Egypt. J. Chem. Environ. Health, 1 (1):729-748 (2015)

Various Analytical Techniques Involved In Mycotoxin Detection and Estimation.

Samia A. El-Zeiny; Magda Sh. Taha; M. K. Abo El-Magd; M. M. Arafa; Nehad A. Badrawy; Abeer, S. Abd El-Rahman and Eman Sh. Laz

Biochemistry, Toxicology and Feed Deficiency Diseases Dep.
Animal Health Research Institute.

Abstract

The aim of this review was to discuss the various analytical techniques involved in mycotoxin detection and estimation. Mycotoxins to be analyzed are originally present in contaminated samples. Hence, mycotoxins must be extracted with different extraction methods and cleaned-up prior to detection techniques, if reliable results are to be obtained. Extraction procedures include extraction of mycotoxins from feeds and foodstuffs. SPE and IAC-cleanup will become of increasing importance as sample preparation techniques prior to instrumental analysis. Immunoaffinity cleanup techniques with high-resolution chromatography showed the most selectivity for mycotoxin analysis. Recently, advances using tandem or mixed selectivity immunoaffinity cartridges have demonstrated the feasibility of multi target mycotoxin assays. In the early 1980s, TLC was the most widely used chromatographic technique applied to mycotoxins because of its relatively simple, fast, and inexpensive properties; however, it has some disadvantages, such a slow sensitivity, high detection limit, and lack of potential for automation. Consequently, it is now almost replaced by the HPLC techniques. Among the available detectors, the most frequently used are PDA, UV, and Fl, which have a particular application in the field of mycotoxins. HPLC–MS has all the HPLC advantages for trace level detection and confirmation, especially for complex matrices and it can obtain qualitative data concerning the identity of mycotoxins. The great potential of LC–MS/MS for screening large amounts of samples for the presence of a number of mycotoxins has recently been demonstrated. Immunoassays that deliver quantitative or semi quantitative results, still represent the most frequently used rapid methods. There is an ongoing development toward quick and reliable methods providing rapid yes/no decisions or semi quantitative results. Also, many projects are in progress aiming to avoid purification step, for example, to measure the analytes directly after extraction. Easy to-use methods are often either too expensive or show a lack of sensitivity. In a nutshell, the previously mentioned methods have their advantages and disadvantages, and the desired method selection should be done according to the analytical objective, sample properties, and environmental conditions. Although there are some reports for qualitative and quantitative analysis of mycotoxins, rapid and sensitive quantitative methods are still high on the wish list.

Introduction

It is difficult to define mycotoxin in a few words. All mycotoxins are low-molecular-weight natural products (i.e., small molecules) produced as secondary metabolites by filamentous fungi. These metabolites constitute a toxigenically and chemically heterogeneous assemblage that are grouped together only because the members can cause disease and death in human beings and other vertebrates. Not surprisingly, many mycotoxins display overlapping toxicities to invertebrates, plants, and microorganisms. The term mycotoxin was coined in 1962 in the aftermath of an unusual veterinary crisis near London, England, during which approximately 100,000 turkey poults died. When this mysterious turkey X disease was linked to a peanut (groundnut) meal contaminated with secondary metabolites from *Aspergillus flavus* (aflatoxins), it sensitized scientists to the possibility that other occult mold metabolites might be deadly. Soon, the mycotoxin rubric was extended to include a number of previously known fungal toxins (e.g., the ergot alkaloids), some compounds that had originally been isolated as antibiotics (e.g., patulin), and a number of new secondary metabolites revealed in screens targeted at mycotoxin discovery (e.g., ochratoxin A). The period between 1960 and 1975 has been termed the mycotoxin gold rush because so many scientists joined the well-funded search for these toxigenic agents. Depending on the definition used, and recognizing that most fungal toxins occur in families of chemically related metabolites, some 300 to 400 compounds are now recognized as mycotoxins, of which approximately a dozen groups regularly receive attention as threats to human and animal health (Abent, 2007). Mycotoxicoses are the animal diseases caused by mycotoxins; mycotoxicology is the study of mycotoxins. While all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins. The target and the concentration of the metabolite are both important. Fungal products that are mainly toxic to bacteria (such as penicillin) are usually called antibiotics. Fungal products that are toxic to plants are called phytotoxins by plant pathologists. Mycotoxins are made by fungi and are toxic to vertebrates and other animal groups in low concentrations. Other low molecular weight fungal metabolites such as ethanol that are toxic only in high concentrations are not considered mycotoxins (Curtui, et.al.,1998). Finally, although mushroom poisons are definitely fungal metabolites that can cause disease and death in humans and other animals, they are rather arbitrarily excluded from discussions of mycotoxicology. Molds (i.e., microfungi) make mycotoxins; mushrooms and other macroscopic fungi make mushroom poisons. The distinction between a mycotoxin and a mushroom poison is based not only on the size of the producing fungus, but also on human intention. Mycotoxin exposure is almost always accidental. In contrast, with the exception of the victims of a few mycologically accomplished murderers, mushroom poisons are usually ingested by amateur mushroom hunters who have collected, cooked,

and eaten what was misidentified as a delectable species (Brithielr, 2005). Mycotoxins are not only hard to define, they are also challenging to classify. Due to their diverse chemical structures and biosynthetic origins, their myriad biological effects, and their production by a wide number of different fungal species, classification schemes tend to reflect the training of the person doing the categorizing. Clinicians often arrange them by the organ they affect. Thus, mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, and so forth. Cell biologists put them into generic groups such as teratogens, mutagens, carcinogens, and allergens. Organic chemists have attempted to classify them by their chemical structures (e.g., lactones, coumarins); biochemists according to their biosynthetic origins (polyketides, amino acid-derived, etc.); physicians by the illnesses they cause (e.g., St. Anthony's fire, stachybotryotoxicosis), and mycologists by the fungi that produce them (e.g., *Aspergillus* toxins, *Penicillium* toxins). None of these classifications is entirely satisfactory.

The analysis of mycotoxins has become a global issue, and most countries have already set up regulatory limits or guideline levels for the tolerance of such contaminants in agricultural commodities and products. Approximately 300 to 400 substances are recognized as mycotoxins, comprising a broad variety of chemical structures produced by various mold species on many commodities and processed food and feed.

Globalization of the agricultural product trade has contributed significantly to the discussion about potential hazards and increased awareness of mycotoxins, at the same time as knowledge of safety in food and feed production has risen due to the simple fact that methods for testing residues and undesirable substances have become noticeably more sophisticated and available at all points of the supply chain.

