Development of a LC-MS/MS Multimethod for the Quantification of the most common Mycotoxins in Foodstuff

Entwicklung einer LC-MS/MS Multimethode für die Quantifizierung der gefährlichsten Mykotoxine in Lebensmitteln

E. Guhsl, C. Lesueur, M. Gartner, P. Knittl

Summary
The goal of this study was the development of a multi toxin analysis for the following mycotoxins: aflatoxins B₁, B₂, G₁, G₂, ochratoxin A, HT-2 toxin and T-2 toxin, deoxynivalenol, 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol, the fumonisins B₁, B₂, and zearalenone.

The analysis comprises of a solid-liquid extraction with acetonitrile+water+formic acid (79+20+1), separation by liquid chromatography followed by tandem mass spectrometry detection. An additional sample preparation after the extraction was not necessary. Matrix matched standards were used since the food matrices have shown to interfere with the detection.

The applicability of the method was confirmed on food samples such as cereals and cereal products, bakery products, dried fruits and nuts. Calibration curves were prepared in solvent and in matrix extracts for calculating the amounts of mycotoxins in the samples. Moreover the responses showed good linearity. LODs and LOQs were determined to be under the regulated limit for all toxins. Thus a time saving and attractive multitoxin method was developed for the analysis of the most hazardous mycotoxins.

Keywords:
Mycotoxins, multitoxin method, liquid chromatography, tandem-mass spectrometry, foodstuff

Introduction
Molds and mycotoxins
Mycotoxins are secondary metabolites of molds that can have toxic effects on humans and animals. About 300 different mycotoxins are known so far. They are grouped according to their molecular structure or to the mold species they are produced by [1, 2]. Generally, small amounts do not evoke symptoms immediately, but continuous consumption may provoke long-term effects that can be carcinogenic, nephrotoxic, neurotoxic, immunosuppressive or estrogenic [3]. An important measure in the risk assessment of the occurrence of mycotoxins is keeping molds on agricultural crops, food and feed as low as possible.

Factors like environmental, ecological or storage conditions, temperature, relative humidity influence the occurrence. The use of fertilizers and fungicides can also have a great impact on the growth, production and biosynthesis of mycotoxins, but the mechanisms are not yet fully understood [4].

The aflatoxins (AflaB₁, AflaB₂, AflaG₁, AflaG₂) are produced by the fungi Aspergillus (i.e. A. flavus, A. parasiticus, A. nomius) [5]; they are characterised as group 1 of the human carcinogens by the IARC [8]. AflaB₁ is one of the most toxic substances regarding liver carcinogenicity. The harmful effects of these toxins range from liver to kidney damage but can also target the immune system. [3]. Aspergillus species can attack...
plants, cereals, nuts, maize, dried fruits, oil seeds, ca-
caco and spices [7].

Ochratoxin A (OTA) can also be produced by the so-
called “storage fungi” Aspergillus ochraceus, Peni-
cillium verrucosum and P. veridecatum [5]. The IARC
classified this mycotoxin in group 2B (potential hu-
man carcinogen) [6]. OTA is primarily a kidney toxin
but in sufficient high concentration it can damage the
liver as well. Generally it emerges due to inappro-
priate storage conditions and high humidity. OTA can oc-
cur in coffee, spices, dried fruits, cereals, moldy fruits
and fruit juices [3].

The Fusarium species produce the group of tri-
chothecenes, whereof the A- and B-trichothecenes
are the most important subgroups, mainly contami-
nating cereals like wheat, barley and maize used as
food and feed. Other Fusarium toxins are zearalenone
(ZON) and the fumonisins [4]. The most common A-
trichothecenes are T-2 toxin and HT-2 toxin. T-2 can be
metabolised into HT-2. As all the trichothecenes they
might bind to the 60s ribosomal subunit and inhibit the
enzyme peptidyltransferase causing disruption of the
formation of peptide bonds [3]. The most important
B-trichothecenes are deoxynivalenol (DON), nivalen-
ol (NIV), fusarenon X, 3-acetyldeoxynivalenol (3-Ac-
DON) and 15-acetyldoxynivalenol (15-AcDON). DON is
also called vomitoxin because of its emesis effect in
animals. Exposure to small amounts of DON can lead
to upregulation of protein synthesis and polyclonal
immunoglobulin production. No carcinogenic, tera-
genetic or mutagenic effects are known so far. ZON,
due to its competitive binding to estrogen receptors,
can influence the reproductive system. ZON is mainly
found in maize, wheat, oats, barley and sorghum [3].
ZON, DON and T-2 are ordered in category 3 (not clas-
sified relating to carcinogenicity for humans) by the
IARC [6]. The fumonisins (FumB1, FumB2) are natural
contaminants of cereals, maize and maize products,
evoked by species like Fusarium and Alternaria [5].
They are classified as potential human carcinogens
group 2B [6] and can for instance cause disorders of
the sphingolipidmetabolism or hepatotoxic impacts
[3].

