COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Qualitative and Quantitative Analysis of Mycotoxins

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ABSTRACT: Mycotoxin toxicity occurs at very low concentrations, therefore sensitive and reliable methods for their detection are required. Consequently, sampling and analysis of mycotoxins is of critical importance because failure to achieve a suitable verified analysis can lead to unacceptable consignments being accepted or satisfactory shipments unnecessarily rejected. The general mycotoxin analyses carried out in laboratories are still based on physicochemical methods, which are continually improved. Further research in mycotoxin analysis has been established in such techniques as screening methods with TLC, GC, HPLC, and LC–MS. In some areas of mycotoxin method development, immunoaffinity columns and multifunctional columns are good choices as cleanup methods. They are appropriate to displace conventional liquid–liquid partitioning or column chromatography cleanup. On the other hand, the need for rapid yes/no decisions for exported or imported products has led to a number of new screening methods, mainly, rapid and easy-to-use test kits based on immuno-analytical principles. In view of the fact that analytical methods for detecting mycotoxins have become more prevalent, sensitive, and specific, surveillance of foods for mycotoxin contamination has become more commonplace. Reliability of methods and well-defined performance characteristics are essential for method validation. This article covers some of the latest activities and progress in qualitative and quantitative mycotoxin analysis.

Introduction

Mycotoxins are secondary metabolites of fungi. Due to the widespread distribution of fungi in the environment, mycotoxins are considered to be one of the most important contaminants in foods and feeds. According to the Food and Agriculture Organization (FAO), more than 25% of the world's agricultural production is contaminated with mycotoxins, resulting in economic losses in the grain industry (Cazzaniga and others 2001). Toxic compounds can contaminate food and feedstuffs and these contaminated materials may be pathogenic for animals and humans; therefore, one of the most effective measures to protect the public health is to establish reasonable regulatory limits of these toxins. Consequently, guidelines regarding the allowed levels of mycotoxins present in food and feed products and in raw materials have been established by the FAO (FAO 1995).

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.

Although a considerable number of studies have been done, more research on mycotoxin detection is highly needed to provide a sound scientific basis for recommendations for both pre- and postharvest measures. The Codex Committee on Food Additives and Contaminants (CCFAC) has developed codes of practice to reduce contamination of food and animal feed with mycotoxins, such as aflatoxins, ochratoxin A (OTA), and patulin (Stuart and Slorach 2002). For public health protection and international trade, more sensitive and accurate analytical methods for mycotoxins are needed. Furthermore, there is a concern at national and international levels to prevent and reduce mycotoxin contamination in food and feedstuffs. This article reviews the analytical methods of mycotoxins.

Mycotoxins

Mycotoxins are toxic substances naturally produced by molds (fungi) that may contaminate agricultural commodities by growing on them. Despite efforts to control fungal contamination, toxigenic fungi are everywhere in nature and they can contaminate a wide range of agricultural products due to mold infestation both before and after harvest wherever humidity and temperature are sufficient. Thousands of mycotoxins exist, but only a few present considerable food safety hazards. *Aspergillus*, *Fusarium*, and *Penicillium*, all known field fungi, are the natural fungal flora associated with foods. The most prominent mycotoxins are aflatoxins, deoxynivalenol (DON), zearalenone (ZEA), ochratoxin, fumonisin, and patulin (Gaag and others 2003). These compounds

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cause adverse health effects such as kidney and liver damage (deterioration), mutagenic and teratogenic effects, birth defects, and cancers (specially liver cancer) that result in symptoms ranging from skin irritation to immuno suppression, neurotoxicity, and death (Bennett and Klich 2003). Aflatoxin B1 (AFB1) and fumonisins are human carcinogens, and patulin is suspected as human carcinogens. DON and other trichothecenes, as well as AFB1, are likely to exert immunosuppressive effects, and fumonisin B1 (FB1) may contribute to neural tube defects. Renal dysfunction due to OTA exposure (suspected in Balkan endemic nephropathy) is also a potentially significant problem, especially as this could exacerbate impaired renal function in individuals with diabetes, a burgeoning worldwide epidemic that is highly likely to grow. There is also uncertainty related to the effects of chronic, low-level, long-term exposure to single and/or multiple mycotoxins, which may be the case even for individuals consuming a diverse diet (Lopez-Garcia and others 1999).

The Intl. Agency for Research on Cancer's (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans and the U.S. Natl. Toxicology program have reviewed hundreds of chemicals, mixtures, and natural products and then graded the cancer risk posed to humans (Abnet 2007). All analytical procedures include 3 steps: extraction, purification (if necessary), and determination (Gobel and Lusky 2004; Ren and others 2007).

Analysis of Mycotoxins

With the decrease of restrictive levels of mycotoxins in foods and feeds defined by the European Union (EU) and other developed countries (EC466 2001; EC472 2002), development and validation of more robust analytical methods for the determination of mycotoxins are urgently requested. Current analytical techniques mainly include fast screening methods and confirmatory quantification. Standardized methods for aflatoxins (EN12955 1999; EN14123 2001), ochratoxin A (EN14132 2003), fumonisins (EN13585 2001; EN14352 2004), and patulin (EN14177 2003) in various foods are available, and methods of analysis for trichothecenes in food and various other mycotoxins in feed are planned to be developed in the near future. A full set of official methods of analysis for mycotoxins has been reported by Gilbert and Anklam (2002).

Sampling

The obvious reason for sampling of a material is to obtain a portion for the estimation or observation of attributes of the particular lot; the sample must be representative of the lot. Based on the measured sample concentration, some decision is made about the edible quality of the bulk lot or the effect of a treatment or a process on reducing mycotoxins in the lot. Sampling is a general requirement for all methods in mycotoxin analysis. Due to the high heterogeneity of mycotoxins, sampling plans are very important to obtain representative samples (JECFA 2001).

Traditional methods of sampling and sample preparation of agricultural crops and foodstuffs are usually not adequate for mycotoxin analyses because mycotoxin contamination is usually heterogeneous, which creates problems in obtaining a representative sample for analysis. Studies on a wide variety of agricultural products such as peanuts and shelled corn indicate that a very small percentage (0.1%) of the kernels in the lot is contaminated and the concentration on a single kernel may be extremely high (Cucullu and others 1966; Johansson and others 2000a).

A common feature of all sampling plans is that the whole primary sample must be ground and mixed so that the analytical test portion has the same concentration of toxin as the original sample. A mycotoxin-sampling plan is defined by a mycotoxin test procedure and a defined accept/reject limit. Sampling for mycotoxin detection specifies how the sample will be selected or taken from the bulk lot and the size of the sample. Sample selection procedures used to take a sample from a bulk lot is extremely important. Every individual item in the lot should have an equal chance of being chosen (called random sampling) (Parker and others 1982; Hurburgh and Bern 1983).

Two types of mistakes cause inconsistency among mycotoxin test results: First, good lots (in the range of regulatory limits) that may be rejected; the so-called sellers' risk (false-positives). Second, bad lots (over the regulatory limits) that may be accepted by the sampling program; the so-called buyers' risk (false-negatives). A plot of the acceptance probability $P(M)$ compared with the lot concentration M is called an operating characteristic (OC) curve. As M approaches 0, $P(M)$ approaches 1% or 100%, and as M becomes large, $P(M)$ approaches zero. The shape of the OC curve is uniquely defined for a particular sampling plan design with designated values of sample size, degree of comminution, subsample size, analytical method type, number of analyses, and the accept/reject limit. Increasing the size of a sample decreases both the buyers' and sellers' risks but it will be very expensive. The best plan will minimize both sellers' and buyers' risks (FAO 1993; Whitaker and others 1995; EC 1998, 2002; Johansson and others 2000b, 2000c).

The majority of sampling plans used in the mycotoxins area have been concerned with the control of aflatoxin concentrations in peanuts (Whitaker and others 1995), while others have studied aflatoxins in pistachios (Schatzki 1995), maize (Jewers and others 1988), and figs (Sharman and others 1994). Modeling and a subsequent simulating study for retail-sampling strategies have been carried out by MacArthur and others (2006). The number of containers sampled can vary from one-fourth in small lots (less than 20 metric tons) to the square root of the total number of containers for large (greater than 20 metric tons) lots (FAO/WHO 2001). The maximum sample result can be 4 to 5 times the lot concentration. Therefore, the average of the 10-sample result is the best estimate of the lot concentration. Furthermore, the distribution of the 10-sample result for each lot is not always symmetrical about the lot concentration (Whitaker 2006).