It is important to develop rapid, sensitive, and reproducible assays to detect the presence of mycotoxins. The accurate and rapid qualitative and quantitative analysis for mycotoxins has been topic of interest by many researchers. Different analytical methods having different sensitivity and accuracy which could be used for different purposes have been developed. Commonly used methods to analyze mycotoxins are thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) with UV or fluorescence detection (FD), and enzyme immunoassays (EIAs). Recently, liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS) techniques have become accessible for the qualitative and quantitative determination of mycotoxins.

Requirements of Modern Mycotoxin Analysis

The most important target analytes are aflatoxins, trichothecenes, zearalenone and its derivatives, fumonisins, ochratoxins, ergot alkaloids, and patulin.1 Various mycotoxins may occur simultaneously, depending on environmental and substrate conditions. Considering this coincident production, humans and animals are likely exposed to mixtures rather than to individual compounds. Recently, the natural occurrence of masked mycotoxins, in which the toxin is conjugated, has been reported, requiring even more selective and sensitive detection principles(Sulyok, et al., 2006)

So far, most analytical methods deal with single mycotoxins or mycotoxin classes, including a limited number of chemically related target analytes. But as additive and synergistic effects have been observed concerning the health hazards posed by mycotoxins, the need for multi-toxin methods for the simultaneous screening of different classes of mycotoxins has risen.

Colorimetric technique

Further separation in mycotoxin detection can be performed with IAC, followed by liquid chromatographic (LC) quantitation, either off-line or online in an automated system, or by fluorometry. Some mycotoxins like aflatoxins, ochratoxins, and citrinin have a conjugated, planar structure that gives them natural fluorescence ability, which makes it feasible for qualitative and quantitative determination using a fluorometer. Commercial IAC, Aflatest P, is used as the cleanup step in an LC method and in a solution-fluorometry method for corn, peanuts, and peanut butter that was adopted as an AOAC Intl. Official Method (Scott and Trucksess 1997). A comparative study of 3 different methods using HPLC, fluorometry, and ELISA for the determination of aflatoxins in sesame butter has been carried out by Nilufer and Boyacioglu (2002). In this study, an immunoaffinity column was used for cleanup and purification of extracts prior to detection by HPLC and fluorometry. The fluorometric determination method was found to be highly correlated with the HPLC method (r = 0.978). Both fluorometry and ELISA methods had high recoveries and low variance (Nilufer and Boyacioglu 2002). In addition, this technique allows the high throughput analysis of a large number of industrial samples for automation by means of a microplate system.

However, this analysis procedure is one semi quantitative, although it is rapid analysis and low cost for analyzing a large number of samples.

Chromatographic technique

Chromatography analysis is based on distribution or partition of a sample solute between 2 phases: stationary phase and mobile phase. Most common chromatography techniques used today in the field of food analysis are gas chromatography (GC), HPLC, and supercritical fluid chromatography (SFC). These methods, when connected to another instrument such as mass spectrometer, work as a separation method. In the 1980s and the early of 1990s, various reviews on the chromatography of mycotoxins were published (Betina 1993).

TLC technique.

Thin-layer chromatography (TLC) is a technique that can be used for the separation, purity assessment, and identification of organic compounds. First reports of this technique were in the 1930s and after that it becomes a very useful and easy technique for the analysis of a wide range of compounds (Betina 1993). TLC also identified as flat-bed chromatography or planar chromatography, is one of the most widely used separation techniques in aflatoxin analysis. Since 1990, it has been considered as an AOAC official method and the method of choice to identify and quantify aflatoxins at levels as low as 1 ng/g. According to reports and articles, mycotoxins were easily separated by TLC using several solvents (Odhav and Naicker 2002), assessed in the multitoxin detection part of this article.

Normal-phase TLC consists of a stationary phase like silica, alumina, and cellulose immobilized on a glass or plastic plate and a solvent as the mobile phase (Betina 1993). The sample, either liquid or dissolved in solvent, is deposited as a spot on the stationary phase. The constituent of a sample can be identified by simultaneously running standards with the unknown spot. Then one edge of the plate is vertically placed in a solvent tank and the solvent moves up the plate by capillary action. After the solvent reaches other edge, the plate is removed from tank and the separated spots (because of different partitioning behavior of the components) are visualized by UV, fluorescence, MS, or other techniques. Pittet and Royer (2002) used this method for the determination of ochratoxin A in green coffee.

Sometimes the plate is dried after first-development and rotated through 90◦ and developed in another solvent. This model is called 2-dimensional TLC and is used for better resolution or removal of interfering compounds (Betina 1985). A further development in TLC is high-performance thin-layer chromatography (HPTLC). Reduction of layer thickness (down to 100 microns) and particle size (2 to 10 microns) of the stationary phase leads to an improved separation within a shorter time. Modern HP-TLC is a precise and accurate analytical tool with efficiency, which is comparable to that of HPLC and ELISA methods. Therefore, quantitative and qualitative analysis of aflatoxins has been

developed using multidimensional–HPTLC–fluorescence excitation, such as applied in peanut butter samples by Liang et al., (1996). Recently, Toteja et al., (2006) determined aflatoxin B1 of rice samples, using HPTLC after extraction with water/chloroform and silica gel column cleanup. The over pressured-layer chromatography (OPLC) method has the advantages of the HPTLC and HPLC methods. The linear OPLC is a forced flow technique, using external pressure on chromatoplate sealed on the edges and a pump system for the admission of mobile phase into the stationary phase. Comparing with the HPLC, it requires less mobile phase, using off-line method, and allows faster examination with the possibility of parallel analysis. OPLC is more efficient than TLC, providing better resolution and more compact spots. OPLC methods were developed for the measurement of aflatoxin (B1, B2, G1, and G2) contamination in various foodstuffs (maize, wheat, peanut, fish meat, rice, sunflower seeds, and red paprica) (M´ oricz et al., 2007).

