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Analysis of Moniliformin in Maize Plants Using Hydrophilic Interaction Chromatography

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A novel HPLC method was developed for detection of the *Fusarium* mycotoxin, moniliformin in whole maize plants. The method is based on hydrophilic interaction chromatography (HILIC) on a ZIC zwitterion column combined with diode array detection and negative electrospray mass spectrometry (ESI^--MS). Samples were extracted using acetonitrile–water (85:15), and the extracts were cleaned up on strong anion exchange columns. By this procedure we obtained a recovery rate of 57–74% moniliformin with a limit of detection at 48 ng/g and a limit of quantification at 96 ng/g using UV detection at 229 nm, which is comparable to current methods used. Limit of detection and quantification using ESI^--MS detection was 1 and 12 ng/g, respectively. Screening of maize samples infected with the moniliformin producing fungi *F. avenaceum, F. tricinctum*, or *F. subglutinans* detected moniliformin levels of 1–12 ng/g in 15 of 28 samples using ESI^--MS detection. To our knowledge this is the first example of HILIC separation in mycotoxin analysis.

KEYWORDS: Moniliformin; *Fusarium*; hydrophilic interaction chromatography; zwitterionic detection; HPLC; maize

INTRODUCTION

Moniliformin (**Figure 1**) is a frequently occurring mycotoxin in cereals and maize world wide (*1*), and produced by several *Fusarium* species including *F. avenaceum, F. proliferatum, F. subglutinans, F. tricinctum* and *F. verticillioides* (*2, 3*). Moniliformin has a weak cytotoxicity (*4*), but it has an acute toxicity comparable to that of other *Fusarium* derived mycotoxins, such as the type A trichothecenes diacetoxyscirpenol and T-2 toxin (*5*). Currently, the level of moniliformin is not regulated in food or feed in the EC, USA or any other country.

The mode of action of monilifomin has been linked to inhibition of enzyme systems and glucogenesis (*6*). Moniliformin has been suggested to be associated with Keshan disease, a human myocardial impairment occurring in areas of China with large consumption of moniliformin contaminated maize (*7, 8*), although others failed to link it to the disease (*9*). The symptoms of Keshan disease are similar to those of animals suffering from moniliformin contamination (*10*). Scandinavian studies have shown that grains from Finland and Norway contain up to 0.81 mg/kg and 0.95 mg/kg moniliformin (*11, 12*), respectively. Worldwide analysis of maize and grain samples have detected moniliformin levels of 2 mg/kg in Austrian cereal grains (*13*) and 3.2 mg/kg in Gambian and South African maize and maize products (*1*). During the last four years there have been numerous cases of ill thrift and health problems especially in dairy cows in Denmark, which has been claimed to be caused by mycotoxins in the maize silage used as feed. The problem has been associated with both *Penicillium* metabolites (*14, 15*) and/or *Fusarium* metabolites.

Surveys have shown that *F. avenaceum* is one of the most frequently observed species in Scandinavian cereal grains (*12, 16*). We therefore hypothesized that moniliformin could be formed in high levels in maize plants.

Determination of moniliformin is very different from other mycotoxins since it is a small, highly polar, acidic molecule with pK_a value of 0.5 (*17–19*). Subsequently, it is thus not well retained on reversed phase chromatography (RP), which is the most powerful HPLC separation mode as it gives very sharp peaks and is compatible with atmospheric pressure ionization mass spectrometric (MS) techniques such as electrospray ionisation (ESI). Positively charged ion-pairing reagent can be added to increase retention (*12, 20–22*), also for MS detection (*11, 16*). However it is our experience that such ion-pair reagent will impair positive ionization on the instrument for many weeks.

For some time we have used hydrophilic interaction chromatography (HILIC) for highly polar substances such as sugars and small acids since it can be interfaced with atmospheric pressure ionization-MS. However HILIC methods seem not to have been used for mycotoxins, even though many mycotoxins like nivalenol, patulin, moniliformin, 3-nitropropionic acid, and terrestric acid are quite difficult to separate by RP chromatography. The only HILIC-like method is that published for cyclopiazonic acid that resembles a mixed ion-exchange–ion-pair chromatographic method as the retention increased with increasing buffer concentration (*23*). HILIC has previously been used for detection of other highly polar compounds such as

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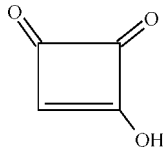

Figure 1. Structure of moniliformin.

carbohydrates, glycopeptides, nucleic acids, and shellfish toxins (*24*, *25*).

