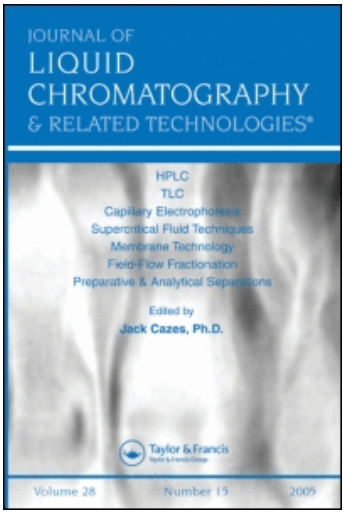


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Review of Recent Trends in Applications of Liquid Chromatography-Mass Spectrometry for Determination of Mycotoxins

P. Songsermsakul[a]; E. Razzazi-Fazeli[b]
[a] Department of Toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand [b] Department of Veterinary Public Health, Institute of Nutrition, VetOMICS Core Facility for Research/University of Veterinary Medicine, Vienna, Austria

Online publication date: 22 June 2010

To cite this Article Songsermsakul, P. and Razzazi-Fazeli, E.(2008) 'A Review of Recent Trends in Applications of Liquid Chromatography-Mass Spectrometry for Determination of Mycotoxins', Journal of Liquid Chromatography & Related Technologies, 31: 11, 1641 — 1686
To link to this Article: DOI: 10.1080/10826070802126395
URL: http://dx.doi.org/10.1080/10826070802126395

Journal of Liquid Chromatography & Related Technologies®, 31: 1641–1686, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802126395

A Review of Recent Trends in Applications of Liquid Chromatography-Mass Spectrometry for Determination of Mycotoxins

P. Songsermsakul[1] and E. Razzazi-Fazeli[2]

[1]Department of Toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand
[2]Department of Veterinary Public Health, Institute of Nutrition, VetOMICS Core Facility for Research/University of Veterinary Medicine, Vienna, Austria

Abstract: Monitoring, control, risk assessment, and prevention of contaminants in food and in feed are important issues associated with public health, agricultural production, food processing, and trade. Food and feed safety, as well as carry over of contaminants into animal tissue, are of major public concern nowadays, especially if considering the global challenge and world trade liberalization. Mycotoxins are toxic secondary metabolites of moulds and one of the major contaminants of agricultural products. Their presence in food and feed is harmful to the health of humans and animals. Therefore, analytical methods for the identification and determination of mycotoxins need to be sensitive, selective, and robust to provide accurate data especially for monitoring, risk assessment, quality control, and research. The application of hyphenated techniques especially HPLC-MS and HPLC-MS-MS has, in many cases, revolutionized the analysis of mycotoxins. The LC-MS methods offer a new tool for the sensitive, selective, and accurate analysis of contaminants in agricultural commodities. Tandem mass spectrometry provides the maximum scale of confidence in analyte identification. However, LC-MS-MS is definitely not a trouble free solution for the analysis of mycotoxins in complex biological matrices. Sample clean-up and chromatography still remain

Correspondence: Razzazi-Fazeli, Department of Veterinary Public Health, Institute of Nutrition, VetOMICS Core Facility for Research/University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria. E-mail: ebrahim.razzazi@vu-wien.ac.at

the important and necessary steps for obtaining reliable analytical results. This paper provides some general aspects regarding mycotoxins in food and emphasizes the analytical techniques with regard to LC-MS technology.

Keywords: Aflatoxins, Ergot alkaloids, Fumonisins, High-performance liquid chromatography, LC-MS, LC-MS-MS, Mass spectrometry, Mycotoxins, Patulin, Trichothecenes, Zearalenone

INTRODUCTION

Mycotoxins are naturally occurring, toxic metabolites which can be produced by fungi infecting agricultural crops during the growth, drying, and subsequent storage. Mycotoxin contamination of human food and animal feed is a serious problem throughout the world. About 200 identified filamentous fungi species have been identified yet, which grow under a wide range of climatic conditions on agricultural commodities. However, the natural fungal flora associated with foods is dominated by three genera Aspergillus, Fusarium, and Penicillium.[1]

Especially, environmental and biological factors such as water activity, temperature, humidity, and insect damage can have a great influence on growth of certain fungi and, therefore, on the spectrum of produced secondary metabolites. Mycotoxins are small molecules with various chemical structures and, therefore, various biological effects.

At the farm level, mycotoxin contamination can result in reduced crop yields, as well as livestock productivity stemming from health problems due to consumption of contaminated feed. Generally, mycotoxins are stable chemical compounds and cannot be destroyed during processing and heat treatment. When present in food in sufficiently high amounts, these fungal metabolites can have toxic effects that range from acute to chronic symptoms. Some mycotoxins were shown to be mutagenic, teratogenic, or/and carcinogenic. Symptoms of intoxications range from skin irritation to immunosuppression, hepatotoxicity, and nephrotoxicity.[1] The well-known historical example of mycotoxicosis in human is ergotism, which was responsible for a disease in the Middle Ages known as "St. Anthony's Fire." However, after the outbreak of turkey X disease in the 1960s, the field of mycotoxicology has become an important issue in food and feed safety. Mycotoxins were realized as a hazard to human and animal health.

Although it is impossible to calculate the exact economic costs of mycotoxins in agriculture, the U.S. Food and Drug Administration (FDA) has performed a computer model to estimate the economic losses of some selected mycotoxins. According to this estimate in the United States only, the potential annual cost of feed and food losses caused by aflatoxins, fumonisins, and deoxynivalenol are estimated to be $932

million.[2] Due to toxic effects on human and animals the risk assessment of mycotoxins is of high relevance.[3] Over a hundred mycotoxins have been identified; however, only a few present significant food safety challenges. The human exposure is possible through intake of contaminated agricultural products like grains, dried fruits, grapes, etc. Another way of exposure are the mycotoxin residues due to carry over and/or metabolization products occurring in foods of animal origin such as, animal tissue, milk, and eggs.

Mycotoxin contamination of food and feed remains a world-wide problem. Different countries have set regulatory maximum limits and guidelines for relevant mycotoxins such as aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, and patulin. Additionally monitoring and control programs for mycotoxins in food and feed have been implemented in many countries, especially in the European Union. Based on toxicological studies in animals, there exist maximum tolerable intake for all major mycotoxins, which allow performing appropriate risk assessment. The International Agency for Research on Cancer (IARC) has classified aflatoxins as carcinogenic to humans, while ochratoxins and fumonisins were classified as possibly carcinogenic. Tichothecenes and zearalenone are those mycotoxins which were classified to be non carcinogenic.[4]

Especially in globalisation era and internationalization in the food and feed trades, restrict regulation strategies have to be set to protect consumers from the mycotoxin exposure. European Food Safety Authorities (EFSA) and US FDA have set regulatory maximum levels for some mycotoxins in food and feed. The products with mycotoxin contaminations exceeding the maximum levels are rejected before import or are removed from the market.

The analytical methods for the identification and determination of mycotoxins in food and feed and biological samples should be accurate and should provide reliable data. Many efforts have been made to improve sensitivity, selectivity, and accuracy, as well as robustness of mycotoxin analysis. Different intercomparison studies and ring trials have been carried out.[5–9] An important point is the use of well characterized reference materials to insure the accuracy and trueness of analytical results. One of the most crucial steps in the analysis of mycotoxins is the sampling, which is strongly associated with a high degree of error. Actually, it is not possible to determine real concentrations of mycotoxins in a bulk with high confidence.[10] Especially aflatoxins and ochratoxins can often occur in hot spots within agricultural products, which would be the main source of error by jugging the analytical results. As a consequence, EU has set regulatory sampling plans for the analysis of ochratoxin A and aflatoxins in food and feed samples.

Another important and critical step in the analysis of mycotoxins is sample preparation and sample clean-up. Different strategies have been performed, including solid phase extraction (SPE), liquid-liquid

partitioning, but also multifunctional columns (MFC). However, in the analysis of mycotoxins, selective and specific antibodies for isolation and purification from the matrix compounds have been extensively used. In immunoaffinity columns (IAC), the purified monoclonal or polyclonal antibodies are immobilized into cyanobromide activated Sepharose gel. The gel with covalently bonded antibodies against an appropriate mycotoxin will be then filled into plastic columns. After preconditioning of the column, diluted sample extract will be poured into the column. While the mycotoxin will selectively bind to antibodies immobilized in the IAC, the matrix compounds pass through the column and can be washed in a further step. The bound analyte-antibody will then be disrupted by treating the column with an organic solvent such as methanol or acetonitrile. The mycotoxins will be released and eluted from the column. The eluate, which is very clean, can be then injected into the chromatographic system. There are several IACs manufactured by different companies which are commercially available and can be used for clean-up of mycotoxins in food and feed samples. If compared with other clean-up strategies, the IACs have the enormous advantage of high selectivity and specificity and can deliver, in most cases, chromatograms free from matrix interferences.

Analytical methods for determination and identification of mycotoxins in food and feed include thin layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC).[5–9,11] TLC Methods have been used, either as screening or for quantitation purposes.[5–9,11] GC with electron-capture (ECD) and mass spectrometric (MS) detection has been used for the analysis of some mycotoxins.[6–9,11,12] However, a derivatization step is necessary to be able to perform the analysis by GC. Immunochemistry based methods are other very selective and sensitive assays. Enzyme-linked immunosorbent assays (ELISA) have been used for screening purposes as well as for sensitive quantification of mycotoxins in various samples.[5–7,9,11] ELISA tests are rapid and easy to use and do not need any sample clean-up step. Recently, dipstick enzyme immunoassays have been introduced into the market for semi quantitative analysis of mycotoxins in food and feed samples. In this method, carrier membranes such as polyvinylidene difluoride (PVDF), nitro-cellulose, or nylon are used to immobilize either the antibody or the antigen. Due to possible cross reactivities with structurally similar derivatives, immunological assays show, often, certain limitations for accurate quantification of mycotoxins in real food and feed samples.

In recent decades, HPLC has become the most important method for the analysis of the known mycotoxins in food and feed, as well as in biological samples. Among different detectors for HPLC, the mass spectrometer is very sensitive, selective, and specific. In contrast to GC-MS, there is no need of derivatisation when analyzing by LC-MS. Since

the availability of LC-MS coupling techniques, mass-spectrometry has become an important analytical tool for routine analysis of samples in complex matrices because of unambiguous analyte identification and accurate quantification possibilities. Especially, the possibility of tandem mass spectrometry has opened a new perspective in the determination and identification of mycotoxins in food and feed samples, as well as their metabolic products in biological samples (e.g., urine, blood, and feces). However, appropriate sample preparation and chromatographic separation of analyte from matrix compounds seems to be necessary, even if performing LC-MS-MS, to insure accurate quantification as well as unambiguous identification.

Different LC-MS interfaces have been used for the analysis of mycotoxins in food and feed, as well as in biological samples such as urine, plasma, serum, and feces. Direct liquid introduction, thermospary, and particle beam were used, having only a historical value. Modern LC-MS instrumentation, nowadays, is mostly based on atmospheric pressure chemical ionization (APCI), atmospheric pressure photo-ionization (APPI), and electrospray ionization (ESI) interfaces. Mainly tandem MS instruments based on either triple quadrupoles, ion trap, and time of flight analyzers (TOF) are used. An advantage of mass spectrometry as detector for HPLC is its enormous selectivity, which allows analysing many mycotoxins simultaneously. Additionally, the use of selective MS detectors can reduce or, at least in some cases, exclude the application of a specific clean-up procedure. Recently, some review papers have been published showing the capacity of LC-MS for mycotoxin analysis.[9,12]

This paper presents a comprehensive literature overview of the application of modern LC-MS techniques in the analysis of mycotoxins in complex matrices such as food, feed, or biological samples. The influence of the matrix on quantification results, as well as sample preparation strategies, will be discussed. The use of LC-MS technology in the analysis of major mycotoxins produced by Aspergillus, Penicillium, Fusarium, and Claviceps species such as aflatoxins, ochratoxins, fumonisins, trichothecenes, zearalenone, and patulin and ergot alkaloids will be discussed.

AFLATOXINS

Aflatoxins are a group of difuranocoumarin derivatives, of which about 20 different types have been identified. The major aflatoxins, due to their occurrence and toxicity, are B_1, B_2, G_1, and G_2 (Fig. 1). These secondary metabolites are produced by different Aspergillus species such as *Aspergillus flavus* and *Aspergillus parasiticus*, as well as *Aspergillus nomius*. Widespread contaminations in warm and humid regions of the world have been reported.[2] Aflatoxins are found in a wide variety of foodstuffs

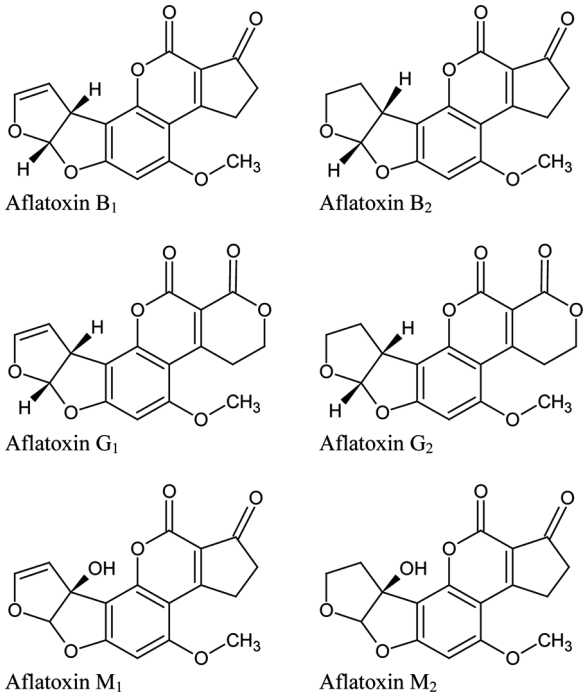


Figure 1. Chemical structure of aflatoxins.

including nuts, cereals, dried fruit, and many other agricultural commodities. "B" and "G" refer to the "blue" or "green" fluorescence observed upon exposure of the toxin to ultraviolet irradiation. The B group contains a cyclopentanone ring, while G group has a lactone ring. Aflatoxins are procarcinogens and, after metabolism by the P450 system in the liver, the 2,3-epoxide derivates are highly carcinogenic, mutagenic, and hepatotoxic agents.[1,2] After ingestion of contaminated feed or food by animals and humans, aflatoxin B_1 (AFB_1) and B_2 (AFB_2) will be metabolized into hydroxylated derivates M_1 (AFM_1) and M_2 (AFM_2), which will be excreted into the milk. According to the IARC, naturally occurring aflatoxins are considered to be human carcinogens and are, therefore, classified in Group 1. AFM_1 is classified in Group 2B as being possibly a human carcinogen.[4] Among all mycotoxins, aflatoxins are the most toxic and the strongest natural carcinogens. Mainly, liver cancer has been observed in laboratory animals and particularly, aflatoxin B_1 is a potent hepatocarcinogenic agent in rats. As a consequence, the most restricted and lowest regulations regarding maximum limits in food and feed have been set for aflatoxins within the EU (4 ng/g for direct consumption). In the United States, there exist action levels of 20 ng/g

for all foods.[13,14] Fortunately, aflatoxin molecules show strong fluorescence in ultraviolet light at 365 nm, which provides the possibility to detect these compounds by using a fluorescence detector. Therefore, aflatoxin contaminations in food and feed can be determined, after proper clean-up and HPLC separation, with fluorescence detection. To be able to analyse AFB_1 and AFG_1 down to ppt levels, fluorescence detection should be enhanced by either performing pre-column derivatization with trifluoroacetic acid or post-column derivatization with bromide and iodide, respectively. Another important issue is the use of a very selective sample cleanup, such as immunoaffinity chromatography. IACs are commercially available for single use only. The combination of HPLC with immunoaffinity columns has become a reference method for the determination of aflatoxins in food and feed matrices. Additionally, very sensitive ELISA tests have been used for either screening purposes or quantitative analysis of aflatoxins in feed and food.