For the chemical confirmation of mycotoxins, there are 2 treatment methods. First, TLC plates were formerly impregnated with acidic-organic solution; second, the TLC plates, with the developed chromatogram, were exposed to vapors of pyridine or acetic anhydride or dipped into aluminum chloride reagent. After these treatments, mycotoxins were converted into new fluorescent compounds, and then the TLC plates were observed under 365 nm light. The combination of TLC with mass spectrometry (MS) has been carried out without an adsorbent elution step (Scott 1993). TLC was an extremely powerful, rapid, and low-cost separation technique in mycotoxicology before HPLC techniques became popular. Several TLC methods were developed for mycotoxin quantitation (Odhav and Naicker 2002; Caldas and Silva 2007) and qualitative determination (Odhav and Naicker 2002). Alhough there are some reports on comparable results between TLC and HPLC for the dtermination of aflatoxin M1 in raw, pasteurized, and UHT milk (recovery values ranged from 85.83% to 73.86% at levels of 0.010 to 0.50 μg/L) (Shundo and Sabino 2006) and aflatoxins (B1, B2, G1, and G2) in products with quantification limit (LOQ) of 2 μg/kg, these TLC methods are generally suitable for qualitative analysis at best (Caldas and Silva 2007).

Liquid chromatography:

Liquid chromatography methods for the determination of mycotoxins in foods include normal-phase LC (NPLC), reversed-phase LC (RPLC) with pre- or before-column derivatization (BCD), RPLC followed by postcolumn derivatization (PCD), and RPLC with electrochemical detection (Calleri et al., 2007). Pre- and postcolumn derivatizations are used for improvement of sensitivity (Hu et al., 2006). LC can be classified into 3 parts, column chromatography, mini-column chromatography, and HPLC. Column chromatography is used for cleanup. Many factors like particle size, particle size distribution, and surface area, packing density, pH, and many other factors affect its

performance. Therefore, columns have been replaced by commercial prepacked cartridges. Mini-columns were used for screening of different mycotoxins (usually aflatoxins, ochratoxin A, and ZEA). After first publishing about the application of HPLC (Betina 1993), the same researcher stated that the usage of HPLC has increased and it has been used for separation, detection, andquantification of mycotoxins.

High-performance liquid chromatography:

HPLC is the most popular method for the analysis of mycotoxins in foods and feeds. Actually it is a quantitative technique that is suited for online cleanup of sample extract and could be combined with different detectors. During the last decades, several reviews have been written in this area. There are some reports of successful application of HPLC techniques for the analysis of mycotoxins in grains (Eke et al., 2004; Visconti et al., 2005), fungal cultures (Delmulle and others 2006), cheese (Kokkonen et al., 2005), milk (Sorensen and Elbaek 2005), bee pollen (Garcia- Villanova et al., 2004), cereal products (Aresta et al., 2003), and feeds (Krska et al., 2007). These HPLC methods differed significantly in the choice of normal-phase or reversed-phase columns of different types, elution mixtures and gradients, detection methods, and sample preparation and purification procedures. Among those, most chromatography techniques were performed in the form of reversed-phase based on acidic mobile phase with ortho-phosphoric acid and fluorescence detection (FD) (Odhav and Naicker 2002; Sobolev 2007), or UV detection (Hayashi and Yoshizawa 2005); the ion pair techniques with UV detection. HPLC in a normal phase mode on a buffered silica gel column was also proposed. Calleri et al., (2007) determined aflatoxin B1 by antiaflatoxin B1 immunoaffinity monolithic disk. Polyclonal anti-AFB1was covalently immobilized in batch on an epoxy-activated monolithic Convective Interaction Media (CIM) disk by a 1-step reaction via epoxy groups of the polymer surface. A weight of 0.96 mg of antibody was immobilized and the CIM disk was coupled through a switching valve to a reversed-phase column.

The fully automated HPLC method with fluorescence detection has a limit of detection of 50 ng/mL (S/N = 3) and a limit of quantitation of 100 ng/mL (Calleri et al., 2007).

HPLC–UV technique:

The reversed-phase HPLC–UV technique was an early method used for the determination of mycotoxins in grains (Cahill et al., 1999), which was established based on an acidic mobile phase with phosphoric acid. However, even using the same type of columns, the retention times were highly variable. In sequence, a general method (Frisvad and Thrane 1987) for mycotoxin analysis was developed, based on HPLC with an alkyl phenone retention index and photodiode array (PDA) detection in 2 different eluents. Application of the PDA technique allowed the simultaneous qualitative detection and

identification of multi-mycotoxins. By analyzing the organic solvent extracts of fungal cultures, this system was found effective for comparison of chemotaxonomic data and for precise identification of fungi. Based on RP–HPLC–UV–PDA techniques, multi-mycotoxin estimations were further developed using linear gradient elution with an acetonitrile/water solvent system (Kuronen 1989). The toxins were characterized by retention times and online UV spectra produced by a diode array detector (DAD). In a simple method, DON in cereal was extracted using methanol, then the solvent was evaporated, and the residue was re-dissolved with water; the extract was then cleaned up by immunoaffinity column and DON was determined using HPLC-UV. The limits of detection (LOD) and quantification

(LOQ) were 10 and 50 ng/g, respectively (Czerwiecki and Wilczy ´nska 2003). In another study for the investigation of DON in wheat, HPLC–DAD has been used after sample cleaning with

immunoaffinity column. The detection limit was 0.03 ng/g and recovery was almost 90% (Danicke et al., 2004). Abdulkadar et al., (2004) used HPLC–UV for the determination of DONin foods after extraction by acetonitrile :water (15:85). Also, Briones-Reyes et al., (2007) developed and optimized an RPHPLC- UV method for determination of zearalenone in corn for human consumption. In this method, zearalenon was extracted by methanol : water (85:15) and cleaned up by Florisil column, defatted by n-hexane and re-extracted by chloroform. Recovery was 90% and LOD was 0.7 ng/g (Briones-Reyes et al., 2007).