Because we were unable to find any studies on the presence of moniliformin in whole maize plant parts, it was decided to develop a method for moniliformin for this. As a result of this we present this HPLC-HILIC-UV method with the possibility to combine with negative ESI-MS for the quantification of moniliformin in maize plants. The method uses a zwitterion stationary phase (ZIC-HILIC) column for analytical separation, and this is to our knowledge this is the first report of a HILIC method for a mycotoxin.

MATERIALS AND METHODS

Chemicals. All chemicals and standards were purchased from Sigma-Aldrich (St. Louis, MO). Solvents were gradient grade and other chemicals analytical grade. Water was purified with a Milli-Q system (Millipore, Bedford, MA).

Moniliformin was purchased as the sodium salt and a 73 μg/mL stock solution of moniliformin in acetonitrile (MeCN)–water (85:15) was made and stored at −20 °C. Purity was confirmed by HPLC-UV-MS using ESI^- and ESI^+ (*26*).

Identification of Viable Fusaria in the Maize Plant Parts. Chopped maize samples, 28 in all, were collected at harvest from different farms across Denmark. Four or five pieces of maize (5–10 mm × 10–30 mm) were placed on 10 Czapek-Dox iprodion dichloran agar (CzID) (*27*) plates per sample. The agar plates were incubated for 7 days at 25 °C below alternating black light and cool white light tubes (12 h on / 12 h off). The emerged *Fusarium* colonies were visually grouped. Colonies representing the different groups were isolated on potato dextrose agar (PDA) with 7 days incubation at 25 °C in darkness. For morphological identification the isolates were grown on PDA, yeast extract sucrose agar (YES) and Spezieller nährstoffarmer agar (SNA) with filter paper. Preparation of PDA, YES, and SNA has been described elsewhere (*28*). The isolated *Fusarium* cultures were identified morphologically (*28*).

Chemical Analyses. *Sample Preparation and Extraction.* Chopped maize plant pieces (5–10 mm × 10–30 mm) from a *Fusarium*-free sample also shown to be moniliformin-free by HPLC-MS were spiked in three replicas with 50 μL of pure solvent, or 0.96; 1.92; 5.72; or 11.5 μg moniliformin/mL to obtain spiked moniliformin levels of 0, 48, 96, 288, and 576 ng/g for HPLC-UV determination. The experiments were performed on three different days to establish day to day variation.

For HPLC-MS analysis spiked samples were prepared in the same way in levels of 3, 6, 12, and 24, 48, and 96 ng/g. Spiked maize samples were incubated at room temperature 30 min prior to extraction, allowing moniliformin to enter plant material. Spiked maize pieces, 4 g, were placed in four 5 mL cryo tubes together with 3 mL MeCN-water (85:15) and approximately 10 3 mm stainless steel balls. The maize pieces were then homogenized using a mini-bead beater (Biospec Products Inc.; Bartlesville, OK) for 1 min. The four 1 g subsamples were pooled in a 50 mL tube together with an additional 28 mL of MeCN-water (85:15). The mashed maize samples were extracted on a rotary shaker at 120 rpm for 30 min and filtered though Whatman no. 1 filters (Brentford, UK). Extracts, 20 mL equivalent to 2 g maize sample, were evaporated to dryness under a stream of nitrogen and dissolved in 2 mL methanol.

Moniliformin Detection of Naturally Contaminated Maize Samples. Chopped pieces of maize from the 28 samples used in the *Fusarium* analysis were analyzed for moniliformin contamination. Ten g maize sample were ground and extracted with 160 mL MeCN–water (85:15). A subsample, 32 mL (equivalent to 2 g maize sample), of the filtered extract was evaporated to dryness and dissolved in 2 mL methanol.

Solid Phase Extraction Clean Up. The SPE clean up was performed with Strata SAX strong anion exchange (500 mg) columns (Phenomenex, Torrance, CA), which were placed in a vacuum manifold. The clean up procedure was adapted from Filek and Lindner (*13*) with minor adjustments, as we excluded a water washing step due to loss of moniliformin from the column. The columns were sequentially activated with 2 mL methanol, 2 mL water, and 2 mL 0.1 M HCl. The dissolved maize extracts were then added to the SAX columns and allowed to percolate through by gravity. The cartridge was sequentially washed with 2 mL methanol–water (50:50) and 2 mL 0.1 M HCl. Moniliformin was eluted with 2 mL 1.0 M HCl, which was evaporated under a stream of nitrogen. The dried moniliformin fraction was dissolved in 100 μL MeCN–water (85:15) and transferred to a HPLC vial.

