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A New High-Performance Liquid Chromatography−Tandem Mass Spectrometry Method Based on Dispersive Solid Phase Extraction for the Determination of the Mycotoxin Fusarin C in Corn Ears and Processed Corn Samples

Karin Kleigrewe, Anna-Carina Söhnel, and Hans-Ulrich Humpf*

Institute of Food Chemistry, University of Münster, Münster, Germany

Supporting Information

ABSTRACT: Fusarin C is a mycotoxin that is produced by a variety of *Fusarium* species and is therefore a possible contaminant in food and feed. For this reason, a reliable high-performance liquid chromatography−tandem mass spectrometry (HPLC-MS/MS) method for the determination of fusarin C in food and feed samples was developed based on dispersive solid phase extraction (DSPE). This method has a limit of detection (LOD) of 2 μg/kg, a limit of quantitation (LOQ) of 7 μg/kg, and a recovery rate of 80%. Fifty different corn samples were analyzed, and fusarin C was detected in 40 of them. The fusarin C level varied in kernels of corn ears from not detectable up to 83 mg/kg and in food samples from not detectable up to 28 μg/kg. The co-occurrence of further structural analogues of fusarin C was confirmed by high-performance liquid chromatography Fourier transformation mass spectrometry (HPLC-FTMS). In addition, the stability of fusarin C under storage conditions was evaluated.

KEYWORDS: Fusarin C, mycotoxin, QuEChERS, dispersive solid phase extraction, *Fusarium*, high-performance liquid chromatography−tandem mass spectrometry, Fourier transformation mass spectrometry, corn

INTRODUCTION

Fusarium fungi produce a wide variety of toxic secondary metabolites that pose a potential health risk for animals and humans. For the most common *Fusarium* toxins like fumonisins, zearalenone, and trichothecenes, maximum limits have been set in the European Union and other countries. The occurrence of regulated mycotoxins in food and feed is well monitored. In contrast, only little is known about the occurrence of fusarin C, even though it is classified as possibly carcinogenic by the International Agency for Research on Cancer. These insufficient data are certainly due to the instability of fusarin C to light and the possible rearrangement to various isomers, which pose a challenge for quantitation.[1,2] Furthermore, analytical standards of fusarin C are not commercially available.

The typical structure of fusarin C consists of a 2-pyrrolidone moiety substituted with an epoxide group and a pentaene moiety (Figure 1).[3,4] This pentaene chain can easily rearrange under UV light to form several stereoisomers like (10*Z*)-, (8*Z*)-, and (6*Z*)-fusarin C.[5−7] In addition, an intramolecular ring formation of the ethanolic side chain leads to fusarins D and A.[8,9] Savard and Miller[8] also reported a rearrangement of fusarin C under reversed phase conditions, and Lu and Jeffery[10] elucidated the structure of fusarin X, which is a 1-hydroxy analogue of fusarin C. All relevant structures are shown in Figure 1.

Fusarin C is mutagenic in the Ames test using *Salmonella* strains activated by S-9 mix liver homogenate.[3,4,11,12] The C13−C14 epoxide of fusarin C is essential for the mutagenicity because fusarins containing no epoxide like fusarins A and D are not mutagenic.[9] In the presence of a microsomal activation system, fusarin C induces sister chromatid exchange, micronuclei, chromosomal aberrations, and the formation of 6-thioguanine resistant mutants in V79 cells as well as asynchronous replication of polyoma DNA sequences.[12,13] Nevertheless, fusarin C does not cause the formation of DNA adducts as *Fusarium* culture extracts do.[14] Some indications are present for an acute toxicity of fusarin C as a high dose of 100 mg fusarin C/kg body weight was lethal to all five female Wistar rats tested, whereas only two out of five male BD IX rats receiving this dosage level died.[15] A recent report also describes mycoestrogenic properties of fusarin C.[16]

The occurrence of fusarin C was already reported in visibly *Fusarium* infected and healthy corn kernels in the Transkei region, South Africa,[11] in Pennsylvania, United States,[17] and in Linxian county, China.[12] Because of that, fusarin C is also discussed as a cause of the high incidence of esophageal cancer in these regions.[12,18]

Because fusarin C is produced by several *Fusarium* species like *Fusarium verticillioides*, *Fusarium acuminatum*, *Fusarium crookwellense*, *Fusarium nygamai*, *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium poae*, *Fusarium sambucinum*, *Fusarium sporotrichioides*,[19−22] it is a potential contaminant in food and feed.