HPLC-fluorescence technique:

HPLC with fluorescence detection (FD) becomes the method of choice because of the available short and high-resolution columns and of the sensitivity of fluorescence detectors, and its potential for automation (Valenta 1998). Extraction is normally performed in acetonitrile-water, methanol-water, or even chloroform. An effective cleanup of the raw extract is required for purification of the analytes. An early RP–HPLC–FD method coupled with solid phase extraction (SPE) cleanup and concentration procedure was developed for the analysis of citrinin from hydrolyzed human urine (Orti et al., 1986). By this method, the detection limit for citrinin

was achieved to a level of 10 ng/g. Liquid chromatography using reversed-phase columns and fluorescence detection was effectively used to quantify different mycotoxins in grains (Abdulkadar et al., 2004; Zinedine et al., 2006), feeds (Charoenpornsook and Kavisarasai 2006), beverages (Abdulkadar and others 2004; Varelis et al., 2006), high-pigmentcontent samples such as chili powder, green bean, black sesame, and other spices (Fazekas et al., 2005), nuts (Abdulkadar et al., 2004), coffee (Ventura et al., 2003), ginseng and ginger (Trucksess et al., 2008), and bee pollen (Gonzalez et al., 2005). Optimizations for selective separations were generally done using ternary or even quaternary eluent

systems. Water, methanol, and acetonitrile were mostly used as a ternary system. The retention of mycotoxins depended on the content of water, whereas the composition and ratio of methanol and acetonitrile determined the elution order and resolution of mycotoxin with other analytes. To obtain fine peak forms and resolution, RP-ion-pair HPLC techniques have also been applied for the determination of mycotoxins. Later, an improved ion pair RP–HPLC coupled with postcolumn fluorometric detection technique for mycotoxin determination was developed. Spectroscopic studies demonstrated that the fluorescence of this metabolite was influenced by the pH of the environment. In the meantime, another ion-pair RP– HPLC procedure coupled with a postcolumn technique and timeresolved luminescence (TRL) detection were developed (Vazquez and others 1996). Some of the mycotoxins such as fumonisins are not fluorescent, so, prior derivatization of these compounds is needed to make fluorescent derivatives (Caldas and Silva 2007). To this aim, different reagents can be used for derivatization, such as fluorescamine, o-phthaldialdehyde (OPA) (Hinojo et al., 2006), 4- fluoro-7-nitrobenzofurazan (NBD-F) (Jimenez and Mateo 1997), 9-fluorenilmethyl chloroformate (Holcomb et al., 1993), or naphthalene 2,3-dicarboaldehyde (Bennett and Richard 1994). Fumonisins (FB1 and FB2) were determined in different corn-based food products using HPLCfl with quantification limit (LOQ) of 0.020 mg/kg (Caldas and Silva 2007). Trebstein et al., (2009) developed an HPLC flourescence

method for the determination of T-2 toxin and HT-2 toxin in different cereals and cereal products after derivatization with 1-anthroylnitrile.

Liquid chromatography with fluorescence detection (LC–FLD) is one of the most widely used techniques for the analysis of mycotoxins. LC using other detection methods, such as photodiode array (Danicke et al., 2004) or mass spectrometry (LC–MS–MS) (Delmulle et al., 2006) has also been reported. However, these 2 detection methods are less sensitive than FLD in some cases but can aid as confirmative tools (Saez et al., 2004). On the other hand, in contrast to HPLC-UV methods, GC-ECD enables the determination of several trichothecenes, even in complex food matrices, in the lower milligram per kilogram range. However, reversed-phase ion-pair HPLC provides good peaks, whereas those in the native fluorescence of mycotoxins were somewhat lost. In that way, the sensitivity and selectivity of this detection method were decreased. This weakness can be solved by acidifying the eluate from the HPLC column before fluorescence detection. So, RP-HPLC is widely used because of its advantages instead of conventional normal-phase HPLC. Although these RP–HPLC–fluorescence detection methods have relatively good sensitivity and recovery, in practice, application of all these methods to various complex matrices was considered boring and time-consuming. Extensive cleanup procedures were generally necessary, and sometimes deficient in specificity. Some problems, such as low reproducible

LC retention times still existed when normal-phase columns were used (Zimmerli et al., 1989), and decreased sensitivity and accuracy resulting from stability of citrinin in organic eluents, ion-pair, reagents, and acid environment.

High-performance liquid chromatography and gas chromatography have traditionally been the favored choices for the analyst when sensitive, reliable results with minimum variability are required. The major disadvantage of mycotoxin analysis using GC is the necessity of derivatization, which can be time-consuming and prone to error; as a result, GC methods are used less frequently.

Bioassay technique:

In general, the previously mentioned physicochemical detection methods required tedious sample extract and cleanup. Besides, there is loss of mycotoxins during sample treatment, unstable chromatographic behavior of mycotoxins, or relative low sensitivity and recovery. Therefore, bioassays have become increasingly useful for mycotoxin detection as a precursor of chemical analysis (Yates 1986). Bioassay by biosensor is designed as an inhibition assay. In these methods a fixed concentration of mycotoxin-specific antibody is mixed with a sample containing an unknown amount of mycotoxin. The antibody and mycotoxin form a complex. Then the sample is passed over a sensor surface to which mycotoxin has been immobilized. Non complexed antibodies are measured as they bind to the mycotoxin on the sensor surface. The responses generated over a range of standard mycotoxin concentrations are used to create a calibration curve and table. Finally, unknown samples are determined by referring to the calibration curve. Advances in biotechnology have made it possible to develop highly specific antibody-based tests. Commercially available test kits can identify and measure aflatoxins in food in less than 10 minutes. For illustration, ochratoxin A, aflatoxin B1, and T-2 toxin in cereal grains can be determined by monoclonal antibodies specific for detection limits of 1 ng/mL ochratoxin A, 0.1 ng/mL aflatoxin B1, and 10 ng/mL T-2 toxin after simple liquid– liquid cleanup procedure. These tests are based on the affinities of the monoclonal or polyclonal antibodies for aflatoxins. The 3 types of immunochemical methods are radioimmunoassay (RIA), ELISA, and immuno-affinity column assay (ICA).

T-2 toxin determination in cereals has been done by membrane-based flow-through enzyme immunoassay. Immunodyne ABC and membrane was coated with 2 microliter of goat anti-horseradish peroxidase (HRP) and rabbit anti-mouse (test spot) (undiluted) immunoglobulins, and the free binding sites were blocked. In one study recovery was between 16% and 82% (Sibanda et al., 2000). After that, Charoenpornsook and Kavisarasai(2006) developed another method for T-2 toxin and DON determination, using ELISA on animal feeds. Although enzyme immunoassay (EIA) methods for major mycotoxins have been known for many years, only recently, EIAs for mycotoxin

