at 65 ng g<sup>-1</sup>). For details of the calibration parameters obtained for spiked maize  
45  
46 extracts see Table 3.  
47  
48

49  
50 The same extraction procedure (only minor adaptations) as in Sulyok et al.  
51  
52 (2006) was used. Therefore, comparison of the results regarding extraction efficiencies  
53  
54 as well as matrix effects is possible, the latter giving information about differences of  
55  
56 the ion source setup regarding its susceptibility to matrix effects. Matrix effects were  
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3 similar to those found on the QTrap 4000 triple quadrupole system. SRM measurements  
4  
5 show lower LODs for most of the analytes, which is in accordance with the findings of  
6  
7 van der Heeft et al. (2009), who investigated hormone and veterinary drug residues  
8  
9 using UPLC-HR-MS on an FT-Orbitrap at 60,000 resolving power and compared  
10  
11 results with those of an MRM UPLC-QqQ-MS/MS confirmatory method.  
12  
13

#### 14 15 *Accuracy (trueness and precision) of the method*

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17  
18 Measurements of well defined test materials were performed, showing the principal  
19  
20 suitability of this method for wheat and maize based food commodities. In addition to  
21  
22 the food test materials (maize (3), breakfast cereal (1), wheat flour (1), wheat draff (1),  
23  
24 grinded wheat (1)), one animal feed (1) test material was included for evaluating the  
25  
26 trueness of the method. Results were corrected for matrix effects in case of maize  
27  
28 materials. For the other matrices no correction of SSE effects was done, since the extent  
29  
30 was not known. For a method to be routinely used, SSE effects should be carefully  
31  
32 evaluated and corrected if significant. As estimate for the method precision, the RSDs  
33  
34 of the recovery functions were used (Validata, Wegscheider et al., 1999). Values were  
35  
36 multiplied with two in order to obtain a 95 % confidence level. To compare  
37  
38 measurement results with assigned concentrations of the inter-laboratory comparison  
39  
40 matrix test materials, the procedure according to European Reference Materials'  
41  
42 Application Note 1 of the European Commission (Linsinger, 2010) was applied. No  
43  
44 significant differences between the measured concentration values and the assigned  
45  
46 concentration values were observed, not even for two concentration values that slightly  
47  
48 exceeded the highest concentration levels used for instrument calibration (DON and  
49  
50 ZON in maize #3). Furthermore, from the results shown in Figure 3 (for test materials  
51  
52 see materials and methods section), it can be concluded that for the tested toxin/matrix  
53  
54 combination no severe matrix effects occurred. Otherwise, matrices other than maize  
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3 would not have resulted in an acceptable outcome. In conclusion, the results obtained  
4  
5 clearly demonstrate the high potential of LC-HR-MS on FT-Orbitrap instruments for the  
6  
7 quantitative determination of mycotoxins and its principle applicability to food samples.  
8  
9

### 10 ***Suspects screening for 200 fungal and bacterial metabolites***

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12  
13 In contrast to MS/MS based quantitative methods such as SRM used with QqQ  
14  
15 instruments, HR full scan measurements offer the opportunity to detect metabolites  
16  
17 initially not intended to be monitored. In this respect, we wanted to explore and show  
18  
19 the principal suitability of the Orbitrap for the screening of compounds without  
20  
21 availability of standards.  
22  
23

24  
25 In order to estimate the effect of the criteria, those were applied to the full scan  
26  
27 measurements of standards solutions (in pure solvents and in presence of matrix maize  
28  
29 (non-contaminated)) that lead to signal intensities of approx.  $10^4$ - $10^6$  counts. Searching  
30  
31 for the exact masses of the protonated molecules or ammonia-/sodium-adducts of  
32  
33 standard solutions (minimum intensity heights  $10^4$  counts, mass deviation  $\pm 3$  ppm) led  
34  
35 to the finding of all 20 standards, but also to 18 (standards in pure solvent) and 41  
36  
37 (standards in presence of matrix maize) false-positive findings. This indicates, that if a  
38  
39 screening for the monitoring of regulated substances (e.g. 2002/657/EC,  
40  
41 SANCO/10684/2009) was to be conducted, searching for the exact masses with a  
42  
43 certain allowed mass deviation would be sufficient, since a following confirmatory  
44  
45 method would have to proof the trueness of these findings. However, for the suspects  
46  
47 screening approach, we applied further strict criteria in order to minimize the number of  
48  
49 false positive findings. Applying criteria 1 and 2 led to the reduction to 13 (pure  
50  
51 solvent) and 37 (matrix maize) false-positives and 0 false-negatives. Application of  
52  
53 criteria 1-3 led to only 3 false positives but also 2 (pure solvent) and 12 (matrix maize)  
54  
55 false negatives. Applying criteria 1 - 4 to standards in pure solvents and standards in  
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3 matrix maize led to 0 false-positives in both cases and to 9 (pure solvents) and 16  
4  
5 (matrix maize) false negatives.  
6

7 Applying the criteria 1-4 to the test materials, several assigned compounds could  
8  
9 not be found. The most limiting criterion turned out to be the maximum tolerated  
10  
11 deviation from the expected (calculated) intensity ratio of the monoisotopic to the first  
12  
13  $^{13}\text{C}$  isotopologue peak of - 35 % to + 5 % (criterion 3). Low intense mass peaks often  
14  
15 show no isotopologue peaks at all (since they show only a fractional amount of the  
16  
17 intensity of the monoisotopic peak). Also, the error of the expected relative intensity  
18  
19 with which those can be determined can be considerably large for single spectra.  
20  
21 Therefore, criterion 3 had not to be fulfilled in five consecutive scans but rather the  
22  
23 occurrence of at least 5 scans within a time window of 25 seconds was permitted  
24  
25  
26  
27 (criterion 4).  
28