Multitoxin method

Since mold species like Fusarium, Penicillium or Al-
ternaria produce different kinds of mycotoxins clas-
sified into diverse toxin groups, the development of
multitoxin methods for the detection of several myco-
toxins with a conjoint sample preparation and follow-
ing analysis is highly desirable. The aim of a success-
ful multitoxin method is to detect groups of mycotox-
ins, which could eventually occur together, in a single
chromatographic run. The combination of liquid chro-
matography (LC) and mass spectrometry (MS) shows
the advantage of being able to separate and detect all
types of toxins. Presently, only a few procedures exist
due to the multitude of food, feed and other matrices
concerned. These methods concentrate on Fusarium
toxins or aflatoxins [8, 9, 10].

Pioneer work in mycotoxin analysis was carried out
by Frisvad and Thane in 1987. They created a meth-
ods for the detection of 182 metabolites using LC-UV
[11]. Berthiller et al. developed a method for type A-
and B-trichothecenes and ZON in maize with LC-MS/MS.
They used MycoSep 226® columns for the purification
[12]. Sulyok et al. determined 39 mycotoxins in wheat
and maize with an advanced method, also using LC-
MS/MS [13]. In 2007, this method was extended to 87
analytes. They used an acidified acetonitrile+water
(ACN+H2O) mixture for the extraction, totally renounc-
ing clean-up cartridges. For the avoidance of matrix ef-
effects, they applied matrix calibrations. The ionisation
was performed both in positive and negative mode
[14]. Today this method includes the determination of
more than 180 substances [15]. Spanjer et al. developed another attractive multimeth-
ood for 33 mycotoxins. As extracting agent they used
ACN+H2O. After a 30 min chromatographic run, de-
tection in multiple reaction monitoring (MRM) mode
with an exclusively positive ionisation followed. The
application of matrix matched standards was found
to be inevitable [16]. Tanaka et al. used a LC-TOF-MS
for the analysis of 13 mycotoxins for achieving a bet-
ter sensitivity. They applied MultiSep 226® columns
for the purification step [17]. Mol et al. compared dif-
ferent extraction procedures for pesticides, mycotox-
ins and plant toxins in food and feed. They evaluated
ACN, methanol (MeOH) and acetonitrile, whereof the
mixture of ACN+H2O was the most successful. Some
substances showed better extraction results with
MeOH, but these extracts were quite turbid because of
incomplete protein precipitation [18].

Mycotoxins are regulated by the European Com-
mission with the Commission Regulations (EC) No.
and regulations of mycotoxins in food and feed for
the European Union, United States and other coun-
tries can also be seen in Murphy et al. [2].

Materials and methods

Chemicals

The standards were bought from Biopure (Tulln, Aus-
tria). Methanol and 10 % formic acid (LC-MS grade)
were purchased from J.T. Baker (Deventer, Nether-
lands), Acetonitrile (LiChrosolv, LC-grade) from Merck
(Darmstadt, Germany) and ammonium formate
(Analar Normapur, MS-grade) from VWR (West Ches-
ter, PA). Water was purified by a Milli-Q plus system
from Millipore (Billerica, MA).
Instrumentation
The chromatographic separation with subsequent detection was performed on an Agilent Technologies 1200 Series HPLC coupled to an Agilent Technologies 6460A Triple Quadrupole with electrospray ionisation jetstream (Agilent Technologies, Waldbronn, Germany).