determination have been developed (Vrabcheva et al., 2000). The antibodies developed for EIA were also used for immunoaffinity columns for HPLC extract cleanup. For detection of ochratoxin A and citrinin, cereal samples including foods (wheat, corn) and feeds (barley, oats, and wheat bran) were mixed with HCl and dichloromethane. After centrifuging, the lower organic layer was extracted by magnetic stirring with aqueous NaHCO3 solution, centrifuged again, and the upper layer was used for EIA analysis. Detection limits were 0.5 and 5 ng/g, for ochratoxin A and citrinin, respectively (Vrabcheva et al., 2000). A radio immunochemical method was used for aflatoxin B1 and ochratoxin in wheat and barely. Detection limit of the RIA method was 0.3 μg/kg (Sedmikova and others 2001). Further, Korde et al., (2003) determined aflatoxin B1 in agricultural commodities rice, wheat, and soy beans by radio-immunoassay (RIA) using AfB1-bovine serum albumin conjugate as immunogen. The recovery values obtained ranged between 92% and 107%. The assay system was optimized in the range of 0.2 to 5 ng/mL. On the other hand, using the electrochemical immunosensor ELISA, a calibration plot for AFB1of grains was obtained over the concentration range 0.15 to 2.5 ng/mL, which gave a detection limit of around 0.15 ng/mL in buffer solution (Pemberton et al., 2006). ELISA, flow-through membrane-based immunoasays, chromatographic techniques, nucleic acid amplification on assays, biosensors, and microarrays were studied for the detection of mold and mycotoxins by Foong-Cunningham and others (2006) have written a review on bioassay methods for fumonisins in fungal cultures and cereals. Many experiments have been conducted on immunoassay methods for mycotoxins; for example a study on fumonisin B1 and B2 in maize (Paepens et al., 2004) and aflatoxin M1 in milk and aflatoxin B1 in feed (Decastelli et al., 2007) and also for multi-mycotoxin detection. Among different mycotoxins Curtui and others 1998 selected deoxynivalenon, 3-acetylDON, 15- acetyl DON, fusarenone X (FX), T-2 toxin (T-2), diacetoxyscirpenol (DAS), ZEA, FB1, AFB1, OTA, and citrinin (CIT) in wheat, maize as feed for their study. Developing rapid and innovative methods offer the microtiterplate immunoassay (ELISA format) has made them the most frequently used rapid tests for mycotoxins. ELISAs are commercially available for important mycotoxins like aflatoxins, fumonisins, trichothecenes, ZEA, OTA, citrinin (Sangare-Tigori et al., 2006). They are useful tools for screening and quantification and offer benefits with respect to speed and sensitivity. These days ELISA is well known as a useful semi quantitative method for mycotoxins and commercial enzyme-linked immunosorbent assays (ELISAs) are widely used (Papadopoulou-Bouraoui et al., 2004). For example, aflatoxins like aflatoxin B1 in groundnut, corn, wheat, cheese, and chili have been determined after dilution of the aqueous methanol extracts without sample cleanup. In this study, recoveries from different food samples were between 91% and 104% and detection limit were 0.25 pg/spot, 0.01

ng/mL (Pal aet al., 2004). In addition, the results of aflatoxin determination in red-scaled, red and black pepper determined by ELISA showed a good correlation with HPLC, since ELISA (in terms of simplicity, rapidity, reliability, cost effectiveness) can be used in the routine screening of aflatoxin contamination in spices (Colak et al., 2006). Aflatoxin M1 in milk has been determined by ELISA by some researchers (Decastelli and others 2007). In a competitive ELISA with monoclonal antibody, dichloromethane/citric acid mixture was used for extraction of cereal ochratoxin A. This cleanup procedure proved to be as effective for OTA extraction as protocols using strong acids. Recovery from cereals infected with 5 to 100 ng/mL ochratoxin A varied between 90% and 130% in different cereals, and the results were confimed by HPLC fluorescence detector (Barna-Vetro et al., 1996). Later, Thirumala et al., (2002) extracted ochratoxin A (OTA) from chilies with methanol-water and KCl. This step is followed by dilution to 1:4 with PBS-T-BSA for processing by ELISA. The mean recoveries from OA-free chilies spiked with 1 to 100 μg of OA per kilogram of chili sample were 90% and 110% (Thirumala-Devi and others 2002). In another experiment, chratoxin A (OTA) in soybean samples was extracted using extract solvent and loaded onto a C18 Sep- Pak cartridge, then determined by competitive direct enzymelinked immuno-sorbent assay (cdELISA), and a competitive indirect ELISA (ciELISA) was used. Efficacy of cdELISA was confirmed by the HPLC method. Recovery rate of OTA was found to be 85.9% in the cdELISA (Yu and others 2005). Simultaneous estimation of aflatoxin B1 and ochratoxin A have been done using membrane-based immunoassay consisting of a membrane with immobilized anti-AFB1 and anti-OA antibodies and a filter paper attached to a polyethylene card below themembrane. In an experiment on chili samples the limit of quantification obtained was 2 and 10 μg/kg for AFB1 and OA, respectively (Saha et al., 2007). At the same time, aflatoxin and ochratoxin were determined in barley and wheat flour by Adanyi et al., (2007). After immobilizing the antibody or antigen conjugate for the direct and indirect measurement, respectively, a sensor chip was used in the flow-injection analyzer (FIA) system. The regression coefficient between the 2 methods for ochratoxin and aflatoxin was determined to be 0.96 and 0.89, respectively. Sensitivity detection range of the competitive detection method was between 0.5 and 10 ng/mL in both cases (Adanyi et al., 2007). In the case of spices, ginger, pepper, and chili, simultaneous detection of aflatoxin B1 and ochratoxin has been done by tandem immunoassay after one cleanup. Cutoff levels were 5 and 10 μg/kg. Results were confirmed by LC–MS/MS with immunoaffinity column cleanup (Saha et al., 2007). Recently, Iacumin et al., (2009) used an ELISA kit for OTA determination in Italian sausages after digesting with hydrochloric acid and extracting with dichloroethane; the method was the same as reported by Matrella et al., (2006). Also, Wang and Gan (2009) developed a flow through quartz crystal microbalance (QCM) immunoassay method based

on aflatoxin B1 antibody. The proposed immunoassay system was simple and rapid without multiple labeling and separation steps. Bioassay methods provided a rapid means for screening samples and allowed the analyst to make an informed decision. Immunochemical methods provided a convenient and sensitive alternative for detecting many mycotoxins (Chu 1991). Ease of operation and high throughput, associated with their use, are the best advantages of ELISA method. Like other methods, these methods have some weakness, which includes cross-reactivity andmatrix dependence, often resulting in overestimation (Krska et al., 2007). Besides the common ELISA procedures, there is an increasing demand for immunoassay techniques for field use, offering protocols for quick and reliable results. Multi analyte dipstick immunoassays for various mycotoxins have been developed, however, with limited sensitivity. One of the recently developed methods for mycotoxin detection is polymerase chain reaction (PCR) method. The main principles of PCR are as follows: first, denaturing (separating the individual strands) DNA by heat. Second, a small segment of DNA will be taken as a probe that will target to anneal with the piece of DNA of interest (the target). Then, it will be amplified and yield doubled DNA. After that, the process will cycled around 40 times to give the desired quantity of DNA product. Finally, the negatively charged DNA will be separated through the gel based on size (Jurado et al.,2006). Small pieces of DNA can be amplified and detected routinely. It is rapid, and does not need to culture organisms prior to their identification. They are specific, since identification of species is made on the basis of genotypic differences, and are highly sensitive, detecting target DNA molecules in complex mixtures even when the mycelia are no longer viable (Russell and Paterson 2006).