Number of incremental samples to be taken depends on the weight of the lot and kind of food such as cereals and cereal products, dried fruits, nuts, and spices that have been tabulated in EC401/2006. For example in more than 50 tons lot weight of cereals we need to take 100 incremental samples from sub lots that make 10 kg aggregate sample weight; however, for less than 50 tons lot weight of cereals we need 3 to 10 incremental samples depend on lot weight to make 1- to 10-kg aggregate sample. The weight of the incremental sample shall be about 300 g in case of dried figs, groundnuts and nuts. In the case of lots in retail packings, the weight of the incremental sample depends on the weight of the retail packing (EC401/2006).

The accumulation of many small incremental portions is called a bulk sample. If the bulk sample is larger than desired, the bulk sample should be blended and subdivided until the desired sample size is achieved. The smallest sample size that is subdivided from the bulk sample and comminuted in a grinder in the sample preparation step is called the test sample.

The main economic factors involved with the overall cost of a mycotoxin testing program are the cost of sampling, the cost of sample preparation, the cost for the actual analysis, and in some instances the cost of sample shipment from the point of sampling to the laboratory that the analysis is performed (Campbell and others 1986).

Sample preparation

Extraction methods. The extraction from a sample depends on both physicochemical properties of the sample matrix and the

toxin. Only exceptional methods such as infrared spectroscopic techniques are able to draw data from the ground and homogenized sample (Kos and others 2003). In all other cases, the sample or ground sample should be blended with extraction solvent in a high-speed blender or mechanical shaker. Then the slurry must be filtered and will be ready for subsequent purification procedures, if necessary. Diatomaceous earth is sometimes included in the solvent system to enhance the filtration step.

Extraction can be performed by liquid–liquid extraction (LLE) using 2 immiscible liquid-phases or solid phase extraction (SPE) using a solid and a liquid phase. In the extraction step, depending on the conditions, the analyte (and any compounds with similar properties) will migrate into the extraction solvent until equilibrium is established. This way, the desired compounds can be concentrated in a solvent and interferences can be removed.

In some cases, multiple extractions are necessary for the analysis of mycotoxins. At the end all fractions are pooled for further treatment (cleanup). Ideally, the extraction solvent is able to remove only the mycotoxin of interest from the sample matrix. Due to the absence of such a completely specific extraction solvent, the solvents chosen are those that can remove as much mycotoxin as possible, while removing as little as possible of any interfering compounds. Also, it should easily be recoverable, nontoxic and nonflammable, and other aspects, such as volatility, stability, transparency to UV light, and environmental impact of solvents are also important. Volatile organic compounds (VOC) may cause ozone depletion for example; previously, chlorofluorocarbons (CFCs) were the great solvent choice for many market segments due to their effectiveness, low cost, and nonflammability. But today, there are serious restrictions on CFCs and chlorinated solvent use due to environmental concerns. Consequently, the solvent market has changed dramatically over the past decade. Evaluations of extraction techniques for trichothecenes from plant materials such as wheat using liquid–liquid extraction (LLE) and SPE procedures have shown that these techniques were competitive with those using commercially available high-quality SPE materials (Stecher and others 2007). The following are some important methods of extraction.

Liquid extraction. For solid samples like cereals, polar solvents can dissolve mycotoxins and extract them from the ground sample. Water is a polar solvent that can be used for extraction of some mycotoxins such as DON (Lisa and others 1999). Polar analytes favor polar solvents and pH plays a key role during extraction. Solvent extraction is also a useful technique for the analysis of liquid samples. Examples of the use of this technique include the analysis of aflatoxin M1 in milk (Cavaliere and others 2006). The most efficient solvents that have been used for extracting mycotoxins are the relatively polar solvents, such as methanol (Juan and others 2005), acetone, acetonitrile (Hinojo and others 2006), ethyl acetate, diethyl ether (Hayashi and Yoshizawa 2005), toluene (Sangare-Tigori and others 2006), and chloroform (Saez and others 2004; Ferracane and others 2007), or mixtures of them (Zinedine and others 2006). Small amounts of water will wet the substrate and offer higher extraction efficiencies, by increasing penetration of the solvent (mixture) into the hydrophilic material (Hinojo and others 2006). An acid solution as the aqueous phase can help the extraction process by breaking interactions between the toxins and sample constituents such as proteins. For example, Dunne and others (1993) have described a multi-mycotoxin method which used hydrochloric acid and dichloromethane for the extraction of mycotoxins from animal feed. Whereas Barna-Vetro and others (1996) used dichloromethane/citric acid for the extraction of ochratoxin A from cereals and Monbaliu and others (2009) used ethyl acetate/formic acid for multiextraction of trichothecenes from sweet pepper. Also, formic acid has been reported as a good aid for simultaneous extraction of fumonisins from maize (Zitomer and others 2008). On the other hand, in another study on co-occurrence of ochratoxin A and aflatoxin B1 in dried figs, higher toxin recovery was reported when alkaline extraction was used instead of conventionally acidic extraction (Senyuva and others 2005). Recently, Reddy and others (2009) used 0.5% KCl in 70% methanol for the extraction aflatoxin B1 from rice before using enzyme-linked immunosorbent assay (ELISA) for mycotoxin detection.

Solid phase extraction. One of the most significant recent improvements in the purification step is the use of SPE. Test extracts are cleaned up before instrumental analysis (thin-layer or liquid chromatography) to remove co-extracted materials that often interfere with the determination of target analytes.

SPE based on molecularly imprinted polymers is an intriguing concept for specific sample preparation and pre-concentration and it has been gaining increased interest in the fields of environmental, clinical, and food/beverage analysis. Synthetic receptors for the mycotoxins such as aflatoxin (Egner and others 2006), OTA (Stander and others 2000; Jodlbauer and others 2002; Sibanda and others 2002), DON, and ZEA (Weiss and others 2003) have been reported for use in solid phase extraction using the noncovalent self-assembly imprinting approach. SPE, especially combined polar and nonpolar materials, becomes a preferred technique as a selective and time-saving sample cleanup technique enabling almost complete removal of possibly interfering matrices (Stecher and others 2007). Using an SPE cartridge that contained a polymeric sorbent, aflatoxins B1, G1, B2, G2, and ochratoxin A, were extracted in 1 step (Ventura and others 2006; Ferracane and others 2007).

SPE of wine and beer ochratoxin A has been carried out on a C18 cartridge to achieve a 100-fold sample concentration for GC–MS extraction in dichloromethane and derivatization with bis [trimethylsilyl] trifluoroacetamide (Soleas and others 2001). Instead of off-line SPE, online SPE has been introduced and was able to offer a series of advantages. The use of online SPE techniques has made possible the development of faster methods by reducing the sample preparation time and thus increasing the sample throughput. Conditioning, washing, and elution steps can be performed automatically and some systems also permit to extract 1 sample while another one is being analyzed by LC (Rodriguez-Mozaz and others 2007).

Accelerated solvent extraction (ASE). In ASE, solvents are used at relatively high pressure and temperatures at or above the boiling point. In this case, parameters like temperature, pressure, static time, cell size, and solvent used are very important (Adou and others 2001; Salces and others 2001; Gentili and others 2004). In this method, a solid sample is placed and sealed in a cell after being filled with an extraction solvent. At static conditions, the fluid is held in the cell under elevated temperature and pressure for short periods, then fresh solvent is flushed through the cell and compressed gas is used to purge the sample extract from the cell into a collection vessel. The high temperature and pressure cause the solvent to be in the liquid state, therefore fast extraction can be achieved (Richter and others 1996). As an example, Juan and others (2005) used ASE (at 1500 psi, 40 °C, and 5 min) with methanol for extraction of ochratoxin A from rice. A rapid and simple supercritical fluid extraction method has been used for the direct screening of macrocyclic lactone mycotoxins (zearalenone and its derivatives), in maize flour samples, by continuous-flow electrochemical detection after cleanup on a Florisil adsorption cartridge (Zougagh and Rios 2008). Further studies to improve mycotoxin extraction of foods and feedstuffs are still needed.