Several other SPE cartridges were also tested including Oasis MAX (strong anion-RP phase, Waters Milford, MA), but it was not possible to elute moniliformin from it using HCl, NaH_2PO_4 or NH_4OH. Moniliformin could, however, be eluted with ion-pair modifiers including tetrabutyl ammonium hydroxide and tetra methyl ammonium hydroxide. Because these ion-pair modifiers are not volatile we abandoned the use of Oasis MAX columns in our SPE clean up. We also tested Strata NH_2 (Phenomenex) columns from which we were able to elute moniliformin with HCl, NaH_2PO_4 or ammonium hydroxide. Moniliformin did not show stable recoveries from this column and was therefore abandoned. Strata-X-AW (weak anion exchanger-RP phase) columns were also tested but did not give cleaner extracts than the SAX columns.

HPLC-UV. The HPLC analyses were performed on an Agilent (Torrance, CA) 1100 HPLC system controlled by Chemstation v 1.01 B. The system was equipped with a diode array detector (DAD) containing a 6-mm flow-cell collecting approximately two UV/vis spectra per second from 190 to 900 nm with a bandwidth of 4 nm. Moniliformin was detected and quantified at 229 ± 2 nm and confirmed by the full UV spectrum, after background subtraction. Samples of 3 μL were injected.

Separation was made on a 150 × 4.6 mm i.d., 3.5 μm ZIC HILIC (SeQuant, Umeå, Sweden) column using flow of 0.5 mL/min and a linear gradient system of MeCN-water system starting at 5% water increasing this to 15% water in 15 min, then increasing flow to 1 mL/min and the water to 50% in 1 min keeping this for 4 min, then reverting to 5% water in 1 min and keeping this for 2 min and then decreasing the flow to 0.5 mL/min. The water was buffered with 100 mM ammonium formate (pH 6.4).

HPLC-MS Confirmation. For confirmation of positive samples as well as to increase sensitivity, selected samples were also analysed by HPLC coupled to high resolution mass spectrometry. This was done on a Micromass HPLCT orthogonal time of flight mass spectrometer (Micromass Manchester, UK) equipped with a Z-spray ESI source and a LockSpray probe (*29*). The HPLC system was the same as described above. Two gradient systems were used: same as for HPLC-UV; and a faster gradient system, starting with 12% 100 mM formic acid increasing this to 30% in 6 min and then increased to 50% in 1 min, maintaining this for 3 min, and then reverting to the start conditions in 5 min. Samples were analyzed in negative electrospray mode as described previously (*26*) except that the desolvation flow was 650 L/hr (nitrogen 99.9%) and that only one scan function was used with a potential difference of 17 V between the cones (skimmers). The $[M–H]^-$ ion at *m/z* 96.9926 was used for detection using an interval of m/z 96.9726–96.9946 (mass −0.02 amu to +0.002 amu, due to dead time correction of the MCP detector) (*29*).

Statistical Assessment. The relative standard deviations of spiked and pure samples quantified with HPLC-UV and HPLC-MS were determined using a linear calibration form.

RESULTS AND DISCUSSION

SPE. SAX columns have previously been applied successfully in SPE clean up procedures from maize and cereal grains (*1*, *13*, *21*). With this column we were able to elute moniliformin with HCl

