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Chromatographic methods for the simultaneous determination of mycotoxins and their conjugates in cereals

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Abstract

About 300–400 mycotoxins are known today. To some extent these compounds show very different physicochemical properties, which led to a vast quantity of analytical methods for single toxins or certain classes of mycotoxins in a variety of matrices. Due to synergistic effects of co-occurring toxins, endeavors have been made to simultaneously detect and quantify several classes of mycotoxins. This paper discusses several of the published LC-MS/MS multi-mycotoxin-methods and also introduces a new method, which allows the concurrent detection and quantification of 90 major mycotoxins and other secondary fungal metabolites in cereals. Even more, known plant derived metabolites of mycotoxins, like zearalenone-4-glucoside or deoxynivalenol-3-glucoside are included in this method. The significance of mycotoxin conjugates is briefly discussed as well.

Keywords: Mycotoxins; Multi-toxin analysis; Conjugated mycotoxins; Mass spectrometry; LC-MS/MS

1. Introduction

More than 31,000 different fungal metabolites were known in 2005 (Antibase, 2005), but many more are expected to be found in the future. Fortunately, just a fraction of them is hazardous to humans and animals at naturally occurring concentrations. About 300–400 mycotoxins are known today, depending on the classification of the compounds (reviewed e.g. by Hussein and Brasel (2001), Bennet and Klich (2003)). Two main routes of exposure have been identified, which are the inhalation of mycotoxins (often associated to spores) produced by indoor moulds and the consumption of mycotoxins with food that has been spoiled by toxigenic molds either before harvest or at storage.

While the chemical diversity of fungal metabolites definitely is a boon for drug discovery programs (reviewed by Larsen et al. (2005)), it could be a bane for analytical chemists. For instance, it was speculated for some time, whether the co-occurring *Fusarium* mycotoxins moniliformin and beauvericin act synergistic. Even if very recent data (Kamyar et al., 2006) demonstrates the contrary, such synergistic effects cannot be completely ruled out for other combinations of mycotoxins. Even if co-occurring toxins do not act synergistically, individual toxicities demand the determination of a variety of mycotoxins. To stick with the example from above, moniliformin is a very small (MW: 98 amu) and polar, acidic molecule while beauvericin is a rather big (MW: 783 amu) cyclohexadepsipeptide that is hardly soluble in water. While a single method for the determination of both toxins would save a lot of sample preparation, measurement time and analytical equipment, it is rather difficult to develop such a method.

Still, most analytical procedures for the determination of mycotoxins have the following steps in common: sampling, homogenization, extraction and clean-up which might include sample concentration. The final separation and detection of compounds of interest is usually achieved by either chromatographic techniques followed by various detection methods or by immunochemical methods. While immunochemical methods rely on specific antibodies for each mycotoxin, chromatographic techniques can separate a huge number of analytes. HPLC-MS/MS has become the most emerging analytical tool for the determination of mycotoxins and their metabolites. In contrast to GC based methods, polar compounds are quickly accessible without the need of derivatization. Further advantages include low detection limits, the ability to generate structural information of the analytes, the requirement of minimal sample treatment and the possibility to cover a wide range of analytes differing in their polarities. Finally, mass spectrometers are rather general detectors

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that are not so dependent on chemical characteristics like UV-absorbance or fluorescence. Matrix effects, however, limit the potential of LC-MS. Ion suppression (or enhancement) might be encountered due to matrix components that co-elute with the analyte of interest. If available, internal standards can often successfully amend these effects. Possible other strategies include the use of matrix matched standards or very careful validation of certain toxin/matrix combinations to exactly determine the matrix effect. Such multi-mycotoxin methods can only be a compromise since extraction and clean-up conditions have to be selected that are away from the optimum for certain analytes. Still, such methods can provide a huge decrease in analytical labor compared to multiple conventional methods and also greatly increase the analytical output. It could be shown that the latest generation of LC-MS/MS instruments using electrospray ionization allows the analysis of crude plant extracts without any previous clean-up (Spanjer et al., 2006; Sulyok et al., 2006).