Stable Isotope Dilution Assay

To overcome matrix effects and related quantification problems, external matrix calibration for each commodity tested has so far been recommended. This process is extremely time consuming and impractical under routine conditions, in which a variety of matrices are present every day. An alternative approach, the use of stable isotope-labeled internal standards, has recently been introduced (Häubl et al., 2006 a).

These substances are not present in real-world samples but have properties identical to the analytes. Internal standards are substances that are highly similar to the analytical target substances: Their molecular structure should be as close as possible to the target analyte, while the molecular weight is different. Within the analytical process, internal standards are added to both the calibration solutions and analytical samples. By comparing the peak area ratio of an internal standard and the analyte, the concentration of the analyte can be determined.

Ideal internal standards are isotope-marked molecules of a respective target analyte, which are usually prepared using organic synthesis by exchanging some of the hydrogen atoms with deuterium or exchanging carbon-12 with carbon-13 atoms. Physicochemical properties of such substances, especially ionization potential, are very similar to or nearly the same as their naturally occurring target analytes, but because of their higher molecular weight (due to the incorporated isotopes), distinction between the internal standard and target analyte is possible.

Considering the wide range of polarities of the analytes, the seemingly highly selective MS/MS detection could lead to the misperception that matrix interferences could be eliminated effectively and quantitative results be obtained without any cleanup and with very little chromatographic separation.

Variations during sample preparation and cleanup, as well as during ionization, are compensated for so that methods with especially high analytical accuracy and precision can be developed. Optimally, these isotope-labeled analogues must have a large enough mass difference to nullify the effect of naturally abundant heavy isotopes in the analyte. This mass difference will generally depend on the molecular weight of the analyte itself; in the case of molecules with a molecular weight range of 200 to 500, a minimum of three extra mass units might be required.

Isotope-labeled standards supplied by Biopure are fully labeled, providing an optimum mass unit difference between the labeled standard and target analyte. For example, the [13C15]-DON standard, which is available as a liquid calibrant (25mgl-1), was thoroughly characterized by Häubl and colleagues with regard to purity and isotope distribution and substitution, the latter being close to 99%.9 Fortification experiments with maize confirmed the excellent suitability of [13C15]-DON as an internal standard, indicating a correlation coefficient of 0.9977 and a recovery rate of 101% +/- 2.4%. When the same analyses were run without considering the internal standard, the correlation coefficient was 0.9974 and the recovery rate was 76% +/- 1.9%, underlining the successful compensation for losses due to sample preparation and ion suppression effects by the isotope-labeled internal standards (Häubl et al., 2006 a,b).

References

Abdulkadar AHW, Al-Ali AA, Al-Kildi AM, Al-Jedah JH. 2004. Mycotoxins in food products available in Qatar. Food Control 15(7):543–8.

Abnet C. 2007. Carcinogenic food contaminants. Cancer Invest 25(3):189–96.

Adanyi N, Levkovets IA, Rodriguez-Gil S, Ronald A, Varadi M, Szendro I. 2007. Development of immunosensor based on OWLS technique for determining aflatoxin B1 and ochratoxin A. Biosens Bioelectron 22(6):797–802

Aresta A, Cioffi N, Palmisano F, Zambonin CG. 2003. Simultaneous determination of ochratoxin A and cyclopiazonic, mycophenolic, and tenuazonic acids in cornflakes by solidphase microextraction coupled to high-performance liquid chromatography. J Agric Food Chem 51(18):5232–7.

Barna-Vetro I, Solti L, Teren J. 1996. Sensitive ELISA test for determination of ochratoxin A. Agric Food Chem 44:4071–4.

Bennett GA, Richard JL. 1994. Liquid chromatographic method for analysis of the naphthalene dicarboxaldehyde derivative of fumonisins. AOAC Int 77:501–5.

Betina V. 1985. Thin-layer chromatography of mycotoxins. J Chromatogr 334(3):211–76.

Betina V. 1993. Chromatography of mycotoxins, techniques and applications. Elsevier 36–7:210–3.

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.

Briones-Reyes D, G´omez-Martinez L, Cueva-Rolon R. 2007. Zearalenone contamination in corn for human consumption in the state of Tlaxcala, Mexico. Food Chem 100:693–8.

Cahill LM, Kruger SC, McAlice BT, Ramsey CS, Prioli1 R, Kohn B. 1999. Quantification of deoxynivalenol in wheat using an immunoaffinity column and liquid chromatography. J Chromatogra A 859:23–8.

Caldas ED, Silva AC. 2007. Mycotoxins in corn-based food products consumed in Brazil: an exposure assessment for fumonisins. J Agric Food Chem 55(19):7974–80.

Calleri E, Marrubini G, Brusotti G, Massolini G, Caccialanza G. 2007. Development and integration of an immunoaffinity monolithic disk for the on-line solid-phase extraction and HPLC determination with fluorescence detection of aflatoxin B1 in aqueous solutions. J Pharm Biomed Anal 44(2):396–403.

Charoenpornsook K, Kavisarasai P. 2006. Mycotoxins in animal feedstuffs of Thailand. KMITL Sci Technol J 6(1):25–8.

Chu FS. 1991. Mycotoxins: food contamination, mechanism, carcinogenic potential and preventive measures. Mutat Res 259(3–4):291–306.

Colak H, Bingol EB, Hampikyan H, Nazli B. 2006. Determination of aflatoxin contamination in red-scaled, red and black pepper by ELISA and HPLC. J Food Drug Anal 14(3):292–6.