HPLC, in combination with APCI and tandem MS in positive ion mode, was used for analysis of aflatoxins in different foodstuffs, such as figs, peanut butter, chilli, and pistachio meal by using aflatoxin M_1 as internal standard.[15] Protonated molecular ions $[M+H]^+$ were used as parent ions and, in the MRM mode, and the following daughter ions were selected for quantification: m/z 241 for AFB_1, m/z 259 for AFB_2, m/z 243 for AFG_1, m/z 257 for AFG_2 and m/z 273 for AFM_1. The method delivered satisfactory performance in figs and peanuts after clean-up on silica gel. However, the authors reported large variations of recoveries (40–280%) in spices and interferences with matrix compounds. The detection limits were comparable to those of FLD (0.1 ng/g).

Trucksess et al. used the LC-APCI-MS for confirmation of aflatoxin contaminations for analysis of a high protein feedstuff.[16] The ESI interface was used in a single quadrupole analyzer for the analysis of aflatoxins in medicinal herbs after solid phase extraction.[17] With ESI, a better abundance could be observed in the positive ionization mode. The mass spectra show predominantly sodium adducts $[M+Na]^+$ with less intensive protonated molecules $[M+H]^+$ without fragmentation. In recent years, an alternative ionisation technique, atmospheric pressure photoionization (APPI) was introduced into the market. Takino et al. have used APPI interface in a single quadrupole instrument for the analysis of aflatoxins in peanuts, red pepper, and corn.[18] No sodium or potassium adducts were observed in the mass spectra and the most abundant ions were due to $[M+H]^+$. The authors have compared the linearity of ESI and APPI interfaces for aflatoxins and noted that in APPI no significant matrix effects could be observed. In spiked food samples, the recovery, repeatability, and reproducibility of APPI were tested and LODs for all aflatoxins were three times better than those of ESI. The better performance of analytical results was reported due to much lower

noise and absence of matrix effects. The data revealed APPI to be a much more selective and sensitive interface than ESI for determination of aflatoxins in food matrices. Moreover, APPI seems to show lower susceptibility to matrix effects compared to ESI when analyzing aflatoxins. Furthermore, the ESI interface and MS-MS was used for the analysis of aflatoxins in olive oil after extraction with matrix solid phase dispersion, without any lipid removal.[19] The extract was then injected into the LC-MS directly without further treatment. AFM_1 was used as internal standard and the method recoveries ranged between 92–107% with LOQs of 0.04 and 0.12 ng/g.

More recently, Ventura et al.[20] have applied ultra performance liquid chromatography (UPLC) coupled with tandem MS for the simultaneous analysis of aflatoxins and ochratoxin A in beer (Fig. 2). ESI in positive mode was applied and sample preparation was performed by using SPE cartridge containing polymeric sorbent. The most abundant fragments in positive ionization mode were the protonated molecules $[M+H]^+$. Sodium adducts were observed additionally. The following transitions were selected for quantification: m/z $313.2 \rightarrow 269.2$ for AFB_1, m/z $329.2 \rightarrow 243.2$ for AFG_1, m/z $315.2 \rightarrow 259.2$ for AFB_2, m/z $331.0 \rightarrow 245.2$ for AFG_2. Different parameters of sample extraction were optimized. Data show that, especially for the recovery of aflatoxins, the pH of the extract plays an important role. Recoveries for all aflatoxins in

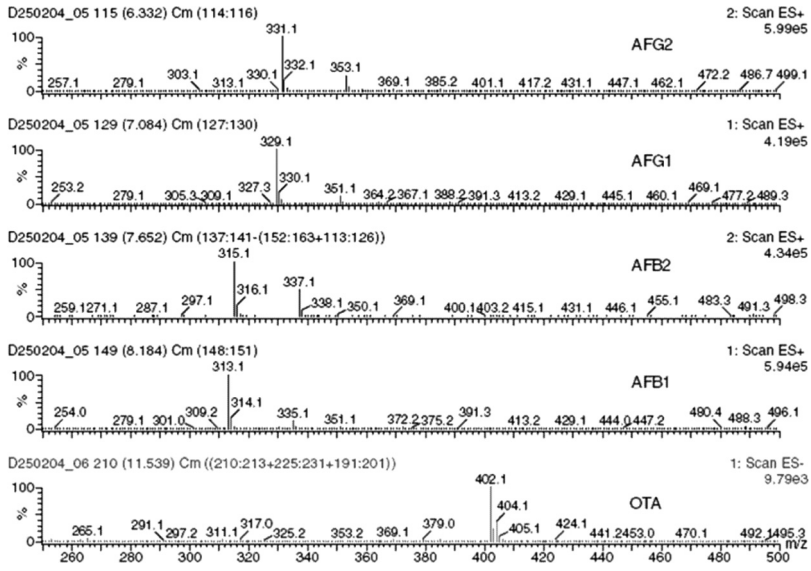


Figure 2. UPLC/MS/MS full scan mass spectra of AFB1, AFG1, AFB2, AFG2 and OTA. *Source*: Reproduced from Ref. [20].

beer ranged between 85–93% with LOD of 0.1 ng/mL and LOQ of 0.2 ng/mL.

Recently, Liau et al.[21] have performed LC/APCI-MS-MS in an ion trap system combined with supercritical fluid extraction for quantification of aflatoxins in a traditional Chinese medicine plant. $[M+H]^+$ and ammonium adducts $[M+NH_4]^+$ were used as precursor ions and the fragmentations in MS^2 and MS^3 were used for confirmation. Detection limits of the method were between 0.17 and 0.32 ng/g. In addition, ESI and APCI performance regarding sensitivity were compared. In APCI, better LODs and LOQs were observed than those in the ESI interface.

Catharino et al. have performed, for the first time, MALDI-TOF in analysis of aflatoxins.[22] By using an ionic liquid matrix based on α-cyano-4-hydroxycinnamic acid and addition of NaCl, mainly Na^+ adducts of aflatoxins were observed. By applying the method to real samples in a fungus contaminated peanut after extraction, sodium adducts could be detected. The authors suggested the use of the MALDI-TOF MS for screening purposes.

For the analysis of AFM_1 in milk, LC-ESI-MS-MS in positive ionization mode was applied by Sorensen and Elbæk.[23] Protonated molecular ion $[M+H]^+$ m/z 329 as the precursor and transitions of m/z $329 \rightarrow 273$ and $329 \rightarrow 259$ were used for quantification. Ammonium acetate was added post-column and the LOD of 10 ng/L and LOQ of 20 ng/L have been reported. Kokkonen et al.[24] have also performed positive ESI combined with tandem MS for the quantification of AFM_1 in cheese. The limit of quantification was reported to be 0.6 ng/g. g. In contrast, Chen et al.[25] applied, for the first time, negative ESI-MS-MS for AFM_1 analysis in milk and milk powder. Signal-to-noise ratio of deprotonated molecular ions $[M\text{-}H]^-$ m/z 327 was reported to be 6–7 times higher than those of $[M+H]^+$, which could be explained by the weak acidity of AFM_1. The authors used the transition, m/z $327 \rightarrow 312$, for quantitative determination and have additionally compared the IAC with multifunctional columns (MFC) as sample clean-up strategies. Significant ion suppression was observed in the case of MFC sample preparation and no matrix effect was reported for IAC clean-up. Cavaliere et al.[26] have compared the performance of positive ESI and APPI interfaces for the analysis of AFM_1 in milk using a tandem MS and after a clean-up with a carbograph-4 cartridge. A QTRAP instrument was used with APPI interface and the following transition pairs were selected: m/z $329 \rightarrow 285$ and m/z $329 \rightarrow 259$. A better sensitivity was reported for APPI compared to ESI. However, due to the latter investigations, ESI would also be acceptable since the LOD lies below the regulatory levels of aflatoxin M_1 in milk. A new generation of mass spectrometers have now allow reaching sensitivities comparable to fluorescence detection, however, with possibilities for confirmation of analytes.

Nevertheless, LC-MS seems not to be used widely in the routine analysis of aflatoxins in food and feed matrices. HPLC-FLD, in combination with immunoaffinity chromatography, still remains the method of choice and is used as a reference method, since this method is easy, robust, and delivers accurate and reliable data.

OCHRATOXIN A

Ochratoxins are a group of structurally related derivatives of dihydroisocoumarin linked to L-phenylalanine (Fig. 3). There are ochratoxin A (OTA), ochratoxin B (OTB) and ochratoxin C (OTC), of which OTA is the most common mycotoxin. A range of fungal species from the genera Aspergillus and Penicillium are able to produce ochratoxins. Mostly *Aspergillus ochraceus* and *Penicillium verrucosum*, as well as *Aspergillus carbonarius*, were reported to be found.

Besides aflatoxins, OTA is one of the important mycotoxins, due to its high toxicity. OTA has been reported to have potent nephrotoxic effects. Additionally, genotoxicity, teratogenicity, and immunosuppression were observed[27,28] OTA has been classified by the IARC as probable human carcinogen in Class 2B carcinogen.[29] The toxin has also been linked to human Balkan nephritis.[27] OTA has been implicated in kidney damage in pigs, causing porcine nephropathy by degradation of the proximal tubules followed by interstitial fibroses. Unlike aflatoxins, the ochratoxin A producing fungi can thrive in the temperatures and humidities commonly found in temperate countries, such as those in Europe and North America. OTA has been reported to be a natural contaminant of various agricultural commodities, such as cereals and their products, coffee, wine, beer, grape juice, and other products. An important issue is the carry-over of OTA into the food consumed by humans. OTA has been found in the blood and organs of slaughtered animals, e.g., in pig kidney and blood sausages. The bioavailability of OTA in monogastric species is high and about 40–60% of orally ingested toxin

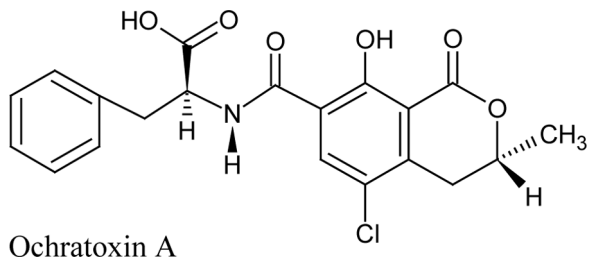


Figure 3. Chemical structure of ochratoxin A.

is absorbed by the gastrointestinal tract.[27] The elimination of OTA via urine and bile is, due to its high binding activity to serum albumin relatively slowly. In general, the toxin seems to have a high affinity for blood, kidney, and liver and especially accumulations in kidneys have been observed.[27] Furthermore, the analysis of OTA in human blood serum can be taken into consideration as an end-point indicator for exposure to OTA. Regulations concerning maximum limits of OTA in food have been set in the EU for baby food between 0.5–10 ng/g, for wine and grape juice at 2 ng/g and 5–10 for different foodstuffs.[30] There are no regulatory limits on OTA in food and feed proposed by US FDA.

For determination of OTA in food and feed matrices, as well as in tissues, primarily ELISA and HPLC have been used. In humans, animal serum, and in animal tissues, however, HPLC and/or LC-MS were performed.[9,12,31] In analysis of OTA, typically, reversed phase HPLC with fluorescence detection is used. As with analysis of aflatoxins, immunoaffinity columns for sample clean up, combined with HPLC-FLD analysis, has become the reference method, which allows quantitative determination of OTA down to ppt.

Different LC-MS interfaces, such as ESI and APCI combined with single and tandem MS, have been employed for the analysis of OTA in different matrices. Becker et al.[32] have performed LC-ESI-MS-MS for the first time for determination of OTA in beer, coffee, and wheat. A positive ion mode was applied and abundant molecular ion $[M+H]^+$ at m/z 404 and an additional sodium adduct $[M+Na]^+$ at m/z 426 were observed. An additional signal at m/z 406 was observed for ^{17}Cl-OTA. In MS-MS mode, fragment ions resulting from the loss of phenylalanine were (m/z 239, $[M+H\text{-Phe}]^+$) registered followed by m/z 358 $[M+H\text{-}H_2O\text{-CO}]^+$ and m/z 386 for $[M+H\text{-}H_2O]^+$. For sample clean-up, SPE based on a silica cartridge was used; the method showed good recoveries with an LOD of 0.01 ng/g by an external calibration method. Lau et al.[33] have compared performance of ESI and APCI interfaces in positive LC-MS-MS and studied the fragmentation pattern extensively. The authors noted that the APCI interface was not as sensitive as ESI. Therefore, the LC-ESI-MS-MS method was applied to the analysis of OTA in human plasma and also in coffee. Monitored transitions were m/z 404 → 239 and m/z 404 → 358. Additionally, the fragmentation pattern of OTB was studied and was used as internal standard. The internal and external standard methods were compared with each other. However, the use of OTB can be seen as a compromise since, in many cases, food or feed samples could contain OTB. A stable isotope dilution method with ESI-MS-MS in positive ion mode for the quantification of OTA in food samples was presented by Lindenmeier et al.[34] [$^2H_5$]-OTA was used as internal standard after clean-up on IAC or SPE based on silica. The detection limit was reported to be 0.5 ng/g and quantification limit of 1.4 ng/g.

Sorensen et al. have performed negative ion mode ESI for the analysis of OTA in milk.[23] The deprotonated molecules, as m/z 402 $[M-H]^-$, were noted in the ESI interface, which were used for the quantification of OTA. In the latter case, transitions of m/z 402 → 358 and m/z 402 → 167 were used. By applying low energy collision induced dissociation and argon, the most abundant product was ion m/z 358. Bacaloni et al.[35] have analysed OTA in beer and wine using an on-line solid-phase extraction and ESI-MS-MS (Fig. 4). The authors applied a negative ionization mode in a QTRAP instrument and compared the enhanced product ion (EPI) with MRM. They found that the S/N ratio in MRM was 3.5 times better than with EPI. However, the EPI mode provides more stringent confirmatory data. Additionally, in EPI mode, the signal response for OTA was found to be linear in the range between 0.05–25 ng/mL. The major fragment for OTA due to loss of CO_2 (m/z 358) $[M\text{-}CO_2\text{-}H]^-$ was used for quantification, whereas the less intense m/z 211 was selected for identification purposes. The LOD and LOQ in wine were about 0.01 ng/mL and for beer 0.08 ng/mL. Recoveries in red (74%) and white wines (76%) were comparable and, in beer, a recovery of 82% was noted.

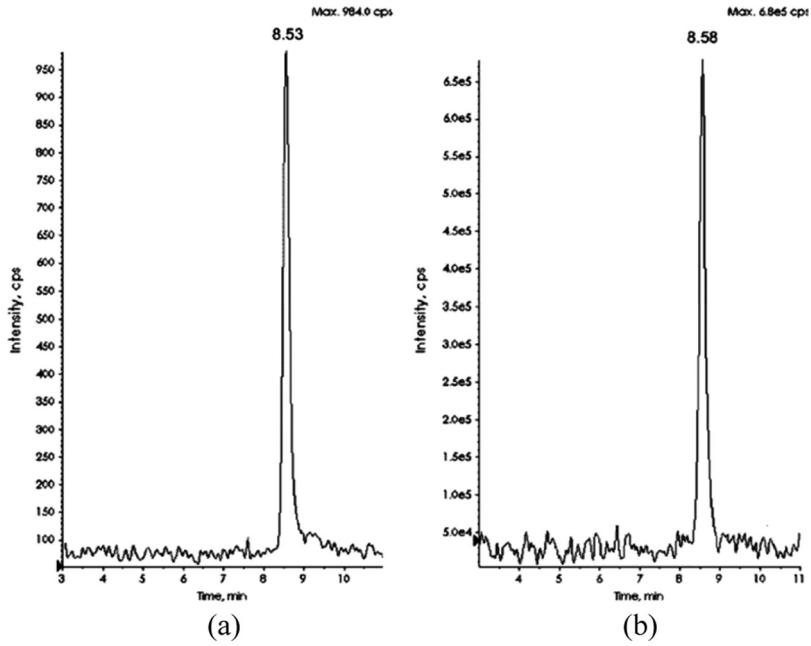


Figure 4. (a) 402 → 358 m/z MRM and (b) EPI LC-MS-MS chromatograms obtained by analyzing a red wine spiked at 0.05 ng mL-1 OTA. *Source*: Reproduced from Ref. [35].