29 Nevertheless, screening for 200 metabolites in the full scan chromatograms of  
30  
31 the test materials (applying criteria 1-4), further highly likely “suspected” metabolites  
32  
33 could be found. A list of the 13 suspects found in addition to the expected target  
34  
35 compounds (as specified by distributor of the respective samples) in the data of the test  
36  
37 materials is given in Table 4. Those include metabolites, which are known to be  
38  
39 produced by the most prevalent food colonizing fungi such as *Alternaria* (macrosporin),  
40  
41 *Aspergillus* (asperlactone, cyclopiazonic acid), *Fusarium* (antibiotic Y, aurofusarin,  
42  
43 beauvericin, chlamydosporol, enniatin B, enniatin B1, fusarielin A), *Penicillium*  
44  
45 (decarestrictine, penicillic acid) and *Claviceps pupurea* (ergosine). All test materials  
46  
47 under investigation were naturally contaminated. Since all of them had assigned  
48  
49 concentration values for *Fusarium* toxins, it is very likely to find further *Fusarium*  
50  
51 metabolites to be present in these samples, supporting the putative identification of  
52  
53 seven more *Fusarium* metabolites. It also has to be noted that both, ergosine and  
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3 macrosporin, usually are accompanied by related compounds such as other ergot  
4  
5 alkaloids and alternaria toxins, respectively, which indicates the need for further  
6  
7 measurements in order to confirm the identity of the suspected metabolites. Additional  
8  
9 confirmatory measures might include the measurement of authentic standards and the  
10  
11 consideration of retention times and MS/MS spectra.  
12

13  
14 The results obtained clearly show the potential of high-resolution mass  
15  
16 spectrometry for the screening of compounds in full scan data. The positive list of  
17  
18 suspects can be adjusted or extended deliberately, according to the expected range of  
19  
20 metabolites. Criteria need to be carefully chosen in order to minimize the number of  
21  
22 false positives and false negatives. Further adaptations and improvements as well as the  
23  
24 establishment of further criteria (e.g. automated EIC correlation of signals that belong  
25  
26 together; MS/MS fragmentation patterns) are subject of ongoing investigations.  
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### 31 **Conclusion**

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34 We have established a LC-HR-MS method for the simultaneous quantification of  
35  
36 selected mycotoxins and screening of fungal metabolites in food samples. A novel  
37  
38 approach for the estimation of LODs of FT-Orbitrap data recorded in “full profile  
39  
40 mode” was successfully applied, leading to reasonable chromatographic peak shapes  
41  
42 and concentration values. LODs were within the limits of the European Regulations  
43  
44 (Commission Regulation (EC) No. 1126/2007 and No. 1881/2006) for FB<sub>1</sub>, FB<sub>2</sub>, DON,  
45  
46 ZON (with few exceptions for baby food, “bread, pastries and biscuits” and processed  
47  
48 cereal-based food). The basic applicability of the method could be shown by the  
49  
50 quantification of several test materials, also including one animal feed sample. The  
51  
52 performance of the method for analysis of animal feed as highly complex matrix shall  
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54 be further investigated in the future.  
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3 Additionally, the potential of HR-MS for retrospective analysis was shown.  
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5 Since no common, accepted criteria for the screening/identification by LC-HR-MS  
6  
7 exist, defined identification criteria were chosen. According to these criteria, 13  
8  
9 different metabolites were detected in proficiency testing materials in addition to the  
10  
11 target toxins for which standards were available. Criteria for the confirmation of  
12  
13 suspects need to be carefully chosen in order to minimize the possibilities for both false  
14  
15 positive and false negative findings. Further investigations for the screening and  
16  
17 determination of suspect and non-target compounds shall be conducted in the future to  
18  
19 fully exploit the possibilities which FT-Orbitrap high-resolution mass spectrometry can  
20  
21 offer.  
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### 27 **Acknowledgements**

28  
29 The authors thank the Federal Country Lower Austria and the European Regional  
30  
31 Development Fund (ERDF) of the European Union for financial support. Miroslav  
32  
33 Flieger and Marika Jestoi are acknowledged for providing mycotoxin standards. This  
34  
35 work contributes to the PhD thesis of Sylvia M. Lehner.  
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Table 1. Assigned values according to material provider and expanded uncertainty (95 % confidence level) for test materials in  $\text{ng g}^{-1}$ . Concentration values  $< \text{LOD}$  in presence of matrix maize ( $\text{LOD}_{\text{matrix}}$ , see Table 3) are not provided.

Test Material	Provider, Number, Year	Analyte	Assigned value [ $\text{ng g}^{-1}$ ]	Expanded uncertainty [ $\text{ng g}^{-1}$ ]
Animal feed	Bipea, 2-3031-0052, 2010	DON	$32.3 \cdot 10^1$	$5.6 \cdot 10^1$
Grinded wheat	Bipea, 05-0631-0080, 2010	DON	$22.7 \cdot 10^2$	$2.5 \cdot 10^2$
Maize #1	FAPAS, T2262, 2010	DON	$17.1 \cdot 10^2$	$1.3 \cdot 10^2$
Maize #3	Bipea, 3-0731-0095, 2010	DON	$40.9 \cdot 10^2$	$4.6 \cdot 10^2$
Maize #2	FAPAS, T2246, 2008	FB <sub>1</sub>	$16.5 \cdot 10^2$	$1.1 \cdot 10^2$
Maize #3	Bipea, 3-0731-0095, 2010	FB <sub>1</sub>	$21.7 \cdot 10^1$	$9.2 \cdot 10^1$
Maize #2	FAPAS, T2246, 2008	FB <sub>2</sub>	$46.1 \cdot 10^1$	$3.2 \cdot 10^1$
Wheat draff	Bipea, 2-2831-0055, 2011	HT-2	$7.2 \cdot 10^1$	$1.0 \cdot 10^1$
	Biopure Referenzsubstanzen			
Wheat flour	GmbH, -, -	HT-2	$8.9 \cdot 10^2$	$2.7 \cdot 10^2$
Wheat draff	Bipea, 2-2831-0055, 2011	T-2	$7.6 \cdot 10^1$	$1.8 \cdot 10^1$
	Biopure Referenzsubstanzen			
Wheat flour	GmbH, -, -	T-2	$7.5 \cdot 10^1$	$3.6 \cdot 10^1$
Breakfast cereal	FAPAS, T2257, 2010	ZON	$70 \cdot 10^0$	$7.0 \cdot 10^0$
Maize #3	Bipea, 3-0731-0095, 2010	ZON	$39.9 \cdot 10^2$	$6.7 \cdot 10^2$

Table 2. Retention time, calibration range, number of evaluated levels, relative standard deviation of the lowest calibration level (RSD low) and LODs obtained for standards in pure solvent for the respective analytes.