Cleanup methods. Cleanup of sample is the removal of substances in the sample extract that may interfere with the detection of the analyte. As a large number of interfering compounds such

as pigments originally are present in a sample, the primary sample extracts must be cleaned-up in some determination methods to get more accurate and precise results. A variety of cleanup methods have been used.

There are a few ways to remove such additional compounds. As an example, for high levels of lipids present in certain commodities (nuts, cereals), which would interfere with subsequent analytical procedures, nonpolar solvents such as hexane can be included in the original solvent system, or they can be added after the homogenization and filtration steps to remove lipid constituents. Primary extracts in mixtures of acetone with water contain proteins that can be precipitated with lead acetate. Sometimes various pigments need to be removed from primary extracts. Cleanup methods which include extract application, a washing step, and application of chromogenic substrate have been carried out for cleanup of OTA from high-colored matrices such as licorice, ginger, nutmeg, black pepper, white pepper, and Capsicum spp. For spices, tandem immunoassay columns coupled with simple methanol-based extraction have been used (Goryacheva and others 2007a).

Most of the rapid methods based on immunochemical techniques usually do not require any further cleanup or analyte-enrichment steps. Thus, the diluted extracts can be used directly for immune-analytical methods, such as ELISA (Barna-Vetro and others 1997; Krska and Molinelli 2007). Also, there are some mycotoxin determination method with LC–MS/MS, which did not use cleanup (Delmulle and others 2006).

Liquid–liquid partitioning, SPE, column chromatography, immunoaffinity columns (IAC), and multifunctional cleanup columns can be used for the purification of extracts in mycotoxin analysis prior to instrumental analysis.

Liquid–liquid separation. Formerly, liquid–liquid partitioning was employed to remove unwanted matrix components in the sample extract. It is based on the partition between immiscible solvents, one of which contains the analyte. Hayashi and Yoshizawa (2005) used diethyl ether for the cleanup of corn and rice. The analyte then migrates into the other phase until equilibrium has been reached. This step can be performed several times with fresh solvent to extract the analyte quantitatively, for example, the determination of aflatoxin G1 in maize (Castegnaro and others 2006). Although the method is simple and easy to perform, it is used less frequently nowadays because it is time-consuming, labor-intensive, and large volumes of solvent are required.

Solid phase extraction (SPE). An SPE technique is based on partitioning of analytes and interfering compounds between a mobile and a stationary phase. The stationary phase, contained within the cartridge, is composed of a solid adsorbent or an immobilized (bonded) liquid phase. Available bound phases include ethyl (C2), octyl (C8), octadecyl (C18), cyclohexyl (CH), phenyl (PH) cyanopropyl (CN), diol (2OH), aminopropyl (NH2), and a selection of ion exchange phases. C18 is the most practical SPE column for mycotoxin detection (Medina and others 2004; Saez and others 2004). SPE is a more rapid, efficient, reproducible, and safer method than the traditional liquid–liquid extraction techniques and offers a wide range of selectivity.

Three different applications have been reported for the SPE process: sample cleanup, sample concentration, and matrix removal. In the sample cleanup mode, the SPE column retains the mycotoxin and allows impurities to pass through the column. In the sample concentration mode, large sample volumes are passed through the column and the retained mycotoxin is concentrated by eluting it with a small volume of solvent. In the matrix removal mode, it is used to retain interfering impurities and the mycotoxin is allowed to pass through the column (Betina 1993).

A typical SPE sequence starts with a conditioning step (namely, activating it with solvent). Then the aqueous sample extract is applied to the conditioned column and the analyte is trapped together with the matrix. After that, a rinsing step removes matrix compounds and, finally, the analyte is eluted from the column with an organic solvent and a further preconcentration step is employed by evaporating excess solvent with nitrogen gas.

Ion-exchange columns. Ion-exchange mechanisms are another kind of cleanup that are employed, if the analyte can be made present as an ion (such as moniliformin, MON). Anionic compounds isolate on strong anion exchange-bonded silica columns. The retention is based on the electrostatic attraction of a charged functional group of the analyte to the charged group on the silica surface of the column. To elute the analyte the bond to the sorbent must be broken. The electrostatic force is disrupted and the compound is eluted. Alternatively, a solution with high ionic strength is used for elution because of its higher affinity to the sorbent. It is important to note that the packing should not dry up between conditioning and sample addition and that, after regeneration, columns can be used again several times. SAX columns can be used for the determination of ochratoxin A and fumonisin (Betina 1993). In a recent study on the multi-mycotoxin detection of sweet pepper using LC–MS–MS, a strong anion-exchange column was used for cleanup of one split of extract while the other split was cleaned up by an aminopropyl column followed by an octadecyl column (Monbaliu and others 2009).

Immunoaffinity columns. Immunoaffinity columns (IACs) for cleanup purposes have become increasingly popular in recent years because they offer high selectivity (Cahill and others 1999; Abdulkadar and others 2004; Danicke and others 2004; Saez and others 2004; Zinedine and others 2006; Calleri and others 2007). They are easy to use for purification of samples which are contaminated with different mycotoxins. The analyte molecules (the mycotoxins) are bound selectively to the antibodies on the column after a preconditioning step. As matrix components do not interact with the antibodies, a rinsing step removes most of the possible interferences and the toxin can be eluted by antibody denaturation.

IACs feature a higher recovery than standard liquid–liquid partitioning. There are also commercially available columns for the aflatoxins, fumonisins, and type A and B trichothecenes such as DON and OTA that are used in some experiments; the method has been used for cleanup of aflatoxins (B1, B2, G1, and G2), patulin, and ergosterol in dried figs (Karaca and Nas 2006). Selectivity of the IAC cleanup was proven by comparison with nonspecific solid phase extraction using octadecylsilica (ODS) sorbent (Mhadhb and others 2006). Regeneration of IACs for reuse in aflatoxin, ochratoxin A, fumonisin, and zearalenone analyses has been investigated by (Scott and Trucksess 1997). Columns are prepared by binding antibodies specific for a given mycotoxin to a specially activated solid phase support and packing the support suspended in aqueous buffer solution into a cartridge. The mycotoxin in the extract or fluid binds to the antibody, while impurities are removed with water or aqueous solution; and then the mycotoxin is desorbed with a miscible solvent such as methanol.

There are many experiments on comparison of IAC to other methods like SPE (Hu and others 2006) and comparison of different kinds of IACs. A study of Trucksess and others (2006) showed 80% recovery for aflatoxin IAC and 70% by multifunction aflaochra IAC. Cleanup using IAC was more sensitive than a Mycosep multifunctional column (MFC) for milk samples (Chen and others 2005). IAC is a well-known method for cleanup and enrichment techniques. The Association of Official Analytical Chemists and the European Union have validated methods which address cleanup of few food commodities using conventional and IAC approaches (Scott and Trucksess 1997; Castegnaro and others

2006); however, in some studies SPE was preferred because with large amounts of mycotoxins these levels would surpass the capacity of IAC columns (Hinojo and others 2006). Using IACs, even small amounts of mycotoxins can be detected with confidence. Visconti and others (1999) stated that the use of immunoaffinity chromatography in the purification step provides a number of advantages over conventional methods, such as clean extracts due to the high specificity of the antibodies for one toxin or a group of related toxins, high precision and accuracy over a wide concentration range of interest, rapidity of the purification step, and reduction in the use of hazardous solvents.

Multifunctional columns for the simultaneous determination of OTA and ZEA are also available. Wang and others (2008) used AOZ multitoxin method detection based on HPLC in the determination of 6 kinds of mycotoxins (aflatoxins B1, B2, G1, and G2, OTA, and ZEA) of air samples. The fact that columns can only be used once and their relative high costs are major disadvantages. Columns are commercially available.

Mycosep™ columns. Rapid multifunctional Mycosep columns remove matrix components efficiently and can produce a purified extract within a very short time. The Mycosep multifunctional cleanup columns consist of adsorbents such as charcoal, celite, polymers, and ion-exchange resins which are packed in a plastic tube and used to remove the entire matrix leaving the desired compound in solution on top of the column (Akiyama and others 2001; Mateo and others 2002). A rubber flange, a porous frit, and a 1-way valve on the lower end ensure that the extract is forced through the packing material when the column is inserted into the culture tube. On top of the plastic tube, the purified extract appears within seconds. Large molecules, proteins, fats, carbohydrates, and pigments are all adsorbed on the solid phase.