Recently UPLC was applied to the analysis of OTA, together with aflatoxins in beer[20] after sample clean-up with polymeric SPE. The effect of pH and washing steps on recovery values of OTA during the clean-up procedure were investigated having no significant influence. Recovery of OTA was about 87% with a LOD of 0.1 ng/mL while a LOQ of 0.2 was noted. The main advantage of UPLC is the tremendous time and efficiency by using an external standard method. OTA and aflatoxins could be analysed within 3.2 min.

An ion trap instrument in positive ion mode was utilized by Reinisch et al. using an ESI interface.[36] The same ions and transitions were monitored as described before. For quantification of OTA in beer, an MRM mode was used with a method of external standard. The ion trap showed linearity between 3.5 ng/g and 38 ng/g with correlation coefficient up to 0.998. A precipitation step, followed by combined ion exchange/reversed phase SPE, was performed for sample clean-up. Limit of detection was 0.4 ng/g and the LOQ has been found to be 0.8. ng/g with satisfactory recoveries between 86–119%. The precipitation step has been shown to have a major effect on method performance.

Timperio et al. have applied nano-RP-LC-ESI-MS to the analysis of OTA in grapes.[37] As sample pretreatment, chloroform extraction and lyophilisation steps were performed. Positive ESI was used on an ion trap instrument. No matrix effect was reported and recoveries were in the range of 93–96% with LOD and LOQ were in 1–2 pg/g range. Almera et al.[38] have analysed OTA in red paprika using ESI ion trap MS in positive mode for confirmatory purposes. The limit of detection of LC-MS ion trap has been found to be comparable to HPLC-FDL combined with IAC having additional possibility for unambiguous confirmation of OTA.

One of the crucial points in trace analysis contaminants in complex matrices such as blood, urine, food, and feed samples is the use of an appropriate internal standard. Lindenmeier et al. have described this fact as an excellent example when they analysed OTA in coffee samples after SPE by using stable isotope dilution and MS-MS technique. A highly selective method with an appropriate internal standard could not eliminate interfering matrix compounds. However, when IAC was used for sample clean-up, the problem could be solved.[34] In another case, Zöllner et al. mentioned[39,40] the importance of judging the results critically, even if highly sophisticated methods such as MS-MS are used. The authors observed that the slopes of the calibration curves of OTA in wine from various origins were different. The use of internal standard could compensate matrix effects within each wine sort, but not between wine sorts of various origins.[40,41] For this reason, many scientists have used immunoaffinity as a clean-up strategy to eliminate matrix effects in MS-MS analysis.[33,39–41] However, this makes the analysis much more

expensive, since both clean-up and the analysis are performed using costly approaches. At least in some cases, the immunoaffinity columns have been shown to be indispensable to eliminate matrix effects. However, according to the literature, there were no significant differences between LC-MS results and those of LC-FLD (combined with IAC) as reported in analysis of OTA in wine.[41] Leitner et al. reported the sensitivity of HPLC-MS-MS combined with SPE having a LOD of 0.15 μg/L wine compared to HPLC-FLD and IAC having a LOD of 0.03 μg/L.[41]

The LC-MS technique is an excellent tool for studying the toxicokinetics of mycotoxins in either animals or for in-vitro studies. Especially, LC-MS was often used for structural elucidation of OTA metabolites in animal tissues as well as in cell lines. Zepnik et al. have applied HPLC-MS-MS for the toxicokinetic studies in rats. OTA and its metabolites in urine, plasma, liver, kidney, and feces were identified.[42]

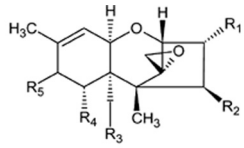
In another study, Mally et al. have used ESI-LC-MS-MS for characterization of DNA-adducts in rat kidney and in-vitro studies.[43] Richard et al. have performed LC-MS for the confirmation of accumulated dust samples in workers indicating the occurrence of OTA in dust.[44] ESI-LC-MS-MS in negative mode was applied for unambiguous confirmation of analytes. In another study, the OTA and OTB metabolites were analysed and identified in kidney cell cultures by using nano-HPLC-ESI-MS.[45] An ion trap instrument was used for MS^2 and MS^3 experiments for identification of de-chlorinated derivates and open ring OTA in negative ion mode.

Although there are limitations for the LC-MS-MS technique, however, due to its high selectivity and sensitivity, this method has been shown to be appropriate. Nevertheless, it is necessary to use a suitable sample clean-up and chromatography to be able to provide reliable results, when analyzing OTA in biological matrices; food, and feed. However, HPLC-FLD can also be used for the separation and detection of OTA in food and feed samples and can deliver accurate data.

FUSARIUM TOXINS

Trichothecenes

Trichothecenes are a group of toxins produced by fungi of the genus Fusarium. Approximately 180 trichothecenes are known to exist, but only a few are significant to human health.[1] Trichothecenes are tetracyclic sesquiterpene alcohols (Fig. 5) and can be divided into subclasses: type A, B, C, and D. Type A is represented by HT-2 toxin, T-2 toxin, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), and neosolaniol (NEO); type B includes deoxynivalenol (DON), 3-acetyl-DON



	R_1	R_2	R_3	R_4	R_5
Neosolaniol	OH	$OCOCH_3$	$OCOCH_3$	H	OH
Monoacetoxyscirpenol	OH	$OCOCH_3$	H	H	H
Diacetoxyscirpenol	OH	$OCOCH_3$	$OCOCH_3$	H	H
T-2 Toxin	OH	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$
HT-2 Toxin	OH	OH	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$
Acetyl T-2 Toxin	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$
Nivalenol	OH	OH	OH	OH	=O
Deoxynivalenol	OH	H	OH	OH	=O
3-Acetyl-Deoxynivalenol	$OCOCH_3$	H	OH	OH	=O
15-Acetyl-Deoxynivalenol	OH	H	$OCOCH_3$	OH	=O
Fusarenon X	OH	$OCOCH_3$	OH	OH	=O

Figure 5. Chemical structure of A-Trichothecens and B-Trichothecens.

(3-Ac-DON), 15-acetyl-DON (15-Ac-DON), nivalenol (NIV), and fusarenon X (FUS-X). DON is the most prevalent trichothecene in food and feed and is primarily produced by *Fusarium graminearum* and *Fusarium culmorum*. It is commonly found in barley, corn, rye, oat, and wheat.[46] When ingested in high doses by farm animals, it causes nausea, vomiting, and diarrhea; at lower concentrations in feed, pigs and other farm animals exhibit weight loss and feed refusal.[46] The FDA advisory level for DON is 1 ppm in finished wheat products for human consumption. European Union established maximum level for cereals intended for direct human consumption at 750 μg/kg.[47]

GC-ECD and GC-MS have been widely used for the analysis of type A- and B-trichothecenes.[11] The limitation of the GC method is the necessity of derivatization and determination of type A- and B-trichothecenes in food and feed matrices have been shown to be associated with many analytical problems.[11,48] The use of HPLC with UV for detection of trichothecenes is limited, due to poor or unspecific absorptions and can be applied only for the analysis of B-trichothecenes.[49] In contrast to the B-trichothecenes, the A-trichothecenes do not have a conjugated double bond group and are, therefore, not amenable to ultraviolet detection. The analysis of B-trichothecenes using HPLC-UV is possible only if a selective clean-up is used. SPE, IAC, and multifunctional columns (MFC) were used for clean-up of trichothecenes in food and feed. Recently, extraction and clean-up techniques for B-trichothecenes and macrocyclic trichothecenes on chromatographic determination has been evaluated.[50] Biselli et al. reported the improvement of A-trichothecenes recoveries, when MFC were additionally washed before using.[51] However, in comparison to IACs, the selectivity

of the Mycosep® method is substantially lower. The use of MS as a selective detector has been shown to offer considerable advantages and could compensate for the non-selective sample clean-up procedures.[52–54] Among B-trichothecenes, only DON analysis in food and feed IACs are available. This clean-up strategy allows the analysis of DON with HPLC-UV in an appropriate concentration range.[55]

ESI and APCI-MS have been widely used in the analysis of trichothecenes.[9,12] The derivatization step can be omitted but an appropriate sample clean-up is still a necessity to eliminate or reduce matrix interferences. However, some authors reported the use of multistage MS without any sample clean-up. The crude grain extract was injected directly into the LC-MS-MS system.[56,57]

For B-trichothecenes, mainly an APCI interface has been used for quantitative determination.[52,53] A negative ionization mode has been found to be more sensitive for B-trichothecenes. In contrast to A-trichothecenes, the positive ion mode was found to provide higher signal intensities. Depending on the structure of B-trichothecenes, deprotonated molecular ions $[M\text{-}H]^-$ and fragments $[M\text{-}H\text{-}CH_2O]^-$ have been observed. When buffers such as acetate and formate were added into the mobile phase, mostly adduct ions $[M + HCOO]^-$, $[M + CH_3COO]^-$ were observed. Analysis of DON, NIV, 15-AcDON, 3-AcDON, and Fus-X was performed using LC-APCI-MS in maize.[58] Additionally, the feasibility of the APCI-MS interface for the determination of DON and its de-epoxy-metabolite in pig urine has been shown by

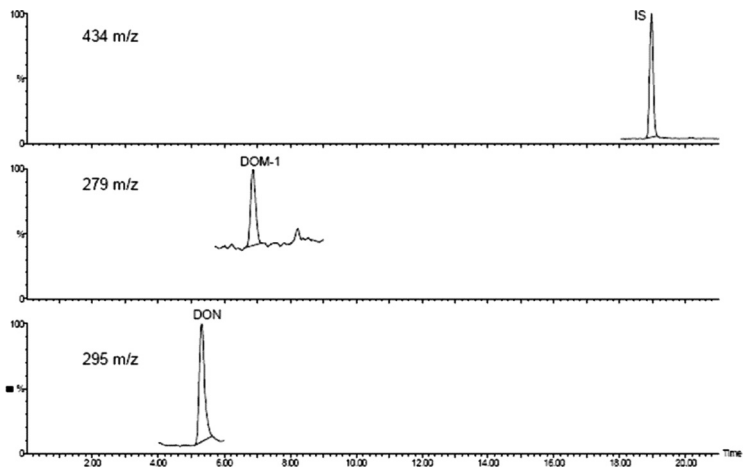


Figure 6. LC-APCI-MS chromatogram of a contaminated pig urine sample containing 185 ng/ml DON and 80 ng/ml DOM-1 in negative ionization mode. *Source*: Reproduced from Ref. [58].

Razzazi-Fazeli et al.[58] (Fig. 6). Recently, tandem mass spectrometry has been employed for the analysis of trichothecenes in food.[54,59] After collision induced dissociation, the acetate daughter ions $[CH_3COO]^-$ (m/z 59) were used for measurement and qualifier in MRM mode for analysis of B-trichothecenes. By applying MS-MS, a chromatographic separation of two acetyl derivatives of DON is not needed, due to different fragmentation patterns in the collision cell. Multi-analysis for A- and B-trichothecenes has been performed in maize using APCI, in both positive and negative ion mode, using a triple quad instrument.[54]

In the case of A-trichothecenes, APCI and ESI interfaces in positive ionisation mode have been reported for the analysis of these toxins in cereals.[53,54,59] The mass spectra of T-2 and HT-2 as the major A-trichothecenes show protonated molecular ions $[M + H]^+$ and ammonium adducts $[M + NH_4]^+$. Fragment ions due to the loss of iso-valeryl side chain $[M + H\text{-}(CH_3)_2CHCH_2COOH]^+$ were reported. For other A-trichothecenes, similar fragment ions were observed in the APCI interface using a single quadrupole instrument.[53]

The MS-MS experiments in the case of A-trichothecenes show, also, a better signal-to-noise in MRM mode and, therefore, better sensitivity in comparison to the single quad instrument. Analysis of T-2 in maize using APCI (+) triple quad showed LOD of 0.3 ng/g,[54] whereas LOD in wheat using APCI (+) single quad showed LOD of 50 ng/g.[53] Mainly daughter ions due to loss of acetate and/or iso-valeryl side chains (m/z 484 in the case of T-2) could be registered and were used as qualifier. Ion trap instruments seem not to be as sensitive as tandem MS. Analysis of T-2 in wheat using APCI (+) on ion trap provided LOD of 3 ng/g,[60] whereas in maize using APCI (+) on triple quad showed LOD of 0.3 ng/g.[54]

Simultaneous determination of A- (NEO, DAS, T-2, HT-2) and B-trichothecenes (DON, 15-Ac-DON, NIV, FUSX) has been also performed by Dall'Asta et al.[61] The recovery calculated for the certified reference material was 95% for DON. Satisfactory sensitivities (LOD ranged from 20–50 ppb) using single quadrupole with ESI (+/−) were noted.[61] Klötzel et al. have developed the method for simultaneous determination of 12 trichothecenes by LC-ESI-MS-MS. Mean recovery values obtained from corn ranged from 48 to 93% at spiking levels of 400 ng/g. LOD in corn ranged from 0.2–4.4 ng/g.[55] Bretz et al. reported the synthesis of stable isotope labeled 15-d_1-deoxynivalenol (15-d_1-DON) from its natural precursor 3-AcDON.[62] The authors developed a method for the analysis of DON and 3-AcDON in tortilla chips, bread, corn flakes, and pretzels using LC-ESI (+/−) MS-MS with stable isotope labeled 15-d_1-DON and 3-d_3-AcDON as internal standards. The limits of quantitation were not reported. Häubl et al. used ($^{13}C_{15}$)DON as an internal standard for determination of DON in maize and wheat without

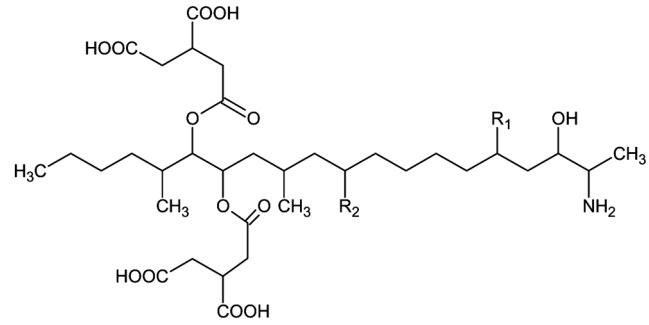
any clean-up step.[63,64] Quantification of DON in the maize reference material (specified DON concentration, $470 \pm 30\,\mu g/kg$) without internal standard led to a measurement result of $176 \pm 22\,\mu g/kg$ DON (apparent recovery, 37%). The method of the IS yielded DON concentration of $463 \pm 16\,\mu g/kg$ corresponding to an average apparent recovery of 99%.[63]

The simultaneous determination of NIV, DON, T-2, and HT-2 in different food matrices, including wheat, maize, barley, cereal-based infant foods, snacks, biscuits, and wafers using LC-APCI-MS on a triple quadrupole has been published recently.[65] A clean-up procedure, based on reversed phase SPE Oasis(R) HLB columns, was used, allowing good recoveries for all studied trichothecenes. LODs in the various investigated matrices ranged 2.5–4.0 μg/kg for NIV, 2.8–5.3 μg/kg for DON, 0.4–1.7 μg/kg for HT-2, and 0.4–1.0 μg/kg for T-2. Mean recovery values, obtained from cereals and cereal products spiked with NIV, DON, HT-2, and T-2 toxins at levels from 10 to 1000 μg/kg, ranged from 72 to 110%.