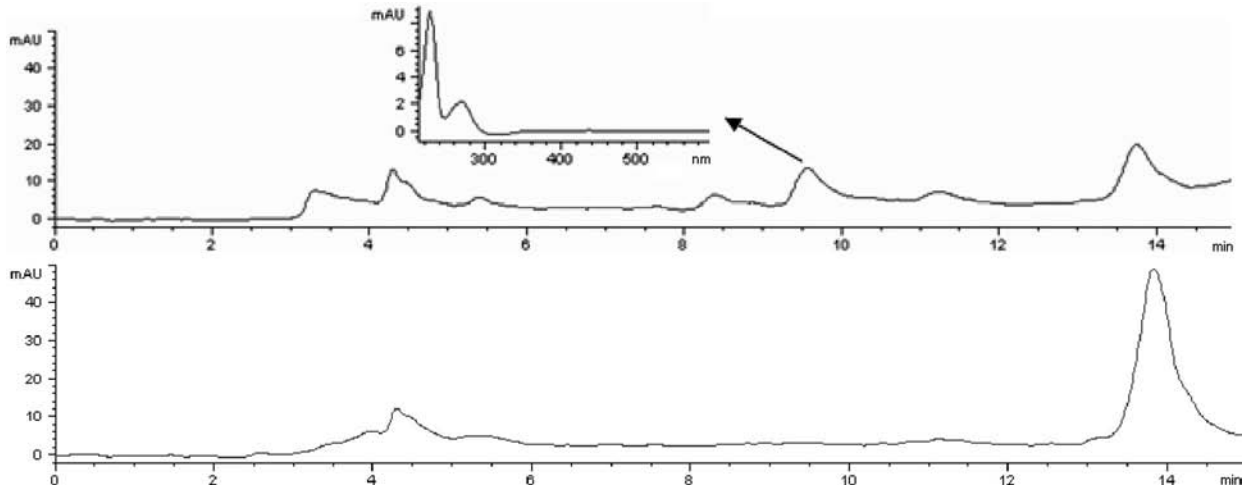

Figure 2. Chromatograms of maize sample spike with 288 ng/g moniliformin (top) and moniliformin free natural sample (bottom). Confirmation spectrum of moniliformin in the spiked sample is inserted.

and NaH_2PO_4 but not with NH_4OH. Because phosphate is not volatile we chose to use HCl as our elution reagent in our final protocol.

The present SPE clean up protocol was based on that of Filek and Lindner (*13*) who reported a consistent recovery of moniliformin in cereal grain samples of 70%. Likewise, from the maize samples spiked to contain moniliformin levels of 48, 96, 288, and 576 ng/g, moniliformin was recovered in a concentration independent manner ranging from 57 to 74% in our experiments indicating that the clean up procedure can be applied to the more complex maize matrix. No differences were observed in recovery rate between the three days, indicating a stable recovery.

HPLC-UV. The detection limit by HPLC-UV was approximately 48 ng/g ($s/n > 5$ at 229 nm, and full UV spectrum confirmation possible after background subtraction) and the quantification limit 96 ng/g (standard deviation from all three rounds of triplicate validation <20% at this level). This is comparable to currently used HPLC-UV methods, in which the quantification limit ranged from 20 to 120 ng/g (*12, 20, 30*).

When using a linear calibration R^2 was determined from the 0.994, 0.991, and 0.965 in spiked extracts from the three validation rounds (four levels in triplicate), 0.999 on the pure standards. For complete separation of moniliformin from UV interfering maize compound, a long run time of 15 min was used in HPLC-UV analysis. With this method moniliformin had a consistent retention time of 9.6–9.7 min in an area with little inference from maize compounds (**Figure 2**).

HPLC-MS. With MS detection we could detect moniliformin below 1 ng/g from spiked maize samples; however, limit of quantification was 12 ng/g (<20% standard deviation on lowest point). Moniliformin could be recovered from the samples spiked with 12–96 ng/g (four levels in triplicate) in a linear manner and a R^2 of 0.933. With the fast HILIC gradient program the retention time was 5 min with a runtime to 12 min. This could not be reduced as it was necessary to elute with 50% 100 mM formic acid to elute stronger retained compounds than moniliformin to avoid build up of contaminants on the column.

Since no qualifier ion was observed using in-source fragmentation, the mass accuracy was vital for specificity, which was demonstrated in two extracts where a peak partly coeluting at 9.55 min at *m/z* 96.56 was detected (moniliformin $[M\text{-}H]^-$ 96.9926 amu) when using a wide ion range; however, when using a narrow ion trace of *m/z* −0.02 to +0.002 this was not observed.

HILIC is not as sensitive as RP when using UV detection due to the broader peaks. However, when using HILIC-MS, the higher concentration of organic solvent at the point of elution gives a significantly better spray and thus compensates for the wider peaks compared to HPLC-RP-MS. This effect will probably vary between instruments. On our Micromass Z-spray source (Mark II) the same column in a 2 mm i.d. format tested at flows of 0.1–0.2 mL/min yielded far poorer sensitivity than the 4.6 mm i.d. column with a flow of 0.5 mL/min. Moniliformin showed very strong ionization, actually so strong that the TIC trace was lowered during peak elution (**Figure 3E**) in the high concentration samples indicating that it may not be very susceptible to ion-suppression. This was further supported by the same recovery rate obtained by ESI-MS and UV detection. However, the ESI tip became quite dirty during the run and had to be cleaned every 2 days, mainly due to the high flow rate of solvent into the source.