Analyte	Retention Time [min]	Calibration Range [ng g <sup>-1</sup> ]	Number of evaluated levels	RSD low [%]	LOD <sub>solvent</sub> [ng g <sup>-1</sup> ]
DON	5.9	160-3200	4	15	160
3ADON	8.8	162-3232	4	13	110
Ergocryptine	9.2	10-960	8	16	8
AFG <sub>2</sub>	9.7	13-643	6	14	12
AFM <sub>1</sub>	9.7	30-1536	6	10	32
RFC	9.7	8-162	7	14	8
AFG <sub>1</sub>	10.0	13-643	6	14	12
AFB <sub>2</sub>	10.3	13-637	6	13	12
AFB <sub>1</sub>	10.7	13-212	5	13	12
FB <sub>1</sub>	10.7	86-4281	9	5	8
HT-2	11.6	32-1078	7	17	24
FB <sub>2</sub>	11.7	86-4264	9	6	8
Tentoxin	11.9	16-794	6	17	16
T-2	12.3	16-1066	8	17	16
MPA	12.4	64-3200	6	9	64
Alternariol	12.8	54-1600	5	12	24
ZON	13.5	41-3232	7	15	40
OTA	13.7	26-1318	6	19	24
EnnB	14.8	22-214	4	18	12
EnnB <sub>1</sub>	14.9	87-1728	4	22	16

Table 3. Performance characteristics in presence of the matrix maize: concentration range, number of evaluated levels, relative standard deviation of the recovery function ( $RSD_{matrix}$ ) at medium concentration of the calibration [ $ng\ g^{-1}$ ], signal suppression/enhancement (SSE), LOD in presence of matrix maize ( $LOD_{matrix}$ ) and recovery of the extraction step ( $R_E$ ).

Analyte	Calibration Range [ $ng\ g^{-1}$ ]	Number of evaluated levels	$RSD_{matrix}$ [%] at conc. [ $ng\ g^{-1}$ ]	SSE [%]	$LOD_{matrix}$ [ $ng\ g^{-1}$ ]	$R_E$ [%]
DON	160-3200	4	11 (1520)	115	160	98*
3ADON	162-3232	4	12 (1535)	113	150	89*
Ergocryptine	19-960	6	11 (471)	94	16	72**
AFG <sub>2</sub>	32-643	4	12 (306)	77	32	110*
AFM <sub>1</sub>	51-1536	5	9 (743)	96	40	100**
RFC	32-162	4	60 (65)	39	8	***
AFG <sub>1</sub>	21-643	5	12 (311)	77	20	107*
AFB <sub>2</sub>	32-637	4	18 (303)	69	24	102*
AFB <sub>1</sub>	21-212	4	16 (96)	55	16	95*
FB <sub>1</sub>	121-2414	4	8 (1147)	114	8	57*
HT-2	65-1078	5	11 (507)	77	40	108*
FB <sub>2</sub>	120-2404	4	10 (1142)	124	12	67*
Tentoxin	27-794	5	14 (384)	102	24	110**
T-2	40-1066	6	19 (513)	81	32	105*
MPA	64-3200	6	5 (1568)	105	64	103**
Alternariol	80-1600	4	6 (760)	88	56	107**
ZON	65-3232	6	12 (1584)	99	64	93*
OTA	44-1318	5	7 (637)	96	24	100*
EnnB	22-214	4	5 (96)	109	12	103*
EnnB <sub>1</sub>	87-1728	4	11 (821)	104	20	103*

Note: Recovery of the extraction step from: \*Sulyok et al., 2006: model matrix maize

\*\*Sulyok et al., 2007: model matrix breadcrumbs

\*\*\* Not evaluated for cereals. It has been evaluated for the matrix dust (94 %) by Vishwanath et al., 2009.

Table 4. Putative compounds detected in test materials found via suspects screening. Only metabolites additionally found to the target toxins are shown. Compound name, test material, most intense ion species (I),  $m/z$  found, retention time, intensity, relative mass deviation and ratio of measured to calculated relative isotope abundance of the first carbon isotopologue (one  $^{13}\text{C}$  instead of  $^{12}\text{C}$ ).