Columns are usually suitable for one analyte only, such as Mycosep 229 Ochra column for the determination of OTA (Buttinger and others 2003, 2004) and DON in maize and wheat, and ZEA in maize (Krska and others 2005), and for a range of mycotoxins such as DON and other A- and B-trichothecenes (Weingartner and others 1997).

Qualitative and Quantitative Analysis

Surveillance of foods for mycotoxin contamination has become more common since analytical methods for detecting mycotoxins have become more widespread. A mycotoxin analysis method should be simple, rapid, robust, accurate, and selective to enable simultaneous determination. Above all, low tolerance levels in feed and food require sensitive methods. The analytical results have to be fit for the purpose and the method has to be chosen accordingly. Analytical methods for the determination of mycotoxins commonly have the following steps: sampling, homogenization, extraction, and cleanup which might include sample concentration. The final separation and detection of compounds of interest is usually achieved by either chromatographic techniques followed by various detection methods or by immunochemical methods. While immunochemical methods rely on specific antibodies for each mycotoxin, chromatographic techniques can separate a huge number of analytes.

HPLC has become the main method for mycotoxin analysis. Coupled with a variety of detectors, practically all mycotoxins have been separated and detected by HPLC. Fumonisins, aflatoxins, ZEA, and OTA are routinely analyzed by HPLC (Shephard 1998; Coker 2000).

There are some reports of validated methods for the analysis of mycotoxins, such as DON, T2 toxin and HT-2 toxin in cereals by gas chromatographic (GC) methods coupled with flame ionization detector (FID) and mass spectrometry (MS) (Eskola and others 2001; Krska and others 2001; Eke and others 2004; Melchert and Pabel 2004). An European Committee (EC)-funded project within the framework of the SMT-programme was organized by Pettersson and Langseth (2002), in which gas chromatographic methods for nivalenol, DON, HT-2, and T-2 toxin were assessed. However, several method problems were identified in that study: higher trichothecene response for calibrants in the presence of matrix than for pure calibrants, nonlinear calibration curves, drifting response for trichothecenes, and carry-over or memory effects from previous samples and matrix interference.

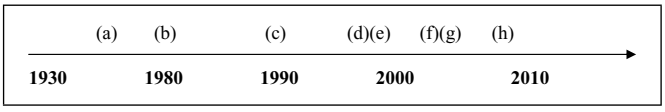

Figure 1—Historical perspective of analytical methods in toxin detection (Hawkins 2005).

Nowadays, ELISAs (enzyme linked immuno-sorbent assays) have also become widespread in mycotoxin determination. Test kits are available for practically all relevant mycotoxins. Not only cleanup methods are continuously optimized, but also instrumental methods.

HPLC–MS/MS has become the most rising analytical tool for the determination of mycotoxins and their metabolites (Berthiller and others 2007; Spanjer and others 2008). In contrast to GC-based methods, polar compounds are quickly reachable without the need of derivatization. Further advantages include low detection limits, the ability to generate structural information of the analytes, the minimal requirement of sample treatment, and the possibility to cover a wide range of analytes differing in their polarities. Finally, mass spectrometers are rather general detectors that are not so dependent on chemical characteristics like UV absorbance or fluorescence.

There are many different methods that have been developed for mycotoxin detection: (a) TLC, (b) mouse bioassay, (c) HPLC and GC, (d) protein phosphatase inhibition, (e) HPLC/MS, GC/MS, (f) ELISA, (g) HPLC/MS-MS, (h) PCR (see Figure 1). Summary of some analytical methods have been tabulated in Table 1.

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 (Trantham and Wilson 1984; 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 semiquantitative, although it is rapid analysis and low cost for analyzing a large number of samples.

Table 1—Multi mycotoxin detection.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Trichothecenes, aflatoxins(B1, B2, G1, and G2), OTA, ZEA, fumonisins and alternaria toxins	Liquid chromatography tandem mass specrometry	Sweet pepper	Monbaliu and others	Development of a multi-mycotoxin liquid chromatography/tandem mass spectrometry method for sweet pepper analysis	Rapid Commun Mass Spectrom 23(1):3–11	2009
Aflatoxin B1	Indirect competitive ELISA	Rice	Reddy and others	Detection of Aspergillus spp. and aflatoxin B1 in rice in India	Food Microbiology 26:27–31	2009
Fumonisins	Liquid chromatography (LC) with fluorescence (FD) and mass spectrometry (MS) detectors	Corn-based food	Silva and others	Analysis of fumonisins in corn-based food by liquid chromatography with fluorescence and mass spectrometry detectors.	Food Chemistry 112:1031–37	2009
OTA		Dry sausages	Iacumin and others	Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages.	Food Microbiology 26:65–70	2009
Aflatoxin B1	Flow through quartz cristal microbalance (QCM) immunoassay	–	Wang and Gan	Biomolecule-functionalized magnetic nanoparticles for flow-through quartz crystal microbalance immunoassay of aflatoxin B(1)	Bioprocess Biosyst Eng 32(1):109–116	2009
Aflatoxins(B1, B2, G1, and G2), Alternaria toxins, cyclopiazonic acid, fumonisins, ochratoxin, patulin, trichothecenes, ZEA	Review paper on sampling and analysis of mycotoxins	–	Shephard and others	Developments in mycotoxin analysis: an update for 2007–2008	World Mycotoxin Journal 2(1):3–21	2009
OTA	HPLC-MS/MS	Cheese	Zhang and others	Direct monitoring of ochratoxin A in cheese with solid-phase microextraction coupled to liquid chromatography-tandem mass spectrometry	J chromatography A, in press	2009
Aflatoxins and OTA	Reversed-phase liquid chromatography	Dietary supplements	Trucksess and others	Sampling and Analytical Variability Associated with the Determination of Total Aflatoxins and Ochratoxin A in Powdered Ginger Sold As a Dietary Supplement in Capsules	Journal of agricultural and food chemistry 57(2):321–325	2009

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins B1, B2, G1, G2; OTA; ZEA and fumonisins FB1 and FB2, DON	HPLC - postcolumn photochemical derivatization	Corn	Ofitserova and others	Multiresidue Mycotoxin Analysis in Corn Grain by Column High-Performance Liquid Chromatography with Postcolumn Photochemical and Chemical Derivatization: Single-Laboratory Validation	J AOAC int 92(1): 15–25	2009
OTA	LC-FD LC-MS-MS	Dry pasta	NG and others	Survey of Dry Pasta for Ochratoxin A in Canada	J food Prot 72(4): 890–3	2009
Aflatoxins(B1, B2, G1, and G2)	LC	Peanut butter sesame paste	Li and others	Natural Occurrence of Aflatoxins in Chinese Peanut Butter and Sesame Paste	J. Agric. Food Chem. 57(9):3519–3524	2009
Aflatoxins (B1, B2, G1, and G2)	Liquid chromatography–mass spectrometry	Nuts, cereals, dried fruits, and spices	Nonaka and others	Determination of aflatoxins in food samples by automated on-line in-tube solid-phase microextraction coupled with liquid chromatography–mass spectrometry	Journal of Chromatography A 1216(20): 4416–4422	2009
Aflatoxins (B1, B2, G1, and G2), ochratoxin, ZEA DON, fumonisins, T-2, HT-2,	Ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry (UHPLC/MS/MS)	Different cereal food	Beltran and others	Determination of mycotoxins in different food commodities by ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry	Rapid Commun Mass Spectrom 23(12):1801–1809	2009
OTA	Solid-phase microextraction (SPME)- LC-FD	Green coffee	Vatinno and others	Determination of ochratoxin A in green coffee beans by solid-phase microextraction and liquid chromatography with fluorescence detection	J Chromatogr A 1187(1-2):145–50	2008
Aflatoxins B1, B2, G1, and G2 and ochratoxin A	Multitoxin immunoaffinity column cleanup with liquid chromatography (LC)	Ginseng and ginger	Trucksess and others	Determination of aflatoxins B1, B2, G1, and G2 and ochratoxin A in ginseng and ginger by multitoxin immunoaffinity column clean-up and liquid chromatographic quantitation: Collaborative study	Journal of AOAC International 91(3):511–523	2008