2. Multi-mycotoxin methods

Early qualitative multi-mycotoxin methods, measuring several groups of mycotoxins have been developed using HPLC-UV (Frisvad, 1987; Frisvad and Thrane, 1987). In their breakthrough paper, Frisvad and Thrane describe an HPLC method to identify 182 mycotoxins and other fungal metabolites based on their alkylphenone retention indices and diode array spectra. Ever since, the separation power of modern HPLC columns greatly increased as new stationary phase materials were introduced and the particle size of the materials decreased. Also, using mass selective detectors even co-eluting analytes can be easily separated in the chromatogram.

GC-MS methods usually need derivatization before analysis of polar compounds, which is time-consuming and a potential source of error. Nonetheless, the first quantitative methods for the determination of the co-occurring trichothecenes and zearalenone (ZON) were based on GC-MS (Onji et al., 1998; Tanaka et al., 2000).

Technical improvements in the field of LC-MS led to several multi-mycotoxin methods in the last few years. Continuing the work of Frisvad and Thrane, Nielsen and Smedsgaard (2003) developed a qualitative LC-UV-MS method for screening of fungal metabolites and mycotoxins in culture extracts. Positive mode electrospray ionization (ESI) combined with high resolution time of flight (TOF) mass spectrometry was used after HPLC separation and diode array detection of mycotoxins. The detection of 474 fungal metabolites make this method unparalleled to this day.

One of the first quantitative LC-MS/MS multi-mycotoxin methods was presented by Rundberget and Wilkins (2002). Their method allows the simultaneous determination of mycophenolic acid, griseofulvin, roquefortine C, chaetoglobosin B, verruculogen and penitrem A in food and feed. After extraction with acetonitrile/water/formic acid (900/99/1, v/v/v), the sample clean-up just contained a defatting step with hexane. Positive mode atmospheric pressure chemical ionization (APCI) on an iontrap instrument was used for quantification.

An early LC-MS/MS method for the determination of mycotoxins from multiple fungal genera was introduced by (Driffield et al., 2003). Aflatoxin B_1, ochratoxins A and alpha, ZON, alpha-zearalenol, beta-zearalenol, nivalenol and deoxynivalenol (DON) were measured in animal offal food products using a triple quadrupole mass spectrometer in both (fast switching) ESI polarities.

Royer et al. used stable isotopically labeled and structural analogues as internal standards for determination of the *Fusarium* mycotoxins DON, fumonisin B_1 and ZON (Royer et al., 2004). APCI-iontrap-MS with polarity switching was applied for the measurement of maize samples.

Similar triple quadrupole LC-MS/MS methods for the quantification of trichothecenes and ZON in cereals were presented in 2005. After extraction with acetonitrile/water (84/16, v/v) and MycoSep® clean-up, samples were measured using ESI (Biselli and Hummert, 2005) or APCI (Berthiller et al., 2005b) interfaces. The elution order of the analytes allowed for switching of ionization polarities within the run from negative ion mode (type B-trichothecenes) to positive ion mode (type A-trichothecenes) and back to negative ion mode (ZON) to achieve optimum ionization yields. Tanaka et al. (2006) used the same clean-up but also included the *Aspergillus* mycotoxins aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 to their method. APCI-TOF-MS was used to determine a total of 13 mycotoxins in corn, wheat, cornflakes and biscuits. Cavaliere et al. (2005) presented their method for the determination of eight trichothecenes, three fumonisins, ZON and alpha-zearalenol. Again corn samples were measured using ESI triple quadrupole MS in both polarity modes.

Aflatoxins B_1, B_2, G_1, G_2 and M_1, ochratoxin A, mycophenolic acid, penicillic acid and roquefortine C can be determined simultaneously with an ESI positive — triple quadrupole MS method in cheese (Kokkonen et al., 2005).

A positive ion mode ESI triple quadrupole MS method for the simultaneous determination of 16 mycotoxins possibly related to "sick building syndrome" was developed by Delmulle et al. (2006). Nivalenol, DON, ZON, diacetoxyscirpenol, T-2 toxin, verrucarol, verrucarin A, neosolaniol, sterigmatocystin, roridin A, ochratoxin A, aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 can be determined with this method directly on scrapped off fungal material or after vacuum blotting onto cellulose filters.