Curtui V, Usleber E, Dietrich R, Lepschy J, M¨artlbauer E. 1998. A survey on the occurrence of mycotoxins in wheat and maize from western Romania. Mycopathologia 143(2):97103.

Czerwiecki L,Wilczy ´nska G. 2003. Determination of deoxynivalenol in cereals by HPLC-UV. Mycotoxin Res 19(1):31–4.

Häubl G, Berthiller F, Krska R, Schuhmacher R. Suitability of a 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.

Häubl G, Berthiller F, Rechthaler J, et al. Characterization and application of isotope-substituted (13C15)-deoxynivalenol (DON) as an internal standard for the determination of DON. *Food Addit Contam*. 2006;23(11):1187-1193.

Scott PM, Trucksess MW. 1997. Application of immunoaffinity columns to mycotoxin analysis. J AOAC Int 80(5):941–9.

Liang Y, Baker ME, Yeager T, Denton MB. 1996. Quantitative analysis of aflatoxins by high-performance thin-layer cphy utilizing a scientifically operated charge-coupled device detector Anal Chem 68:3885–91.

Nilufer D, Boyacioglu D. 2002. Comparative study of three different methods for the determination of aflatoxins in tahini. J Agric Food Chem 50(12):3375–9.

Odhav B, Naicker V. 2002. Mycotoxins in South African traditionally brewed beers. Food Addit Contam 19(1):55–61.

M´ oricz ´ AM, Fat´er Z, Otta KH, Tyih´ak E, Mincsovics E. 2007. Overpressured layer chromatographic determination of aflatoxin B1, B2, G1 and G2 in red paprika. Microchem J 85(1):140–4.

Pittet A, Royer D. 2002. Rapid, low-cost thin-layer chromatographic screening method for the detection of ochratoxin A in green coffee at a control level of 10 microg/kg. J Agric Food Chem 50(2):243–7.

Toteja GS, Mukherjee A, Diwakar S, Singh P, Saxena BN, Sinha KK, Sinha AK, Kumar N,

Nagaraja KV, Bai G, Krishna Prasad CA, Vanchinathan S, Roy R, Sarkar S. 2006. AflatoxinB(1) contamination of parboiled rice samples collected from different states of India: multi-centre study. Food Addit Contam 23(4):411–4.

Shundo L, Sabino M. 2006. Aflatoxin M1 in milk by immunoaffinity column cleanup with TLC/HPLC determination. Braz J Microbiol 37:164–7.

Danicke S, Ueberschar KH, Valenta H,Matthes S, Matthaus K, Halle I. 2004. Effects of graded levels of Fusarium-toxin-contaminated wheat in Pekin duck diets on performance, health and metabolism of deoxynivalenol and zearalenone. Br Poult Sci 45(2):264–72.

Delmulle B, De Saeger S, Adams A, De Kimpe N, Van Peteghem C. 2006. Development of a liquid chromatography/tandem mass spectrometry method for the simultaneous determination of 16 mycotoxins on cellulose filters and in fungal cultures. Rapid Commun Mass Spectrom 20(5):771–6.

Eke Z, Kende A, Torkos K. 2004. Simultaneous detection of A and B trichothecenes by gas chromatography with flame ionization ormass-selective detection. Microchem J 78(2):211–6.

Frisvad JC, Thrane U. 1987. Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection). J Chromatogr 404(1):195–214.

Garcia-Villanova RJ, Cordon C, Gonzalez Paramas AM, Aparicio P, Garcia Rosales ME.2004. Simultaneous immunoaffinity column clean-up and HPLC analysis of aflatoxins and ochratoxin A in Spanish bee pollen. J Agric Food Chem 52(24):7235–9.

Hayashi Y, Yoshizawa T. 2005. Analysis of cyclopiazonic acid in corn and rice by a newly developed method. Food Chem 93:215–21.

Hu YY, Zheng P, Zhang ZX, He YZ. 2006. Determination of aflatoxins in high-pigment content samples by matrix solid-phase dispersion and high-performance liquid chromatography. J Agric Food Chem 54(12):4126–30.

Kokkonen M, Jestoi M, Rizzo A. 2005. Determination of selected mycotoxins in mould cheeses with liquid chromatography coupled to tandem with mass spectrometry. Food Addit Contam 22(5):449–56.

Krska R, Molinelli A. 2007. Mycotoxin analysis: state-of-the-art and future trends. Anal Bioanal Chem 387:145–8.

Kuronen P. 1989. High-performance liquid chromatographic screening method for mycotoxins using new retention indexes and diode array detection. Arch Environ Contam Toxicol 18(3) :336–48.

Scott P. 1993. Recent developments in analysis for mycotoxins in foodstuffs. Trends Anal Chem 12:373–81.

Sobolev V. 2007. Simple, rapid, and inexpensive clean-up method for quantitation of aflatoxins in important agricultural products by HPLC. J Agric Food Chem 55(6):2136–41.

Sorensen LK, Elbaek TH. 2005. Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 820(2):183–96.

Visconti A, Lattanzio VM, Pascale M, Haidukowski M. 2005. Analysis of T-2 and HT-2 toxins in cereal grains by immunoaffinity clean-up and liquid chromatography with fluorescence detection. J Chromatogr A 1075(1–2):151–8.

Fazekas B, Tar A, Kovacs M. 2005. Aflatoxin and ochratoxin A content of spices in Hungary. Food Addit Contam 22(9):856–63.

Gonzalez G, Hinojo MJ, Mateo R, Medina A, Jimenez M. 2005. Occurrence of mycotoxin producing fungi in bee pollen. Int J Food Microbiol 105(1):1–9.

Hinojo MJ, Medina A, Valle-Algarra F, Gimeno-Adelantado J, Jimenez M, Mateo R. 2006. Fumonisin production in rice cultures of Fusarium verticillioides under different incubation conditions using an optimized analytical method. Food Microbiol 23(2):119–27.

Holcomb M, Sutherland J, Chiarelli M, Korfmacher W, Thompson H Jr, Lay J Jr, Hankins L, Cerniglia C. 1993. HPLC and FAB mass spectrometry analysis of fumonisins B1 and B2 produced by Fusarium moniliforme on food substrates. J Agric Food Chem 41(3):357– 60.

Jimenez M, Mateo R. 1997. Determination of mycotoxins produced by Fusarium isolates from banana fruits by capillary gas chromatography and high-performance liquid chromatography. J Chromatogr A 778(1–2):363–72.