Published methods for the quantitation of fusarin C in corn samples used solvent extraction followed by fractionation on silica gel or amino-bonded solid phase columns. The analysis was performed with either normal phase or reversed phase high-performance liquid chromatography with ultraviolet detection (HPLC-UV).[2,11,17] To study fungal culture extracts, a high-performance

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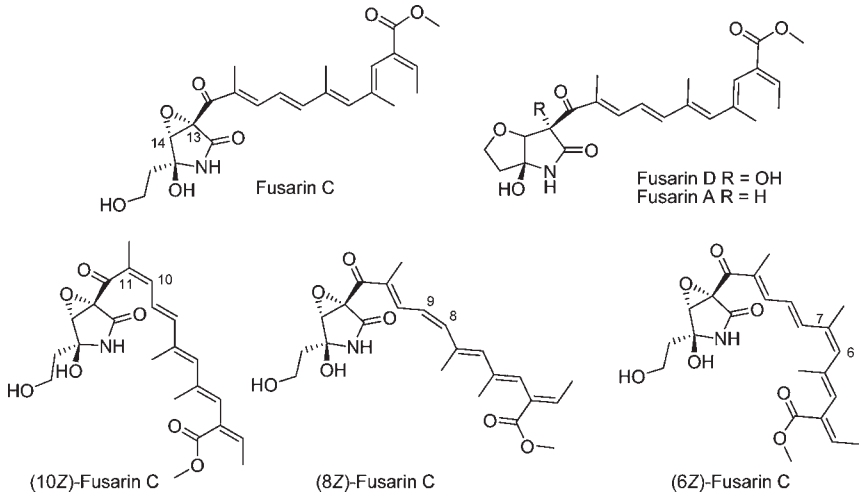

Figure 1. Structures of fusarins.

liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method was published recently.[23] Furthermore a competitive indirect enzyme-linked immunosorbent assay was developed for the detection of fusarins in food, but up to now, no food samples were analyzed with this technique.[24] Therefore, a new method was developed that allows the quick and reliable quantitation of fusarin C in food and feed samples even at low concentrations. Nowadays, dispersive solid phase extraction (DSPE), better known as QuEChERs, is used for sample preparation for various analytes ranging from pesticides to mycotoxins.[25,26] In this study, this method was adapted for the analysis and quantitation of fusarin C in corn samples.

MATERIALS AND METHODS

Chemicals and Materials. All chemicals were purchased from Sigma-Aldrich GmbH (Seelze, Germany), Carl Roth GmbH + Co. KG (Karlsruhe, Germany), or VWR International GmbH (Darmstadt, Germany). Water for HPLC separation was purified by a Milli-Q Gradient A 10 system (Millipore, Schwalbach, Germany). Before usage, $MgSO_4$ was heated for 10 h to 500 °C in a muffle furnace to remove residual water. Bondesil-PSA, 40 μm, was purchased from Varian now Agilent Technologies (Böblingen, Germany).

Isolation of Fusarin C Standard. Fusarins are not commercially available. Therefore, *F. verticillioides* MRC 0712 was used for the isolation of fusarins C and D standard material. Five day old submerge cultures (MYRO medium) of *F. verticillioides* MRC 0712 were extracted by solid phase extraction using C18-SPE cartridges and further purified by preparative normal phase HPLC.[1,20,27] NMR and FTMS measurements were in agreement with literature data of fusarins C and D.[7,9] The purity of the fusarin C stock solution was confirmed by HPLC with UV and evaporative light scattering detection (HPLC-UV-ELSD) with a LC-20AT system (Shimadzu, Kyoto, Japan) on a 150 mm × 4.6 mm i.d., 5 μm, Zorbax Eclipse XDB-C18 column (Agilent Technologies) using isocratic conditions of 58% MeOH and 42% H_2O for 40 min. The wavelength of the UV detector was set to 363 nm. The temperature of the ELSD was set to 40 °C, and 2.5 bar of pressurized air was used. Data acquisition was performed with LCsolution Version 1.21 SP1, LabSolutions (Shimadzu, Kyoto, Japan).