Compound Name	Test Material	Ret. Time [min]	Most int. ion species (I)	Intensity [counts]	$m/z$ found [Th]	Rel. mass dev. [ppm]	$[\text{Int}(\text{I}+1)_{\text{meas}}]/[\text{Int}(\text{I}+1)_{\text{calc}}]$
Antibiotic Y	Maize 3	12.0	$[\text{M}+\text{H}]^+$	$6.5 \cdot 10^4$	319.045	0.05	0.71
Asperlactone	Wheat draff	8.2	$[\text{M}+\text{H}]^+$	$1.3 \cdot 10^5$	185.081	-1.62	0.67
Aurofusarin	Maize 3	13.5	$[\text{M}+\text{H}]^+$	$6.4 \cdot 10^5$	571.087	-0.01	0.71
Beauvericin	Maize 2	15.0	$[\text{M}+\text{Na}]^+$	$3.3 \cdot 10^5$	806.398	-0.25	0.85
Chlamydosporol	Wheat draff	8.5	$[\text{M}+\text{H}]^+$	$8.2 \cdot 10^4$	227.091	-1.34	1.02
Cyclopiazonic acid	Maize 1	11.8	$[\text{M}+\text{H}]^+$	$8.9 \cdot 10^5$	337.155	0.16	0.91
Cyclopiazonic acid	Maize 2	11.8	$[\text{M}+\text{H}]^+$	$8.2 \cdot 10^5$	337.155	0.62	0.82
Cyclopiazonic acid	Maize 3	11.8	$[\text{M}+\text{H}]^+$	$1.2 \cdot 10^6$	337.154	-0.56	0.84
Decarestrictine	Maize 1	7.7	$[\text{M}+\text{H}]^+$	$3.1 \cdot 10^4$	217.107	0.10	0.68
Decarestrictine	Maize 3	7.8	$[\text{M}+\text{H}]^+$	$4.2 \cdot 10^4$	217.107	-0.33	0.86
Enniatin B	Animal feed	14.8	$[\text{M}+\text{Na}]^+$	$8.5 \cdot 10^5$	662.397	-2.24	0.70
Enniatin B	Breakfast cereal	14.8	$[\text{M}+\text{Na}]^+$	$6.2 \cdot 10^4$	662.398	-1.14	1.02
Enniatin B	Grinded wheat	14.7	$[\text{M}+\text{Na}]^+$	$8.7 \cdot 10^5$	662.398	-1.41	0.68
Enniatin B	Maize 3	14.7	$[\text{M}+\text{Na}]^+$	$2.4 \cdot 10^5$	662.399	-0.21	0.85
Enniatin B	Wheat draff	14.8	$[\text{M}+\text{Na}]^+$	$3.0 \cdot 10^6$	662.397	-2.06	0.86
Enniatin B	Wheat flour	14.7	$[\text{M}+\text{Na}]^+$	$3.4 \cdot 10^5$	662.398	-0.40	0.81
Enniatin B1	Animal feed	15.0	$[\text{M}+\text{Na}]^+$	$4.7 \cdot 10^5$	676.413	-2.41	0.86
Enniatin B1	Grinded wheat	15.0	$[\text{M}+\text{Na}]^+$	$1.8 \cdot 10^5$	676.413	-2.05	0.92
Enniatin B1	Wheat draff	15.0	$[\text{M}+\text{Na}]^+$	$2.0 \cdot 10^6$	676.413	-1.69	0.79
Enniatin B1	Wheat flour	14.9	$[\text{M}+\text{Na}]^+$	$3.9 \cdot 10^5$	676.414	-0.34	0.74
Ergosine	Maize 3	12.9	$[\text{M}+\text{Na}]^+$	$1.8 \cdot 10^4$	570.268	-0.88	0.79
Fusarielin A	Maize 2	13.8	$[\text{M}+\text{Na}]^+$	$1.4 \cdot 10^4$	425.265	-2.79	0.76
Macrosporin	Animal feed	11.2	$[\text{M}+\text{H}]^+$	$6.5 \cdot 10^4$	285.076	-0.13	0.68
Penicillic acid	Wheat draff	3.5	$[\text{M}+\text{H}]^+$	$2.6 \cdot 10^4$	171.065	-1.07	0.82

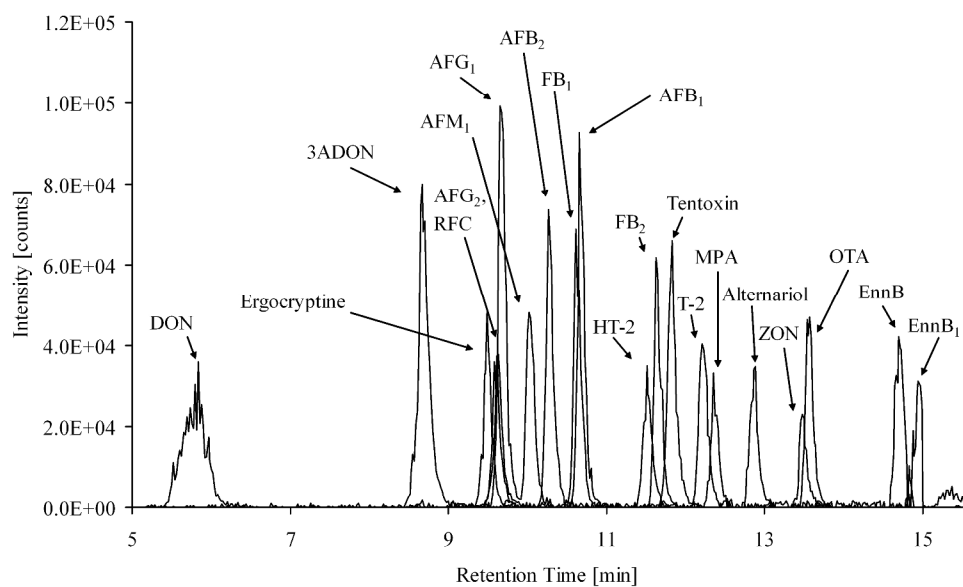


Figure 1. Chromatogram for the separation of 20 selected mycotoxins (abbreviations see text). EICs were generated from FT-Orbitrap full scan data using a mass extraction window of  $\pm 5$  ppm.  
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Supplement Table 1. List of fungal metabolites for screening purpose.