Berthiller et al. have developed a method for the analysis of masked mycotoxins by using LC-ESI-MS-MS.[66] The authors have detected DON-3-glucopyranoside in wheat and maize and suggested that masked mycotoxins should not be ignored with regard to food and feed safety. Additionally, LC-MS and MS-MS was used further in the analysis of DON in various biological samples such as pig urine,[58] human urine,[67] pig serum,[68] eggs,[69] and milk.[23]

Fumonisins

Fumonisins are a group of mycotoxins produced by the maize pathogens, *Fusarium verticillioides* (formerly *F. moniliforme*) and *F. proliferatum*, at very low levels by *Alternaria*[70] Their chemical structures are primary amines with 2 tricarballylic groups, which contribute to their water-solubility. Maize-based food and feed have been found to be the major source of fumonisins. Three groups of fumonisins (A–C) have been identified; groups A and B are characterized by the presence of an amide and amine group, respectively (Fig. 7). Group C is similar to the B-group, except for the absence of the methyl group at the C_1-terminal.[70] Fumonisin B_1 (FB_1) was shown to be the cause of leukoencephalomalacia in horses and pulmonary edema in swine. Hepatotoxicity and nephrotoxicity have also been reported in connection with fumonisin intoxications.[1] The fumonisins are weakly carcinogenic among different rodent species and are probable human carcinogens (group 2B), associated with increased incidence of esophageal cancers in South Africa and China.[71] The European Union set up a regulatory maximum level of



	R_1	R_2
Fumonisin B_1	OH	OH
Fumonisin B_2	OH	H
Fumonisin B_3	H	OH

Figure 7. Chemical structure of fumonisins.

fumonisins in maize intended for direct human consumption at 1,000 μg/kg.[47] Baking, frying, and extrusion cooking of corn at high temperatures (≥190°C) can reduce fumonisin concentrations in foods.[72] FB_1 is transformed into its N-carboxymethyl analogue when it is heated with reducing sugar.[73] Different analytical techniques, such as TLC, ELISA, GC-MS, HPLC, and HPLC-MS were used for determinaiton of fumonisins in food, feed, and biological samples.[9] ELISA methods are easy, rapid, and adapted for rapid screening purposes. ELISA generally overestimates the concentration of fumonisins present in samples due to the cross reactivity of antibodies.[74] GC-MS has been used to a limited extent in the analysis of FBs, it was usually used to confirm the identity of fumonisin metabolites.[74] HPLC-FLD is a method of choice for determination of fumonisins in food and feed. However, due to the lack of UV chromophore in the molecules, derivatisation is neccessary. o-Phthalaldehyde (OPA) is used for pre-column derivatization and fluorescence detection. LC-MS and LC-MS-MS have been mainly used for detection of fumonisins and their metabolites in biological samples.[75–78] The clean-up step has been performed using RP-SPE on C_{18}, strong anion-exchange (SAX) cartridges or IAC.

HPLC-MS provides high sensitivity and selectivity in the analysis of fumonisins without the need of derivatization. As fumonisins are ionic, abundant signal in both positive and negative ion modes can be achieved. However, the positive ion mode has been used more often (Fig. 8), where predominantly base peaks of m/z 722 as $[M+H]^+$ for FB_1 and m/z 706 as $[M+H]^+$ for FB_2 have been registered for quantitative determination.[79–81] In the negative ion mode, deprotonated molecular ions at

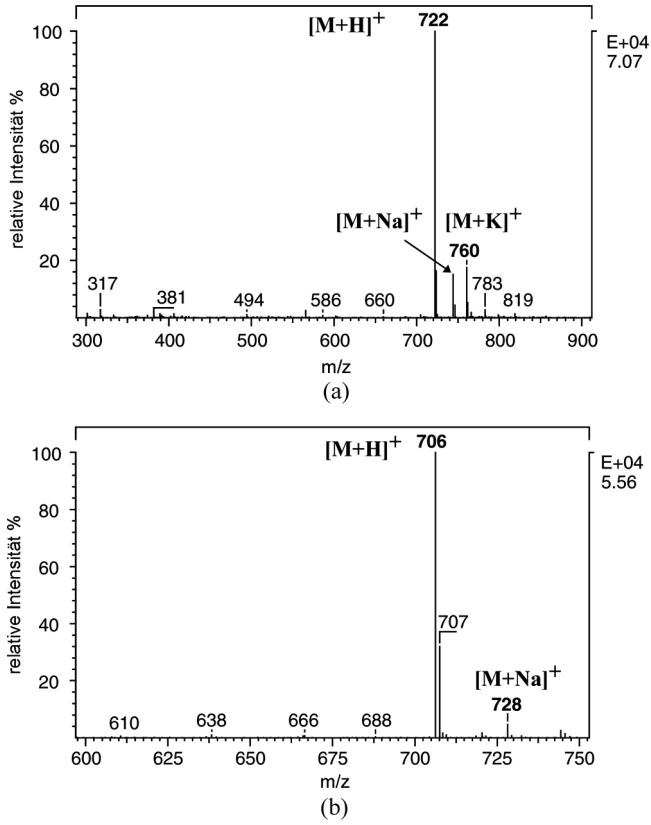


Figure 8. Electrospray mass spectra of fumonisin B_1 (a) and fumonisin B_2 (b) in positive mode. *Source*: Reproduced from Ref. [79].

m/z 720 and m/z 704 were observed with lower sensitivities than those in the positive ion mode. In low energy CID using argon as collision gas, predominantly consecutive loss of the tricarballylic acid (TCA) and water groups were observed in the spectra of the daughter ions of FB_1 and FB_2. Generally, the fragmentation patterns of all fumonisins are similar. As an example, for FB_1 following daughter ions, m/z 686, and m/z 668 represent the consecutive losses of water molecules, which correspond to the number of hydroxyl groups on the fumonisin backbone. The elimination of TCA from both side chains leads to fragmentation, which corresponds to m/z 370 $[M + H\text{-}2TCA]^+$[81]

LC-MS/MS using d_6-labeled FB_1 as internal standard was applied to investigate hydrolyzed degradation products of FB_1 (HFB_1).[82] For HFB_1 molecular ion as m/z 406 has been noted as the base peak and additional Na^+ and K^+ adducts were observed. Similar to FB_1, loss of

water molecules were registered after collision dissociation. The sensitivity of ESI was reported as LODs for FB_1 and HFB_1 about 5 ng/g and 8 ng/g, respectively.[82] LC-MS-MS methods have been used for structural elucidation/confirmation and trace quantification by Bartok et al. using an ion-trap instrument.[83] The fate of fumonisins during the production of fried tortilla chips could be studied and different hydrolyzed products were confirmed using LC-ESI-MS-MS.[84] The occurrence of fumonisin in asparagus spears and cornflakes was confirmed and quantitatively analysed using LC-ESI-MS-MS.[85,86]

Additionally, the APCI (+) interface has been used in analysis of FB_1 in maize. However, the sensitivity was worse than with ESI. LOD and LOQ were reported to be 20 μg/kg and 50 μg/kg, respectively.[87] Since fumonisins undergo an in-source fragmentation which is similar to collision activated MS-MS fragmentation; APCI single stage MS could also provide comparable structural information.[9]

If compared with HPLC-FLD, the LC-MS-MS shows similar LODs in a range between 5–20 ng/g. Limits of detection and quantification of published LC-MS-MS methods are well below those required according to the maximum and action limits in foodstuffs.[88]

ESI is the most suitable interface for ionization and detection of fumonisins in biological samples. FB_1, FB_2 and FB_3 as well as their hydrolysis products, HFB_1 and HFB_2, were detected in monkey hair.[76] Tandem MS yielded product ion mass spectra, which served as diagnostic indicators of FB_1, FB_2, and FB_3 as well as HFB_1 and HFB_2.[76] The same method has been applied in assessing human exposure to fumonisin mycotoxins in South Africa.[77] Recoveries from spiked hair samples after extraction with methanol, clean-up with strong anion column, and detection by HPLC-ESI(+)MS ranged from 81% to 101%. Single stage MS (ESI + single quadrupole) has been successfully applied in analysis of FB_1 in pig's tissue and biological fluids. Identification of FB_1 corresponded to m/z 722, 388, 300.[75] This method was also used for the study of distribution and elimination of fumonisin after oral administration of FB_1, FB_2 and FB_3 in weaned piglet.[78]

Zearalenone

Zearalenone (ZON) belongs to the group of fusariotoxins produced by several field fungi such as *Fusarium graminearum* and *Fusarium culmorum*. ZON is a resorcylic acid lactone chemically described as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid lactone and has been shown to have estrogenic activities. It is a stable compound during storage, milling, processing, and cooking of food and is heat stable.[89] ZON is common in maize, also in cereal crops such as barley, oats,

wheat, rice, sorghum and soy beans. The crops can be contaminated both in the temperate and warmer climate zones. Zearalenone can disrupt reproductive function in domestic animals. Pigs are especially sensitive to this mycotoxin and the ingestion of ZON causes hyperestrogenism, which is associated with severe reproductive problems. When analysing biological samples such as urine, plasma, faeces, and animal tissues, different metabolites of ZON, like α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL) and zearalanone (ZAN) (Fig. 9) have to be investigated for obtaining the metabolic profile and the carry-over. The latter metabolites are considered to be endocrine disruptors.

There are various analytical techniques for ZON such as TLC, ELISA, GC-MS, HPLC, LC-MS. The ELISA test for the determination of ZON is now available from several suppliers, providing LODs between 50–250 μg/kg. The use of IAC followed by HPLC-FLD has become the predominant analytical method for analysis of ZON in food and feed because of its convenience.[90] Conventional extraction and clean-up methods for the analysis of ZON and its metabolites are mainly based on liquid–liquid partitioning with chloroform–aqueous sodium hydroxide followed by SPE. Another approach includes the use of IAC, which has become popular nowadays. Analysis of ZON and its metabolites by use of IAC is simple and robust. However, the commercially available

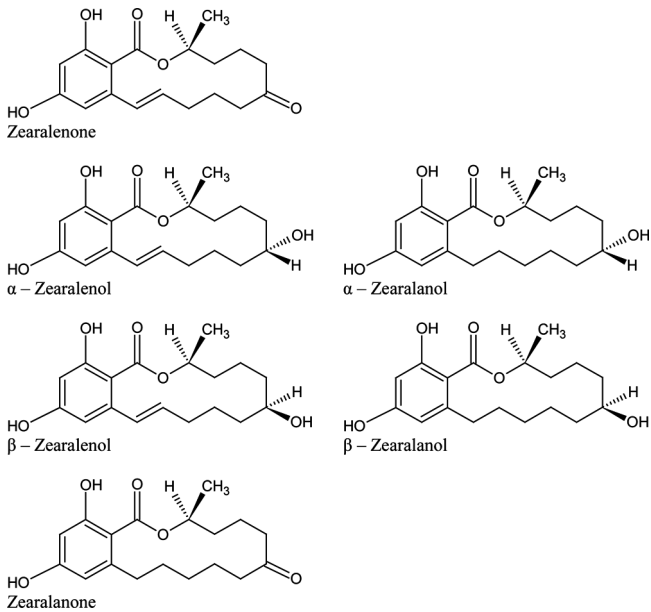


Figure 9. Chemical structure of zearalenone and its metabolites.

IACs are expensive and cannot be re-used. Another alternative clean-up technique is the use of multifunctional Mycosep® columns. Analysis of spiked corn samples gave a mean recovery of 99.4% for ZON. The LOD of the method was found to be 3.6 mg/kg by HPLC-FLD.[91] The analytical process for biological samples is similar to those used in food and feed, including sample extraction, clean-up, separation, and detection of the mycotoxins. Liquid–liquid extraction, SPE, and IAC were used in the clean-up and extraction step. Recently, Erbs et al.[92] determined the cross-reactivities for ZON, α-ZOL, β-ZOL, α-ZAL, β-ZAL, and ZAN on three commercial immunoaffinity columns targeting ZON. IAC in combination with liquid-liquid extraction and SPE have been used for extraction and clean-up strategies in complex biological samples.[93,94]

Rosenberg et al.[95] developed a method based on LC-APCI-MS for the determination of ZON in maize. The best response for ZON was obtained by a negative ionization mode with the most abundant ion as deprotonated molecular ion $[M\text{-}H]^-$. Furthermore, Zöllner et al. proposed a robust LC-APCI(-)MS-MS method for the determination of ZON in grains and beer samples, using ZAN as internal standard.[96,97] A LOD of 0.5 mg/kg and LOQ of 1 mg/kg in maize could be achieved.[96] Recently, Cramer et al.[98] have reported the use of 3,5-d_2-ZON as internal standard in the stable isotope dilution analysis of zearalenone by ESI(−)MS-MS in cereal products. The extraction and clean-up were performed by using acetonitrile/water mixture and a Bond Elut Mycotoxin column. The eluate was then analyzed by HPLC-MS-MS. The recovery rates were 103–104%. However, LOD and LOQ were not reported. Highly selective and sensitive techniques like HPLC-MS and HPLC-MS-MS are methods of choice for determination of ZON and its metabolites in biological samples. Jodlbauer et al.[99] developed a method for simultaneous analysis of ZON, α-ZOL, β-ZOL, α-ZAL, and β-ZAL in urine and tissues of bovine and pigs using HPLC-APCI(−)MS-MS using ZAN as an internal standard. One step clean-up with RP-18 SPE was performed. The recovery of 91–102% in urine and 86–91% in tissue samples were reported. This method was also applied for the analysis of ZON and its metabolites in pig liver with recoveries ranging between 55–85% and 94–105% in urine.[100] However, ZAN was no longer used as internal standard, since it could occur in animal tissues; thus, the quantification was performed using d_2-ZAN (MW 322) as internal standard. For the quantification in MRM mode, the deprotonated molecular ions of ZON (m/z 317), α- and β-ZOL and ZAN (m/z 319), α- and β-ZAL, and d_2-ZAN (m/z 321) were selected as precursor ions. The quantification was based on the following transitions: m/z 277/303 for α- and β-ZAL, m/z 207 for D_2-ZAN, m/z 160/174 for α- and β-ZOL, m/z 205/275 for ZAN, and m/z 131/175

for ZON. The method achieved LODs of 0.1 μg/kg for ZON, α-ZOL, α-ZAL and ZAN, 0.3 μg/kg for β-ZOL, and 1 μg/kg for β-ZAL in pig liver. The described method was also applied to the analysis of ZON and its metabolites in urine, liver, and muscle of heifers.[101]

Bily et al.[102] developed a method for analysis of ZON, DON and 15-AcDON in fungal liquid cultures, maize grain, insect larvae, and pig serum. The authors used an LC-MS method based on single quadrupole in positive ionization mode. However, the levels of other ZON derivatives were not determined. Songsermsakul et al. have used LC-APCI(−)MS using a single quadrupole instrument coupled with IAC and a combination of IAC and SPE for determination of ZON and its metabolites in urine, plasma, and faeces of horses.[94] The recovery ranged between 84–101% in plasma. LOD and LOQ ranged from 0.1–0.5 μg/L or μg/kg and 0.5–1.0 μg/L or μg/kg, respectively.[94] Figure 10 shows LC-MS chromatograms of urine sample obtained in single ion recording mode.

As a growth promoter, α-ZAL (zeranol) is of interest to be analysed in animal tissues. Although its application has been banned in the European Union, it is still used in other parts of the world.[46] LC-ESI-MS/MS in negative ion mode was developed for detection and identification of zeranol in chicken or rabbit liver.[103] A C_{18} SPE cartridge was used for clean-up and recoveries in the range of 61–90% were obtained. The LOQ based on the assay validation was 1 μg/kg. van Bennekom et al. have applied HPLC-ESI(-)MS-MS for the determination of six resorcylic acid lactones ZON-related in bovine tissues using

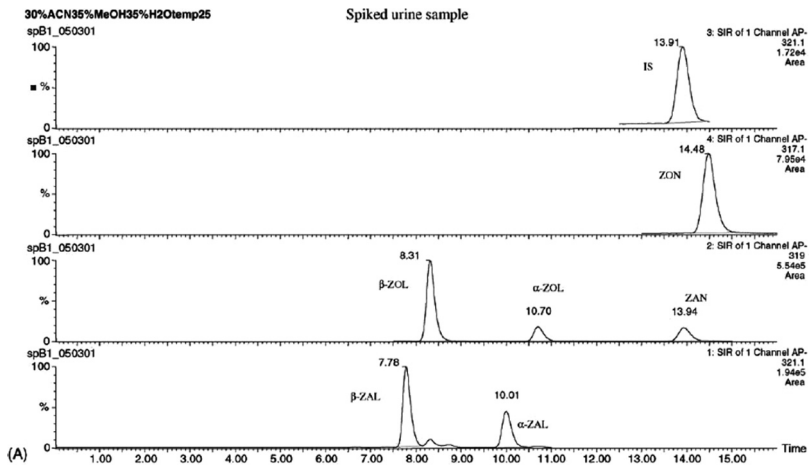


Figure 10. LC-APCI/MS chromatogram of horse's urine: Spiked urine sample with ZON and its metabolites using D2 ZAN as internal standard (IS). *Source*: Reproduced from Ref. [94].