Stability Studies. The stability of fusarins C and D was confirmed in a long-term experiment. Therefore, samples containing 0.26 mg/mL fusarins C or D and 0.42 mg/mL phenothiazine as an internal standard were stored at −80 °C in amber screw cap vials. Phenothiazine as an internal standard was already used as in a previous study.[1] As a solvent, MeOH/H_2O (50/50; v/v) was used. Before measurement, the samples were defrosted and measured in triplicate weekly about a time period of 8 weeks with a LC-10ATVP HPLC-DAD system (Shimadzu). The ratio of the peak areas of fusarin C or D and phenothiazine was plotted over the time. For quantitation of fusarin C or D, the wavelength of 363 nm was used, and for phenothianzine, 254 nm was used. For stability testing, the same chromatographic conditions were used as mentioned above.

HPLC-MS/MS. An API 4000 QTrap mass spectrometer (Applied Biosystems, Darmstadt, Germany) coupled to an Agilent 1100 series HPLC was used for the detection of fusarin C in multiple reaction monitoring mode (MRM). Data acquisition was performed with Analyst 1.4.2 software (Applied Biosystems).

HPLC Parameters. Chromatographic separation was carried out on a 150 mm × 2.1 mm i.d., 5 μm, Zorbax Eclipse XDB-C18 column (Agilent Technologies) using a linear binary gradient at a column temperature of 40 °C. The injection volume was 30 μL, and the autosampler was cooled to 4 °C. The flow rate was 300 μL/min. Solvent A was MeOH with 5% tetrahydrofuran (v/v), and solvent B was H_2O. The HPLC was programmed isocratically for the first 16 min at 55% A. Afterward, the column was washed with 100% solvent A and equilibrated at starting conditions.

MS/MS Parameters. For electrospray ionization, the ion voltage was set to +5500 V in the positive mode, and nitrogen was used as the curtain gas (20 psi). Zero-grade air was used as a nebulizer gas (35 psi) and as a drying gas (45 psi) heated to 350 °C. The declustering potential was set for all transitions to 61 V. For quantitation, transition reactions were monitored for a duration of 100 ms each. The proton adduct of fusarin C was measured with the following collision energy (CE) and collision cell exit potential (CXP): Fusarin C $[M + H]^+$ 432.3−115.0 (CE 129 V, CXP 18 V), $[M + H]^+$; 432.3−141.0 (CE 89, CXP 18). Both quadrapoles were set at unit resolution. The first MRM transition listed was used as a quantifier, and the second was used as a qualifier.

Sample Preparation. Five grams of freeze-dried, ground, and homogenized kernels of corn ears collected from fields were weighed into a 50 mL centrifuge tube. Five milliliters of H_2O and 15 mL of acetonitrile were added. Then, they were shaken vigorously for 1 min by using a Vortex mixer at maximum speed. Afterward, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added, and the samples were immediately mixed for 1 min on a Vortex mixer to prevent the formation of $MgSO_4$ conglomerates. Subsequently, the samples were centrifuged at 3000 rpm for 5 min. Five milliliters of the supernatant was added to 750 mg of

$MgSO_4$ and 125 mg of Bondesil-PSA (primary and secondary amine) as a cleanup step. After 1 min of shaking, the sample was centrifuged at 3000 rpm for 5 min. To concentrate the samples, 3 mL of the DSPE cleanup solution was evaporated to dryness and redissolved in 500 μL of MeOH/H_2O (50/50; v/v). To remove insoluble particles, the samples were centrifuged at 9000 rpm for 10 min, and the supernatant was analyzed by HPLC-MS/MS. Highly contaminated samples were diluted prior to measurement.

