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A new approach using micro HPLC-MS/MS for multi-mycotoxin analysis in maize samples

Sebastian Hickert · Johannes Gerding · Edson Ncube · Florian Hübner · Bradley Flett · Benedikt Cramer · Hans-Ulrich Humpf

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Abstract Using micro high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) a simple and fast method for the quantitative determination of 26 mycotoxins was developed. Sample preparation consists of a single extraction step and a dilute-and-shoot approach without further cleanup. With a total run time of 9 min and solvent consumption below 0.3 mL per chromatographic run, the presented method is cost-effective. All toxins regulated by the European Commission with maximum or guidance levels in grain products (fumonisins B_1 and B_2 (FB_1 and FB_2)); deoxynivalenol (DON); aflatoxins B_1, G_1, B_2, and G_2 (AFB_1, AFG_1, AFB_2, and AFG_2); ochratoxin A (OTA); T-2 and HT-2 toxins; and zearalenone (ZEN) can be quantified with this method. Furthermore, the enniatins B, B1, A, and A1 (EnB, EnB1, EnA, and EnA1); beauvericin (BEA); 3-acetyl-deoxynivalenol (3-AcDON); fusarin C (FusC); sterigmatocystin (STC); gliotoxin (GT); and the Alternaria toxins alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), tentoxin (TEN), and altertoxin I (ATX I) can also be quantified. For all regulated compounds, recoveries ranged between 76 and 120 %. For all other toxins, the recovery was at least 51 %. The method was applied for the analysis of 42 maize samples from field trials in South Africa.

Keywords Micro LC · Mass spectrometry · Capillary chromatography · Aflatoxin · Multi-method

Introduction

Some mycotoxins are under regulation by the European Commission. These toxins include trichothecenes (deoxynivalenol (DON), T-2, and HT-2 toxins); aflatoxins B_1, G_1, B_2, and G_2 (AFB_1, AFB_2, AFG_1, AFG_2); fumonisins B_1 and B_2 (FB_1 and FB_2); ochratoxin A (OTA); and zearalenone (ZEN). Maximum levels for grain products for human and animal consumption have been set (EC 2006a, b, 2013). In South Africa, where the analyzed samples originate from, maximum levels in food and feed for some toxins have been set as well (e.g., aflatoxins, patulin) (Department of Health and Government of South Africa DoH 2004). The *Codex Alimentarius* Committee established by the *World Health Organization* and the *Food and Agricultural Organization* of the United Nations provides also maximum levels for some mycotoxins (e.g., trichothecenes, OTA, ZEN, and fumonisins (Codex Alimentarius Committee CAC 1995)) and recommends procedures to minimize contamination (Codex Alimentarius Committee CAC 2003). To control these maximum levels, different methods can be applied. The methods commonly used for single mycotoxin determination are based on immunochemical reactions (ELISA) (Goryacheva et al. 2007), or chromatographic separation (GC or HPLC) coupled to UV, FLD, FID, or mass spectrometric detection (Tanaka et al. 2000; Herzallah 2009; Pearson et al. 1999; Lukacs et al. 1996).

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S. Hickert · J. Gerding · F. Hübner · B. Cramer · H.-U. Humpf (✉)
Institute of Food Chemistry, University of Münster, Corrensstraße 45, 48149 Münster, Germany
e-mail: humpf@uni-muenster.de