Selection of mycotoxins and representative food samples
The selection of the mycotoxins and food samples that should be integrated in the method development was arranged according to the Commission Regulation. Thus, the regulated mycotoxins AflaB1, B2, G1, G2, OTA, DON, ZON, FumB1 and FumB2 were chosen, as well as the not yet regulated toxins 3-AcDON, 15-AcDON (co-occurrence with DON), HT-2 and T-2 (probably regulated in the near future). Representing the most affected food matrices, cereals, maize, bread, dried fruits and nuts were selected.

Optimisation of MS parameters
The selection of precursor and product ions, fragmentor voltage and optimisation of collision energy were performed with single standards using direct injection without column. The influence of the buffer (ammonium formate) was tested in order to optimise the ionisation and the formation of precursor ions. The gas temperature was regulated at 350 °C and the sheath gas temperature at 400 °C. The gas flow was set to 11 l/min and the sheath gas flow to 12 l/min. The nebulizer was used at 60 psi, the capillary was adjusted to 4000 V, the nozzle voltage to 500 V. A dwell time of 20 milliseconds was set in MRM mode.

Optimisation of LC parameters
The optimisation of the chromatographic separation included the selection of the column, mobile phases, gradients and flow rates. The column ZOR-BAX StableBond C18, 4.6 * 150 mm (1.8 µm) (Agilent Technologies, Waldbronn, Germany) was selected. The length of the column facilitated the separation of the thirteen toxins while the density of the packing material allowed better separation efficiency and higher flow rates. The choice of a gradient with two different mobile phases was important for the separated elution of the toxins. Mobile phase A consisted of H2O+MeOH+HCOOH (90+9+1), mobile phase B of MeOH+H2O+HCOOH (90+9+1). The addition of the buffer ammonium formate (5 mM) was also a test alternative. Different HPLC gradients were tested. The injection volume was 4 µl and the flow rate 1 ml/min.

Sample preparation and extraction optimisation
The extraction, like the whole method, should be time saving and economic. A mixture of ACN+H2O+HCOOH (79+20+1) was tested as extraction solvent and compared to the extraction efficiency of the mixture of MeOH+H2O+HCOOH (79+20+1). Initially, samples were homogenised in a blender except for the raisins that were milled in a coffee grinder. 10 g were weighed into 50 ml Falcon tubes. Subsequently, 20 ml of the extraction solvent were added. The mixture was shaken on a vortex for a few seconds and then extracted for 15 min in an ultrasonic bath. After centrifugation (6000 rpm, 3 min), an aliquot of the supernatant was filtered (0.45 µm) and collected into a HPLC vial.

Spiking experiments for the evaluation of the extracting agents
Samples of organic wheat, maize and bread were taken as test samples. 10 g of each matrix (in replicates) were spiked with 100 µl of a 5 µg/ml standard mix containing all mycotoxins, thus producing samples enriched with 50 µg/kg of each toxin. Samples were shaken for a short time and left at room temperature for 30 min. Blank samples and spike replicates were then extracted, once with MeOH+H2O+HCOOH and once with ACN+H2O+HCOOH (79+20+1).

Measurement of external standards in solvent and in matrix
External standards in ACN were prepared for all mycotoxins to calculate the amount of toxins in the samples. Additionally, matrix matched standards were used to show the influence of matrix effects during the detection by ion signal enhancement or suppression. Samples of wheat, maize, bread, walnuts and raisins were extracted by the mentioned protocol. Afterwards the extracts were used for diluting a 5 µg/ml standard mix containing all toxins. For the aflatoxins, concentrations between 0.05 and 20 µg/l were selected, for OTA between 0.1 and 100 µg/l. Calibrations for all other toxins were established between 1 and 1000 µg/l. The lower concentrations should indicate possible limits of detection. For the external standards in ACN and the matrix matched standards, separate dilutions for each food matrix were carried out, taking into account the regulated limits of the mycotoxins. Bread, wheat and maize were used for all mycotoxins, nuts only for the aflatoxins, raisins for OTA and the aflatoxins.

Determination of LODs and LOQs
For the determination of the limits of detection (LODs) and limits of quantification (LOQs), the relevant matrices were spiked with the following concentrations of the mycotoxins (aflatoxins with 0.2 and 2 µg/kg, OTA with 0.2 as well as 2 and 10 µg/kg, B-trichothecenes, fumonisins and ZON with 10, 20 und 40 µg/kg, A-trichothecenes with 2, 10 und 20 µg/kg). Considering the signal/noise ratios from the lowest spikes, the LODs
(S/N = 3) and LOQs (S/N = 10) were calculated according to DIN 32645 for chemical analysis [21].