For food and feed samples, the preparation was slightly different. Ten grams of the homogenized corn samples was suspended with 5 mL of H_2O and 15 mL of acetonitrile. Again, they were shaken vigorously for 1 min by using a Vortex mixer at maximum speed. Afterward, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added, and the samples were mixed again. After centrifugation, 5 mL of extract was added to 1.5 g of $MgSO_4$ and 250 mg of PSA, and the sample was shaken again for 1 min. After the centrifugation step, only 2.8 mL was evaporated to dryness and dissolved in 500 μL of MeOH/H_2O (50/50; v/v). Insoluble particles were removed by centrifugation, and the supernatant was analyzed by HPLC-MS/MS. All laboratory work was carried out under reduced light conditions due to the instability of fusarin C. The centrifuge tubes were wrapped with aluminum foil, and for analysis, amber screw-cap vials were used.

Matrix Calibration. Because of the lack of an internal standard, the quantitation of fusarin C was done by matrix-matched calibration. Therefore, blank samples, which were corn samples containing no fusarin C, were prepared as mentioned above. Instead of dissolving the evaporated samples with 500 μL of MeOH/H_2O, they were spiked with 10, 30, 50, 70, 90, 110, 130, or 150 ng/mL fusarin C. For quantitation, the calibration curves measured before and after the samples were averaged.

Validation Parameters of the HPLC-MS/MS Method for the Determination of Fusarin C in Corn Products. According to the calibration method of the German Standard DIN 32645, the limit of detection (LOD) and the limit of quantitation (LOQ) were calculated.[28] An α error of 0.05 and a confidence range of ±33.3% ($k = 3$) were applied (see eqs 1 and 2).

$$x_{LOD} = s_{x0} \cdot t_{f,\alpha} \cdot \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{\bar{x}^2}{Q_{xx}}} \quad (1)$$

$$x_{LOQ} = k \cdot s_{x0} \cdot t_{f,\alpha} \cdot \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(k \cdot x_{LOD} - \bar{x})^2}{Q_{xx}}} \quad (2)$$

x_{LOD} = LOD, s_{x0} = standard deviation of the procedure, t = critical value of the Student distribution, f = degrees of freedom, α = level of significance, n = number of measurements per calibration point, m = number of calibration standards, $\bar{x}$ = mean of all x_i, Q_{xx} = the sum of $(x_i - \bar{x})^2$, x_{LOQ} = limit of quantification, and k = confidence range.

Five grams of blank sample was spiked at the beginning of the sample preparation with 25, 75, 125, 175, 225, 275, and 325 μL of 1 μg/mL fusarin C stock solution, respectively. The sample preparation was done as described above. Each calibration point was prepared in duplicate, and each sample was measured twice. Calibration curves were calculated by linear regression. The recovery was determined by the function of recovery from the matrix matched calibration curve and from the calibration curve of the spiked samples, which were spiked at the beginning of sample preparation, so that the recovery is calculated over the entire working range.[29]

HPLC-FTMS. Random selected samples, which contained detectable amounts of fusarin C, were analyzed by high-performance liquid chromatography with Fourier transformation mass spectrometry detection (HPLC-FTMS) to analyze the fragmentation of the fusarin C isomers. Therefore, an Accela LC 60057-60010 system (Thermo Fisher Scientific, Bremen, Germany) was linked to a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific). Data acquisition was performed with Xcalibur 2.07 SP1 (Thermo Scientific). Separation was carried out on 150 mm × 2.1 mm i.d., 5 μm, Zorbax Eclipse XDB-C18 column (Agilent Technologies) using a binary gradient at a column temperature of 30 °C. The injection volume was 10 μL, and the autosampler was cooled to 7 °C. The flow rate was set to 250 μL/min. Solvent A was MeOH with 5% tetrahydrofuran (v/v), and solvent B was H_2O. The HPLC was programmed as follows: linear gradient from 55 to 56.5% A in 18 min. Afterward, the column was washed with 100% solvent A and equilibrated at starting conditions. The mass spectrometer was operated in the positive ion mode, and ionization was performed with heated electrospray ionization. Further conditions were as follows: capillary temperature, 225 °C; APCI vaporizer temperature, 250 °C; sheath gas flow, 35; aux gas flow, 10; source voltage, 4 kV; capillary voltage, 8 V; tube lens, 120 V; multiple 00 offset, −4.00 V; lens 0 voltage, −4.20; gate lens offset, −35.00 V; multipole 1 offset, −8.00 V; and front lens, −5.25. The first scan event contained a total ion scan of a mass range from m/z 200 to 800 with a resolution of 60000. Second, the product ion spectrum of the sodium adduct of fusarin C was monitored: m/z 454.18 $[M + Na]^+$, CID 35%, isolation width 1.5, resolution = 7500, and activation $Q = 0.250$.