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
ZEA	Direct competitive enzyme-linked immunosorbent assay (DC-ELISA)	Cereal	Thongrussamee and others	Monoclonal-based enzyme-linked immunosorbent assay for the detection of zearalenone in cereals	Food Addit Contam 25(8):997–1006	2008
OTA	HPLC-FD	Wine	Tafuri and others	A rapid high-performance liquid chromatography with fluorescence detection method developed to analyze ochratoxin A in wine	J Food Prot 71(10):2133–7	2008
OTA	HPLC-FD	Grapes, dried vine fruits, and winery byproducts	Solfrizzo and others	Determination of ochratoxin A in grapes, dried vine fruits, and winery byproducts by high-performance liquid chromatography with fluorometric detection (HPLC-FLD) and immunoaffinity cleanup	J Agric Food Chem 56(23):11081–6	2008
33 mycotoxins include of Aflatoxins (B1, B2, G1, and G2) OTA, DON, ZEA, T-2 toxin, HT-2 toxin and others	LC-MS/MS	Peanut, pistachio, wheat, maize, cornflakes, raisins, figs	Spanjer and others	LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs	Food Addit Contam 25(4):472–89	2008
Fumonisin B2, HT-2 toxin, patulin, and ZEA	Liquid chromatography combined with time-of-flight mass spectrometry (LC-TOF-MS)	Dried figs	Senyuva and Gilbert	Identification of fumonisin B2, HT-2 toxin, patulin, and zearalenone in dried figs by liquid chromatography-time-of-flight mass spectrometry and liquid chromatography-mass spectrometry	J Food Prot 71(7):1500–4	2008
Macrocyclic lactone mycotoxins (zearalenone, ZON; alpha-zearalenol, alpha-ZOL; and beta-zearalenol, beta-ZOL)	Supercritical fluid extraction (SFE) and clean-up on Florisil adsorption cartridge before Chromatography	Maize flour	Zougagh and Rios	Supercritical fluid extraction of macrocyclic lactone mycotoxins in maize flour samples for rapid amperometric screening and alternative liquid chromatographic method for confirmation	J Chromatogr A 1177(1):50–7	2008
T-2 and HT-2 toxins	LC-FD	Cereals	Trebstein and others	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):4968–4975	2008

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Fumonisins	LC-MS-MS	Maize	Zitomer and others	A single extraction method for the analysis by liquid chromatography/tandem mass spectrometry of fumonisins and biomarkers of disrupted sphingolipid metabolism in tissues of maize seedlings	Anal Bioanal Chem 391:2257–63	2008
Aflatoxins (B1, B2, G1, and G2), OTA, ZEA	HPLC-FD	Poultry house	Wang and others	Simultaneous detection of airborne aflatoxin, ochratoxin and zearalenone in a poultry house by immunoaffinity clean-up and high-performance liquid chromatography	Environ Res 107(2):139–44	2008
For 25 contaminants	ACQUITY UPLC separation and detection with a Waters Quattro Premier XE tandem quadrupole mass spectrometer	A variety of sample types	Kok and others	Rapid multi-mycotoxin analysis using ACQUITY UPLC and Quattro Premier XE	Waters Applications Note 2007 Volume: Page: 5 pp	2007
Aflatoxins, ochratoxin, fumonisins, trichothecenes		Tropical cereals	Magan and Aldred	Postharvest control strategies: Minimizing mycotoxins in the food chain.	Int J Food Microbiol 2007 Jul 31	2007
Aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and zearalenone.		Cereal grains	Bullerman and Bianchini	Stability of mycotoxins during food processing	Int J Food Microbiol 2007 Jul 31	2007
Aflatoxins	HPLC aflatoxins were quantified by HPLC equipped with a C18 column, a photochemical reactor, and a fluorescence detector.	Agricultural commodities ground sample	Sobolev	Simple, rapid, and inexpensive cleanup method for quantitation of aflatoxins in important agricultural products by HPLC	J Agric Food Chem 2007; 55:2136–41	2007
Ochratoxin A (OTA) and 4-deoxynivalenol (DON)	Results of OTA and DON occurrence from the database gathered in Belgium	Beer	Harcz and others	Intake of ochratoxin A and deoxynivalenol through beer consumption in Belgium	Food Addit Contam, August 2007; 24(8):910–6	2007
Simultaneous estimation of aflatoxin B(1) [AFB(1)] and ochratoxin A (OA)	Membrane-based immunoassay consisting of a membrane with immobilized anti-AFB(1) and anti-OA antibodies and a filter paper attached to a polyethylene card below the membrane	Chili samples	Saha and others	Simultaneous enzyme immunoassay for the screening of aflatoxin B(1) and ochratoxin A in chili samples	Anal Chim Acta 2007 Feb 19; 584(2):343–9	2007
		Cereal	Berthiller and others	Chromatographic methods for the simultaneous determination of mycotoxins and their conjugates in cereals	Int J Food Microbiol 2007 Jul 31	2007

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Simultaneous aflatoxins (B(1), B(2), G(1), G(2)), ochratoxin A, fumonisins (B(1), B(2)), deoxynivalenol, zearalenone, T-2 and HT-2 toxins	Liquid chromatography/ tandem mass spectrometry -reversed-phase liquid chromatography coupled with electrospray ionization triple quadrupole mass spectrometry (LC/ESI-MS/MS) using, as chromatographic mobile phase,	Maize	Lattanzio and others	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 Sep 10; 21(20):3253–61	2007
Aflatoxins aflatoxins (B1, B2, G1, and G2)	Enzyme-linked immunosorbent assay (ELISA).	Rice artificially contaminated hull, bran, polished broken grains, and polished whole kernels)	Castells and others	Distribution of total aflatoxins in milled fractions of hulled rice	J Agric Food Chem 2007; 55:2760–4	2007
Simultaneously aflatoxins, type A trichothecenes, type B trichothecenes, OTA, zearalenone, fumonisins, and patulin	Comprehensive LC/MS/MS in a single run	Analysis of corn flake extracts	Rudrabhatla and others	Multicomponent mycotoxin analysis by LC/MS/MS	The 10th annual meeting of the Israel Analytical Chemistry Society Conference & Exhibition, January 23–4	2007
Simultaneously measure mycotoxins (NIV), (DON), AFG1, AFG2, AFB1, AFB2, FB1, FB2, Diacetoxyscripenol (DAS), T2-Toxine, Ochratoxin A, and ZEN	LC–MS/MS method HPLC (Thermo Scientific, San Jose, Calif).	Cattle Forages and Food Matrices	Huls and others	Analysis of mycotoxins in various cattle forages and food matrices with the TSQ Quantum Discovery MAX	30 Mass spectrometry advertising supplement the application notebook March 2007	2007
Reduced up to 88% aflatoxin B1, 44% zearalenone, and 29% for fumonisins ochratoxin. Standard Q/FIS was ineffective in reducing DON uptake			Avantaggiato and others	Assessment of the multi-mycotoxin-binding efficacy of a carbon/ aluminosilicate-based product in an in vitro gastrointestinal model	J Agric Food Chem 2007 May 19	2007