Compound	Molecular formula	CAS	Exact monoisotopic mass (M) [g mol <sup>-1</sup> ]	<i>m/z</i> [M+H] <sup>+</sup>	<i>m/z</i> [M+NH <sub>4</sub> ] <sup>+</sup>	<i>m/z</i> [M+Na] <sup>+</sup>	<i>m/z</i> [M-H] <sup>-</sup>
3-Acetyl-deoxynivalenol	C17H22O7	50722-38-8	338.13655	339.14383	356.17038	361.12577	337.12928
15-Acetyl-deoxynivalenol	C17H22O7	88337-96-6	338.13655	339.14383	356.17038	361.12577	337.12928
15-Monoacetoxyscirpenol	C17H24O6	2623-22-5	324.15729	325.16456	342.19111	347.14651	323.15001
16-Ketoaspergillimide	C20H27N3O4	199784-50-4	373.20016	374.20743	391.23398	396.18938	372.19288
2-Amino-14,16-dimethyloctadecan-3-ol	C20H43ON	540770-33-0	313.33447	314.34174	331.36829	336.32369	312.32719
3-O-Methylviridicatin	C16H13NO2	6152-51-4	251.09463	252.10191	269.12845	274.08385	250.08735
AAL-TA1 Toxin	C25H47NO10	79367-52-5	521.32000	522.32727	539.35382	544.30922	520.31272
Actinomycin D	C62H86N12O16	50-76-0	1254.62847	1255.63575	1272.66230	1277.61770	1253.62120
Aflatoxin B1	C17H12O6	1162-65-8	312.06339	313.07066	330.09721	335.05261	311.05611
Aflatoxin B2	C17H14O6	7220-81-7	314.07904	315.08631	332.11286	337.06826	313.07176
Aflatoxin G1	C17H12O7	1165-39-5	328.05830	329.06558	346.09213	351.04752	327.05103
Aflatoxin G2	C17H14O7	7241-98-7	330.07395	331.08123	348.10778	353.06317	329.06668
Aflatoxin M1	C17H12O7	6795-23-9	328.05830	329.06558	346.09213	351.04752	327.05103
Aflatoxin M2	C17H14O7	6885-57-0	330.07395	331.08123	348.10778	353.06317	329.06668
Agroclavine	C16H18N2	548-42-5	238.14700	239.15428	256.18082	261.13622	237.13972
Alamethicin F30	C92H150N22O25	27061-78-5	1963.11425	1964.12152	1981.14807	1986.10347	1962.10697
alpha-Zearalenol	C18H24O5	36455-72-8	320.16237	321.16965	338.19620	343.15160	319.15510
alpha-Zearalenol-4-O-glucoside	C24H34O10	135626-94-7	482.21520	483.22247	500.24902	505.20442	481.20792
Altenuene	C15H16O6	29752-43-0	292.09469	293.10196	310.12851	315.08391	291.08741
Altenusin	C15H14O6	31186-12-6	290.07904	291.08631	308.11286	313.06826	289.07176
Alternariol	C14H10O5	641-38-3	258.05282	259.06010	276.08665	281.04204	257.04555
Alternariolmethylether	C15H12O5	26894-49-5	272.06847	273.07575	290.10230	295.05769	271.06120
Altersolanol	C16H16O7	22268-16-2	320.08960	321.09688	338.12343	343.07882	319.08233
Altertoxin-I	C20H16O6	56258-32-3	352.09469	353.10196	370.12851	375.08391	351.08741
Antibiotic Y	C15H10O8	102426-44-8	318.03757	319.04484	336.07139	341.02679	317.03029
Apicidin	C34H49N5O6	183506-66-3	623.36828	624.37556	641.40211	646.35751	622.36101
Ascomycin	C43H69NO12	104987-12-4	791.48198	792.48925	809.51580	814.47120	790.47470