S. Hickert · J. Gerding · H.-U. Humpf
NRW Graduate School of Chemistry, Wilhelm-Klemm-Str. 10, 48149 Münster, Germany

E. Ncube · B. Flett
ARC-Grain Crops Institute, P. Bag X1251, Potchefstroom 2520, South Africa

B. Flett
Unit of Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

Modern methods rely on liquid chromatography with mass spectrometric detection, making this the method of choice. There are several single-analyte methods published for the detection and quantification of mycotoxins at trace levels. Sample preparation (e.g., extraction, cleanup by SPE, or immunoaffinity columns (Pearson et al. 1999; Cramer et al. 2007) is optimized for the specific target substance in these methods. These sample preparation strategies are in most cases only suitable for one toxin or for a specific class of toxins. Newest generation mass spectrometers provide enhanced sensitivity, which allows less sample preparation and thereby makes multi-analyte methods possible. Mostly, these methods rely on dilute-and-shoot approaches. There are several multi-mycotoxin methods published (Sulyok et al. 2006, 2010; Sulyok et al 2007; Ren et al. 2007; Monbaliu et al. 2010; Oueslati et al. 2012; Rasmussen et al. 2010; Spanjer et al. 2008); however, these methods involve long chromatographic runs, usually of more than 20 min. In this publication, we describe the use of a micro high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method for accelerated chromatographic separation. This technology uses columns with an inner diameter <1 mm and reduced flow rates compared to conventional HPLC systems. The smaller diameter of micro HPLC columns causes less diffusion, resulting in better resolution of the chromatographic peaks (Takeuchi and Ishii 1980; Ishii et al. 1977). This allows chromatographic separation equivalent to state-of-the-art UHPLC separations with short chromatographic run times, but with less solvent consumption, making it environmentally beneficial as well as cost-effective. A further benefit of micro HPLC systems is the improved ionization ratio due to the low flow rate of 10–50 μL/min which increases mass spectrometric signal intensities (Legido-Quigley et al. 2002). The new method presented in this manuscript focuses not only on regulated mycotoxins but also on toxins which are commonly found in cereals and are not yet regulated due to a lack of occurrence and toxicity data as for example Alternaria toxins (alternariol (AOH), alternariol monomethyl ether (AME), AAL toxin (AAL), tentoxin (TEN), altertoxin I (ATX I), altenuene (ALT) (European Food Safety Authority EFSA 2011), or sterigmatocystin (STC) (European Food Safety Authority EFSA 2013). The enniatins B, B1, A, and A1 (EnB, EnB1, EnA, and EnA1) and beauvericin (BEA) were also included as they are currently under investigation by the European Food Safety Authority (European Food Safety Authority EFSA 2014).

Experimental

Chemicals and reagents

All solvents were of gradient grade and if not stated otherwise purchased from VWR (Darmstadt, Germany). Water was purified with a Milli-Q Gradient A10 system from Millipore (Schwalbach, Germany). Enniatins, BEA, aflatoxins (as a mixture of AFB_1/AFB_2/AFG_1 and AFG_2), STC, TEN, and AAL toxin were purchased from Sigma-Aldrich (Steinheim, Germany). Gliotoxin was obtained from VWR (Darmstadt, Germany). AOH, AME, and ALT were isolated from fungal cultures (unpublished data). HT-2 and T-2 toxins were isolated, and stable isotope derivatives were synthesized according to Beyer et al. in our working group (Beyer et al. 2009). FB_1 and FB_2 were isolated according to Hübner et al. (Hübner et al. 2012), and d_6-FB_1 was synthesized according to Lukacs et al. (Lukacs et al. 1996). ZEN isolation and d_2-ZEN production are described by Cramer et al. (Cramer et al. 2007). DON and 3-AcDON isolation and synthesis of d_1-DON and d_3-3-AcDON were performed according to Bretz et al. (Bretz et al. 2006). OTA was isolated and d_5-OTA prepared as described by Cramer et al. (Cramer et al. 2008). Fusarin C was isolated by Kleigrewe et al. (Kleigrewe et al. 2012).

Preparation of standard solutions

Mycotoxin standards (purity>95 %) were dissolved in acetonitrile (ACN) to obtain stock solutions of 250 or 200 μg/mL. All toxins were combined in one working solution in ACN. Altenuene and enniatin B1 were combined in a separate working solution as they were later added to the method. The resulting concentrations are shown in Table 1. The estimation of the limit of detections (LODs) and the limit of quantifications (LOQs) was performed by dilution of the standards in a blank matrix extract based on a signal to noise ratio (S/N) of 3 for the LOD and S/N>10 for the LOQ. The resulting values are given in Table 1. The working solution was stored at −80 °C in the dark until further use. All calibration and spiking solutions and samples were directly analyzed after preparation and kept in amber glassware to avoid the decomposition of FusC (Kleigrewe et al. 2012).