Sorensen and Elbaek (2005) developed an LC-ESI-MS/MS method for determination of 18 mycotoxins and their metabolites in milk. DON, 3-acetyl-DON, 15-acetyl-DON, deepoxy-DON, ochratoxin A, ZON, alpha-zearalenol, beta-zearalenol, alpha-zearalanol and beta-zearalanol were detected in negative ESI mode. Using different chromatographic conditions, T-2 toxin, HT-2 toxin, T-2 triol, diacetoxyscirpenol, 15-monoacetoxyscirpenol, fumonisin B_1, fumonisin B_2 and aflatoxin M_1 were detected in positive ESI mode.

Already back in 2004, Spanjer presented a method for the simultaneous determination of aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A, DON, 3-acetyl-DON, 15-acetyl-DON, fumonisins B_1 and B_2, diacetoxyscirpenol, ZON, T2-toxin, HT2-toxin, roquefortine and sterigmatocystin in various foodstuffs like peanuts, cornflakes, wheat or figs (Spanjer et al., 2006). The method uses a positive ESI triple quadrupole setup without any clean-up. Ever since their first report, the method was continuously developed, allowing the determination of even more mycotoxins.

Sulyok et al. (2006) published an LC-ESI-MS/MS method for the determination of 39 mycotoxins in cereals. A- and B-trichothecenes, ZON and related derivatives, fumonisins, enniatins, ergot alkaloids, ochratoxins, aflatoxins and moniliformin are covered with this method. Two consecutive chromatographic runs, one for each ESI polarity, have been chosen to obtain best sensitivities for all analytes. Clean-up was avoided, therefore raw extracts (e.g. maize extracts) had to be diluted before injection to achieve good overall recoveries.

Reviews about the determination of mycotoxins with liquid chromatography-mass spectrometry have been published recently (Sforza et al., 2005; Zöllner and Mayer-Helm, 2006).

3. Conjugated mycotoxins

Mycotoxins, as all other xenobiotics, can be partly metabolized by living plants. Humans and animals consuming parts of mycotoxin contaminated plants (e.g. cereals, nuts, raisins, etc.) are therefore not just exposed to the native mycotoxins, but also to their metabolites formed by the plants. Still just little is known about the occurrence, bioavailability and further metabolism of these compounds, which additionally also escape established analytical detection techniques.

In 1988, zearalenone-4-glucoside (Z4G) was shown to be a metabolite of plants, after ZON was transformed by maize cell suspension cultures to its glucoside (Engelhardt et al., 1988). A mini-survey of 24 wheat samples — 22 of them were contaminated with ZON above the limit of quantification — resulted in 10 samples (42%) also positive for Z4G (Schneweis et al., 2002). Approximately 10–20% of the total ZON content of these samples was detected as Z4G. Several papers describe the metabolism of mycotoxins in plants, especially the phase II transformation of ZON (Gareis, 1994; Wallnöfer et al., 1996; Engelhardt et al., 1999; Berthiller et al., 2006). Both, direct and indirect methods for the qualitative and quantitative determination of mycotoxins and their conjugates exist. With indirect methods the precursor toxin is quantified before and after acidic, basic or enzymatic hydrolization of the conjugate. In this regard, beta-glucosidase sample treatment was used to indirectly quantify Z4G concentrations (Gareis et al., 1990). Direct techniques employed HPLC separation with fluorescence (Zill et al., 1990) or MS (Schneweis et al., 2002) detection. Z4G is completely cleaved to ZON during digestion in swine (Gareis et al., 1990). In that same study the term “masked mycotoxin” has been forged to emphasize a substance that is usually not detected in routine analysis of feed but contributes to the total mycotoxin content.

Ochratoxin A has also been shown to be transformed in wheat and maize cell suspension cultures to ochratoxin α, ochratoxin A methyl ester, two isomers of hydroxyochratoxin A and the glucosides and methyl esters of both hydroxyochratoxin A isomers (Ruhland et al., 1994, 1996).