A 100 × 2 mm i.d., 3 μm polyhydroxyethyl aspartamide column was also tested for HILIC-MS, and from pure standards it did give better detection limits due to sharper peaks and lower buffer concentration needed for elution of moniliformin. However when analyzing real samples numerous impurities coeluted with moniliformin and obscured its detection even at the highest calibration levels.

Naturally Contaminated Samples. One or more *Fusarium* species could be isolated from all of the 28 examined maize samples. The predominant species were *F. avenaceum* and *F. graminearum*, which both were present in 13 samples (**Table 1**). *F. culmorum* and *F. equiseti* were also frequently isolated, occurring in 12 and 10 samples, respectively. Two isolates of *F. tricinctum* and three isolates of *F. subglutinans*, which are known moniliformin producers, were also identified.

On one hand, moniliformin could not be detected in any of the samples by HPLC-UV indicating that the contamination levels were below 48 ng/g. The samples were therefore analyzed by HPLC-MS and moniliformin was successfully detected in 15 out 28 samples; however, they were all below level of quantification. Moniliformin was detected in 11 of the 16 samples which contained *F. avenaceum, F. subglutinans*, and/or *F. tricinctum*, whereas it was only detected in 4 of the 12 samples without a potential moniliformin producing species. This indicates that the moniliformin producing fungi are unevenly distributed in the maize plant as the mycological examination of the randomly selected chopped maize pieces did not detect potential moniliformin

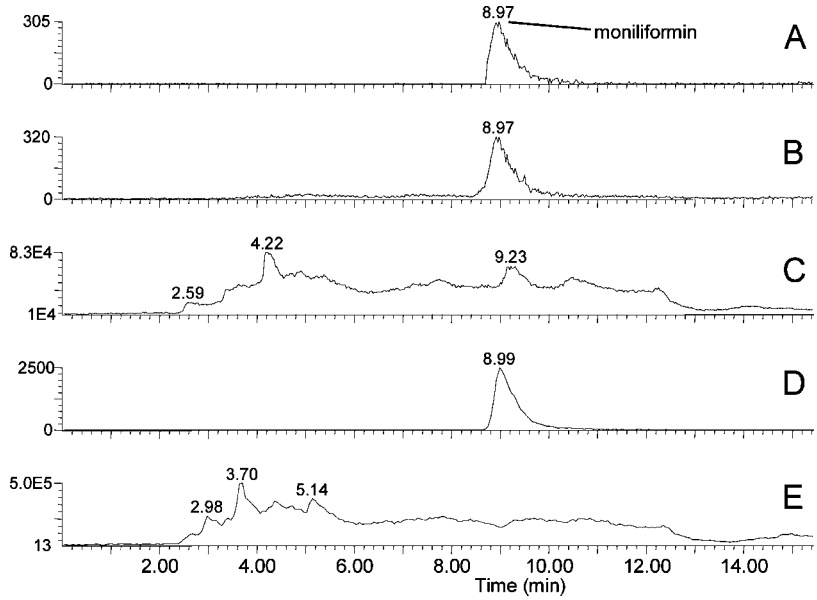

Figure 3. HPLC-ESI$^-$ chromatograms of anion exchanged maize plant extract: **A**, naturally contaminated with moniliformin (approx 10 ng/g) m/z 96.9726–96.9946 ([M-H]$^-$, −0.02 to +0.002 amu); **B**, the same but ± 0.5 amu; and **C**, same but TIC trace. **D**, spiked sample (96 ng/g) of [M-H]$^-$ (−0.02 to +0.002 amu); and **E** TIC of the same. Gradient system was the same as used for HPLC-UV.

Table 1. Presence of Fusarium Species and Moniliformin in 28 Maize Samples

F. avenaceum	*F. crookwellense*	*F. culmorum*	*F. equiseti*	*F. flocciferum*	*F. graminearum*	*F. sambucinum*	*F. subglutinans*	*F. sporotrichioides*	*F. tricinctum*	moniliformin (ng/g)
X										<12
X			X		X					<12
X		X								<12
X		X			X					<12
X		X				X				<12
X										nd
		X	X				X			nd
X		X								<12
X					X					nd
X				X	X					<12
					X					nd
X			X		X		X			<12
			X		X					nd
		X	X					X		nd
	X									<12
X		X	X							<12
					X			X		nd
X								X	X	nd
					X					<12
	X				X			X	X	<12
	X	X	X							nd
X		X								nd
								X		nd
		X			X			X		<12
		X	X							nd
			X		X					<12
		X	X							nd
	X						X			<12

producers. On the other hand, in most cases moniliformin-producing species were detected in moniliformin containing samples.