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Af B1, B2, G1, G2, M1, trichothecenes, DON diacetoxyscirpenol, T-2 toxin and HT-2 toxin), FB1, B2, B3, agaric acid, ergot alkaloids, OTA, ZEA patulin, phomopsins, sterigmatocystin,	Review		van Egmond and others	Regulations relating to mycotoxins in food : Perspectives in a global and European context	Anal Bioanal Chem 2007 May 17	2007
DON, ZEN, and fumonisins B1 and B2 (FB1, FB2)	HPLC system consisted of a P1000XR pump HPLC–MS/MS system.	Corn silage	Niderkorn and others	Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol, zearalenone and fumonisins in an in vitro simulated corn silage model	Food Addit Contam, April 2007; 24(4):406–15	2007
Fumonisins (FB1 and FB2) also analyzed for aflatoxins (B1, B2, G1, and G2) one by one	FB1 FB2:HPLC/fluorescence following naphthalene-2,3 dicarboxaldehyde (NDA) derivatization AFs onTLC) plate underUV light	Different corn-based food products	Caldas and Silva	Mycotoxins in corn-based food products consumed in Brazil: an exposure assessment for fumonisins	J Agric Food Chem *55*(19):7974–80	2007
Aflatoxin and ochratoxin	Optical waveguide lightmode spectroscopy (OWLS) technique in competitive and in direct immunoassays.	Barley and wheat flour	Ad́anyi and others	Development of immunosensor based on OWLS technique for determining aflatoxin B1 and ochratoxin A	Biosens Bioelectron 2007; 22:797–802	2007
Aflatoxin B1	HPLC CIM diskwas coupled through a switching valve to a reversed-phase column, namely, Chromolith Performance RP-18e. A fully automated HPLC fluorescence detection		Calleri and others	Development and integration of an immunoaffinity monolithic disk for the online solid phase extraction and HPLC determination with fluorescence detection of aflatoxin B1 in aqueous solutions	J Pharma Biomed Anal 2007; 44:396–403	2007
Extended multi-mycotoxin method, for 25 contaminants	Separation and detection with a Waters Quattro Premier XE tandem quadrupole mass spectrometer	A variety of sample types	Kok and others	Rapid multi-mycotoxin analysis using ACQUITY UPLC and Quattro Premier XE	Waters Applications Note 2007, Page: 5 pp	2007
Aflatoxin Bi (AFBi)	HPLC isocratic reverse-phase liquid chromatography (HPLC) using a LiChrospher 100 RP-18 (5 mm column 25 × 4.6 mm i.d.) EcoPack (Merck, Portugal), with post column derivatisation confirm by TLC	Cattle feed collected from 7 dairy cow's farms from Portugal	Martins and others	Occurrence of aflatoxin Bi in dairy cow's feed over 10 y in Portugal (1995 to 2004)	Rev Iberoam Micol 2007; 24:69–71	2007

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin B_1, citrinin, deoxynivalenol, fumonisin B_1, gliotoxin, ochratoxin *A*, and zearalenone	HPLC–MS, Zorbax SB-C18 column (Agilent Technologies, Palo Alto, USA) with a 1 mm Optiguard C18 precolumn. Mass spectrometry was performed on a quadrupole analyser equipped with electron spray ionization (ESI).	Corn silage	Richard and others	Toxigenic fungi and mycotoxins in mature corn silage	Available online 22 June 2007	2007
Ochratoxin A (OTA)	Immunoassay	High-colored matrices liquorice, ginger, nutmeg, black pepper, white pepper *Capsicum* spp. spices	Goryacheva and others	Rapid all-in-one three-step immunoassay for non-instrumental detection of ochratoxin A in high-coloured herbs and spices	Talanta Volume 72, Issue 3, 15 May 2007, Pages 1230–34	2007
Fusarium toxins fumonisins (FBs), moniliformin (MON), zearalenone (ZEA), and type-A and -B trichothecenes	HPLC or GC in combination with a variety of detectors Screening mycotoxins is performed by (TLC) ELISA	Feeds	Krska and others	Analysis of Fusarium toxins in feed	Animal Feed Science and Technology 137(3-4):241–64	2007
Aflatoxin B_1, citrinin, deoxynivalenol, fumonisin B_1, gliotoxin, OTA and zearalenone	High-performance liquid chromatography coupled to mass spectrometry (HPLC–MS)	Corn silage		Toxigenic fungi and mycotoxins in mature corn silage		2007
Aflatoxins; ochratoxins; fumonisins, deoxynivalenol; zearalenone	Analyzed by HPLC ochratoxin A and aflatoxin B1 was performed using a reversed phase Symmetry C18 column (15 cm _ 4.6 mm, 5Ìm particles) preceded by a Rheodyne guard 0.5 Ìm filter. The fluorescence detector emission for ochratoxin A emission for aflatoxin B1.	A blend of naturally contaminated grains	Avantaggiato and others	Assessment of the multi-mycotoxin-binding efficacy of a carbon/ aluminosilicate-based product in an *in vitro* gastrointestinal model	J Agric Food Chem 2007; 55:4810–9	2007
Fusariotoxin analysis DON, ZEA, FB1, ...	Liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS),	Maize meal	Cavaliere and others	Mycotoxins produced by Fusarium genus in maize: determination by screening and confirmatory methods based on liquid chromatography tandem mass spectrometry	Food Chem 105(2):700–10	2007

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Ochratoxin A (OTA)	Cleanup tandem immunoassay column	Ginger, nutmeg, black pepper, and white pepper		Rapid all-in-one 3-step immunoassay for non-instrumental detection of ochratoxin A in high-coloured herbs and spices	Talanta Vol. 72, Issue 3, 15 May 2007; 1230–4	2007
Simultaneous detection of aflatoxin B1 and ochratoxin A	Tandem immunoassay 1 mL column with 1 cleanup layer and two detection immunolayers ELISA Results confirmed byHPLC-fluorescence detection. LC–MS/MS with immunoaffinity column cleanup	Spices Ginger, pepper, chili	Goryacheva and others	Simultaneous noninstrumental detection of aflatoxin B1 and ochratoxin A using a cleanup tandem immunoassay column	Anal Chim Acta 2007; 590:118–24	2007
Aflatoxin B1 (AFB1), citrinin (CIT) and ochratoxin A (OTA)	HPLC with fluorimetry detection equipped with an injector 20 μL loop, a C18 spherisorb column (3 lm C18, 0.46 $*$ 25 cm), and a fluorescence detector (Spectra physic 2000), was used. Different excitation and emission fluorescence parameters	Rice	Nguyen and others	Occurrence of aflatoxin B1, citrinin and ochratoxin A in rice in.ve provinces of the central region of Vietnam	Food Chem 2007; 105:42–7	2007
	Contaminants with the highest level of evidence include aflatoxin, alcoholic beverages, 2,3,7,8-tetracholordibenzo-p-dioxin		Abnet	Carcinogenic food contaminants	Cancer Invest 2007 Apr–May; 25(3):189–96.	2007
Aflatoxin M_1 (AFM_1) and ochratoxin A (OTA)		Raw bulk milk	Boudra and others	Aflatoxin M_1 and ochratoxin A in raw bulk milk from french dairy herds	J Dairy Sci 2007; 90:3197–201	2007
Simultaneous determination of trichothecenes (NIV, DON, F-X, T-2 Ro-A, Ve-A)	HPLC coupled to UV and mass spectrometric (MS) detection.	Plant material such as wheat, wheat	Stecher and others	Evaluation of extraction methods for the simultaneous analysis of simple and macrocyclic trichothecenes	Talanta 2007; 73:251–7	2007
Zearalenone, a- and b-zearalenols, fumonisin B1	High-performance liquid chromatography coupled with mass spectroscopy (HPLC/MS) LC analysis by Varian system, 2 pumps, polar modified RP-18 column	Maize	Adejumo and others	Survey of maize from south-western Nigeria for zearalenone, α- and β-zearalenols, fumonisin B1 and enniatins produced by Fusarium species	Food Addit Contam, September 2007; 24(9):993–1000	2007

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins and ochratoxin A.	were determined by TLC and HPLC methods	Poultry feeds	Fraga and others	Potential Aflatoxin and Ochratoxin A production by *Aspergillus* species in poultry feed processing	Vet Res Commun,Volume 31, Number 3-/343-53/23 Dec 2006	2007
Simultaneous determination of aflatoxin B1 (AFB1) and ochratoxin A (OTA)	HPLC column was Bio-sil C18 HL 90-5 S (5 mm, 4.6 * 150 mm) 200 ng/g with a corresponding limit of detection	Olive oil	Ferracane and others	Simultaneous determination of aflatoxin B_1 and ochratoxin A and their natural occurrence in Mediterranean virgin olive oil	Food Addit Contam, Vol. 24. 2:173–80	2007
Simultaneous, aflatoxins (AFL), i.e., B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2), and ochratoxin A (OTA)	AF reversed-phase liquid chromatography (RPLC) with fluorescence detection after postcolumn UV photochemical derivatization. OTA was separated and determined by RPLC with fluorescence detection.	Ginseng and ginger	Trucksess and others	Use of multitoxin immunoaffinity columns for determination of aflatoxins and ochratoxin A in ginseng and ginger.	J AOAC Int 2007 Jul to Aug; 90(4):1042–9	2007
Aflatoxins or ochratoxins	Review	Tree nuts (almonds, pistachios, and walnuts)	Molyneux and others	Mycotoxins in edible tree nuts	Int J Food Microbiol 2007 Jul 31	2007
Aflatoxins, deoxynivalenol, fumonisins, zearalenone, T-2 toxin, ochratoxin and certain ergot alkaloids	Review	Crop plants	Richard	Some major mycotoxins and their mycotoxicoses: an overview.	Int J Food Microbiol 2007 Jul 31	2007
Aflatoxin B1, citrinin, deoxynivalenol, fumonisin B1, gliotoxin, ochratoxin A and zearalenone	High-performance liquid chromatography coupled to mass spectrometry (HPLC–MS).	Corn silage mycotoxins on nutrient agar	Richard and others	Toxigenic fungi and mycotoxins in mature corn silage	Food Chem Toxicol (2007) Jun 22	2007
13 trichothecenes, (SCIRP), 15-monoacetoxyscirpenol, 4,15-diacetoxyscirpenol, T-2 tetraol, HT-2 toxin, (DON), 15-, 3-acetyl DON, ZEA, α- and β-ZOL	Gas chromatography/mass spectrometry, zearalenone (ZEA), α- and β-zearalenol (α- and β-ZOL) by high-performance liquid chromatography (HPLC) with fluorescence and UV-detection.	Whole beans, roasted soy nuts, flour and flakes, textured soy protein, tofu, proteinisolate including infant formulas and fermented products (soy sauce)	Schollenberger and others	Natural occurrence of Fusarium toxins in soy food marketed in Germany	Int J Food Microbiol 2007; 113:142–6	