A lot of speculation was arising about possible conjugation products of DON. Already back in 1983, it was shown that the DON concentration of *Fusarium graminearum* infected wheat reached a maximum and then declined until harvest (Miller et al., 1983). A year later it was reported that the DON content of yeast fermented food products was higher than that of the contaminated flour used for their production (Young et al., 1984). Deoxynivalenol-3-glucoside (D3G) was identified as the main DON metabolite after treatment of maize cell suspension cultures with DON (Sewald et al., 1992). However, it took until 2005, until D3G was also found in naturally contaminated wheat and maize samples (Berthiller et al., 2005a). The fate of this compound after digestion (in particular the cleavage to DON) by mammals is still unknown and therefore D3G should be regarded as potentially hazardous for human and animal health.

Besides metabolism, processing of food (e.g. cooking) is another source of mycotoxin conjugate formation. *N*-(carboxymethyl)-fumonisin B_1 (Howard et al., 1998) and *N*-(1-deoxy-D-fructos-1-yl)-fumonisin B_1 (Poling et al., 2002) have been identified as conversion products of FB_1 after heating with reducing sugars. Model experiments yielded evidence, that FB_1 might be bound to other saccharides, amino acids (Seefelder et al., 2003) and proteins (Kim et al., 2003; Park et al., 2004). In this context, a review about the effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins was published (Humpf and Voss, 2004).

4. An enhanced LC-MS/MS method for the determination of about 90 mycotoxins

The LC-MS/MS method from Sulyok et al. (2006) for the determination of 39 mycotoxins was improved to allow the unambiguous detection and quantification of about 90 mycotoxins and some of their naturally occurring metabolites. MS conditions for all analyzed substances were optimized to gain multiple reaction monitoring (MRM) transitions. One MRM transition per analyte is used for quantification, while another one is used as a qualifier.

Sample preparation is rather simple and avoids any clean-up. Ground wheat or maize kernels are extracted with acetonitrile/water/acetic acid (79/20/1, v/v/v) on a rotary shaker. The mixture is centrifuged and the raw extract diluted 1/1 (v/v) with acetonitrile/water/acetic acid (20/79/1, v/v/v) to adjust the solvent strength. ESI (turbo ion spray) is used in both modes of polarity to achieve optimum ionization yields for all analytes. Two successive LC runs are required for each sample. The run time of a single LC run is 21 min. With injection times of about 1.5 min (including needle wash), this amounts to a total analysis time of 45 min for both required runs to analyze for 90 mycotoxins.

In positive ESI mode, the following analytes are detected (in their order of elution): ox-elymoclavine (1), ox-Luol (2), ergine (3), elymoclavine-fructoside (4), elymoclavine (5), ergometrine (6), dihydrolysergol (7), lysergol (8), chanoclavine (9), ergometrinine (10), agroclavine (11), festuclavine (12), neosolaniol (13), methysergide (14), verrucarol (15), gibberellic acid (16), 15-acetyl-DON (17), 3-acetyl-DON (18), ergovaline (19), ergosine (20), 15-mono-acetoxyscirpenol (21), dihydroergosine (22), aflatoxin G_2 (23), aflatoxin M_1 (24), ergosinine (25), meleagrin (26), ergotamine (27), ergocornine (28), dihydroergotamine (29), ergotaminine (30), aflatoxin G_1 (31), aflatoxin B_2 (32), ergocryptine (33), sulochrin (34), diacetoxyscirpenol (35), ergocristine (36), hydrolyzed fumonisin B_1 (37), altenuene