Calibration

Matrix-matched calibration was applied using blank polenta. The matrix-matched standard solution was obtained by extracting the polenta analog to the samples and analyzing the obtained solution with the method presented here. The working solution was diluted 4000- to 40-fold to obtain seven calibration points within the working range. Generally, the calibration points were chosen close to the LOQs for each analyte. The calibration points for the fumonisins were higher due to the higher levels of fumonisins commonly found in maize samples. FB_3 was quantified using the same multiple reaction monitoring (MRM) transitions as FB_2 due to the structural similarities of FB_2 and FB_3 varying only in the position of one OH group. Only six out of seven calibration points were used for AFB_2 and AFG_2 as aflatoxins were applied as a mixture, already reflecting the typical ratio of AFB_1

Table 1 Concentrations in stock solution [ng/mL], LODs [ng/mL], working range [ng/mL], and average $w_{1/2}$ values for the quantifiable toxins

Toxin	Concentration in stock solution [ng/mL]	LOD [ng/mL]	LOQ [ng/mL]	Working range [ng/mL]	Average $w_{1/2}$ [s]
AAL	1000	0.10	0.50	0.5–50	3.0
AFB_1	97.5	0.025	0.05	0.05–4.89	2.4
AFB_2	28.8	0.025	0.05	0.05–1.44	2.7
AFG_1	97.5	0.025	0.05	0.05–4.89	2.7
AFG_2	28.8	0.025	0.05	0.05–1.44	3.4
ALT[a]	1000	0.30	1.00	1.00–100	2.4
AME	10000	1.00	5.00	5–500	4.8
AOH	10000	1.00	5.00	5–500	3.0
ATX I	15000	3.00	7.50	7.5–750	2.3
BEA	10000	1.50	5.00	5–500	3.9
DON	12400	2.49	6.25	6.25–625	2.0
3–AcDON	5090	0.51	2.50	2.50–250	1.5
EnA	1000	0.20	0.50	0.5–50	3.6
EnA1	1000	0.20	0.50	0.5–50	4.2
EnB	500	0.07	0.25	0.5–50	4.0
EnB1[a]	1000	0.20	0.5	0.5–50	4.5
FB_1	5000	0.33	1.00	5–500	2.2
FB_2	2000	0.13	0.50	1–100	2.1
FusC	10000	1.00	3.00	5–500	3.3
GT	2000	0.40	1.00	1–100	2.6
OTA	97.6	0.02	0.05	0.05–4.88	3.1
STC	200	0.025	0.05	0.05–5.00	3.5
T-2	199	0.04	0.10	0.1–10	2.9
HT-2	2000	0.20	1.00	1–100	2.6
TEN	200	0.02	0.10	0.1–10	2.5
ZEN	990	0.15	0.50	0.5–50	3.5

[a] Toxins combined in a second stock solution

to AFB_2 and AFG_1 to AFG_2. Deuterated internal standards of FB_1, OTA, ZEN, DON, 3-AcDON, and T-2 toxin were available and added to each sample at constant concentrations (d_2-ZEN, 70 μg/kg; d_1-DON, 840 μg/kg; d_5-OTA, 7.00 μg/kg; d_3-T-2, 56 μg/mL; d_3-3-AcDON, 420 μg/kg; and d_6-FB_1, 350 μg/kg). If internal standards were added, calibration was carried out using the intensity ratios of labeled and unlabeled mycotoxin as described by Cramer et al. (Cramer et al. 2007). The calibration solutions were measured twice each day and the average of both calibrations used for calculations.

Method performance

Recovery rates for all analytes were evaluated by spiking 5 g of blank polenta in duplicate at three different levels. These spiked samples were extracted analog to the samples. Repeatability was calculated by analyzing the spiked blank matrix extracts at three different levels on two separate days. Average repeatability, recovery, and regression coefficients are given in Table 2. AAL, EnB, STC, and AME show recoveries below 70 %. However, the recovery rates are reproducible, and as there are no legal limits for these substances, these recovery rates were regarded as acceptable for the aim of this study according to CEN/TR 16059:2010 (CEN 2010). TEN showed a high recovery rate of 160 %. The high recovery rate is reproducible and is probably caused by signal enhancing matrix effects. It is regarded as acceptable for this study, as there are no positive samples for TEN. It is a common drawback of multi-analyte methods that not all analytes can be extracted with good efficiency due to the diverse range of polarity (Sulyok et al. 2007).