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins, type A trichothecenes, type B trichothecenes, Ochratoxin A, Zearalenone Fumonisins, and Patulin	LC/MS/MS	Corn flake		Multicomponent mycotoxin analysis by LC/MS/MS	The 10th annual meeting of the Israel analytical chemistry society 2007	2007
Mycotoxins within 12 minutes: (NIV), (DON), AfG1, AFG2, AFB1, AfB2, FB1, FB2, Diacetoxyscripenol (DAS), T2- Toxine, OTA, and (ZEN)	HPLC LC–MS/MS method for the determination of mycotoxins	Various cattle forages.		Analysis of mycotoxins in various cattle	Mass spectrometry	2007
Zearalenone, glucosides, malonylglucosides, di-hexose- and hexose–pentose disaccharides of zearalenone, and _- and _-zearalenol, were detected	LC coupled to tandem mass spectrometry (LC–MS/MS). analysis by (HPLC)-MS/MS. Aquasil C18 column (100_4.6 mm, 3 mm)	Using the model plant rabidopsis thaliana. After treatment of plant seedlings	Berthiller and others	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 Additives and Contaminants, November 2006; 23(11):1194–1200	2006
Aflatoxins	HPLC-fluorescence detection (FLD) with postcolumn electrochemical derivatization in a Kobra cell.	Chili powder, green bean, and black sesame.	Hu and others	Determination of aflatoxins in high-pigment content samples by matrix solid phase dispersion and high-performance liquid chromatography	J Agric Food Chem 2006, 54, 4126–30	2006
Aflatoxin B1, fumonisin B1, zearalenone, ochratoxin A	Aflatoxin B1, fumonisin B1, zearalenone using immunoassays, and ochratoxin A using a validated HPLC method with fluorescence detector	Rice, maize and peanuts	Sangare-Tigori and others	Co-occurrence of aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone in cereals and peanuts from Côte d'Ivoire	Food Additives and Contaminants, October 2006; 23(10):1000–1007	2006
Aflatoxins	ELISA HPLC All positive samples were also analyzed and confirmed by HPLC.	Red scaled, red and black pepper.	Colak and others	Determination of aflatoxin contamination in red-scaled, red and black pepper by ELISA and HPLC	Journal of Food and Drug Analysis, Vol. 14, No. 3, 2006, Pages 292–96	2006
Trichothecenes, ochratoxins, zearalenone, fumonisins, aflatoxins, enniatins, moniliformin	Atmospheric pressure ionisation (API) techniques in the late 80s, LC/MS has become a routine technique also in food analysis		Zöllner and Mayer-Helm		J Chromatogr A 2006 Nov 4	2006

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin; Ochratoxin A; Patulin; *Fusarium* toxins	PCR review		Paterson	Identification and quantification of mycotoxigenic fungi by PCR	Process Biochemistry Volume 41, Issue 7, July 2006, Pages 1467–74	2006
Trichothecenes, ochratoxins, zearalenone, fumonisins, aflatoxins, enniatins, moniliformin	LC–(API)MS review			Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography–atmospheric pressure ionisation mass spectrometry	Journal of Chromatography A Volume 1136, Issue 2, Pages 123–69	2006
Aflatoxin M1 in milk and B1 in feed	ELISA immunoassay, used as screening test, positive samples confirmed by HPLC	Milk and feed	Decastelli and others	Aflatoxins occurrence in milk and feed in Northern Italy during 2004 to 2005	Available online 27 October 2006	2006
Aflatoxins B1, G1, B2, G2 and ochratoxin A	Ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS), mass spectrometer used an electrospray ionization source operated in the positive mode to detect aflatoxins and in the negative mode to detect ochratoxin	Beer	Guillén and others	Ultra-performance liquid chromatography/tandem mass spectrometry for the simultaneous analysis of aflatoxins B1, G1, B2, G2 and ochratoxin A in beer	Rapid Communications in Mass Spectrometry Volume 20, Issue 21, Pages 3199–204	2006
Mycotoxins OTA, DON, AFB1, and FB were detected simultaneously	ELISA	Food sample		Rapid detection of foodborne contaminants using an Array Biosensor	Sensors and Actuators B 113 (2006) 599–607	2006
	Review		Malir and others	Monitoring the mycotoxins in food and their biomarkers in the Czech Republic.	Mol Nutr Food Res 2006 Jun; 50(6):513–8	2006
AFL and ochratoxin A (OTA)	Liquid chromatographic separation, and fluorescence detection	Ginseng and other selected botanical roots	Trucksess and others	Determination of aflatoxins and ochratoxin A in ginseng and other botanical roots by immunoaffinity column cleanup and liquid chromatography with fluorescence detection	J AOAC Int 2006 May-Jun; 89(3):624–30	2006

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Analysis of mycotoxins analysis of three mycotoxins. Aflatoxins (AFs): Aflatoxin G1	Aflatoxin G1 has been detected by liquid-liquid partitioning methods with HPLC detection as false-positive in some maize Fumonisins (FB): Compounds interfering with the FB's antibodies were also observed while analysing breakfast cereals leading to underestimation of FB. Ochratoxin A (OTA)	Maize	Castegnaro and others	Advantages and drawbacks of immunoaffinity columns in analysis of mycotoxins in food	Mol Nutr Food Res 2006 May; 50(6):480–7	2006
Ochratoxin (OT) and aflatoxin (AF)		Barley rootlets (BR)	Ribeiro and others	Influence of water activity, temperature, and time on mycotoxins production on barley rootlets.	Lett Appl Microbiol 2006 Feb; 42(2):179–84	2006
Trichothecenes, ochratoxins, zearalenone, fumonisins, aflatoxins, enniatins, moniliformin and several other mycotoxins	LC–(API)MS	Review		Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography–atmospheric pressure ionisation mass spectrometry	Journal of Chromatography A Volume 1136, Issue 2, 15 December 2006, Pages 123–69	2006
Simultaneously NIV, DON, ZEN, diacetoxyscirpenol, T-2 toxin, verrucarol, verrucarin A, neosolaniol, sterigmatocystin, roridin A, OTA,AFB1, AFB2,AFG1, AFG2	HPLC fluorescence detector, injector, gradient and data handling capability is required. The fluorescence detector settings: excitation 315 nm, emission >415 nm liquid chromatography/ tandem mass spectrometry (LC/MS/MS) method	Two fungal media were used as samples	Delmulle and others	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):771–6	2006
Aflatoxin; Ochratoxin A; Patulin; *Fusarium* toxins	PCR	Food stuff review		Identification and quantification of mycotoxigenic fungi by PCR	Process Biochemistry Volume 41, Issue 7, July 2006, Pages 1467–74	2006