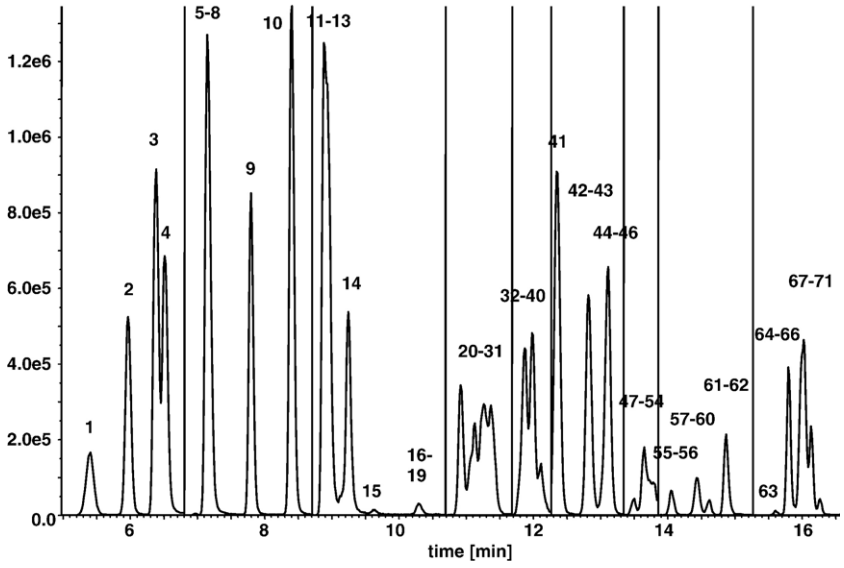

Fig. 1. Total ion MRM chromatogram of 71 mycotoxins and derivatives measured in positive ESI mode. Vertical lines indicate different time periods.

(38), aflatoxin B_1 (39), gliotoxin (40), ergocorninine (41), ergocryptinine (42), fumonisin B_1 (43), HT-2 toxin (44), ergocristinine (45), griseofulvin (46), fumonisin B_3 (47), antibiotic Y (48), T-2 toxin (49), mycophenolic acid (50), verrucarin A (51), ochratoxin B (52), fumitremorgin C (53), roridin A (54), fumonisin B_2 (55), cytochalasine E (56), citrinin (57), chetomin (58), chaetoglobosin A (59), ochratoxin A (60), sterigmatocystin (61), verruculogen (62), penitrem A (63), mevilonin (64), enniatin B (65), paxillin (66), enniatin B1 (67), beauvericin (68), alamethicin (69), enniatin A1 (70), enniatin A (71). The total ion HPLC-MS/MS chromatogram measured in positive ion mode is shown in Fig. 1.

Using a second LC run in negative ion mode, the following analytes are detected: moniliformin, nivalenol, patulin, DON, D3G, fusarenone-X, deepoxy-DON, 15-acetyl-DON, 3-acetyl-DON, Z4G, ochratoxin alpha, alternariol, beta-zearalenol, alpha-zearalenol, ZON-4-sulfate, ZON, alternariol methylether, emodin.

The method will be validated for wheat and maize (Sulyok et al., in preparation). So far, the lower limits of detection are between 0.03 (enniatin B) and 30 μg/kg (nivalenol) for all analytes.

5. Conclusion

Methods for the simultaneous determination of several groups of mycotoxins are highly desirable. Besides overall faster analysis times, a screen for a variety of different mycotoxins can considerably help to increase food safety. Although some mycotoxins will never co-occur in a single matrix, often matrices are mixed to produce food or feed. A constant screening program using such methods will also allow for monitoring of changes in fungal predominance in certain countries, which is especially interesting in times of climatic changes. Furthermore, the future food analyst might analyze almost any kind of matrix with a single method for mycotoxins. The rise of LC-MS has also found its way into mycotoxin analyses and more and more multi-mycotoxin-methods are developed. Some of these methods can easily be expanded as is shown here with a novel method that allows the quantification of about 90 mycotoxins in cereals. Besides the mycotoxins, also their metabolites, often formed by plants, might be hazardous for man. Such conjugated or masked mycotoxins can also be easily analyzed with LC-MS methods and should be included into multi-mycotoxin analysis. LC-MS methods are also very well suited for high sample throughput. While the investment for LC-MS instruments is still a big one, it is believed that both commercial companies and national food monitoring agencies sooner or later will renew their analytical equipment with LC-MS instruments for economical as well as analytical reasons.

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References

AntiBase 2005 (software), Hartmut Laatsch, ISBN: 3-527-31721-X.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497–516.

Berthiller, F., Dall'Asta, C., Schuhmacher, R., Lemmens, M., Adam, G., Krska, R., 2005a. Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. 53, 3421–3425.

Berthiller, F., Schuhmacher, R., Buttinger, G., Krska, R., 2005b. Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr., A 1062, 209–216.

Berthiller, F., Werner, U., Sulyok, M., Krska, R., Hauser, M.T., Schuhmacher, R., 2006. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) determination of phase II metabolites of the mycotoxin zearalenone in the model plant *Arabidopsis thaliana*. Food Addit. Contam. 23, 1194–1200.