Samples

Forty-two ground maize samples from South Africa were provided by the Agricultural Research Council, South Africa.

Table 2 Method performance characteristics of all toxins analyzed in maize matrix

Toxin	Repeatability [%]	Regression coefficient	Recovery [%]
AAL	7.8	0.9960	60.±9.7
AFB_1	5.5	0.9966	120±8.3
AFB_2	7.8	0.9979	120±8.7
AFG_1	5.0	0.9971	120±22.1
AFG_2	12	0.9912	86±13
ALT	5.2	0.9932	110±8.5
AME	9.0	0.9999	70±13
AOH	5.6	0.9998	76±8.2
ATX I	3.8	0.9994	110±8.5
BEA	13	0.9921	99±9.2
DON	4.3	0.9980	97±11
3-AcDON	2.3	0.9987	87±13
EnA	14	0.9840	89±4.7
EnA1	15	0.9915	70±2.1
EnB	14	0.9974	67±20
EnB1	19	0.9998	80±20.1
FB_1	5.5	0.9970	100±6.3
FB_2	4.2	0.9957	76±8.5
FusC	8.1	0.9997	80±6.5
GT	7.5	0.9980	110±8.6
OTA	8.6	0.9967	100±17
STC	10	0.9974	51±13
T-2	13	0.9994	120±14
HT-2	2.8	0.9983	100±6.4
TEN	4.0	0.9964	160±10
ZEN	6.9	0.9987	120±16

Samples were extracted using a modified approach described by Sulyok et al. (Sulyok et al. 2006). Approximately 5 g of ground samples was weighed in a 40-mL polypropylene tube. Twenty milliliters of an extraction solvent (ACN/H_2O/FA, 79+20+1, *v*/*v*/*v*) was added, and the samples were extracted for 1 h at 150 rpm on a laboratory shaker. Afterwards, the samples were allowed to settle for 30 min. One hundred twenty-five microliters of the supernatant was mixed with 25 μL of the internal standard mix and 850 μL of water resulting in an eightfold dilution of the raw extract. Samples were directly used for micro HPLC-MS/MS analysis. The samples were extracted in duplicate, and the results given represent the mean analyte concentration of both individual samples (standard deviation indicated).

Micro HPLC-MS/MS settings

Chromatographic separation was carried out using an Eksigent™ MicroLC 200 System (Eksigent, Darmstadt, Germany). The column used was 100×0.5 mm filled with ReproSil-Pur 120 C18-AQ material (3 μm particle size) by Dr. Maisch GmbH (Augsburg, Germany). The column was equipped with a M 538 filter system (IDEX Health and Science, Wertheim-Mondfeld, Germany). A binary gradient consisting of acetonitrile (A) and water (B) (both with 0.1 % formic acid) was applied. Ten microliters of sample solution was injected. Starting conditions were 15 % A at a flow rate of 20 μL/min and held for 0.2 min, followed by a linear gradient to 90 % A and a flow rate of 40 μL/min at 7 min. These