Measurement of real food samples
For the evaluation of the applicability of the method, several “blank” food samples (such as bread, wheat, maize flour, walnuts and raisins) were extracted, analysed and quantified using the respective external standard curves.

Results and discussion
LC-MS/MS optimisation
The optimised precursor and product ions with corresponding fragmentor voltage and collision energy are presented in table 1. Finally, only the positive ion mode was used, because applying this all mycotoxins showed better results. Thus, no change from positive to negative mode was necessary during the detection procedure.

For most of the toxins protonated molecules [M+H]+ were selected. Addition of ammonium formate to the mobile phase showed the general effect that the signals of the protonated molecules were enhanced, compared to the Na+ -adducts. In the case of the aflatoxins, the B-trichothecenes and the fumonisins the buffer was an important measure, otherwise the Na+ -adducts of these toxins could not be fragmented. Only the A-trichothecenes showed better results with Na+ or NH4+ -adducts (coming from the buffer). In the case of HT-2 the signal of the Na+ -adduct was still more intense than the others, additionally the Na+ -adducts of these toxins could not be fragmented. Finally, only the positive ion mode was used, because applying this all mycotoxins showed better results. Thus, no change from positive to negative mode was necessary during the detection procedure.

Optimisation of extraction
The aim of this experiment was comparing two different extracting agents for unpolluted as well as naturally contaminated samples, in unspiked and spiked form. As a result we found out that ACN+H2O+HCOOH showed better extraction efficiency than MeOH+H2O+HCOOH. Moreover, the MeOH+H2O+HCOOH extracts were quite turbid and could hardly be filtrated. In table 2 unspiked wheat samples and 50 µg/kg spiked wheat samples, ex-

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Nominal mass [g/mol]</th>
<th>Precursor Ions (m/z)</th>
<th>Fragmentor-voltage [V]</th>
<th>Product Ions (m/z)</th>
<th>Collision-energy [V]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AflaB1</td>
<td>312</td>
<td>313 [M + H]+</td>
<td>80</td>
<td>285/241</td>
<td>21/39</td>
</tr>
<tr>
<td>AflaB2</td>
<td>314</td>
<td>315 [M + H]+</td>
<td>120</td>
<td>287/259</td>
<td>24/30</td>
</tr>
<tr>
<td>AflaG1</td>
<td>328</td>
<td>329 [M + H]+</td>
<td>100</td>
<td>243/200</td>
<td>24/42</td>
</tr>
<tr>
<td>AflaG2</td>
<td>330</td>
<td>331 [M + H]+</td>
<td>130</td>
<td>245/313</td>
<td>27/24</td>
</tr>
<tr>
<td>OTA</td>
<td>403</td>
<td>404 [M + H]+</td>
<td>90</td>
<td>239/258</td>
<td>21/9</td>
</tr>
<tr>
<td>DON</td>
<td>296</td>
<td>297 [M + H]+</td>
<td>70</td>
<td>203/249</td>
<td>6/6</td>
</tr>
<tr>
<td>3-AcDON</td>
<td>338</td>
<td>339 [M + H]+</td>
<td>80</td>
<td>321/137</td>
<td>0/3</td>
</tr>
<tr>
<td>15-AcDON</td>
<td>338</td>
<td>339 [M + H]+</td>
<td>80</td>
<td>231/203</td>
<td>9/12</td>
</tr>
<tr>
<td>ZON</td>
<td>318</td>
<td>319 [M + H]+</td>
<td>80</td>
<td>283/301</td>
<td>6/3</td>
</tr>
<tr>
<td>HT-2</td>
<td>424</td>
<td>447 [M + Na]+</td>
<td>100</td>
<td>345/285</td>
<td>15/18</td>
</tr>
<tr>
<td>T-2</td>
<td>466</td>
<td>484 [M + NH4]+</td>
<td>80</td>
<td>185/305</td>
<td>18/9</td>
</tr>
<tr>
<td>FumB1</td>
<td>721</td>
<td>722 [M + H]+</td>
<td>150</td>
<td>334/352</td>
<td>45/39</td>
</tr>
</tbody>
</table>