Biselli, S., Hummert, C., 2005. Development of a multicomponent method for *Fusarium* toxins using LC-MS/MS and its application during a survey for

the content of T-2 toxin and deoxynivalenol in various feed and food samples. Food Addit. Contam. 22, 752–760.

Cavaliere, C., Foglia, P., Pastorini, E., Samperi, R., Lagana, A., 2005. Development of a multiresidue method for analysis of major *Fusarium* mycotoxins in corn meal using liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 19, 2085–2093.

Delmulle, B., De Saeger, S., Adams, A., De Kimpe, N., Van Peteghem, C., 2006. Development of a liquid chromatography/tandem mass spectrometry method for the simultaneous determination of 16 mycotoxins on cellulose filters and in fungal cultures. Rapid Commun. Mass Spectrom. 20, 771–776.

Driffield, M., Hird, S.J., MacDonald, J., 2003. The occurrence of a range of mycotoxins in animal offal food products by HPLC-MS/MS. Asp. Appl. Biol. 68, 205–210.

Engelhardt, G., Zill, G., Wohner, B., Wallnöfer, P.R., 1988. Transformation of the *Fusarium* mycotoxin zearalenone in maize cell suspension cultures. Naturwissenschaften 75, 309–310.

Engelhardt, G., Ruhland, M., Wallnöfer, P.R., 1999. Metabolism of mycotoxins in plants. Adv. Food Sci. 21, 71–78.

Frisvad, J.C., 1987. High-performance liquid chromatographic determination of profiles of mycotoxins and other secondary metabolites. J. Chromatogr. 392, 333–347.

Frisvad, J.C., Thrane, U., 1987. Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV–VIS spectra (diode array detection). J. Chromatogr. 404, 195–214.

Gareis, M., 1994. Maskierte mykotoxine. Ubers. Tierernahr. 22, 104–113.

Gareis, M., Bauer, J., Thiem, J., Plank, G., Grabley, S., Gedek, B., 1990. Cleavage of zearalenone-glycoside, a "masked" mycotoxin, during digestion in swine. J. Vet. Med. 37, 236–240.

Howard, P.C., Churchwell, M.I., Couch, L.H., Marques, M.M., Doerge, D.R., 1998. Formation of *N*-carboxymethyl-fumonisin-B_1, following the reaction of fumonisin B_1 with reducing sugars. J. Agric. Food Chem. 46, 3546–3557.

Humpf, H.U., Voss, K.A., 2004. Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins. Mol. Nutr. Food Res. 48, 255–269.

Hussein, H.S., Brasel, J.M., 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 167, 101–134.

Kamyar, M.R., Kouri, K., Rawnduzi, P., Studenik, C., Lemmens-Gruber, R., 2006. Effects of moniliformin in presence of cyclohexadepsipeptides on isolated mammalian tissue and cells. Toxicol. In Vitro 20, 1284–1291.

Kim, E.K., Scott, P.M., Lau, B.P., 2003. Hidden fumonisin in corn flakes. Food Addit. Contam. 20, 161–169.

Kokkonen, M., Jestoi, M., Rizzo, A., 2005. Determination of selected mycotoxins in mould cheeses with liquid chromatography coupled to tandem with mass spectrometry. Food Addit. Contam. 22, 449–456.

Larsen, T.O., Smedsgaard, J., Nielsen, K.F., Hansen, M.E., Frisvad, J.C., 2005. Phenotypic taxonomy and metabolite profiling in microbial drug discovery. Nat. Prod. Rep. 22, 672–695.

Miller, J.D., Young, J.C., Trenholm, H.L., 1983. *Fusarium* toxins in field corn. I. Time course of fungal growth and production of deoxynivalenol and other mycotoxins. Can. J. Bot. 61, 3080–3087.

Nielsen, K.F., Smedsgaard, J., 2003. Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. J. Chromatogr., A 1002, 111–136.

Onji, Y., Aoki, Y., Tani, N., Umebayashi, K., Kitada, Y., Dohi, Y., 1998. Direct analysis of several *Fusarium* mycotoxins in cereals by capillary gas chromatography-mass spectrometry. J. Chromatogr., A 815, 59–65.