Table 3 MRM parameters

Compound	Parent ion [*m*/*z*]	t_R[min]	Quantifier/qualifier [Da]	DP [V]	CE [V]	CXP [V]
AAL	$[M+H]^+$ 522	2.70	328/292	171	31/37	14/14
AFB_1	$[M+H]^+$ 313	3.64	241/285	100	45/45	6/6
AFB_2	$[M+H]^+$ 315	3.45	259/287	100	35/35	6/6
AFG_1	$[M+H]^+$ 329	3.45	243/283	100	35/35	6/6
AFG_2	$[M+H]^+$ 331	3.26	245/275	100	35/35	6/6
ALT	$[M+H]^+$ 293	3.33	257/239	76	19/27	16/14
AME	$[M-H]^-$ 271	4.81	228/213	−135	−40/−50	−11/−15
AOH	$[M-H]^-$ 257	3.79	215/213	−165	−34/−30	−7/−13
ATX I	$[M-H]^-$ 351	3.71	315/263	−165	−20/−38	−10/−11
BEA	$[M+H]^+$ 784	7.31	244/262	295	33/35	14/6
DON	$[M+H]^+$ 297	2.04	203/249	76	17/15	12/16
d_1-DON	$[M+H]^+$ 298	2.04	203/249	76	17/15	12/16
3-AcDON	$[M+H]^+$ 339	2.85	231/203	150	25/25	4/8
d_3-3-AcDON	$[M+H]^+$ 342	2.85	231/203	150	25/25	4/8
EnA	$[M+H]^+$ 682	7.85	210/100	285	37/85	12/14
EnA1	$[M+H]^+$ 668	7.57	210/228	280	35/37	4/6
EnB	$[M+H]^+$ 640	6.98	196/214	87	37/37	10/20
EnB1	$[M+H]^+$ 654	6.28	196/86	150	33/85	13/13
FB_1	$[M+H]^+$ 722	2.98	334/370	121	55/55	8/8
FB_2	$[M+H]^+$ 706	3.38	336/354	121	55/55	8/8
FB_3	$[M+H]^+$ 706	3.24	336/354	121	55/55	8/5
d_6-FB1	$[M+H]^+$ 728	2.98	340/376	121	55/55	8/8
FusC	$[M+H]^+$ 432	4.33	141/115	61	89/129	10/18
GT	$[M+H]^+$ 327	3.41	245/111	56	23/33	4/15
OTA	$[M+H]^+$ 404	4.71	239/102	60	33/101	14/16
d_5-OTA	$[M+H]^+$ 409	4.71	239/102	60	33/101	14/16
STC	$[M+H]^+$ 325	5.07	281/253	211	51/57	16/8
T-2	$[M+NH_4]^+$ 484	4.46	305/215	65	19/24	19/14
d_3-T-2	$[M+NH_4]^+$ 487	4.46	215/308	65	19/24	19/14
HT-2	$[M+NH_4]^+$ 442	3.70	263/215	54	22/18	14/18
TEN	$[M+H]^+$ 415	3.72	312/256	175	31/39	10/8
ZEN	$[M-H]^-$ 317	4.83	175/131	−190	−34/−34	−7/−7
d_2-ZEN	$[M-H]^-$ 319	4.83	177/133	−190	−34/−34	−7/−7

t_R retention time, *DP* declustering potential, *CE* collision energy, *CXP* collision cell exit potential

conditions were held constant for 1 min and then decreased to starting conditions within 0.2 min. The column was equilibrated for 0.8 min prior to the next injection. Temperature was held at 40 °C. The mass spectrometer used was an AB SCIEX (Darmstadt, Germany) QTRAP® 5500 with ESI. The curtain gas was set to 35 psi, the collision activated dissociation gas to "medium," GS1 to 35 psi, and GS2 to 45 psi. The source temperature was 500 °C. The ion spray voltage was 5500 V in positive mode and −4500 V in negative mode. Entrance potentials of 10 and −10 V were used in positive and negative modes, respectively. Unit resolution was applied. The pause between mass ranges has been set to 5.007 ms, and the setting time was 50 ms. Additional detailed MS parameters including extracted ion chromatograms for all MRM transitions can be found in the Supplementary Material. Data analysis was done with Analyst® Software (AB SCIEX, Darmstadt, Germany) (version 1.5.2). All chromatograms were smoothed with a smoothing width of five points within the *Analyst*® software.

Parent and fragment ions (quantifier and qualifier) for each analyte were chosen regarding to the highest possible intensity after direct infusion of the analyte in matrix-matched solution. The potentials given in Table 3 were optimized accordingly.