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5	Aspercolorin	C25H28N4O5	29123-52-2	464.20597	465.21325	482.23980	487.19519	463.19869
6	Aspergillimide	C20H29N3O3	195966-93-9	359.22089	360.22817	377.25472	382.21011	358.21362
7	Asperlactone	C9H12O4	76375-62-7	184.07356	185.08084	202.10738	207.06278	183.06628
8	Asperloxine A	C21H19N3O5	223130-52-7	393.13247	394.13975	411.16630	416.12169	392.12519
9	Aspionene	C9H16O4	157676-96-5	188.10486	189.11214	206.13868	211.09408	187.09758
10	Aspyron	C9H12O4	17398-00-4	184.07356	185.08084	202.10738	207.06278	183.06628
11	Asterric Acid	C17H16O8	577-64-0	348.08452	349.09179	366.11834	371.07374	347.07724
12	Atpenin A5	C15H21Cl2NO5	119509-24-9	365.07968	366.08695	383.11350	388.06890	364.07240
13	Aureobasidin A	C60H92N8O11	127757-30-6	1100.68856	1101.69583	1118.72238	1123.67778	1099.68128
14	Aurofusarin	C30H18O12	13191-64-5	570.07983	571.08710	588.11365	593.06905	569.07255
15	Austdiol	C12H12O5	53043-28-0	236.06847	237.07575	254.10230	259.05769	235.06120
16	Austocystin A	C19H13ClO6	55256-58-1	372.04007	373.04734	390.07389	395.02929	371.03279
17	Avenacein Y	C15H10O8	102426-44-8	318.03757	319.04484	336.07139	341.02679	317.03029
18	Beauvericin	C45H57N3O9	26048-05-5	783.40948	784.41676	801.44331	806.39870	782.40220
19	beta-Zearalenol	C18H24O5	71030-11-0	320.16237	321.16965	338.19620	343.15160	319.15510
20	beta-Zearalenol-4-O-glucoside	C24H34O10	135626-93-6	482.21520	483.22247	500.24902	505.20442	481.20792
21	Brefeldin A	C16H24O4	20350-15-6	280.16746	281.17474	298.20128	303.15668	279.16018
22	Brevicompanine B	C22H29N3O2	215121-47-4	367.22598	368.23325	385.25980	390.21520	366.21870
23	Calphostin C	C44H38O14	121263-19-2	790.22616	791.23343	808.25998	813.21538	789.21888
24	Cephalosporin C	C16H21N3O8S	61-24-5	415.10494	416.11221	433.13876	438.09416	414.09766
25	Chaetocin	C30H28N6O6S4	28097-03-2	696.09532	697.10259	714.12914	719.08454	695.08804
26	Chaetoglobosin A	C32H36N2O5	50335-03-0	528.26242	529.26970	546.29625	551.25164	527.25515
27	Chanoclavin	C16H20N2O	2390-99-0	256.15756	257.16484	274.19139	279.14678	255.15029
28	Chetomin	C31H30O6N6S4	1403-36-7	710.11097	711.11824	728.14479	733.10019	709.10369
29	Chlamydosporol	C11H14O5	135063-30-8	226.08412	227.09140	244.11795	249.07334	225.07685
30	Citreoviridin	C23H30O6	25425-12-1	402.20424	403.21152	420.23806	425.19346	401.19696
31	Citrinin	C13H14O5	518-75-2	250.08412	251.09140	268.11795	273.07334	249.07685
32	Citromycetin	C14H10O7	478-60-4	290.04265	291.04993	308.07648	313.03187	289.03538
33	Cochliodinol	C32H30N2O4	11051-88-0	506.22056	507.22783	524.25438	529.20978	505.21328
34	Curvularin	C16H20O5	10140-70-2	292.13107	293.13835	310.16490	315.12029	291.12380
35	Cycloaspeptide A	C36H43N5O6	109171-13-3	641.32133	642.32861	659.35516	664.31056	640.31406
36	Cycloechinulin	C20H21N3O3	143086-29-7	351.15829	352.16557	369.19212	374.14751	350.15102
37	Cyclophenin	C17H14N2O3	19553-26-5	294.10044	295.10772	312.13427	317.08966	293.09317
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5	Cyclopeptide	C17H16N2O2	50886-63-0	280.12118	281.12845	298.15500	303.11040	279.11390
6	Cyclopiazonic acid	C20H20N2O3	18172-33-3	336.14739	337.15467	354.18122	359.13661	335.14012
7	Cyclosporin A	C62H111N11O12	59865-13-3	1201.84137	1202.84864	1219.87519	1224.83059	1200.83409
8	Cyclosporin C	C62H111N11O13	59787-61-0	1217.83628	1218.84356	1235.87011	1240.82550	1216.82901
9	Cyclosporin D	C63H113N11O12	63775-96-2	1215.85702	1216.86429	1233.89084	1238.84624	1214.84974
10	Cyclosporin H	C62H111N11O12	83602-39-5	1201.84137	1202.84864	1219.87519	1224.83059	1200.83409
11	Cytochalasin A	C29H35O5N	14110-64-6	477.25152	478.25880	495.28535	500.24074	476.24425
12	Cytochalasin B	C29H37O5N	14930-96-2	479.26717	480.27445	497.30100	502.25639	478.25990
13	Cytochalasin C	C30H37O6N	22144-76-9	507.26209	508.26936	525.29591	530.25131	506.25481
14	Cytochalasin D	C30H37O6N	22144-77-0	507.26209	508.26936	525.29591	530.25131	506.25481
15	Cytochalasin E	C28H33O7N	36011-19-5	495.22570	496.23298	513.25953	518.21492	494.21843
16	Cytochalasin H	C30H39NO5	53760-19-3	493.28282	494.29010	511.31665	516.27204	492.27555
17	Cytochalasin J	C28H37NO4	56144-22-0	451.27226	452.27954	469.30608	474.26148	450.26498
18	Decarestrictine	C10H16O5	127393-89-9	216.09977	217.10705	234.13360	239.08899	215.09250
19	Dechlorogriseofulvin	C17H18O6	3680-32-8	318.11034	319.11761	336.14416	341.09956	317.10306
20	Deeopxy-deoxynivalenol	C15H20O5	88054-24-4	280.13107	281.13835	298.16490	303.12029	279.12380
21	Deoxybrevinamide E	C21H25N3O2	34610-68-9	351.19468	352.20195	369.22850	374.18390	350.18740
22	Deoxynivalenol	C15H20O6	51481-10-8	296.12599	297.13326	314.15981	319.11521	295.11871
23	Deoxynivalenol-3-glucoside	C21H30O11	131180-21-7	458.17881	459.18609	476.21264	481.16803	457.17154
24	Diacetoxyscirpenol	C19H26O7	2270-40-8	366.16785	367.17513	384.20168	389.15707	365.16058
25	Dihydroergosine	C30H39N5O5	7288-61-1	549.29512	550.30240	567.32894	572.28434	548.28784
26	Dihydroxyergotamine	C33H37N5O5	511-12-6	583.27947	584.28675	601.31329	606.26869	582.27219
27	Dihydrolysergol	C16H20N2O	18051-16-6	256.15756	257.16484	274.19139	279.14678	255.15029
28	Elymoclavine	C16H18N2O	548-43-6	254.14191	255.14919	272.17574	277.13113	253.13464
29	Elymoclavine fructoside	C22H28N2O6	12379-50-9	416.19474	417.20201	434.22856	439.18396	415.18746
30	Emodin	C15H10O5	518-82-1	270.05282	271.06010	288.08665	293.04204	269.04555