Orti D, Hill R Jr, Liddle J, Needham L, Vickers L. 1986. High-performance liquid chromatography of mycotoxin metabolites in human urine. J Anal Toxicol 10(2):41–5.

Saez JM, Medina A, Gimeno-Adelantado JV, Mateo R, Jimenez M. 2004. Comparison of different sample treatments for the analysis of ochratoxin A in must, wine and beer by liquid chromatography. J Chromatogr A 1029(1–2):125–33.

Saha D, Acharya D, Roy D, Shrestha D, Dhar TK. 2007. Simultaneous enzyme immunoassay for the screening of aflatoxin B1 and ochratoxin A in chili samples. Anal Chim Acta 584(2):343–9.

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.

Trebstein A, Seefelder W, Lauber U, Humpf HU. 2008. Determination of T-2 and HT-2 toxins in cereals including oats after immunoaffinity cleanup by liquid chromatography and fluorescence detection. J Agric Food Chem 56(13):4968–75.

Trucksess MW, Whitaker TB, Weaver CM, Slate A, Giesbrecht FG, Rader JI, Betz JM, USDA ARS. 2009. Sampling and analytical variability associated with the determination of total aflatoxins and ochratoxin A in powdered ginger sold as a dietary supplement in capsules. J Agric Food Chem 57(2):321–5.

Valenta H. 1998. Chromatographic methods for the determination of ochratoxin A in animal and human tissues and fluids. J Chromatogr A 815(1):75–92.

Varelis P, Leong SL, Hocking A, Giannikopoulos G. 2006. Quantitative analysis of ochratoxin A in wine and beer using solid-phase extraction and high-performance liquid chromatography-fluorescence detection. Food Addit Contam 23(12):1308–15.

Ventura M, Vallejos C, Anaya IA, Broto-Puig F, Agut M, Comellas L. 2003. Analysis of ochratoxin Aa in coffee by solid-phase cleanup and narrow-bore liquid chromatographyfluorescence detector-mass spectrometry. J Agric Food Chem 51(26):7564–7.

Decastelli L, Lai J, Gramaglia M, Monaco A, Nachtmann C, Oldano F, Ruffier M, Sezian A, Bandirola C. 2007. Aflatoxins occurrence in milk and feed in Northern Italy during 2004–2005. Food Control 18(10):1263–6.

Foong-Cunningham S, Vasavada PC, Deak T, Fung DYC. 2006. Rapid detection of mycotoxigenic molds and mycotoxins in fruit juice. Bull Istanbul Technol Univ 54(4):28–38.

Iacumin L, Chiesa L, Boscolo D, Manzano M, Cantoni C, Orlic S, Comi G. 2009. Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages. Food Microbiol 26:65–70.

Jurado M, Vazquez C, Marın S, Sanchis V, Gonzalez-Jaen MT. 2006. PCR-based strategy to detect contamination with mycotoxigenic Fusarium species in maize. SystAppl Microbiol 29:681–9.

Korde A, Pandey U, Banerjee S, Sarma HD, Hajare S, Venkatesh M, Sharma AK, Pillai MR. 2003. Development of a radioimmunoassay procedure for aflatoxin B1 measurement. J Agric Food Chem 51(4):843–6.

Matrella R, Monaci L, Milillo MA, Palmisano F, Tantillo MG. 2006. Ochratoxin A determination in paired kidneys and muscle samples from swines slaughtered in southern Italy. Food Control 17:114–7.

Paepens C, Saeger S, Sibanda L, Barna-Vetr ´o I, L´eglise I, Van Hove F, Peteghem C. 2004. A flow-through enzyme immunoassay for the screening of fumonisins in maize. Anal Chim Acta 523(2):229–35.

Pal A, Acharya D, Saha D, Dhar TK. 2004. Development of a membrane-based immunofiltration assay for the detection of T-2 toxin. Anal Chem 76(14):4237–40.

Papadopoulou-Bouraoui A, Vrabcheva T, Valzacchi S, Stroka J, Anklam E. 2004. Screening survey of deoxynivalenol in beer from the European market by an enzyme-linked immunosorbent assay. Food Addit Contam 21:607–17.

Pemberton R, Pittson R, Biddle N, Drago G, Hart J. 2006. Studies towards the development of a screen printed carbon electrochemical immunosensor array for mycotoxins: a sensor for aflatoxin B 1. Anal Lett 39(8):1573–86.

Russell R, Paterson M. 2006. Identification and quantification of mycotoxigenic fungi by PCR. Process Biochem 41:1467–74.

Sangare-Tigori B, Moukha S, Kouadio HJ, Betbeder AM, Dano DS, Creppy EE. 2006. Cooccurrence of aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone in cereals and peanuts from Cote d'Ivoire. Food Addit Contam 23(10):1000–7.

Sibanda L, De Saeger S, Van Peteghem C, Grabarkiewicz-Szczesna J, Tomczak M. 2000. Detection of T-2 toxin in different cereals by flow-through enzyme immunoassay with a simultaneous internal reference. J Agric Food Chem 48(12):5864–7.

Thirumala-Devi K, Mayo MA, Hall AJ, Craufurd PQ, Wheeler TR, Waliyar F, Subrahmanyam A, Reddy DV. 2002. Development and application of an indirect competitive enzymelinked immunoassay for aflatoxin m(1) in milk and milk-based confectionery. J Agric Food Chem 50(4):933–7.

Vrabcheva T, Usleber E, Dietrich R, Martlbauer E. 2000. Co-occurrence of ochratoxin A and citrinin in cereals from Bulgarian villages with a history of Balkan endemic nephropathy. J Agric Food Chem 48(6):2483–8.

Wang L, Gan X. 2009. Biomolecule-functionalized magnetic nanoparticles for flow-through quartz crystal microbalance immunoassay of aflatoxin B(1). Bioprocess Biosyst Eng 32(1):109–16.

Yates I. 1986. Bioassay systems and their use in the diagnosis of mycotoxicoses. Diagnosis of mycotoxicoses. Dordrecht, The Netherlands: Martinus Nijhoff. 33:333–81.

Zimmerli B, Dick R, Baumann U. 1989. High-performance liquid chromatographic determination of citrinin in cereals using an acid-buffered silica gel column. J Chromatogr 462:406–10.

Zinedine A, Brera C, Elakhdari S, Catano C, Debegnach F, Angelini S, Santis BD, Faid M, Benlemlih M, Minardi V. 2006. Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. Food Control 17(11):868–74.