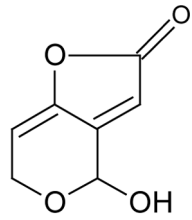
d_4-α-ZAL- and d_4-β-ZAL as internal standard.[104] The method has been applied for the confirmatory assay of α- and β-ZAL in bovine urine.[105] Both methods reached LOQs below 1 ng/g by using a combination of two SPE columns.

Besides quantification, LC-MS has been used for structural elucidation of ZON metabolites in plants. Berthiller et al. reported 17 different metabolites, prominently glucosides, malonylglucosides, di-hexose-, and hexose-pentose disaccharides of zearalenone, α- and β-zearalenol in the model plant, *Arabidopsis thaliana*, which proved the occurrence of masked mycotoxins.[106]

Patulin[1]

Patulin is a mycotoxin with a hemiacetal lactone structure (Fig. 11). It is produced by a number of fungi belonging to *Aspergillus* and *Penicillium* Spp. The most important producer is *Penicillium expansum*, which grows on apple and other fruits. Patulin is considered to be mutagenic, although it may not be carcinogenic.[1] It has also been found in animal trials to have adverse effects on the developing foetus and gastrointestinal tract. Additionally, immunotoxic and neurotoxic effects were also reported.[46] The maximum level of patulin in EU has been defined as 10 μg/kg in apple products and juice for infants and 50 μg/kg in apple juice.[107] In the USA, 50 μg/kg in apple juice has been under discussion.[13]

Liquid–liquid extraction and SPE are the traditional methods of sample preparation in the analysis of patulin in food samples prior the analysis with TLC, HPLC, GC-MS, LC-MS.[108] Recently, a competitive immunofluorescent assay for the detection of patulin has been developed.[109] The method was based on the binding of fluorescence labeled antibodies to the synthetic patulin derivative, covalently immobilized on a sepharose matrix. The assay allowed the detection of 10 μg/L. RP-HPLC, coupled to UV detection, has been found to be most suitable in patulin trace analysis since it exhibits strong UV absorption at



Patulin

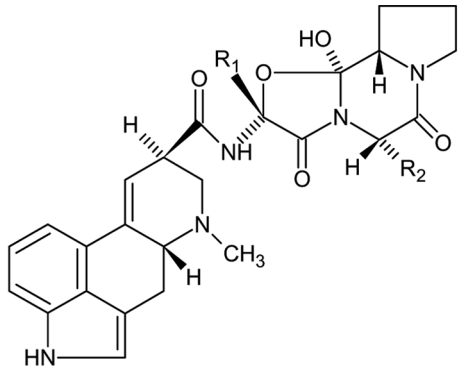
Figure 11. Chemical structure of patulin.

275 nm.[110,111] The analysis of patulin using LC-MS was performed mainly in the negative ionization mode using deprotonated molecular ions by either ESI or APCI (70–74). LC-APCI-MS-MS in negative mode on ion trap instrument was employed for the analysis of patulin in apple juice.[112] Molecular ion of m/z 153 $[M\text{-}H]^-$ and the m/z 135 due to the loss of water followed by m/z 125 due to the loss of carbon monoxide and m/z 109 due to the loss of carbon dioxide or acetaldehyde were observed.[112] The authors performed liquid-liquid extraction and the method performance such as repeatability, recovery, and sensitivity (LOQ = 10 ng/g) were comparable to those of HPLC-UV with the additional possibility of analyte identification. Rychlik et al. have performed stable isotope dilution assay using an electrospray interface and ^{13}C-labeled patulin as internal standard for determination of patulin in apple juice.[113,114] However, the sensitivity and repeatability of the LC-MS method was not as good as the conventional HPLC-UV method. APPI-LC-MS in negative mode has been shown to be an alternative ionisation method for patulin analysis.[115] In comparison to APCI, the APPI interface seems to be much more robust in terms of matrix interferences. The SIM chromatograms in APPI show lower chemical noise and stable base line. Repetitive injections of apple juice spiked with patulin in APPI shows, over the whole day, lower drift (90.3%) than those of APCI (50.3%). This fact has been suggested to be due to the contamination of the corona discharge needle and the source.

Ergot Alkaloids

One of the oldest known mycotoxicoses in man is ergotism, which is caused by the toxic alkaloids produced by different fungi such as Claviceps, Neotyphodium and Epichloe spp. Ergotism was first mentioned in Mesopotamia in 600 BC by Assyrians. However, there were many epidemic outbreaks of ergotism in the middle ages after consumption of contaminated bread. Not only wheat, rye, and other grains can be contaminated, but also grasses play an important role in widespreading of this type of parasitic fungal species. The major producer in terms of frequency of occurrence has been found to be *Claviceps purpurea*. However *Claviceps africanum, Claviceps sorghi and Neotyphodium coenophialum* have also been reported to produce ergot alkaloids.

After infection of plants by fungi, fungal hyphae replace the plant tissue mostly the kernels, which have a black violet colour and is called "ergot" or "sclerotium". The sclerotia of each species contain a certain spectrum of alkaloids, e.g., those alkaloids from *C. purpurea* differ from that produced by other fungi. Additionally, the content of various alkaloids differs also within *C. purpurea* growing on different agricultural



	R_1	R_2
Ergovaline	Methyl	Isopropyl
Ergotamine	$Methyl_3$	Benzyl
Ergocornine	Isopropyl	Isopropyl
Ergocryptine	Isopropyl	Isobutyl
Ergocrystine	Isopropyl	Benzyl

Figure 12. Chemical structure of some major ergot alkaloids.

commodities.[116] Ergot represents a group of alkaloids that grow on the heads of grasses. Ergot alkaloids belong to indol alkaloids having strong biological and pharmaceutical effects. There are 80 or more ergot alkaloids and many of them are suspected of being psychoactive. The ergot alkaloids consist of a tetracyclic ergoline ring system of lysergic acid as its basic structure (Fig. 12). The major ergot alkaloids are divided into three groups, clavine type, the amide type of lysergic acid derivates (water soluble) such as ergometrine, ergobasine, ergonovine, and the peptide derivates of lysergic acid or ergopeptides (water insoluble) such as ergotamine, ergovaline, ergosine, ergostine, ergocornine, ergocryptine and ergocrystine. The clavine type of alkaloids, such as agroclavine, is a potent uterine stimulant and ergovaline is the major alkaloid produced in some infected grasses. The major alkaloids produced by *Claviceps* spp. are ergometrine, ergotamine, ergosine, ergocornine, and ergocristine. The poisoning in human and animals causes both convulsive and gangrenous effects. Nausea and vomiting have also been reported.[117] The vasoconstuctive action of the ergotamine group leads to the gangrenous effect. Toxicoses in pigs and cattle have been reported associated with reproductive disorder and inhibition of prolactin secretion in pregnant swine and, in severe cases, abortion.[2,117] Ergotism shows high mortality up to 34% and 5–10 g of fresh ergot would be lethal to human.

The maximum level in grain in USA and Canada is 300 mg ergot/kg. In the EU, the maximum levels of 0.05% ergot in grain (500 mg/kg) for human consumption and 1,000 mg/kg in grains for animal feed have been set. The total weigh of ergots (sclerotia) within grain seeds is measured gravimetrically, but this method gives no information about the amount of ergot alkaloids, which can be totally different for different ergots. The problem of ergot contaminations in food and feed has been roughly solved technologically in flour mills. Fortunately, modern methods of grain cleaning have almost eliminated ergotism as a human disease.[46]

Various methods, such as TLC, GC, and CE, as well as reversed phase HPLC with fluorescence detection, were performed for the analysis of ergot alkaloids.[117–119] HPLC with UV and fluorescence detection are the predominant methods used for the quantification of ergot alkaloids. HPLC-FLD demonstrates appropriate sensitivity, accuracy, and reproducibility and can be used in routine laboratory analysis of ergot alkaloids; however, the sample preparation procedure is very complicated and time consuming. Using HPLC-FLD, some ergot alkaloids (ergotamine, ergosine, ergocornine, ergocryptine, ergocristine) could be quantified down to 4 ng/g in food matrices after C_{18} SPE clean-up.[120] Also, an ELISA test with focus on ergovalin as an indicator compound was developed. For many of alkaloids, there are no standards available; mass spectrometry is the only tool to be used for identifying unknown chromatographic peaks. LC-MS-MS and LC-MS with electrospray interface in positive ion mode have been applied for the verification, confirmation, and structural elucidation of major ergot alkaloids. After extraction and partial purification of individual ergot compounds, semipreparative HPLC-FLD was used. The fractions were collected and injected further into reversed phase LC-ESI-MS on a double-sector instrument.[121] Mainly, protonated molecular ions $[M+H]^+$ or sodium $[M+Na]^+$ and potassium adducts $[M+K]^+$ were registered. To obtain more structural information, source CID was applied by increasing cone voltage. In the case of ergopeptines, the loss of one or two water molecules as well as other fragments of protonated lysergamide (m/z 268), 9,10-unsaturated ABCD ergoline system (m/z 223) were registered.[121] In another paper, tandem mass spectrometry was used for studying fragmentation patterns of some ergot alkaloid standards.[122] The mechanism of fragmentation was studied and fragments were extensively interpreted for each alkaloid. The lysergic acid peptide ergot derivatives, such as ergovaline (m/z 534), ergotamine (m/z 582), ergocornine (m/z 562), ergocryptine (m/z 576), and ergocrystine (m/z 610) exhibit fragments due to loss of water (–18 amu). In the case of ergovaline and ergotamine, the m/z 320 fragment due to the cleavage of peptide ring system (amide and ether functions) was registered (Fig. 13). For ergocornine, ergocryptine, and ergocrystine, the m/z 348 due to the cleavage within and loss of most

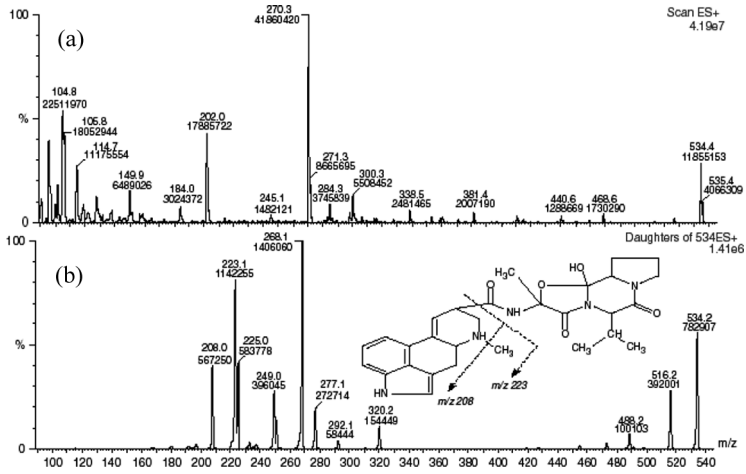


Figure 13. ESI$^+$ mass spectrum of ergovaline $[M+H]^+$ m/z 534.4 (a) and the MS-MS spectrum of daughter ions (b). *Source*: Reprinted from Ref. [122].

of the peptide ring system (amide and ether functions) was observed. In contrast, these types of fragments were not observed in ergonovine amide type lysergic acid derivatives, indicating the reliability of latter interpretations. However, similar to tested ergopeptides, m/z 223 and 208 were observed. On the other hand, in the spectrum of clavine type ergot alkaloids (lysergic acid and lysergol), the fragments of m/z 268, 251 and 225 are absent.[122]

The identification of ergot alkaloids and their specific subtypes is very important for diagnosis of certain clinical cases of toxins. The same authors[123] published an additional paper using the identical ESI-MS-MS method for the elucidation and screening of ergot alkaloids in feed samples. Compound-specific dwell time, as well as the corresponding collision energies, were used for each ergot alkaloid in an MRM method. Feed samples were extracted with chloroform and purified on silica. The authors have also compared LC-ESI-MS-MS with HPLC-FLD. Two HPLC columns with different inner diameters (2 and 4 mm) were used. Using the developed LC-MS method, 11 veterinary clinical cases were investigated. In two cases, horses died after ingestion of feed samples. Not only well characterized, but also unknown ergot alkaloid compounds, were identified and confirmed. In two other cases, feed of cattle with clinical signs of fescus foot were analysed, containing a large number of ergot alkaloids. The authors have studied, comprehensively, the mass fragmentation spectra and the retention time behavior of unknown chromatographic peaks. Chemical variants of the ergot alkaloids are discussed extensively. New ergot alkaloids in a molecular weight

range between 381 and 611 Da with specific pair of peaks corresponding to the ergoline ring system and its demethylated variant were identified. Mohamed et al.[124] have also studied the fragmentation mechanisms of six ergot alkaloids by CID using triple quadrupole and ion trap mass spectrometers. Those alkaloids belonging to the lysergic acid derivatives, such as ergonovine and methysergide hydrogen maleinate and peptide-type derivatives such as ergochristine, ergotamine, ergocornine, and α–ergocryptine were analysed. Again, ESI in positive ion mode was used to postulate fragmentation pathways of peptide-type and lysergic acid derivates. The nature of product ions was confirmed by hydrogen/deuterium exchange experiments. Two specific product ions at m/z 223 and m/z 208 were noted for both classes of molecules corresponding to the lysergic acid moiety. Since m/z 223 was more abundant, this fragment was used for monitoring of ergot alkaloids using precursor ion scanning. By applying the developed method, the authors could confirm the presence of ergosine in naturally contaminated rye flour samples.

The same authors have published a confirmatory and a quantitative method for five ergot alkaloids, ergocristine, ergotamine, ergonovine, ergocornine, and α-ergokryptine, in rye flour.[125] The method is based on LC-ESI-MS-MS in positive mode by using two transition reactions per analyte. Liquid–liquid extraction, followed by a clean-up step on a C_{18} solid-phase extraction (SPE) cartridge was applied. Methysergide hydrogen maleinate was used as internal standard. The method was fully validated, showing LODs ranging between 7–11 ng/g and the LOQs in the range between 23–37 ng/g. The developed method was then applied to analyse fifteen rye flour samples.

A quantitative approach using LC-ESI-MS-MS was performed for the determination of bromocriptine, an ergot derivative with dopamine receptor agonist activity, in human plasma.[126] Quantification levels down to 2 pg/mL could be reached by using α-ergocryptine as internal standard. For bromocriptine $[M+H]^+$ at m/z 656.7 and the corresponding daughter ion at m/z 348.2 was used. For α-ergocryptine, $[M+H]^+$ at m/z 576.7 and m/z 268.3 transitions were selected. Solid phase extraction, followed by RP-HPLC was performed for sample clean-up and separation from the matrix. A mean recovery of 79.6% could be achieved.

Brük et al. have analysed the main alkaloids in ergot (ergometrine, ergotamine, ergochristine ergocornine, and α-ergocryptine) by using a triple quadrupole MS with ESI in positive ionisation mode. Each alkaloid was identified using specific transition and retention time. LOQs were between 0.1 and 1 ng/g and recoveries of 62–97% with poorest values for ergometrine and ergochristine were reached. Samples were extracted with dichloromethane : ethyl acetate : methanol : ammonia mixture and an isocratic RP-HPLC method was used for separation of the alkaloids.