Tab. 1: Selected precursor and product ions
Tab. 1: Ausgewählte Precursor- und Produkt-Ionen
extracted with ACN+H₂O+HCOOH or MeOH+H₂O+HCOOH mixtures, are presented. Results were calculated by using calibration with external standards in ACN. Several unspiked samples showed a more or less intense contamination with mycotoxins, especially the DON concentration was very high in the wheat sample. For spiking experiments, in general, uncontaminated samples should be used, as it was done for the following validation studies (not shown). The spiked 50 µg/kg of the different toxins were recovered for the most part, using ACN+H₂O+HCOOH for the extraction. The experiment was also done for other food matrices for confirming the results. Consequently, it was decided to use the mixture of ACN+H₂O+HCOOH (79+20+1) as the final extracting agent.

Calibrations and matrix effects
Matrix matched standards and external standards in ACN were compared by means of calculating the calibration curves and the ratios of slope_{matrix/slope_{ACN} (Fig. 2). All responses in matrices as well as in ACN showed good linearity. Depending on the differences between the slopes of the standard curves, it was decided whether a correction with matrix matched standards was necessary. A possible alternative to matrix matched standards would be the use of isotope labelled internal standards.

In figure 2, a comparison of matrix matched calibration and standard calibration is shown for T-2. The slopes of the calibration curves in bread, wheat and ACN are quite the same while the slope of the calibra-

<table>
<thead>
<tr>
<th>Toxins</th>
<th>ACN/H₂O/HCOOH (79/20/1)</th>
<th>MeOH/H₂O/HCOOH (79/20/1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unspiked</td>
<td>spiked with 50 µg/kg</td>
</tr>
<tr>
<td></td>
<td>mean in µg/kg</td>
<td>mean in µg/kg</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>RSD</td>
</tr>
<tr>
<td>AfIB1</td>
<td>0.6</td>
<td>39.4</td>
</tr>
<tr>
<td>AfIB2</td>
<td>&lt;0.1</td>
<td>45.45</td>
</tr>
<tr>
<td>AfIG1</td>
<td>0.4</td>
<td>40.1</td>
</tr>
<tr>
<td>AfIG2</td>
<td>&lt;0.1</td>
<td>48.9</td>
</tr>
<tr>
<td>DON</td>
<td>251.5 +/- 9.5</td>
<td>291.4</td>
</tr>
<tr>
<td>3-AcDON</td>
<td>-</td>
<td>50.55</td>
</tr>
<tr>
<td>15-AcDON</td>
<td>-</td>
<td>45.85</td>
</tr>
<tr>
<td>HT-2</td>
<td>8.4 +/- 1</td>
<td>41.3</td>
</tr>
<tr>
<td>T-2</td>
<td>&lt;2</td>
<td>57.85</td>
</tr>
<tr>
<td>ZON</td>
<td>-</td>
<td>50.6</td>
</tr>
</tbody>
</table>

Tab. 2: Comparison of extracting solvents ACN/H₂O/HCOOH and MeOH/H₂O/HCOOH in organic wheat, calibrated with external standards in ACN

Tab. 2: Vergleich der Extraktionslösungen ACN/H₂O/HCOOH und MeOH/H₂O/HCOOH in Bio-Weizen, kalibriert mit externen ACN-Standards
tion curve in maize is much steeper. Consequently, it can be assumed that maize requires a matrix correction due to matrix enhancement, in the case of T-2, whereas bread and wheat do not. The same procedure was repeated for all toxin-matrix combinations. Each toxin showed matrix enhancement for at least one matrix (graphics not shown). For that reason it was decided that matrix matched standards were necessary where ratios showed a deviation higher than 10 %.

Table 3 shows for which matrices and toxins a matrix calibration is inevitable. Fatty matrices like nuts or maize tend to require a matrix correction, as well as bread. Generally, matrix matched standards had to be used for at least one toxin in each matrix. Therefore a matrix correction was incorporated into the multitoxin method anyway.