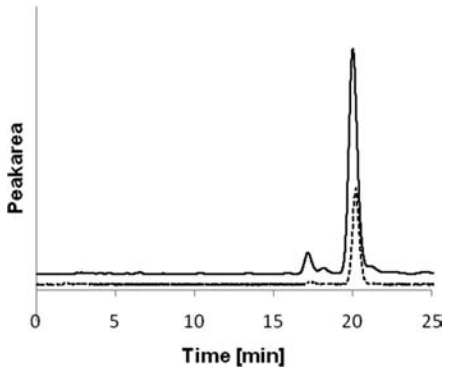

Figure 2. HPLC-UV-ELSD chromatogram of fusarin C standard. The dotted line represents the chromatogram of the ELSD, and the continuous line represents the chromatogram of the UV-detector (363 nm).

RESULTS AND DISUCSSION

As fusarin C might pose a potential health risk to humans and animals due to its mutagenic and possibly carcinogenic properties, the determination of the contamination levels of fusarin C in food and feed is substantial for an appropriate risk assessment. This study concentrated on the screening of corn samples, since *F. verticillioides* produces high amounts of fusarin C and is one of the most prevalent fungi associated with corn kernels in most areas of the world.[30] As fusarin C is reported to be unstable, stability studies were performed as first step.

Stability of Fusarin C. Fusarin C rearranges under reversed phase conditions until the formation of equilibrium.[8] In a stability study of fusarins C and A in which conditions were tested and expected to be encountered in immunoassays, the same formation of equilibrium was reported.[24] Hence, the isolation of pure fusarin C under reversed phase conditions is unfeasible. Figure 2 shows a HPLC-UV-ELSD chromatogram of purified fusarin C standard. The chromatogram has an additional peak at the retention time of 17.3 min and therefore a calculated purity of 97% (by ELSD). This peak is due to the rearrangement of fusarin C. In addition to that, UV radiation enhances the formation of (10*Z*)-, (8*Z*)-, and (6*Z*)-isomers.[3] Therefore, it is crucial to use amber glassware and to reduce light to a minimum.

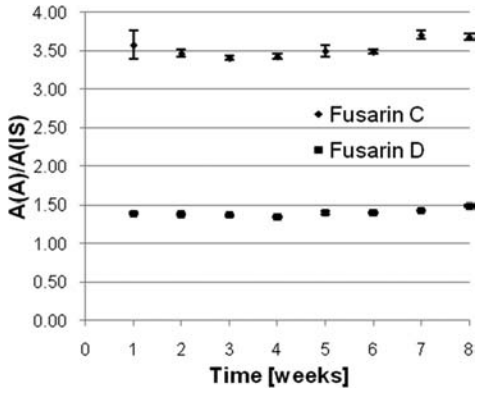

Figure 3. Stability of fusarin C and D during storage at −80 °C. The ratio of the peak area of fusarin C or D (363 nm) and the peak area of phenothiazine (254 nm) were plotted against the time. The measurements were done in triplicate.