Different food samples were analysed using the described method and a significantly high percentage of rye bread samples showed measurable amounts of alkaloids. More recently, Uhlig et al.[128] have studied the major ergot alkaloid components of Norwegian wild grasses. *Claviceps* sclerotia was analysed by different types of mass spectrometers such as triple quadrupole MS, ion trap MS, and high-resolution Q-TOF MS coupled with ESI interface in positive ion mode. Quantitative determination was performed for those alkaloids with available standards. Relatively high amounts of unknown alkaloids were found in analysed samples. Sulyok et al. have also applied LC-ESI-MS-MS for the simultaneous determination of up to 87 different mycotoxins, including 20 ergot alkaloids. The authors performed only an extraction step prior to quantitative determination using LC-MS-MS.[134,135]

SIMULTANEOUS DETERMINATION OF MYCOTOXINS

The advantage of determining several mycotoxins in one run is that one sample could be contaminated by more than 1 mycotoxin. Muti-stage MS plays an important role in this matter because of its sensitivity and specificity. However, the method of analysis is not easy to develop. The differences in chemical and physical properties of mycotoxins challenge sample preparation, chromatographic separation, and detection methods. Positive/negative polarity switching in MS is necessary for the most abundant ionization for each mycotoxin.[20,23,51,59,66,129–137] Biselli et al.[51] developed a method based on ESI (+/−) MS-MS for the analysis of 16 mycotoxins of Aspergillus and Fusarium in various food and feed matrices. Sample clean-up has been performed using Mycosep 226 multifunctional cartridges. LOQ ranged between 1–10 ng/g. The same group developed a method for analysis of A/B-trichothecenes and zearalenone using ESI-MS-MS.[51] The average recoveries for trichothecenes ranged from 65% for NIV up to 96% for DON and 89% for zearalenone (ZON). The limit of quantification varied between 0.02 and 10 ng/g. Other authors have performed multitoxin analyses including aflatoxin, fusarium toxins, and trichothecenes in bovine milk using ESI (+/−) MS-MS.[23] The clean-up method was based on SPE (Oasis HLB cartridges). DON, de-epoxydeoxynivalenol (DOM-1), 3-AcDON, 15-AcDON, OTA, ZON, α-ZOL, β-ZOL, α-ZAL and β-ZAL were detected in negative ion mode after separation on a Hypersil ENV analytical column, while T-2, HT-2, T-2 triol, DAS, MAS, FB_1, FB_2 and aflatoxin M_1 were detected in positive ion mode after separation on a Luna C_{18} column. The LOD ranged between 0.02–0.15 μg/L. The mean recoveries were in the range 76–108%. QTRAP-LC-MS-MS has been used by Berthiller et al. for simultaneous determination of trichothecenes and ZON in maize by

APCI(+/−).[66] The clean-up of maize samples was performed using Mycosep® columns. ZAN was used as an internal standard (IS) for quantification of ZON, whereas no IS was used for the trichothecenes. Detection of the mycotoxins was carried out in MRM mode. The LOD ranged between 0.3 and 3.8 μg/kg and the mean recoveries were in the range between 50 and 99%. Both the screening and confirmation by ESI (+/−) MS-MS method have been recently developed for fusariotoxins.[129] Sample preparation has been performed on a Carbograph-4 cartridge in these studies.[59,137] LC-TOF-MS has been used for multitoxin analysis by Nielsen et al. and Tanaka et al.[131,136] which has a significant advantage over the other instruments as it can detect a wide mass range without losing sensitivity. Nielsen and Smedsgaard[131] used ESI(+) TOF for monitoring simultaneously up to 474 mycotoxins in fungal cultures. Tanaka et al. have performed simultaneous determination of 13 mycotoxins, including trichothecenes, zearalenone and aflatoxins in food stuffs using APCI(+)TOF-MS.[136] Sample preparation has been performed on MFC Sep#226 cartridge. LOD were between 0.1–4.9 ng/g and recoveries ranged from 81–133% in corn samples. Sulyok et al.[135] validated a method for the determination of 39 mycotoxins in wheat and maize using a single extraction without any clean-up step followed by ESI (+/−) MS-MS. LOD ranged from 0.03 to 220 μg/kg. The same group has applied this method for quantification of 87 analytes including metabolites produced by fungi involved in food spoilage. Limits of detection ranged between 0.02 and 225 μg/kg. The developed method was applied to 18 moldy samples including bread, fruits, vegetables, jam, cheese, chestnuts, and red wine from private households.[134] The authors have optimized the extaction procedure for all tested mycotoxins including some ergot alkaloids.[134,135] The same method was applied to crude grain

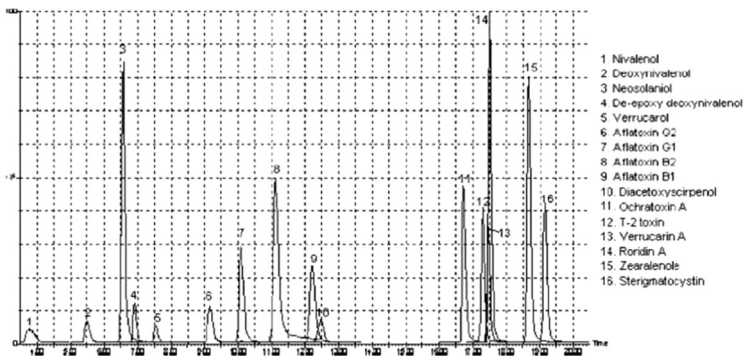


Figure 14. MS-MS chromatogram of the 16 mycotoxins separated on the C18 reversed-phase analytical column. *Source*: Reproduced from Ref. [138].

extracts of spelt, rice, and barley as well.[133] Recently, a new multitoxin immunoaffinity clean-up has also been introduced and used for simutaneous determination of 11 mycotoxins including aflatoxins, OTA and fusarium toxins in maize.[130] All mycotoxins were detected by ESI(+/−)MS-MS having a LOD between 0.3 and 4.2 μg/kg and recoveries were higher than 79% for all tested mycotoxins.

Besides food and feed, simultaneous determination of 16 mycotoxins (Fig. 14), possibly related to sick building syndrome on cellulose filters and in fungal cultures has been developed by Delmulle et al.[138] The analysis has been performed by using ESI(+) MS-MS. Recoveries ranged between 53 and 104% and LODs were between 0.009 and 50 pg/μL. Interestingly, there was no ionization switching performed in this study. Recently, UPLC has been introduced into analytical field. It is a novel solution designed for fast and high throughput analysis with a high speed of analysis, greater resolution, higher peak capacity, and sensitivity. The simultaneous analysis of aflatoxins B1, G1, B2, G2, and OTA in beer was performed by UPLC/MS/MS within 3.2 minutes.[20] In another study, UPLC was combined with ESI and a triple quadrupole tandem mass spectrometer for simultaneous quantification of 17 mycotoxins in various foods and feeds.[132] The clean-up was performed by using Mycosep 226 Aflazon + MFC. Results of linear gradient elution indicated that 10 mycotoxins in positive ion mode and 7 mycotoxins in negative ion mode could be completely analysed within 6.5 and 4 min, respectively.

INFLUENCE OF MATRIX EFFECTS ON LC-MS MEASUREMENTS

LC-MS has been known for its specificity and selectivity. As a result, sample clean-up and chromatographic separation are supposed to be simplified or even eliminated from the procedure. However, it has been shown that the co-eluting matrix components may affect the ionization efficiency. Zöllner et al.[139] proposed that ion-suppression phenomena in complex biological matrices are directly related to insufficient sample clean-up and/or chromatographic separation, reducing the sensitivity and accuracy of the assays. It has been reported that ion suppression is more likely to occur in ESI than in APCI.[140] Matrix effects have to be considered as an ionization phenomenon. In most cases, they are not due to co-eluting isomeric or isobaric compounds or fragments, but are due to unknown matrix constituents that suppress or enhance the ionisation efficiency of the analyte. Improved sample pre-treatment and/or separation can be used to reduce or eliminate these constituents. Matrix-matched standards, isotopically-labeled internal standards, and/or standard addition can be used to reduce the effects on accuracy

and precision. The use of internal standard could be a solution to eliminate matrix effects if a proper compound is available. Ideally, the structural, physical and chemical properties of an internal standard, together with its chromatographic behaviour, should be similar to the analyte of interest. This would provide comparable ionization properties in the presence of co-eluting components for sufficient compensation of matrix effects.[139] Stable isotope-labelled compounds have all these characteristics and have been reported to reduce matrix effects.[63,64,94,141] The use of ($^{13}C_{15}$)DON as an internal standard (IS) for the determination of DON in maize and wheat by ESI-MS-MS showed a recovery of 99% (compare to the maize reference material) without any clean-up step after extraction.[63] Additionally, [D6]-FB_1 has been used as an internal standard for analysis of fumonisins,[23,85,142] which was reported to reduce the matrix effect. The use of a stable isotope labelled internal standard is a great advantage and can provide more reliable data in terms of reproducibility of the response. Furthermore, it can compensate the sensitivity drift of the LC-MS system within a given day. However, stable isotope-labelled internal standards are not always available. Klötzel et al. have studied the matrix effect when A-and B-trichothecenes were analyzed by LC-ESI-MS by applying verrucarol (VER) and deepoxy-deoxynivalenol (DOM-1) as internal standards. With DOM-1 as an internal standard, all toxins were baseline-separated and no interferences were noticed.[55]

Matrix effects, as well as ion suppression phenomena, seem to be not only analyte specific but also strongly matrix dependent. The consequence of the latter effects is the decrease of sensitivity in terms of LOD and LOQ. Another strategy to compensate and improve the linearity, reliability, and accuracy of the analytical results is to perform a calibration curve for each analyte in real sample matrix and not in standard solution. Sulyok et al. reported that, for matrix effect compensation, a model matrix used for matrix-matched calibration should be equivalent to the investigated samples as much as possible.[133] The same opinion has been reported before by determination of ZON, α/β-ZOL in beer samples.[97] Zöllner et al. reported that general calibration curves for all beer brands is not possible, since calibration curves vary considerably from brand to brand. Additionally, Sulyok et al. have studied, extensively, the signal suppression effects in the case of 87 different mycotoxins.[134,135]

Application of only a single proper internal standard is not able to solve all the matrix effects when single stage MS is used. Both internal standard and the use of extensive sample preparation have been recently reported in the analysis of ZON and its metabolites in urine, plasma, and faeces of horses.[94] D_2-ZAN was used as internal standard. The authors have used IAC and a combination of IAC and SPE as a clean-up step. However, those strategies could correct only a part of the matrix effects. Either ion suppression or ion enhancement for each metabolite was still

observed. Due to the complex nature of biological samples, it is difficult to predict that either ion enhancement or ion suppression would occur. Both effective sample preparation and the use of proper internal standard are important to reduce matrix effects. Nevertheless, matrix effects might not be completely eliminated.

CONCLUSION

Sensitive methods are required to provide data for dietary exposure, to protect consumers, perform risk assessment, risk management, and to support research in the toxicokinetic studies. Subsequently, the applied instrumental methods for determination and analysis of toxicologically relevant substances such as mycotoxins should have appropriate selectivity, sensitivity, reproducibility, and robustness. Especially, LC-MS and tandem mass spectrometry have provided a new dimension in the analysis of mycotoxins in food, feed, and in biological samples. Due to its excellent selectivity and high sensitivity, coupled with high discrimination power of MS detection, the LC-MS has significantly extended the use of mass spectrometry in the analysis of naturally occurring substances. However, there are some limitations of the technique. One of the reported limitations is the so called ion suppression phenomenon. This is due to the coeluting matrix compounds. Therefore, in many cases, a suitable chromatographic separation and appropriate clean-up have to be performed prior to analysis. Another strategy to reduce the error sources in trace analysis of mycotoxins in complex matrices is the use of an internal standard identical, or having close retention time, to the analyte. LC-MS and LC-MS-MS are being widely used as routine methods for determination of mycotoxins in different matrices. This hyphenated technique undergoes a very effective and continuous improvement. As a result the instruments of each new generation, methods will become more sensitive as well as robust and will solve many analytical problems we are dealing with at this moment.

ACKNOWLEDGMENTS

The authors want to thank ASEA-UNINET (Asean-European University Network) for supporting Dr. Piyada Songsermsakul. Additionally, we would like to thank the University of Veterinary Medicine in Vienna for providing the scholarship. Dr. Ursula Schober at the bureau for international relations/University of Veterinary Medicine in Vienna is also highly appreciated for her kind support.

REFERENCES

1. Murphy, P.A.; Hendrich, S.; Landgren, C.; Bryant, C.M. Food mycotoxins: An update. J. Food. Sci. **2006**, *71* (5), 51–65.
2. Council for Agricultural Science and Technology (CAST). *Mycotoxins: Risks in Plant, Animal and Human System*; Ames, Iowa, USA.; Task Force Report 2003, No. 139.
3. Kuiper-Goodman, T. Risk Assessment and Risk Management of Mycotoxins in Food, in *Mycotoxins in Food, Detection and Control*; Magan, N., Olsen, M., Eds.; Woodhead Publishing, CRC Press: L Boca Raton 2004; 3–87.
4. International Agency for Research on Cancer (IARC) (1993a): IARC Monographs on the evaluation of carcinogenic risks to humans: Aflatoxins. Lyon, France, *56*, 245–395.
5. Patel, P. Mycotoxin Analysis: current and emerging Technologies, in *Mycotoxins in Food, Detection and Control*; Magan, N., Olsen, M., Eds.; Woodhead Publishing, CRC Press: Boca Raton 2004; 88–110.
6. Wilson, D.M.; Sydenham, E.W.; Laombaert, G.A.; Trucksess, M.W.; Abramson, D.; Bennet, G.A. Mycotoxin analytical Techniques, in *Mycotoxins in Agriculture and Food Safety*; Sinha, K.K., Bhatnagar, D., Eds.; Marcel Dekker, Inc.: New York 1998; 135–182.
7. Scudamore, K.A., Analysis Principles and Applications of Mycotoxin Analysis. in *Mycotoxin Blue Book*; Diaz, D., Ed.; Nottingham University Press: Nottingham 2005; 157–187.
8. Betina, V. *Chromatography of Mycotoxins Techniques and Applications*, Journal of Chromatography Library; Elsevier: Amsterdam/Netherlands, 1993; Vol. 54.
9. Zöllner, P.; Mayer-Helm, B. Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionisation mass spectrometry. J. Chromatogr. A **2006**, *1136* (2), 123–169.
10. Whitaker, T.B., Sampling for Mycotoxins, in *Mycotoxins in Food, Detection and Control*; Magan, N., Olsen, M., Eds.; Woodhead Publishing, CRC Press: Boca Raton 2004; 88–110.
11. Krska, R.; Baumgartner, S.; Josephs, R. The state-of-the-art- in the analysis of type A- and B-trichothecene mycotoxins in cereals. Fresen. J. Anal. Chem. **2001**, *371* (3), 285–299.
12. Sforza, F.; Dall'Asta, C.; Marchelli, R. Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic technique/mass spectrometry. Mass. Spectrom. Rev. **2006**, *25* (1), 54–76.
13. Bhatnagar, D., Payne, G.A., Cleveland, T.H., Robens, J.F., Mycotoxins. Current Issues in U.S.A. in *Meeting the Mycotoxin Menace*; Barug, D., van Egmond, H., López-Garciá, R., van Osenbruggen, T., Visconti, A., Eds.; Wageningen Academic Publishers: The Netherlands 2004; 17–40.
14. Commission Regulation (EC) No 466/2001 Official Journal of the European Communities, 2001, http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_077/l_07720010316en00010013.pdf, accessed: May 2005.
15. Vahl, M., Jorgensen, K. Determination of Aflatoxins in Food using LC/MS/MS, Z. Lebensm. Unters. Forsch. A **1998**, *206*, 243–245.