Park, J.W., Scott, P.M., Lau, B.P., Lewis, D.A., 2004. Analysis of heat-processed corn foods for fumonisins and bound fumonisins. Food Addit. Contam. 21, 1168–1178.

Poling, S.M., Plattner, R.D., Weisleder, D., 2002. *N*-(1-deoxy-D-fructos-1-yl) fumonisin B_1, the initial reaction product of fumonisin B_1 and D-glucose. J. Agric. Food Chem. 50, 1318–1324.

Royer, D., Humpf, H.U., Guy, P.A., 2004. Quantitative analysis of *Fusarium* mycotoxins in maize using accelerated solvent extraction before liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. Food Addit. Contam. 21, 678–692.

Ruhland, M., Engelhardt, G., Wallnöfer, P.R., 1994. Transformation of the mycotoxin ochratoxin A in wheat and maize cell suspension cultures. Naturwissenschaften 81, 453–454.

Ruhland, M., Engelhardt, G., Schafer, W., Wallnöfer, P.R., 1996. Transformation of the mycotoxin ochratoxin A in plants: 1. Isolation and identification of metabolites formed in cell suspension cultures of wheat and maize. Nat. Toxins 4, 254–260.

Rundberget, T., Wilkins, A.L., 2002. Determination of *Penicillium* mycotoxins in foods and feeds using liquid chromatography-mass spectrometry. J. Chromatogr., A 964, 189–197.

Schneweis, I., Meyer, K., Engelhardt, G., Bauer, J., 2002. Occurrence of zearalenone-4-β-D-glucopyranoside in wheat. J. Agric. Food Chem. 50, 1736–1738.

Seefelder, W., Knecht, A., Humpf, H.U., 2003. Bound fumonisin B_1: analysis of fumonisin-B_1 glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry. J. Agric. Food Chem. 51, 5567–5573.

Sewald, N., Lepschy von Gleissenthall, J., Schuster, M., Müller, G., Aplin, R.T., 1992. Structure elucidation of a plant metabolite of 4-desoxynivalenol. Tetrahedron 3, 953–960.

Sforza, S., Dall´Asta, C., Marchelli, R., 2006. Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic techniques/mass spectrometry. Mass Spectrom. Rev. 25, 54–76.

Sorensen, L.K., Elbaek, T.H., 2005. Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. J. Chromatogr., B, Biomed. Sci. Appl. 820, 183–196.

Spanjer, M., Rensen, P., Scholten, J., 2006. Multi-mycotoxin analysis by LC-MS/MS in a single sample extract. Proceedings of the XIth International IUPAC Symposium on Mycotoxins and Phycotoxins, Bethesda, Maryland USA, May 17–21, 2004. Wageningen Academic Publishers, pp. 117–124.

Sulyok, M., Berthiller, F., Krska, R., Schuhmacher, R., 2006. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. Rapid Commun. Mass Spectrom. 20, 2649–2659.

Tanaka, T., Yoneda, A., Inoue, S., Sugiura, Y., Ueno, Y., 2000. Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography-mass spectrometry. J. Chromatogr., A 882, 23–28.

Tanaka, H., Takino, M., Sugita-Konishi, Y., Tanaka, T., 2006. Development of a liquid chromatography/time-of-flight mass spectrometric method for the simultaneous determination of trichothecenes, zearalenone and aflatoxins in foodstuffs. Rapid Commun. Mass Spectrom. 20, 1422–1428.

Wallnöfer, P.R., Preiß, U., Ziegler, W., Engelhardt, G., 1996. Konjugatbildung organischer Schadstoffe in Pflanzen. UWSF-Z. Umweltchem. Ökotox. 8, 43–46.

Young, C.J., Fulcher, G.R., Hayhoe, J.H., Scott, P.M., Dexter, J.E., 1984. Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. J. Agric. Food Chem. 32, 659–664.

Zill, G., Ziegler, W., Engelhardt, G., Wallnöfer, P.R., 1990. Chemically and biologically synthesized zearalenone-4-β-D-glucopyranoside: comparison and convenient determination by gradient HPLC. Chemosphere 22, 435–442.

Zöllner, P., Mayer-Helm, B., 2006. Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionization mass spectrometry. J. Chromatogr., A 1136, 123–169.