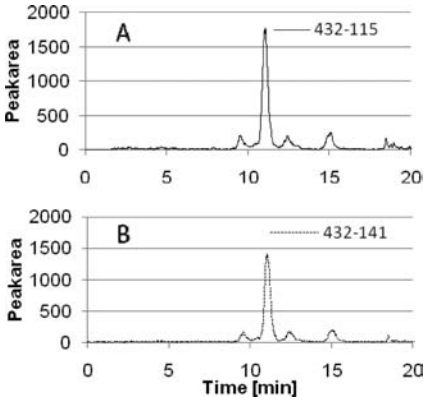

Figure 4. HPLC-MS/MS chromatogram of a corn sample containing fusarin C. (A) Presents the quantifier MRM transition and (B) the qualifier MRM transition.

Furthermore, the stability of fusarin C under storage conditions was tested since in literature a loss of fusarin C under different storage conditions is described.[1,2] Jackson et al.,[1] for example, reported a 25% loss of fusarin C during a time period of 10 days at −20 °C stored as gums. Because quantitation with unstable reference material is impossible, first storage conditions were tested. These experiments were undertaken with fusarin C and fusarin D. Stock solutions of fusarins C and D ($MeOH/H_2O$, 50/50, v/v) were stored at −80 °C, and samples were measured over a time period of 8 weeks by high-performance liquid chromatography with diode array detection (HPLC-DAD). As an internal standard, phenothiazine was used to correct the loss of analyte during sample inlet.[1] Because phenothiazine has an absorption maximum at 254 nm and fusarins C and D at 363 nm, the integration of the peaks was done at different wavelengths. The ratio of the peak area of fusarin C or D and phenothiazine was plotted over the time period of 8 weeks. Figure 3 shows that there is no degradation of fusarins C and D during that time period. The mean ratios differed from 3.71 to 3.41 for fusarin C and from 1.36 to 1.49 for fusarin D, which is due to random errors of the analysis. Because the stability of the fusarin C reference material was confirmed, the basic requirements for establishing a quantitation method of fusarin C were assumed.

Sample Preparation. The sample preparation was done by DSPE according to the QuEChERS protocol, which is used for the simultaneous determination of more than 400 pesticide residues in food and has already been adapted for the mycotoxins deoxynivalenol, zearalenone, T-2 toxin, and HT-2 toxin.[25,26] The advantage of the QuEChERS method is that the sample preparation is done quick, easy, cheap, effective, rugged, and safe and in this case also with reduced light. PSA was used as a dispersive solid phase material, which consists of PSAs and thus reduces the matrix content in the sample. Nevertheless, not all matrix effects during HPLC-MS/MS measurement were prevented, but they were compensated by matrix-matched calibration. The matrix-matched calibration was done with freshly prepared fusarin C calibration solutions ranging from 10 to 150 ng/mL. Therefore, blank samples were spiked at the end of sample preparation. The calibration solutions were measured twice before and after the samples. For quantitation, the resulting two calibration curves were averaged. The LOD was 2 μg/kg (8 ng/mL), and the LOQ was 7 μg/kg (26 ng/mL) according to the calibration method of the German Standard DIN 32645. The recovery rate was determined by the function of recovery from calibration curves of the matrix-matched calibration and from calibration curves of samples spiked at the beginning of sample preparation and yielded a recovery of 80%.[29] In addition, different corn matrices (polenta, corn kernels, cornmeal, and popcorn) were spiked with fusarin C, and their recoveries yielded the same values, demonstrating that the method works for each matrix.

Fusarin C in Corn. At the beginning of this study, kernels of corn ears were analyzed for fusarin C to see if fusarin C occurs in field samples. Therefore, corn ears visibly contaminated by fungi were screened, but also, corn ears that were not visibly contaminated were screened. Figure 4 presents a HPLC-MS/MS chromatogram of a corn kernel sample showing both measured MRM transitions. Because all naturally occurring fusarins except for fusarins A and X have the same exact mass and the same mass transitions in the MRM mode, the chromatograms of samples always reveal a characteristic peak pattern. This similarity was further confirmed by HPLC-FTMS/MS analysis. Figure 5 demonstrates that the extracted ion chromatogram of the sodium adduct of fusarin C at m/z 454.1836 $[FC + Na]^+$ reveals the same peak pattern as the chromatogram of the HPLC-MS/MS in the MRM mode (Figure 4). Furthermore, as can be seen from Figure 5, the product ion spectrum of each peak (m/z 454.2) forms the same fragment of m/z 290 and thus demonstrates the structural similarity. The fragments of m/z 335 and m/z 368 are used to differentiate between fusarin D and fusarin C (Z)-isomers. The spectra shown in Figure 5B,C represent fusarin C and fusarin D. The identity of spectra shown in Figure 5A,D,E cannot be fully confirmed. However, the spectrum in Figure 5A represents most likely a diastereomer and Figure 5D,E (Z)-isomers.