31	Enniatin A	C36H63N3O9	144446-20-8	681.45643	682.46371	699.49026	704.44565	680.44915
32	Enniatin A1	C35H61N3O9	4530-21-6	667.44078	668.44806	685.47461	690.43000	666.43350
33	Enniatin B	C33H57N3O9	917-13-5	639.40948	640.41676	657.44331	662.39870	638.40220
34	Enniatin B1	C34H59N3O9	19914-20-6	653.42513	654.43241	671.45896	676.41435	652.41785
35	Enniatin B2	C32H55N3O9	632-91-7	625.39383	626.40111	643.42766	648.38305	624.38655
36	Enniatin B3	C31H53N3O9	864-99-3	611.37818	612.38546	629.41201	634.36740	610.37090
37	Enniatin B4	C34H59N3O9	19893-21-1	653.42513	654.43241	671.45896	676.41435	652.41785
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5	Enniatin J1	C31H53N3O9	19893-15-3	611.37818	612.38546	629.41201	634.36740	610.37090
6	Enniatin K1	C32H55N3O9	716318-00-2	625.39383	626.40111	643.42766	648.38305	624.38655
7	Equisetin	C22H31NO4	57749-43-6	373.22531	374.23258	391.25913	396.21453	372.21803
8	Ergine	C16H17N3O	478-94-4	267.13716	268.14444	285.17099	290.12638	266.12989
9	Erginine	C16H17N3O		267.13716	268.14444	285.17099	290.12638	266.12989
10	Ergocornine	C31H39N5O5	564-36-3	561.29512	562.30240	579.32894	584.28434	560.28784
11	Ergocorninine	C31H39N5O5	564-37-4	561.29512	562.30240	579.32894	584.28434	560.28784
12	Ergocristine	C35H39N5O5	511-08-0	609.29512	610.30240	627.32894	632.28434	608.28784
13	Ergocristinine	C35H39N5O5	511-07-9	609.29512	610.30240	627.32894	632.28434	608.28784
14	Ergocryptine	C32H41N5O5	511-09-1	575.31077	576.31805	593.34460	598.29999	574.30349
15	Ergocryptinine	C32H41N5O5	511-10-4	575.31077	576.31805	593.34460	598.29999	574.30349
16	Ergometrine	C19H23N3O2	60-79-7	325.17903	326.18630	343.21285	348.16825	324.17175
17	Ergometrinine	C19H23N3O2	479-00-5	325.17903	326.18630	343.21285	348.16825	324.17175
18	Ergosine	C30H37N5O5	561-94-4	547.27947	548.28675	565.31329	570.26869	546.27219
19	Ergosinine	C30H37N5O5	596-88-3	547.27947	548.28675	565.31329	570.26869	546.27219
20	Ergotamin	C33H35N5O5	113-15-5	581.26382	582.27110	599.29764	604.25304	580.25654
21	Ergotaminine	C33H35N5O5	639-81-6	581.26382	582.27110	599.29764	604.25304	580.25654
22	Ergovaline	C29H35N5O5	2873-38-3	533.26382	534.27110	551.29764	556.25304	532.25654
23	Ergovalinine	C29H35N5O5	3263-56-7	533.26382	534.27110	551.29764	556.25304	532.25654
24	Festuclavine	C16H20N2	569-26-6	240.16265	241.16993	258.19647	263.15187	239.15537
25	Fulvic Acid	C14H12O8	479-66-3	308.05322	309.06049	326.08704	331.04244	307.04594
26	Fumagillin	C26H34O7	23110-15-8	458.23045	459.23773	476.26428	481.21967	457.22318
27	Fumigaclavin A	C18H22N2O2	6879-59-0	298.16813	299.17540	316.20195	321.15735	297.16085
28	Fumitremorgin C	C22H25N3O3	118974-02-0	379.18959	380.19687	397.22342	402.17881	378.18232
29	Fumonisin B1	C34H59NO15	116355-83-0	721.38847	722.39575	739.42230	744.37769	720.38119
30	Fumonisin B2	C34H59NO14	116355-84-1	705.39356	706.40083	723.42738	728.38278	704.38628
31	Fumonisin B3	C34H59NO14	136379-59-4	705.39356	706.40083	723.42738	728.38278	704.38628
32	Fusaproliferin	C27H40O5	152469-17-5	444.28757	445.29485	462.32140	467.27680	443.28030
33	Fusarenone X	C17H22O8	23255-69-8	354.13147	355.13874	372.16529	377.12069	353.12419
34	Fusarielin A	C25H38O4	132341-17-5	402.27701	403.28429	420.31084	425.26623	401.26973
35	Fusidic Acid	C31H48O6	6990-06-3	516.34509	517.35237	534.37891	539.33431	515.33781
36	Fusaric acid	C10H13NO2	536-69-6	179.09463	180.10191	197.12845	202.08385	178.08735
37	Geodin	C17H12Cl2O7	427-63-4	397.99601	399.00328	416.02983	420.98523	396.98873
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5	Gibberellic Acid	C19H22O6	77-06-5	346.14164	347.14891	364.17546	369.13086	345.13436
6	Gliotoxin	C13H14O4N2S2	67-99-2	326.03950	327.04677	344.07332	349.02872	325.03222
7	Griseofulvin	C17H17O6Cl	126-07-8	352.07137	353.07864	370.10519	375.06059	351.06409
8	HC-Toxin	C21H32N4O6	83209-65-8	436.23218	437.23946	454.26601	459.22141	435.22491
9	HT-2-Toxin	C22H32O8	26934-87-2	424.20972	425.21699	442.24354	447.19894	423.20244
10	hydrolyzed fumonisin B1	C22H47NO5	145040-09-1	405.34542	406.35270	423.37925	428.33464	404.33815
11	Kojic Acid	C6H6O4	501-30-4	142.02661	143.03389	160.06043	165.01583	141.01933
12	Lolitrein B	C42H55NO7	81771-19-9	685.39785	686.40513	703.43168	708.38707	684.39058
13	Lysergol	C16H18N2O	602-85-7	254.14191	255.14919	272.17574	277.13113	253.13464
14	Macrosporin	C16H12O5	22225-67-8	284.06847	285.07575	302.10230	307.05769	283.06120
15	Malformin C	C23H39N5O5S2	59926-78-2	529.23926	530.24654	547.27309	552.22848	528.23198
16	Marcfortine A	C28H35N3O4	75731-43-0	477.26276	478.27003	495.29658	500.25198	476.25548
17	Meleagrin	C23H23N5O4	71751-77-4	433.17500	434.18228	451.20883	456.16423	432.16773
18	Methysergide	C21H27N3O2	361-37-5	353.21033	354.21760	371.24415	376.19955	352.20305
19	Mevastatin	C23H34O5	73573-88-3	390.24062	391.24790	408.27445	413.22985	389.23335
20	Mevinolin	C24H36O5	75330-75-5	404.25627	405.26355	422.29010	427.24550	403.24900
21	Moniliformin	C4H2O3	71376-34-6	98.00039	99.00767	116.03422	120.98962	96.99312
22	Mycophenolic acid	C17H20O6	24280-93-1	320.12599	321.13326	338.15981	343.11521	319.11871
23	Neosolaniol	C19H26O8	36519-25-2	382.16277	383.17004	400.19659	405.15199	381.15549
24	Neoxaline	C23H25N5O4	71812-10-7	435.19065	436.19793	453.22448	458.17988	434.18338
25	NG012	C32H38O15	141731-76-2	662.22107	663.22835	680.25490	685.21029	661.21379
26	Nidulin	C20H17Cl3O5	10089-10-8	442.01416	443.02143	460.04798	465.00338	441.00688