Results and discussion

Method development

The use of multi-mycotoxin methods is widely spread among analytical laboratories but usually requires HPLC-MS/MS systems with high sensitivity. Thus, as this instrumentation is usually expensive, times for analysis are limited and the use of high throughput methods is often mandatory. Additionally, most laboratories are directed to work environmentally friendly and to reduce solvent consumption. In order to tackle these two aspects, a micro HPLC-MS/MS method for the simultaneous quantification of 26 mycotoxins in grain reaching the threshold levels set by the European Union (EC 2006a, b) was developed. The development of fast and sensitive methods for the analysis of mycotoxins ranging from highly polar to nonpolar compounds is especially challenging, as several aspects have to be considered. When using micro HPLC-MS/MS, the time of a measurement cycle (cycle time) becomes a critical parameter. Early eluting peaks (like DON) result in very narrow peaks ($w_{1/2}$, see Table 1), requiring a cycle time of 0.4 s or less. Therefore, short dwell time for each MRM monitored has to be applied. While it is desirable in conventional HPLC-MS/MS approaches to use dwell times of 10 ms or higher, this is not applicable in micro HPLC-MS/MS. Even dwell times of only 5 ms for each of the 66 MRM transitions monitored lead to a cycle time of about 0.5 s, including polarity switching and thus result in an insufficient number of data points per peak. To overcome this problem, we applied dwell times as low as 2 ms per MRM, accepting a moderate loss of sensitivity. However, this was only possible as a sensitive mass spectrometer with fast polarity switching was applied. Alternatively, if more analytes are to be implemented in this method, algorithms optimizing the period in the HPLC run, when a specific MRM transition is observed, such as *Scheduled MRM*® (AB SCIEX, Darmstadt) should be used.

The method developed in this study shows good performance regarding linearity, recovery, and repeatability. All performance criteria set by the European Union for aflatoxins, OTA, DON, ZEN, FB_1 and FB_2, T-2, and HT-2 are reached (European Commission Regulation EC, EC 2006a, b). If only the regulated toxins are of interest, analysis time can be even shorter as all regulated toxins elute within 5 min, as can be seen from the chromatogram in Fig. 1. Analysis of these selected compounds can be achieved within a total run time of

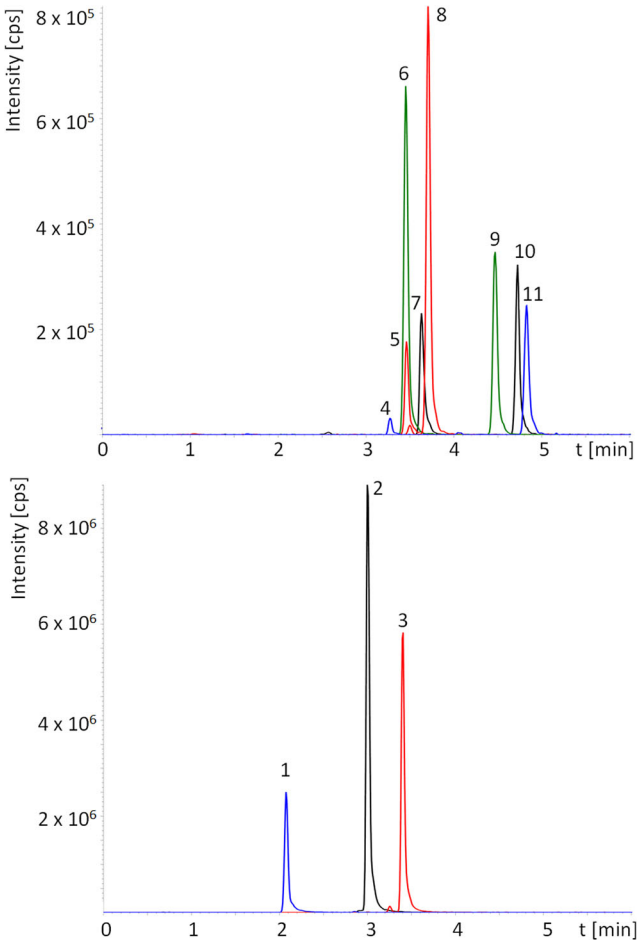

Fig. 1 Micro HPLC-MS/MS chromatogram of all toxins regulated by the European Union. The figure is split in two different panes due to different intensities. The *peak labels* indicate the following toxins: *1* DON, *2* FB_1, *3* FB_2, *4* AFG_2, *5* AFG_1, *6* AFB_2, *7* AFB_1, *8* HT-2, *9* T-2, *10* OTA, *11* ZEN

6 min (including re-equilibration). Advantages of micro HPLC MS/MS are short analysis times as demonstrated here, increased sensitivity due to high ionization ratios, and the usage of less solvents, making it environmentally beneficial. The shortest method in literature for all regulated toxins with comparable LODs and LOQs was described by Frenich et al. (Frenich et al. 2009). This method uses UPLC-MS/MS with chromatographic separation within 8.5 min. Application of micro HPLC MS/MS allows comparable chromatographic separation, LODs, and LOQs but uses less than 10 % of solvent.