Nevertheless, for quantitation, only the peak area of fusarin C was used, because the peak area of fusarin D overlaps with a (Z)-isomer (Figure 5). This overlapping cannot be differentiated by HPLC-MS/MS in the MRM mode, and because fusarins D and A lack the mutagenic effect, the concentration of the toxic compounds might be overestimated by summing up the peak areas of all peaks.[9]

The results of the screening of corn kernels are summarized in Table 1. All repeat determinations were measured twice, and the standard deviation (SD) was calculated. The highest detectable amount of fusarin C was 83 ± 0.6 mg/kg, but fusarin C could also be proven in kernels of corn ears that were not visibly contaminated by molds. Out of 25 corn ears tested, fusarin C was not detectable

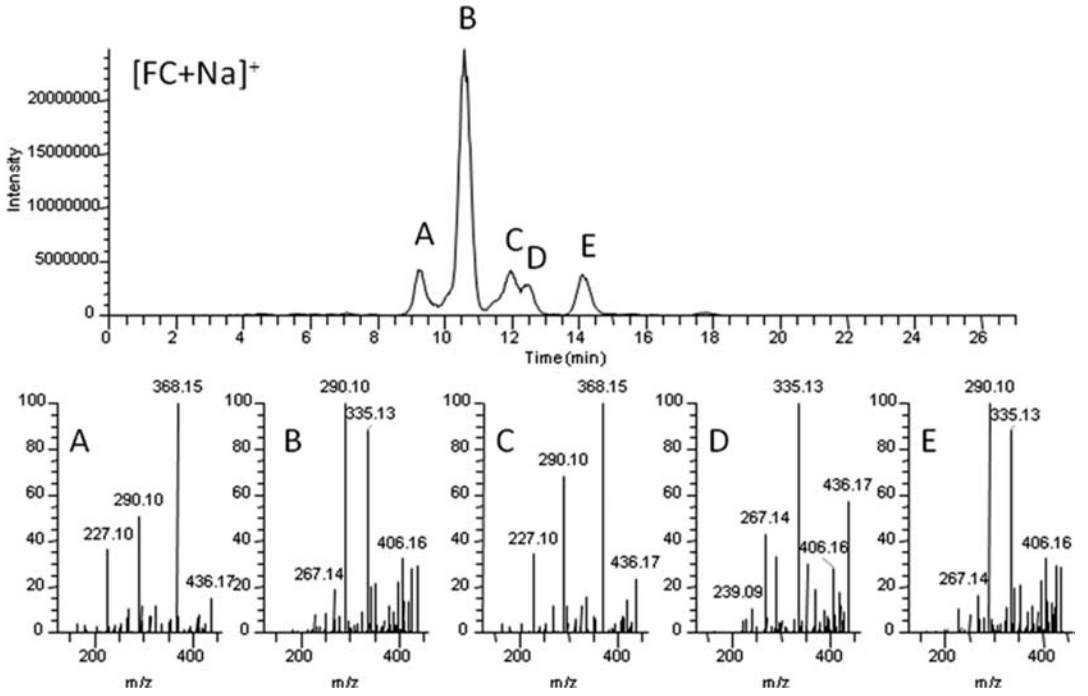

Figure 5. HPLC-FTMS/MS chromatogram of a corn sample. The chromatogram shows the extracted exact mass of the sodium adduct of fusarin C at m/z 454.1836. Below are listed the product ion spectra of m/z 454.18.