27	Nivalenol	C15H20O7	23282-20-4	312.12090	313.12818	330.15473	335.11012	311.11363
28	Nornidulin	C19H15Cl3O5	33403-37-1	427.99851	429.00578	446.03233	450.98773	426.99123
29	Ochratoxin A	C20H18NO6Cl	303-47-9	403.08227	404.08954	421.11609	426.07149	402.07499
30	Ochratoxin alpha	C11H9ClO5	19165-63-0	256.01385	257.02113	274.04768	279.00307	255.00657
31	Ochratoxin B	C20H19NO6	4825-86-9	369.12124	370.12851	387.15506	392.11046	368.11396
32	O-Methylsterigmatocystin	C19H14O6	17878-69-2	338.07904	339.08631	356.11286	361.06826	337.07176
33	Oxaspirodion	C13H14O5	774538-95-3	250.08412	251.09140	268.11795	273.07334	249.07685
34	Paraherquamide A	C28H35N3O5	77392-58-6	493.25767	494.26495	511.29150	516.24689	492.25039
35	Paspaline	C28H39NO2	11024-56-9	421.29808	422.30536	439.33191	444.28730	420.29080
36	Paspalinine	C27H31NO4	63722-91-8	433.22531	434.23258	451.25913	456.21453	432.21803
37	Paspalitrein A	C32H39NO4	63722-90-7	501.28791	502.29519	519.32173	524.27713	500.28063
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5	Paspalitre B	C32H39NO5	63764-58-9	517.28282	518.29010	535.31665	540.27204	516.27555
6	Patulin	C7H6O4	149-29-1	154.02661	155.03389	172.06043	177.01583	153.01933
7	Paxilline	C27H33NO4	57186-25-1	435.24096	436.24824	453.27478	458.23018	434.23368
8	Penicillic acid	C8H10O4	90-65-3	170.05791	171.06519	188.09173	193.04713	169.05063
9	Penicillin G	C16H18O4N2S	61-33-6	334.09873	335.10600	352.13255	357.08795	333.09145
10	Penicillin V	C16H18N2O5S	87-08-1	350.09364	351.10092	368.12747	373.08286	349.08637
11	Penigequinolone A	C27H33NO6	180045-91-4	467.23079	468.23806	485.26461	490.22001	466.22351
12	Penitrem A	C37H44O6NCl	12627-35-9	633.28572	634.29299	651.31954	656.27494	632.27844
13	Pentoxifylline	C13H18N4O3	6493-05-6	278.13789	279.14517	296.17172	301.12711	277.13061
14	Pestalotin	C11H18O4	34565-32-7	214.12051	215.12779	232.15433	237.10973	213.11323
15	Phomopsin A	C36H45ClN6O12	64925-80-0	788.27840	789.28568	806.31222	811.26762	787.27112
16	Physcion	C16H12O5	521-61-9	284.06847	285.07575	302.10230	307.05769	283.06120
17	Pseurotin A	C22H25NO8	58523-30-1	431.15802	432.16529	449.19184	454.14724	430.15074
18	Pyrenophorol	C16H24O6	22248-41-5	312.15729	313.16456	330.19111	335.14651	311.15001
19	Pyripyropene A	C31H37NO10	147444-03-9	583.24175	584.24902	601.27557	606.23097	582.23447
20	Radicicol	C18H17ClO6	12772-57-5	364.07137	365.07864	382.10519	387.06059	363.06409
21	Roquefortine C	C22H23N5O2	58735-64-1	389.18518	390.19245	407.21900	412.17440	388.17790
22	Roridin A	C29H40O9	14729-29-4	532.26723	533.27451	550.30106	555.25645	531.25996
23	Rubellin D	C30H22O10	121325-49-3	542.12130	543.12857	560.15512	565.11052	541.11402
24	Rugulosin	C30H22O10	23537-16-8	542.12130	543.12857	560.15512	565.11052	541.11402
25	Satratoxin G	C29H36O10	53126-63-9	544.23085	545.23812	562.26467	567.22007	543.22357
26	Satratoxin H	C29H36O9	53126-64-0	528.23593	529.24321	546.26976	551.22515	527.22866
27	Scirpentriol	C15H22O5	2270-41-9	282.14672	283.15400	300.18055	305.13594	281.13945
28	Secalonic acid	C32H30O14	56283-72-8	638.16356	639.17083	656.19738	661.15278	637.15628
29	Setosusin	C29H38O8	182926-45-0	514.25667	515.26394	532.29049	537.24589	513.24939
30	Stachybotrylactam	C23H31NO4	163391-76-2	385.22531	386.23258	403.25913	408.21453	384.21803
31	Sterigmatocystin	C18H12O6	10048-13-2	324.06339	325.07066	342.09721	347.05261	323.05611
32	Sulochrin	C17H16O7	519-57-3	332.08960	333.09688	350.12343	355.07882	331.08233
33	T-2 Tetraol	C15H22O6	34114-99-3	298.14164	299.14891	316.17546	321.13086	297.13436
34	T-2 Toxin	C24H34O9	21259-20-1	466.22028	467.22756	484.25411	489.20950	465.21301
35	T-2 Triol	C20H30O7	34114-98-2	382.19915	383.20643	400.23298	405.18837	381.19188
36	Tentoxin	C22H30N4O4	28540-82-1	414.22671	415.23398	432.26053	437.21593	413.21943
37	Tenuazonic Acid	C10H15O3N	610-88-8	197.10519	198.11247	215.13902	220.09441	196.09792
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5	Terphenyllin	C20H18O5	52452-60-5	338.11542	339.12270	356.14925	361.10464	337.10815
6	Territrem B	C29H34O9	70407-20-4	526.22028	527.22756	544.25411	549.20950	525.21301
7	Trichodermin	C17H24O4	4682-50-2	292.16746	293.17474	310.20128	315.15668	291.16018
8	Ustiloxin A	C28H43N5O12S	143557-93-1	673.26289	674.27017	691.29672	696.25211	672.25562
9	Ustiloxin B	C26H39N5O12S	151841-41-7	645.23159	646.23887	663.26542	668.22081	644.22432
10	Ustiloxin D	C23H34N4O8	158243-18-6	494.23766	495.24494	512.27149	517.22689	493.23039
11	Verrucaric acid	C27H34O9	3148-09-2	502.22028	503.22756	520.25411	525.20950	501.21301
12	Verrucarol	C15H22O4	2198-92-7	266.15181	267.15909	284.18563	289.14103	265.14453
13	Verrucofortine	C24H31N3O3	113706-21-1	409.23654	410.24382	427.27037	432.22576	408.22927
14	Verruculogen	C27H33O7N3	12771-72-1	511.23185	512.23913	529.26568	534.22107	510.22457
15	Viomellein	C30H24O11	55625-78-0	560.13186	561.13914	578.16569	583.12108	559.12459
16	Viridicatin	C15H11NO2	129-24-8	237.07898	238.08626	255.11280	260.06820	236.07170
17	Wortmannin	C23H24O8	19545-26-7	428.14712	429.15439	446.18094	451.13634	427.13984
18	Zearalenone	C18H22O5	17924-92-4	318.14672	319.15400	336.18055	341.13594	317.13945
19	Zearalenone-4-glucoside	C24H32O10	105088-14-0	480.19955	481.20682	498.23337	503.18877	479.19227
20	Zearalenone-4-sulfate	C18H22O8S	132505-04-5	398.10354	399.11081	416.13736	421.09276	397.09626
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