The low and presumable nontoxic levels quantities of moniliformin observed in the examined maize samples are due to the examination of maize pieces derived from the entire plant, whereas the high moniliformin levels in maize reported from other studies are mainly based on maize kernels. We examined moniliformin in samples derived from whole maize plants because maize in Denmark is primarily used as silage. Contamination studies of moniliformin solely in maize kernel is therefore not relevant for Danish grown maize.

This is to our knowledge the first report of HIILC based detection of a mycotoxin. Using this method we were able to for the first time to quantify moniliformin in samples derived from entire maize plants. With this method we are able to screen samples for moniliformin contamination above 48 ng/g using HPLC-UV detection or use HPLC-MS to detect moniliformin down to below 1 ng/g.

LITERATURE CITED

(1) Sharman, M.; Gilbert, J.; Chelkowski, J. A survey of the occurrence of the mycotoxin moniliformin in cereal samples

from sources worldwide. *Food Addit. Contam.* **1991**, *8*, 459–466.
(2) Moretti, A.; Mule, G.; Susca, A.; Gonzalez-Jaen, M. T.; Logrieco, A. Toxin profile, fertility and AFLP analysis of *Fusarium verticillioides* from banana fruits. *Eur. J. Plant Pathol.* **2004**, *110*, 601–609.
(3) Schütt, F.; Nirenberg, H. I.; Deml, G. Moniliformin production in the genus *Fusarium*. *Mycotoxin Res.* **1998**, *14*, 35–40.
(4) Morrison, E.; Kosiak, B.; Ritieni, A.; Aastveit, A. H.; Uhlig, S.; Bernhoft, A. Mycotoxin production by *Fusarium avenaceum* strains isolated from Norwegian grain and the cytotoxicity of rice culture extracts to porcine kidney epithelial cells. *J. Agric. Food Chem.* **2002**, *50*, 3070–3075.
(5) Kriek, N. P. J.; Marasas, W. F. O.; Steyn, P. S.; van Rensburg, S. J.; Steyn, M. Toxicity of a moniliformin-producing strain of *Fusarium moniliforme* var. *subglutinans* isolated from maize. *Food Cosmet. Toxicol.* **1977**, *15*, 579–587.
(6) Pirrung, M. C.; Nauhaus, S. K.; Singh, B. Cofactor-directed, time-dependent inhibition of thiamine enzymes by the fungal toxin moniliformin. *J. Org. Chem.* **1996**, *61*, 2592–2593.
(7) Zhang, H.; Li, J. L. Study on toxicological mechanism of moniliformin. *Acta Microbiol. Sin.* **1989**, *29*, 93–100.
(8) Chen, L. Y.; Tian, X. L.; Yang, B. A study on the inhibition of rat myocardium glutathione-peroxidase and glutathione-reductase by moniliformin. *Mycopathologia* **1990**, *110*, 119–124.
(9) Yu, S. R.; Liu, X. J.; Wang, Y. H.; Liu, J. A survey of moniliformin contamination in rice and corn from Keshan disease endemic and non-KSD areas in China. *Biomed. Environ. Sci.* **1995**, *8*, 330–334.
(10) Wu, W. D.; Liu, T. X.; Vesonder, R. F. Comparative cytotoxicity of fumonisin B-1 and moniliformin in chicken primary cell cultures. *Mycopathologia* **1995**, *132*, 111–116.
(11) Jestoi, M.; Rokka, M.; Yli-Mattila, T.; Parikka, P.; Rizzo, A.; Peltonen, K. Presence and concentrations of the *Fusarium*-related mycotoxins beauvericin, enniatins and moniliformin in Finnish grain samples. *Food Addit. Contam.* **2004**, *21*, 794–802.
(12) Uhlig, S.; Torp, M.; Jarp, J.; Parich, A.; Gutleb, A. C.; Krska, R. Moniliformin in Norwegian grain. *Food Addit. Contam.* **2004**, *21*, 598–606.
(13) Filek, G.; Lindner, W. Determination of the mycotoxin moniliformin in cereals by high-performance liquid chromatography and fluorescence detection. *J. Chromatogr., A* **1996**, *732*, 291–298.