16. Trucksess, M.W.; Dombrink-Kurtzman, M.A.; Tournas, V.H.; White, K.D. Occurrence of Aflatoxins and Fumonisins in Incaparina from Guatemala. Food. Addit. Contam. **2002**, *19* (7), 671–675.
17. Ventura, M.; Gómez, A.; Anaya, I.; Díaz, J.; Broto, F.; Agut, M.; Comellas, L. Determination of aflatoxins B_1, G_1, B_2 and G_2 in medicinal herbs by liquid chromatography-tandem mass spectrometry. J. Chromatogr. A **2004**, *1048* (1), 25–29.
18. Takino, M.; Tanaka, T.; Yamaguchi, K.; Nakahara, T. Atmospheric pressure photo-ionization liquid chromatography/mass spectrometric determination of aflatoxins in food. Food. Addit. Contam. **2004**, *21* (1), 76–84.
19. Cavaliere, C.; Foglia, P.; Guarino, C.; Nazzari, M.; Samperi, R.; Lagana, A. Determination of aflatoxins in olive oil by liquid chromatography–tandem mass spectrometry. Anal. Chim. Acta **2007**, *596* (1) 141–148
20. Ventura, M.; Guillén, D.; Anaya, I.; Broto-Puig, F.; Lliberia, J.L.; Agut, M.; Comellas, L. Ultra-performance liquid chromatography/tandem mass spectrometry for the simultaneous analysis of aflatoxins B1, G1, B2, G2 and ochratoxin A in beer. Rapid. Commun. Mass. Spectrom. **2006**, *20* (21), 3199–3204.
21. Liau, B.; Jong, T.; Lee, M.; Chang, C.J. Supercritical fluid extraction and quantification of aflatoxins in Zizyphi Fructus by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. Rapid. Commun. Mass. Spectrom. **2007**, *21* (5), 667–673.
22. Ramos Catharino, R.; de Azevedo Marques, L.; Silva Santos, L.; Baptista, A.S.; Glória, E.M.; Calori-Domingues, M.A.; Facco, E.M.; Eberlin, M.N. Aflatoxin screening by MALDI-TOF mass spectrometry. Anal. Chem. **2005**, *77* (24), 8155–8157.
23. Sorensen, L.K., Elbæk, T.H. Determination of Mycotoxins in Bovine Milk by Liquid Chromatography Tandem Mass Spectrometry. J. Chromatgr. B **2005**, *820*, 183–196.
24. Kokkonen, M.; Jestoi, M.; Rizzo, A. Determination of selected mycotoxins in mould cheeses with liquid chromatography coupled to tandem with mass spectrometry. Food. Addit. Contam. **2005**, *22* (5), 449–456.
25. Chen, C.; Li, W.; Peng, K. Determination of aflatoxin M1 in milk and milk powder using high-flow solid-phase extraction and liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. **2005**, *53* (22), 8474–8480.
26. Cavaliere, C.; Foglia, P.; Pastorini, E.; Samperi, R.; Lagana, A. Liquid chromatography/tandem mass spectrometric confirmatory method for determining aflatoxin M1 in cow milk: Comparison between electrospray and atmospheric pressure photoionization sources. J. Chromatogr. A **2006**, *1101* (1–2), 69–78.
27. Pfohl-Leszkowicz, A.; Manderville, R.A. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. Mol. Nutr. Food Res. **2007**, *51* (1), 61–99.
28. Aish, J.L. Ochratoxin A, in *Mycotoxins in Food, Detection and Control*; Magan, N., Olsen, M., Eds.; Woodhead Publishing, CRC Press: Boca Raton, 2004, 308–338.

29. International Agency for Research on Cancer (IARC) (1993b): IARC Monographs on the evaluation of carcinogenic risks to humans: Ochratoxin A. Lyon, France, *56*, 489–512.
30. Commission Regulation (EC) No 123/2005 Official Journal of the European Communities, 2005 http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l_025/l_02520050128en00030005.pdf, accessed: June 2005.
31. Scott, P.M. Methods of Analysis for Ochratoxin A, in *Mycotoxins and Food Safety, Advances in experimental Medicine and Biology*, Vol. 504; DeVries, J.W., Trucksess, M.W., Jackson, L.S., Eds.; Kluwer Academic/Plenum Publishers: N.Y., USA 2000; 117–134.
32. Becker, M.; Degelmann, P.; Herderich, M.; Scheier, P.; Humpf, H.U. Column liquid chromatography – electrospray ionisation-tandem mass spectrometry for the analysis of ochratoxin. J. Chromatgr. A **1998**, *818* (2), 260–264.
33. Lau, B.P.; Scott, P.M.; Lewis, D.A.; Kanhere, S.R. Quantitative determination of ochratoxin A by liquid chromatography/electrospray tandem mass spectrometry. J. Mass. Spectrom. **2000**, *35* (1), 23–32.
34. Lindenmeier, M.; Schieberle, P.; Rychlik, M. Quantification of ochratoxin A in foods by a stable isotope dilution assay using high-performance liquid chromatography-tandem mass spectrometry. J. Chromatgr. A. **2004**, *1023* (1), 57–66.
35. Bacaloni, A.; Cavaliere, C.; Faberi, A.; Pastorini, E.; Samperi, R.; Lagana, A. Automated on-line solid-phase extraction-liquid chromatography-electrospray tandem mass spectrometry method for the determination of ochratoxin A in wine and beer. J. Agric. Food. Chem. **2005**, *53* (14), 5518–5525.
36. Reinsch, M.; Töpfer, A.; Lehmann, A.; Nehls, I.; Panne, U. Determination of ochratoxin A in beer by LC-MS/MS ion trap detection. Food. Chem. **2007**, *100* (1), 312–317.
37. Timperio, A.M.; Magrob, P.; Chilosi, G.; Zolla, L. Assay of ochratoxin A in grape by high-pressure liquid chromatography coupled on line with an ESI–mass spectrometry. J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. **2006**, *832* (1), 127–133.
38. Almela, L.; Rabe, V.; Sánchez, B.; Torrella, F.; López-Pérez, J.P.; Gabaldón, J.A.; Guardiola, L. Ochratoxin A in red paprika: Relationship with the origin of the raw material. Food. Microbiol. **2007**, *24* (4), 319–327.
39. Zöllner, P.; Leitner, A.; Lubda, D.; Cabrera, K.; Lindner, W. Application of a chromolith speedROD RP-18e HPLC column: Determination of ochratoxin A in different wines by high-performance liquid chromatography-tandem mass spectrometry. Chromatographia **2000**, *52*, 818–820.
40. Zöllner, P.; Leitner, A.; Berner, D.; Kleinova, M.; Jodelbauer, J.; Mayer, B.; Lindner, W. Improving LC-MS/MS analyses in complex food matrices part I. LC-GC Europe **2003**, *16* (3), 163–171.
41. Leitner, A.; Zöllner, P.; Paolillo, A.; Stroka, J.; Papadopoulou-Bouraoui, A.; Jaborek, S.; Anklam, E.; Lindner, W. Comparison of methods for the

determination of ochratoxin A in wine. Anal. Chim. Acta **2002**, *453* (1), 33–41.
42. Zepnik, H.; Völkel, W.; Dekant, W. Toxicokinetics of the mycotoxin ochratoxin A in F 344 rats after oral administration. Toxic. Appl. Pharm. **2003**, *192* (1), 36–44.
43. Mally, A.; Zepnik, H.; Wanek, P.; Eder, E.; Dingley, K.; Ihmels, H.; Volkel, W.; Dekant, W. Ochratoxin A: Lack of Formation of Covalent DNA Adducts. Chem. Res. Toxicol. **2004**, *17* (2), 234–242.
44. Richard, J.L.; Plattner, R.D.; May, J.; Liska, S.L. The occurrence of ochratoxin A in dust collected from a problem household. Mycopathologia **1999**, *146* (2), 99–103.
45. Faucet-Marquis, V.; Pont, F.; Størmer, F.C.; Rizk, T.; Castegnaro, M.; Pfohl-Leszkowicz, A. Evidence of a new dechlorinated ochratoxin A derivative formed in opossum kidney cell cultures after pretreatment by modulators of glutathione pathways: Correlation with DNA-adduct formation. Mol. Nutr. Food. Res. **2006**, *50* (6), 530–542.
46. Bennett, J.W.; Klich, M. Mycotoxins. Clin. Microbiol. Rev. **2003**, *16* (3), 497–516.
47. European Commission, 1126/2007/EC (2007).
48. Pettersson, H.; Langseth, W. Intercomparison of trichothecene analysis and feasibility to produce certified calibrants and reference material. Final report I, Method Studies. BCR Information, Project Report EUR 20285/1. 2002, EN 1–82.
49. Lombaert, G.A. Methods for the Determination of Deoxynivalenol and other Trichothecenes in Foods, in *Mycotoxins and Food Safety, Advances in experimental Medicine and Biology*, Vol. 504; DeVries, J.W., Trucksess, M.W., Jackson, L.S., Eds.; Kluwer Academic/Plenum Publishers: N.Y., USA 2000; 141–153.
50. Stecher, G.; Jarukamjorn, K.; Zaborski, P.; Bakry, R.; Huck, C.W.; Bonn, G.K. Evaluation of extraction methods for the simultaneous analysis of simple and macrocyclic trichothecenes. Talanta **2007**, *73* (2), 251–257.
51. Biselli, S.; Hummert, C. 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. **2005**, *22* (8), 752–760.
52. Razzazi-Fazeli, E.; Böhm, J.; Luf, W. Determination of nivalenol and deoxynivalenol in wheat using liquid chromatography – mass spectrometry with negative ion atmospheric pressure chemical ionisation. J. Chromatgr. A. **1999**, *854* (1–2), 45–55.
53. Razzazi-Fazeli, E.; Rabus, B.; Cecon, B.; Böhm, J. Simultaneous quantification of A-Trichothecene mycotoxins in grains using liquid chromatography atmospheric pressure chemical ionisation-mass spectrometry. J. Chromatgr. A **2002**, *968* (1–2), 129–142.
54. Berthiller, F.; Schuhmacher, R.; Buttinger, G.; Krska, R. 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 **2005**, *1062* (2), 209–216.

55. Klötzel, M.; Gutsche, B.; Lauber, U.; Humpf, H.U. Determination of 12 type A and B trichothecenes in cereals by liquid chromatography-electrospray ionization tandem mass spectrometry. J. Agric. Food. Chem. **2005**, *53* (23), 8904–8910.
56. Plattner, R.D. HPLC/MS analysis of fusarium mycotoxins, fumonisins and deoxynivalenol. Nat. Toxins **1999**, *7* (6), 365–370.
57. Plattner, R.D.; Maragos, C.M. Determination of deoxynivalenol and nivalenol in corn and wheat by liquid chromatography with electrospray mass spectrometry. J. AOAC. Int. **2003**, *86* (1), 61–65.
58. Razzazi-Fazeli, E.; Böhm, J.; Jarukamjorn, K.; Zentek, J. Simultaneous determination of major B-Trichothecenes and the de-epoxy-metabolite of deoxynivalenol in pig urine and maize using high-performance liquid chromatography-mass spectrometry. J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. **2003**, *796* (1), 21–33.
59. Cavaliere, C.; Foglia, P.; Pastorini, E.; Samperi, R.; Laganà, A. Development of a multiresidue method for analysis of major Fusarium mycotoxins in corn meal using liquid chromatography/tandem mass spectrometry. Rapid. Commun. Mass. Spectrom. **2005**, *19* (14), 2085–2093.
60. Berger, U.; Oehme, M.; Kuhn, F. Quantitative determination and structure elucidation of type A- and B-trichothecenes by HPLC/ion trap multiple mass spectrometry. J. Agric. Food. Chem. **1999**, *47* (10), 4240–4245.
61. Dall'Asta, C.; Sforza, S.; Galaverna, G.; Dossena, A.; Marchelli, R. Simultaneous detection of type A and type B trichothecenes in cereals by liquid chromatography-electrospray ionization mass spectrometry using NaCl as cationization agent. J. Chromatgr. A **2004**, *1054* (1–2), 389–395.
62. Bretz, M.; Beyer, M.; Cramer, B.; Humpf, H.U. Stable isotope dilution analysis of the Fusarium mycotoxins deoxynivalenol and 3-acetyldeoxynivalenol. Mol. Nutr. Food. Res. **2006**, *50* (3), 251–260.
63. Häubl, G.; Berthiller, F.; Krska, R.; Schuhmacher, R. Suitability of a fully 13C isotope labeled internal standard for the determination of the mycotoxin deoxynivalenol by LC-MS/MS without clean up. Anal. Bioanal. Chem. **2006**, *384* (3), 692–696.
64. Häubl, G.; Berthiller, F.; Rechthaler, J.; Jaunecker, G.; Binder, E.M.; Krska, R.; Schuhmacher, R. Characterization and application of isotope-substituted ($^{13}C_{15}$)-deoxynivalenol (DON) as an internal standard for the determination of DON. Food. Addit. Contam. **2006**, *23* (11), 1187–1193.
65. Lattanzio, V.M.; Solfrizzo, M.; Visconti, A. Determination of trichothecenes in cereals and cereal-based products by liquid chromatography-tandem mass spectrometry. Food. Addit. Contam. **2007**, *24* (Epub ahead of print).
66. Berthiller, F.; Dall'Asta, C.; Schuhmacher, R.; Lemmens, M.; Adam, G.; Krska, R. Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography-tandem mass spectrometry. J. Agric. Food Chem. **2005**, *53* (9), 3421–3425.
67. Meky, F.A.; Turner, P.C.; Ashcroft, A.E.; Miller, J.D.; Qiao, Y.L.; Roth, M.J.; Wild, C.P. Development of a urinary biomarker of human exposure to deoxynivalenol. Food Chem. Toxicol. **2003**, *41* (2), 265–273.

68. Bily, A.C.; Reid, L.M.; Savard, M.E.; Reddy, R.; Blackwell, B.A.; Campbell, C.M.; Krantis, A.; Durst, T.; Philogene, B.J.; Arnason, J.T.; Regnault-Roger, C. Analysis of Fusarium graminearum mycotoxins in different biological matrices by LC/MS. Mycopathologia **2004**, *157* (1), 117–26.
69. Sypecka, Z.; Kelly, M.; Brereton, P. Deoxynivalenol and zearalenone residues in eggs of laying hens fed with a naturally contaminated diet: Effects on egg production and estimation of transmission rates from feed to eggs. J. Agric. Food Chem. **2004**, *52* (17), 5463–5471.
70. Cole, R.J., Jarvis, B.B., Schweikert, M.A., 2003. *Handbook of Secondary Fungal Metabolites*, vol. III; Academic Press: New York.
71. International Agency for Research on Cancer (IARC) (1993c): IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Toxins derived from Fusarium moniliforme. Fumonisins B1 and B2 and Fusarin C. Lyon, France, *56*, 445–466.
72. Humpf, H.U.; Voss, K.A. Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins. Mol. Nutr. Food Res. **2004**, *48* (4), 255–269.
73. Howard, P.C.; Churchwell, M.I.; Couch, L.H.; Marques, M.M.; Doerge, D.R. Formation of N-(Carboxymethyl)fumonisin B1, following the reaction of fumonisin B1 with reducing sugars. J. Agric. Food Chem. **1998**, *46* (9), 3546–3557.
74. Krska, R.; Welzig, E.; Boudra, H. Analysis of fusarium toxins in feed. Anim. Feed Sci. Technol. **2007**, *137*, 241–264.
75. Meyer, K.; Mohr, K.; Bauer, J.; Horn, P.; Kovács, M. Residue formation of fumonisin B1 in porcine tissues. Food Addit. Contam. **2003**, *20* (7), 639–647.
76. Sewram ,V.; Nair, J.J.; Nieuwoudt, T.W.; Gelderblom, W.C.; Marasas, W.F.; Shephard, G.S. Assessing chronic exposure to fumonisin mycotoxins: the use of hair as a suitable noninvasive matrix. J. Anal. Toxicol. **2001**, *25* (6), 450–455.
77. Sewram, V.; Mshicileli, N.; Shephard, G.S.; Marasas, W.F. Fumonisin mycotoxins in human hair. Biomarkers **2003**, *8* (2), 110–118.
78. Fodor, J.; Meyer, K.; Riedlberger, M.; Bauer, J.; Horn, P.; Kovacs, F.; Kovacs, M. Distribution and elimination of fumonisin analogues in weaned piglets after oral administration of Fusarium verticillioides fungal culture. Food Addit. Contam. **2006**, *23* (5), 492–501.
79. Humpf, H.U. Fumonisine in Lebensmitteln: Analytik, Stereochemie, Toxikologie, Habilitationsschrift, Universität Würzburg, 1999.
80. Musser, S.M.; Eppley, R.M.; Trucksess, M.W. Electrospray mass spectrometry for fumonisin detection and method validation, in *Mycotoxins and Food Safety, Advances in Experimental Medicine and Biology*, Vol. 504; DeVries, J.W., Trucksess, M.W., Jackson, L.S., Eds.; Kluwer Academic/Plenum Publishers: N.Y., USA 2000; 95–105.
81. Josephs, J.L. Detection and characterization of fumonisin mycotoxins by liquid chromatography/electrospray ionization using ion trap and triple quadrupole mass spectrometry. Rapid Comm. Mass. Spectrom. **1996**, *10* (11), 1333–1344.