Samples

Forty-two maize samples from South Africa were analyzed. All samples contained fumonisins B_1, B_2, and B_3 with maximum concentrations of 1600 +/- 1.3 (FB_1), 342±2.7 (FB_2), and 210±0.5 μg/kg (FB_3). No sample exceeded the European regulations for the sum of FB_1 and FB_2 in raw maize samples. The presence of fumonisins was expected as the fumonisin-producing fungi occur in subtropical areas (Soriano and Gragacci 2004). No DON and only low levels of its metabolite 3-AcDON (up to 26±1.7 μg/kg, 8 out of 42 samples positive) could be found. Furthermore, only trace levels of other trichothecenes (T-2, HT-2) were found as these toxins are mainly formed in colder areas (Goswami and Kistler 2004). OTA and ZEN were found continuously (19 out of 42 samples positive for OTA and 7 out of 42 samples positive for ZEN). Five samples exceeded the maximum level for OTA in raw cereals with concentrations of up to 12±4.7 μg/kg. The maximum levels for ZEN were not reached. The highest concentration found was 73±0.2 μg/kg. AFG_1 was found in a few samples at low concentrations (two samples positive, highest level 0.9 ±0.1 μg/kg). FusC occurred commonly (10 out of 42 samples positive) at high levels of up to 1200±6.4 μg/kg. Some less analyzed toxins could be detected in quantifiable amounts. The presence of the Alternaria toxins ATX I and ALT could be shown. ATX I was found in one sample with a concentration of 43±6.7 μg/kg. ALT occurred in three samples (highest level 13±0.3 μg/kg). GT was found in three samples with a maximum level of 20±1.2 μg/kg. The results are summarized in Table 4.

Table 4 Mycotoxin contamination of analyzed maize samples (n=42)

Toxin	Positive samples [%]	Highest [μg/kg]	Mean pos. [μg/kg]	Lowest [μg/kg]
AAL	0	–	–	–
AFB_1	0	–	–	–
AFB_2	0	–	–	–
AFG_1	4.8	0.9±0.1	0.8±0.5	0.8±0.1
AFG_2	0	–	–	–
ALT	7.1	13±0.3	8.7±1.7	6±2.0
AME	7.1	<LOQ	<LOQ	<LOQ
AOH	2.4	<LOQ	<LOQ	<LOQ
ATX I	2.4	43±6.9	43±6.9	43±6.9
BEA	7.1	<LOQ	<LOQ	<LOQ
DON	0	–	–	–
3-AcDON	19	26±1.7	22±1.7	13±1.5
EnA	0	–	–	–
EnA1	0	–	–	–
EnB	0	–	–	–
EnB1	0	–	–	–
FB_1	100	1600±1.3	180±2.6	120±0.7
FB_2	100	340±2.7	36±0.6	25±0.2
FB_3	100	210±0.5	30±0.1	25±0.9
FusC	24	1200±6.7	370±52	32±0.4
GT	7	20±1.2	13±0.1	8.3±0.7
OTA	48	12±4.5	4.4±0.8	0.3±0.1
STC	0	–	–	–
T-2	2.4	<LOQ	<LOQ	<LOQ
HT-2	2.4	<LOQ	<LOQ	<LOQ
TEN	0	–	–	–
ZEN	17	73±0.2	36±2.4	12±0.6

Conclusions

A fast, sensitive, and cost-effective method for the simultaneous quantification of 26 mycotoxins using micro HPLC-MS/MS in maize was developed successfully. All toxins regulated by the European Union can be determined at or below their corresponding maximum levels within 6 min run time including re-equilibration. Furthermore, some emerging mycotoxins, e.g., the enniatins or several toxins of the genus Alternaria, can be quantified. The samples analyzed highlight the use of multi-mycotoxin analysis. Some of the analyzed samples showed contamination with rarely occurring toxins (altenuene, altertoxin I, gliotoxin). As the occurrence data for these toxins is not sufficient for risk evaluation, multi-mycotoxin screening methods can help to add data. Additionally, high levels of the mutagenic but not regulated toxin Fusarin C were found.

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Conflict of interest None

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