Table 1. Fusarin C Levels in Kernels of Corn Ears

sample no.	concn of FC ± SD in kernels of corn ears
1	84 ± 0.6 mg/kg
2	12 ± 0.0 mg/kg
3	1.6 ± 0.0 mg/kg
4	1.5 ± 0.1 mg/kg
5	691 ± 27 μg/kg
6	319 ± 13 μg/kg
7	368 ± 6 μg/kg
8	352 ± 13 μg/kg
9	256 ± 28 μg/kg
10	37 ± 2 μg/kg
11	31 ± 1 μg/kg
12	28 ± 3 μg/kg
13	21 ± 1 μg/kg
14	14 ± 2 μg/kg
15	4.5 ± 1.8 μg/kg
16−22	<LOQ
23−25	ND

in the kernels of three corn ears, seven were below LOQ, six contained up to 40 μg/kg, five contained between 250 and 700 μg/kg, and four samples contained more than 1 mg/kg. No attempts were undertaken to classify the molds on the corn ears. Nevertheless, they seemed to be contaminated not only by *Fusarium* species but also by other molds as for example *Stenocarpella maydis*.[31]

Fusarin C in Food and Feed. Because the occurrence of fusarin C was confirmed in corn ears, the question was if this mutagenic compound is present in food and feed samples. Therefore, cornmeal, polenta, popcorn, tortilla chips, and feed samples were screened (Table 2).

Table 2. Fusarin C Levels in Food Samples

types of food samples	concn of FC ± SD
polenta	2 < LOQ
	2 ND
cornmeal	4 < LOQ
	1 ND
popcorn	28 ± 2 μg/kg
	28 ± 3 μg/kg
	25 ± 1 μg/kg
	1 < LOQ
	2 ND
tortilla chips	2 ND
whole corn	14 ± 2 μg/kg
	11 ± 6 μg/kg
	1 < LOQ

Fusarin C was detectable in four out of five cornmeal samples, but the contents were below LOQ. Two out of five polenta samples showed fusarin C levels below the LOQ. Four out of seven corn samples for the production of popcorn contained fusarin C with 28 ± 7, 28 ± 3, and 25 ± 1 μg/kg, respectively, and one sample was below the LOQ. Two samples of tortilla chips contained no detectable amount of fusarin C. Three samples of corn kernels revealed detectable amounts of fusarin C containing 14 ± 2 and 11 ± 6 μg/kg, and one sample was below the LOQ.

All tested feed samples contained fusarin C: Three samples of corn kernels contained 8.5 ± 1.1, 7.9 ± 2.1, 7.7 ± 0.9 μg/kg fusarin C, and one sample of corn gluten was below the LOQ as well a corn mix sample. All data are summarized in Table 3.

This is the first report about the occurrence of the mutagenic mycotoxin fusarin C in commercially available food products.

Table 3. Fusarin C Levels in Feed Samples

type of feed sample	concn of FC ± SD
corn kernels	8.5 ± 1.1 μg/kg
	7.9 ± 2.1 μg/kg
	7.7 ± 0.9 μg/kg
corn mix	1 < LOQ
corn gluten	1 < LOQ

These samples are not representative but clearly demonstrate the need for further studies. Currently, no tolerable daily intake for fusarin C is available, and no maximum limits have been set. For this reason, any risk assessment based on the detected fusarin C concentrations is not possible. Nevertheless, fusarin C was detectable in 80% of the randomly selected samples.

Taken together, a reliable method for the detection of fusarin C in food and feed samples was developed with a recovery rate of 80%, a LOD of 2 μg/kg, and a LOQ of 7 μg/kg. This report not only reveals the occurrence of fusarin C in corn ears (0−83 mg/kg) but also in food samples like cornmeal, polenta, and popcorn in concentrations ranging from not detectable to 28 μg/kg as well as in feed samples (>LOD to 8.5 μg/kg).

■ ASSOCIATED CONTENT

Supporting Information. Table containing pictures of analyzed corn ears and their fusarin C contents. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +49 251 8333391. Fax: +49 251 8333396. E-mail: humpf@uni-muenster.de.

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■ ABBREVIATIONS USED

DSPE, dispersive solid phase extraction; ELSD, evaporative light scattering detection; PSA, primary and secondary amine

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