82. Hartl, M.; Humpf, H.U. Simultaneous determination of fumonisin B1 and hydrolyzed fumonisin B1 in corn products by liquid chromatography/electrospray ionization mass spectrometry. J. Agric. Food Chem. **1999**, *47* (12), 5078–5083.
83. Bartók, T.; Szécsi, A.; Szekeres, A.; Mesterházy, A.; Bartók, M. Detection of new fumonisin mycotoxins and fumonisin-like compounds by reversed-phase high-performance liquid chromatography/electrospray ionization ion trap mass spectrometry. Rapid Commun. Mass. Spectrom. **2006**, *20* (16), 2447–2462.
84. Voss, K.A.; Poling, S.M.; Meredith, F.I.; Bacon, C.W.; Saunders, D.S. Fate of fumonisins during the production of fried tortilla chips J. Agric. Food Chem. **2001**, *49* (6), 3120–3126.
85. Seefelder, W.; Gossmann, M.; Humpf, H.U. Analysis of fumonisin B1 in fusarium proliferatum-infected asparagus spears and garlic bulbs from Germany by liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem. **2002**, *50* (10), 2778–2781.
86. Paepens, C.; De Saeger, S.; Van Poucke, C.; Dumoulin, F.; Van Calenbergh S.; Van Peteghem, C. Development of a liquid chromatography/tandem mass spectrometry method for the quantification of fumonisin B1, B2 and B3 in cornflakes. Rapid. Commun. Mass. Spectrom. **2005**, *19* (14), 2021–2029.
87. Royer, D.; Humpf, H.U.; Guy, P.A. Quantitative analysis of Fusarium mycotoxins in maize using accelerated solvent extraction before liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. Food Addit. Contam. **2004**, *21* (7), 678–692.
88. Razzazi-Fazeli; E. Analyses and Identification of Mycotoxins in Food Using LC-MS, in *The Encyclopedia of Mass Spectrometry*, Vol. 8; Hyphenated Methods; Elsevier: Amsterdam 2006; 707–721.
89. European Food Safety Authority (EFSA). Opinion of the Scientific Panel on contaminants in the food chain on a request from the Commission related to Zearalenone as undesirable substance in animal feed. The EFSA J. **2004**, *89*, 1–35.
90. Krska, R.; Josephs, R. The state-of-the-art in the analysis of estrogenic mycotoxins in cereals. Fresen. J. Anal. Chem. **2001**, *369* (6), 469–476.
91. Silva, C.M.; Vargas, E.A. A survey of zearalenone in corn using Romer Mycosep^{TM} 224 column and high performance liquid chromatography. Food Addit. Contam. **2001**, *18* (1), 39–45.
92. Erbs, M.; Hartmann, N.; Bucheli T.D. Determination of the cross-reactivities for alpha-zearalenol, beta-zearalenol, zearalanone, alpha-zearalanol, and beta-zearalanol on three commercial immunoaffinity columns targeting zearalenone. J. AOAC. Int. **2007**, *90* (4), 1197–1202.
93. Dänicke, S.; Ueberschär, K.H.; Halle, I.; Valenta, H.; Flachowsky, G. Excretion kinetics and metabolism of zearalenone in broilers in dependence on a detoxifying agent. Arch. Anim. Nutr. **2001**, *55* (4): 299–313.
94. Songsermsakul, P.; Sontag, G,; Cichna-Markl, M.; Zentek, J.; Razzazi-Fazeli, E. Determination of zearalenone and its metabolites in urine, plasma and faeces of horses by HPLC-APCI-MS. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **2006**, *843* (2), 252–261.

95. Rosenberg, E.; Krska, R.; Wissiack, R.; Kmetov, V.; Josheps, R.; Razzazzi, E.; Grasserbauer, M. High-performance liquid-chromatography atmospheric pressure chemical ionization mass spectrometry as a new tool for the determination of the mycotoxin zearalenone in food and feed. J. Chromatogr. A **1998**, *819* (1–2), 277–288.
96. Zöllner, P.; Jodlbauer, J.; Lindner, W. Determination of zearalenone in grains by high-performance liquid chromatography–tandem mass spectrometry after solid-phase extraction with RP-18 columns or immunoaffinity columns. J. Chromatogr. A. **1999**, *858* (2), 167–174.
97. Zöllner, P.; Berner, D.; Jodlbauer, J.; Lindner, W. Determination of zearalenone and its metabolites α- and β-zearalenol in beer samples by high-performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. B. Biomed. Sci. Appl. **2000**, *738* (2), 233–241.
98. Cramer, B.; Bretz, M.; Humpf H.U. Stable isotope dilution analysis of the fusarium mycotoxin zearalenone. J. Agric. Food Chem. **2007**, *55* (21), 8353–8358.
99. Jodlbauer, J.; Zöllner, P.; Lindner, W. Determination of zeranol, taleranol, zearalenone, α- and β-zearalenol in urine and tissue by high-performance liquid chromatography-tandem mass spectrometry. Chromatographia **2000**, *51*, 681–687.
100. Zöllner, P.; Jodlbauer, J.; Kleinova, M.; Kahlbacher, H.; Kuhn, T.; Hochsteiner, W.; Lindner, W. Concentration levels of zearalenone and its metabolites in urine, muscle tissue, and liver samples of pigs fed with mycotoxin-contaminated oats. J. Agric. Food Chem. **2002**, *50* (9), 2494–2501.
101. Kleinova, M.; Zöllner, P.; Kahlbacher, H.; Hochsteiner, W.; Lindner, W. Metabolic profiles of the mycotoxin zearalenone and of the growth promoter zeranol in urine, liver, and muscle of heifers. J. Agric. Food Chem. **2002**, *50* (17), 4769–4776.
102. Bily, A.C.; Reid, L.M.; Savard, M.E.; Reddy, R.; Blackwell, B.A.; Campbell, C.M.; Krantis, A.; Durst, T.; Philogene, B.J.; Arnason, J.T.; Regnault-Roger, C. Analysis of Fusarium graminearum mycotoxins in different biological matrices by LC/MS. Mycopathologia **2004**, *157* (1), 117–126.
103. Fang, X.; Chen, J.; Guo, D.; Wang, G. Detection and identification of zeranol in chicken or rabbit liver by liquid chromatography-electrospray tandem mass spectrometry. J. AOAC. Int. **2002**, *85* (4), 841–847.
104. van Bennekom, E.O.; Brouwer, L.; Laurant, E.H.M.; Hooijering, H.; Nielen, M.W.F. Confirmatory analysis method for zeranol, its metabolites and related mycotoxins in urine by liquid chromatography-negative ion electrospray tandem mass spectrometry. Anal. Chim. Acta **2002**, *473* (1–2), 151–160.
105. Launay, F.M.; Young, P.B.; Sterk, S.S.; Blokland, M.H.; Kennedy, D.G. Confirmatory assay for zeranol, taleranol and the Fusarium spp. toxins in bovine urine using liquid chromatography –tandem mass spectrometry. Food Addit. Contam. **2004**, *21* (1), 52–62.
106. Berthiller, F.; Werner, U.; Sulyok, M.; Krska, R.; Hauser, M.T.; Schuhmacher, R. 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. **2006**, *23* (11), 1194–1200.
107. European Commission 1425/2003/EC (2003).
108. AOAC. 1997. *Official methods of Analysis of AOAC International*. 16th edition; Method 995.10.
109. De Champdoré, M.; Bazzicalupo, P.; De Napoli, L.; Montesarchio, D.; Di Fabio, G.; Cocozza, I.; Parracino, A.; Rossi, M.; D'Auria, S. A new competitive fluorescence assay for the detection of patulin toxin. Anal. Chem. **2007**, *79* (2), 751–757.
110. Brause, A.R.; Trucksess, M.W.; Thomas, F.S.; Page, S.W. Determination of patulin in apple juice by liquid chromatography: collaborative study. J. AOAC. Int. **1996**, *79* (2), 451–455.
111. Frisvad, J.C.; Thrane, U. 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. **1987**, *404* (1), 195–214.
112. Sewram, V.; Nair, J.J.; Nieuwoudt, T.W.; Leggott, N.L.; Shephard, G.S. Determination of patulin in apple juice by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J. Chromatgr. A. **2000**, *897* (1–2), 365–374.
113. Rychlik, M.; Schieberle, P. Synthesis of ^{13}C-Labeled Patulin [4-Hydroxy-4H-furo[3,2-c]pyran-2(6H)-one] to be used as internal Standard in a Stable Isotope Dilution Assay. J. Agric. Food Chem. **1998**, *46* (12), 5163–5169.
114. Rychlik, M.; Schieberle, P. Quantification of the Mycotoxin Patulin by a Stable Isotope Dilution Assay. J. Agric. Food Chem. **1999**, *47* (9), 3749–3755.
115. Takino, M.; Daishima, S.; Nakahara, T. Liquid chromatography/mass spectrometric determination of patulin in apple juice using atmospheric pressure photoionization. Rapid. Commun. Mass Spectrom. **2003**, *17* (17), 1965–1972.
116. Kren, V.; Cvak, L. *Ergot: The Genus Claviceps, Medicinal & Aromatic Plants, Industrial Profiles*; CRC Press: Amsterdam, The Netherlands, 1999.
117. Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission relatet to Ergot as undesireable Substance in Animal Feed. The EFSA J. **2005**, *225*, 1–27.
118. Frach, K.; Blaschke, G. Separation of Ergot Alkaloids and their Epimers and Determination in Sclerota by Capillary Electrophoresis. J. Chromatogr. A **1998**, *808*, 247–252.
119. Durix, A.; Jaussaud, P.; Garcia, P.; Bonnaire, Y.; Bony, S. Analysis of Ergovaline in Milk using High-Performance Liquid Chromatography with Ffluorimetric Detection. J. Chromatgr. B **1999**, *729*, 255–263.
120. Lombaert, G.A.; Pallaers, P.; Roscoe, V.; Mankotia, M.; Neil, R.; Scott, P.M. Mycotoxins in Infant Cereal Foods from the Canadian retail Market. Food Addit. Contam. **2003**, *20*, 494–504.
121. Shelby, R.A.; Olsovska, J.; Havlicek, V.; Flieger, M. Analysis of Ergot Alkaloids in Endophyte-Infected Tall Fescue by Liquid Chromatography/Electrospray Ionisation Mass Spectrometry. J. Agric. Food Chem. **1997**, *45*, 4674–4679.

122. Lehner, A.F.; Craig, M.; Fannin, N.; Bush L.; Tobin, T. Fragmentation patterns of selected Ergot Alkaloids by Electrospray Ionization Tandem Quadrupole Mass Spectrometry, J. Mass Spectrom. **2004**, *39*, 1275–1286.
123. Lehner, A.F.; Craig, M.; Fannin, N.; Bush, L.; Tobin, T. Electrospray[+] tandem quadrupole mass spectrometry in the elucidation of ergot alkaloids chromatographed by HPLC: screening of grass or forage samples for novel toxic compounds. J. Mass Spectrom. **2005**, *40* (11), 1484–1502.
124. Mohamed, R.; Gremaud, E.; Tabet, J.C.; Guy, P.A. Mass spectral characterization of ergot alkaloids by electrospray ionization, hydrogen/deuterium terium exchange, and multiple stage mass spectrometry: Usefulness of precursor ion scan experiments. Rapid. Commun. Mass Spectrom. **2006**, *20* (19), 2787–2799.
125. Mohamed, R.; Gremaud, E.; Richoz-Payot, J.; Guy, P.A. Quantitative determination of five ergot alkaloids in rye flour by liquid chromatography-electrospray ionisation tandem mass spectrometry. J. Chromatogr. A **2006**, *1114* (1), 62–72.
126. Salvador, A.; Dubreuil, D.; Denouel, J.; Millerioux L. Sensitive method for the quantitative determination of bromocriptine in human plasma by liquid chromatography–tandem mass spectrometry. J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. **2005**, *820* (2), 237–242.
127. Bürk, G.; Höbel, W.; Richt, A. Ergot alkaloids in cereal products : Results from the Bavarian Health and Food Safety Authority. Mol. Nutr. Food Res. **2006**, *50* (4–5), 437–442.
128. Uhlig, S.; Vikøren, T.; Ivanova, L.; Handeland, K. Ergot alkaloids in Norwegian wild grasses: a mass spectrometric approach. Rapid. Commun. Mass Spectrom. **2007**, *21* (10), 1651–1660.
129. Cavaliere, C.; Foglia, P.; Guarino, C.; Motto, M.; Nazzari, M.; Samperi, R.; Laganà, A.; Berardo, N. Mycotoxins produced by Fusarium genus in maize: determination by screening and confirmatory methods based on liquid chromatography tandem mass spectrometry. Food Chem. **2007**, *105* (2), 700–710.
130. Lattanzio, V.M.; Solfrizzo, M.; Powers, S.; Visconti, A. Simultaneous determination of aflatoxins, ochratoxin A and fusarium toxins in maize by liquid chromatography/tandem mass spectrometry after multitoxin immunoaffinity cleanup. Rapid. Commun. Mass Spectrom. **2007**, *21* (20), 3253–3261.
131. 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* (1–2), 111–136.
132. Ren, Y.; Zhang, Y.; Shao, S.; Cai, Z.; Feng, L.; Pan, H.; Wang, Z. Simultaneous determination of multi-component mycotoxin contaminants in foods and feeds by ultra-performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A **2007**, *1143* (1–2), 48–64.
133. Sulyok, M.; Krska, R.; Schuhmacher, R. Application of a liquid chromatography-tandem mass spectrometric method to multi-mycotoxin

determination in raw cereals and evaluation of matrix effects. Food Addit. Contam. **2007**, *24* (10), 1184–1195.
134. Sulyok, M.; Krska, R.; Schuhmacher, R. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. Anal. Bioanal. Chem. **2007**, DOI 10.1007/s00216-007-1542-2.
135. Sulyok, M.; Berthiller, F.; Krska, R.; Schuhmacher, R. 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. **2006**, *20* (18), 2649–2659.
136. Tanaka, H.; Takino, M.; Sugita-Konishi, Y.; Tanaka, T. 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. **2006**, *20* (9), 1422–1428.
137. Cavaliere, C.; D'Ascenzo, G.; Foglia, P.; Pastorini, E.; Samperi, R.; Lagana, A. Determination of type B trichothecenes and macrocyclic lactone mycotoxins in field contaminated maize. Food Chem. **2005**, *92* (3), 559–568.
138. Delmulle, B.; De Saeger, S.; Adams, A.; De Kimpe, N.; Van Peteghem, C. 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. **2006**, *20* (5), 771–776.
139. Zöllner, P.; Leitner, A.; Berner, D.; Kleinova, M.; Jodlbauer, J.; Mayer, B.X.; Lindner, W. Improving LC-MS/MS analyses in complex food matrices Part I—Sample preparation and chromatography. LC-GC Europe **2003**, *16* (6), 354–360.
140. Matuszewski, B.K.; Constanzer, M.L.; Chavez-Eng, C.M. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations. Anal. Chem. **1998**, *70* (5), 882–889.
141. Asam, S.; Rychlik, M. Synthesis of four carbon-13-labeled type a trichothecene mycotoxins and their application as internal standards in stable isotope dilution assays. J. Agric. Food Chem. **2006**, *54* (18), 6535–6546.
142. Seefelder, W.; Hartl, M.; Humpf, H.U. Determination of N-(carboxymethyl)fumonisin B(1) in corn products by liquid chromatography/electrospray ionization-mass spectrometry. J. Agric. Food Chem. **2001**, *49* (5), 2146–2151.

Manuscript 6273E