(14) Nielsen, K. F.; Sumarah, M. W.; Frisvad, J. C.; Miller, J. D. Production of metabolites from the *Penicillium roqueforti* complex. *J. Agric. Food Chem.* **2006**, *54*, 3756–3763.
(15) O'Brien, M.; Nielsen, K. F.; O'Kiely, P.; Forristal, P. D.; Fuller, H. T.; Frisvad, J. C. Mycotoxins and other secondary metabolites produced *in vitro* by *Penicillium paneum* Frisvad and *Penicillium roqueforti* Thom isolated from baled grass silage in Ireland. *J. Agric. Food Chem.* **2006**, *54*, 9268–9276.
(16) Jestoi, M.; Rokka, M.; Rizzo, A.; Peltonen, K. Moniliformin in Finnish grains: Analysis with HPLC-MS/MS. *Aspects Appl. Biol.* **2003**, *68*, 211–216.
(17) Scharf, H. D.; Frauenrath, H.; Pinske, W. Synthesis and properties of semisquaric acid and its alkaline-salts (moniliformin). *Chem. Berichte-Recueil* **1978**, *111*, 168–182.
(18) Bellus, D.; Fischer, H.; Greuter, H.; Martin, P. Syntheses of moniliformin, a mycotoxine with a cyclobutenedione structure. *Helv. Chim. Acta* **1978**, *61*, 1784–1813.
(19) Steyn, M.; Thiel, P. G.; Van Schalkwyk, G. C. Isolation and purification of moniliformin. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 578–580.
(20) Kandler, W.; Nadubinska, M.; Parich, A.; Krska, R. Determination of moniliformin in maize by ion chromatography. *Anal. Bioanal. Chem.* **2002**, *374*, 1086–1090.
(21) Munimbazi, C.; Bullerman, L. B. High-performance liquid chromatographic method for the determination of moniliformin in corn. *J. AOAC Int.* **1998**, *81*, 999–1004.
(22) Shepherd, M. J.; Gilbert, J. Method for the analysis in maize of the *Fusarium* mycotoxin moniliformin employing ion-pairing extraction and high-performance liquid-chromatography. *J. Chromatogr.* **1986**, *358*, 415–422.
(23) Monaci, L.; Aresta, A.; Palmisano, F.; Visconti, A.; Zambonin, C. G. Amino-bonded silica as stationary phase for liquid chromatographic determination of cyclopiazonic acid in fungal extracts. *J. Chromatogr., A* **2002**, *955*, 79–86.
(24) Dell'Aversano, C.; Hess, P.; Quilliam, M. A. Hydrophilic interaction liquid chromatography-mass spectrometry for the analysis of paralytic shellfish poisoning (PSP) toxins. *J. Chromatogr., A* **2005**, *1081*, 190–201.
(25) Alpert, A. J. Hydrophilic-interaction chromatography for the separation of peptides, nucleic-acids and other polar compounds. *J. Chromatogr.* **1990**, *499*, 177–196.
(26) Nielsen, K. F.; Grafenhan, T.; Zafari, D.; Thrane, U. Trichothecene production by *Trichoderma brevicompactum*. *J. Agric. Food Chem.* **2005**, *53*, 8190–8196.
(27) Abildgren, M. P.; Lund, F.; Thrane, U.; Elmholt, S. Czapek-Dox agar containing iprodione and dicloran as a selective medium for the isolation of *Fusarium* species. *Lett. Appl. Microbiol.* **1987**, *5*, 83–86.
(28) Samson, R. A.; Hoekstra, E. S.; Frisvad, J. C.; Filtenborg, O. *Introduction to Food-and Airborne Fungi*, 6th ed.; Centraalbureau voor Schimmelcultures: Utrecht, The Netherlands, 2002.
(29) Nielsen, K. F.; Smedsgaard, J. Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. *J. Chromatogr. A* **2003**, *1002*, 111–136.
(30) Munimbazi, C.; Bullerman, L. B. Chromatographic method for the determination of the mycotoxin moniliformin in corn. In *Mycotoxin Protocols*; Trucksess, M. W., Pohland, A. E., Eds.; Humana Press Inc.: Totowa, NJ, 2001; pp 999–1004.

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