**LODs and LOQs**

LODs and LOQs of all toxins in the matrices bread and raisins were calculated from the lowest spiked food samples. Furthermore, MRM chromatograms of different spiked concentrations were compared to the calculated LODs and LOQs and the values regulated by law. All determined LOQs integrated into the multitoxin method are listed in table 4. The quantification limit of the aflatoxins is 0.2 µg/kg. OTA can be quantified down to 2 µg/kg in dried fruits, cereals and bakery products. The B-trichothecenes can be quantified down to 20 µg/kg, ZON to 10 µg/kg, T-2 and HT-2 even lower. Only the fumonisins display higher quantification limits of around 40 µg/kg. Some mycotoxins could be quantified at lower concentrations but depending on the regulated limits, the measurement of such low amounts is not necessary.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>LOQs [µg/kg]</th>
<th>Regulated Limits [µg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AflaB₁</td>
<td>0.2</td>
<td>2–8</td>
</tr>
<tr>
<td>AflaB₂</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>AflaG₁</td>
<td>0.2</td>
<td>Sum Aflatoxins*</td>
</tr>
<tr>
<td>AflaG₂</td>
<td>0.2</td>
<td>4–15</td>
</tr>
<tr>
<td>OTA</td>
<td>2</td>
<td>3–10</td>
</tr>
<tr>
<td>DON</td>
<td>20</td>
<td>200–1750</td>
</tr>
<tr>
<td>3-AcDON</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>15-AcDON</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>ZON</td>
<td>10</td>
<td>20–350</td>
</tr>
<tr>
<td>HT-2**</td>
<td>10</td>
<td>50–500</td>
</tr>
<tr>
<td>T-2**</td>
<td>10</td>
<td>50–500</td>
</tr>
<tr>
<td>FumB₁</td>
<td>40</td>
<td>200–4000</td>
</tr>
<tr>
<td>FumB₂</td>
<td>40</td>
<td>200–4000</td>
</tr>
</tbody>
</table>

*Sum Aflatoxins...AflaB₁ + AflaB₂ + AflaG₁ + AflaG₂
**HT-2, T-2...not yet regulated, but probable prospective limits
--...no regulated limits

Tab. 4: LOQs of all mycotoxins

Tab. 4: LOQs aller Mykotoxine

The possible LOQs by the developed method are compared with regulated limits in the following table.

**Application on food samples**

Some results of the first food samples measured with the developed multitoxin method are listed in table 5. The analysis of the food samples displayed peaks of all four aflatoxins in the chromatograms, but mainly under the determined LOQs. Bread, wheat flour and
maize contained DON and even traces of HT-2. One sample of raisins showed in fact 8 µg/kg OTA, a relatively high amount, being exactly the regulated limit in dried fruits.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>AflaB₁</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>AflaB₂</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>AflaG₁</td>
<td>-</td>
<td>&lt;0.2</td>
<td>0.21</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>AflaG₂</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>0.28</td>
</tr>
<tr>
<td>OTA</td>
<td>-</td>
<td>-</td>
<td>&lt;2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>DON</td>
<td>&lt;20</td>
<td>29</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-AcDON</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-AcDON</td>
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<td></td>
</tr>
<tr>
<td>ZON</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT-2</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>-</td>
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</tr>
<tr>
<td>T-2</td>
<td>-</td>
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Tab. 5: Detected mycotoxins in natural (unspiked) food samples

Tab. 5: In natürlichen Lebensmittelproben (ohne Befall) entdeckte Mykotoxine
<0.2 µg/kg (concerning the aflatoxins) means values below the LOQs

Conclusion

The development of a method for the simultaneous determination of the most common mycotoxins, partly regulated by the EU Commission, was achieved. This includes a conjoint extraction with the subsequent separation and detection by LC-MS/MS. The renunciation of extra purification before the extraction is specific to this method, facilitated by the capability of the tandem-MS. The regulated maximum levels in food can be detected down to 0.2 µg/kg, and OTA down to 2 µg/kg.

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References


Address of the authors:
DI DI Eva Guhs
Dr. Céline Lesueur*
Dr. Michael Gartner
DI Patrik Knittl
LVA GmbH
Blasstraße 29
1190 Vienna
celine.lesueur@lva.co.at
* corresponding author

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t +43 1 715 31 93, f +43 1 715 48 19
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www.ernaehrung-nutrition.at

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