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Ochratoxin A (OTA)	Extracts were subsequently analysed using reverse-phase high-performance liquid chromatography–fluorescence detection with post column ammoniation to improve the limit of detection.	Wine and beer	Varelis and others	Quantitative analysis of ochratoxin A in wine and beer using solid phase extraction and high-performance liquid chromatography–fluorescence detection	Food Additives and Contaminants, December 2006; 23(12):1308–15	2006
Aflatoxins (B1, B2, G1, and G2), patulin and ergosterol one by one	HPLC (Agilent, 1100 series, USA) equipped with a fluorescence detector (G1321A, Agilent, 1100 series, USA) after postcolumn bromination immunoaffinity column (Vicam, Watertown, MA, USA)	Dried figs	Karaca and Nas	Aflatoxins, patulin and ergosterol contents of dried figs in Turkey	Food Additives and Contaminants, May, 2006; 23(5):502–08	2006
Trichothecenes, ochratoxins, zearalenone, fumonisins, aflatoxins, enniatins, moniliformin, and several other mycotoxins	Application of LC–(API)MS atmospheric pressure ionisation (API) techniques			Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography–atmospheric pressure ionisation mass spectrometry	[b]Department of Clinical Pharmacology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Wien, Austria	2006
AFM1	Thin-layer chromatography for determining AFM1.compared with HPLC fluorescence detection	Raw, pasteurized and ultrahigh treated temperature (UHT) milk	Shundo and Sabino	Aflatoxin M1 in milk by immunoaffinity column cleanup with TLC/HPLC determination	Brazilian Journal of Microbiology (2006) 37:164–67	2006
Ochratoxin A (OTA) and aflatoxin B1 (AFB1) one by one	Mediterranean shores. It has a rectangular shape with 10.452 km^2 area. OTA was detected and quantified by reversed-phase HPLC. autosampler (Agilent 1100, G1313A, ALS) and a fluorescence detector A selected RP-18 column HPLC method for aflatoxin B1 analysis both fluorescence and UV detector	Wine-grapes in Lebanon on Czapek yeast extract agar (CYA). culture medium	El Khoury and others	Occurrence of Ochratoxin A- and Aflatoxin B1-producing fungi in lebanese grapes and ochratoxin a content in musts and finished wines during 2004	J Agric Food Chem 2006, 54, 8977–82	2006

Continued

Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
	ELISA		Mhadhbi and others	Generation and characterization of polyclonal antibodies against Microcystins Application to immunoassays and immunoaffinity sample preparation prior to analysis by liquid chromatography and UV detection	Talanta 70 (2006) 225–35	2006
Aflatoxin B1	TLC silica gel as an adsorbent and 7% methanol in chloroform as the developing solvent. And fluorodensitometrically using HPTLC plates aluminum sheets, silicagel 60 F254 precoated,	Rice	Toteja and others	Aflatoxin B1 contamination of parboiled rice samples collected from different states of India: a multicentre study	Food Additives and Contaminants, April, 2006; (23)4:411–14	2006
	enzyme linked immunosorbent assays (ELISA), flow through membrane based immunoasays, chromatographic techniques nucleic acid amplification assays, biosensors, and microarrays for detection of molds and mycotoxins.		Foong-Cunningham and others	Rapid detection of mycotoxigenic molds and mycotoxins in fruit juice	ARI The Bulletin of the Istanbul Technical University VOLUME 54, NUMBER 4	2006
AFB1 and OTA one by one	Quantitated by HPLC using a fluorescence detector.	Black and green olives of Greek origin	Ghitakou and others	Study of aflatoxin B1 and ochratoxin A production by natural micro.ora and Aspergillus parasiticus in black and green olives of Greek origin	Food Microbiology 23 (2006) 612–21	2006
Aflatoxins	High-performance liquid chromatography (HPLC)-fluorescence detection (FD), confirmed using HPLC-electrospray ionization (ESI) -mass spectrometry (MS).	Polished rice	Park and others	Effect of pressure cooking on aflatoxin B1 in rice	J Agric Food Chem 2006, 54, 2431–35	2006

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins (AFs) (B1, B2, G1, and G2), zearalenone (ZEA), and ochratoxin A (OTA)	high-performance liquid chromatography (HPLC) with fluorescence detection	Cereal grains	Li and others	[Simultaneous determination of aflatoxins, zearalenone and ochratoxin A in cereal grains by immunoaffinity column and high-performance liquid chromatography coupled with postcolumn photochemical derivatization]	Se Pu 2006 Nov; 24(6):581–4	2006
Aflatoxin B1 (AFB1)	Screen-printed carbon electrodes (SPCEs) bearing a surface-adsorbed antibody ELISA	Real samples from grain extracts.	Pemberton and others	Studies toward the development of a screen-printed carbon electrochemical immunosensor array for mycotoxins: a sensor for aflatoxin B1	Analytical Letters, 39:1573–86, 2006	2006
Aflatoxin B1, ochratoxin A, deoxynivalenol and T-2 toxin), one by one	Aflatoxin and ochratoxin by HPLC fluorescence. T-2 toxin and deoxynivalenol, by ELISA	Animal feeds.	Charoenpornsook and others	Mycotoxins in animal feedstuffs of Thailand	KMITL Sci. Tech. J. Vol. 6 No. 1 Jan.–Jun. 2006	2006
Aflatoxin B1-*N*7-guanine (AFB1-*N*7-Gua), major human aflatoxin-DNA	Stable isotope-labeled internal standard (AFB1-*N*7-15N5-Gua) HPLC C18 microbore HPLC column	Excreted in the urine.	Egner and others	Quantification of aflatoxin-B1-*N*7-guanine in human urine by high-performance liquid chromatography and isotope dilution tandem mass spectrometry.	Chem Res Toxicol 2006, 19:1191–95	2006
Fusarium metabulits moniliformin, acetamido-butenolide, chlamydosporol,) antibiotic Y, chrysogine, fusarin C, enniatins, 2-AOD-3-ol, aurofusarin	In a rat hepatoma (H4IIE-W), porcine epithelial kidney (PK-15), foetal feline lung.broblast, dog lymphoblast (D3447), and a human hepatocarcinoma (Hep G2) cell line Alamar BlueTM assay. HPLC high-performance liquid chromatography with photodiode array and mass spectrometric detection,	Extracts from rice cultures	Uhlig and others	Multiple regression analysis as a tool for the identification of relations between semiquantitative LC–MS data and cytotoxicity of extracts of the fungus Fusarium avenaceum	Toxicon 48 (2006) 567–79	2006
4-deoxynivalenol (DON or vomitoxin), DON and nivalenol	ELISA	Maize, wheat, and barley.		Production and characterization of a monoclonal antibody that cross-reacts with the mycotoxins nivalenol and 4-deoxynivalenol	Food Additives and Contaminants, August 2006; 23(8):816–25	2006

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin B1, B2, G1, and G2, ochratoxin A, and fumonisin B1, B2, and B3 one by one	HPLC, LC_MS, or high-performance thin-layer chromatography	Peanut butter, corn, peanuts, buckwheat flour, dried buckwheat noodles, rice, sesame oil, oatmeal, wheat flour, rye, buckwheat, green and roasted coffee beans, raisins, beer, wine	Sugita-Konishi and others	Occurrence of aflatoxins, ochratoxin A, and fumonisins in retail foods in Japan	J Food Prot. 2006 Jun;69(6):1365–70	2006
Simultaneous determination of 12 trichothecenes DON, NIV, 3-acetyldeoxy NIV, 15-acetyldeoxy NIV, fusarenon X, T-2 toxin, HT-2 toxin, neosolaniol, monoacetoxyscir-penol, diacetoxyscirpenol, T-2 triol, and T-2 tetraol	Liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS)	Wheat and oat samples	Klotzel and others	Determination of 12 Type A and B trichothecenes in cereals by liquid chromatography-electrospray ionization tandem mass spectrometry	J Agric Food Chem 2005, 53:8904–10	2005
			Whitlow and others	Mycotoxins in dairy cattle: occurrence, toxicity, prevention and treatment	Whitlow and Hagler, 2005. Proc. Southwest Nutr. Conf.: 124–38	2005
FUM1	LCMS		Yu and others	Developing a genetic system for functional manipulations of *FUM1*, a polyketide synthase gene for the biosynthesis of fumonisins in *Fusarium verticillioides*	FEMS Microbiology Letters Volume 248, Issue 2, 15 July 2005, Pages 257–64	2005
OTA, α-ZEA, β-ZEA zearalanol (taleranol), FB1, FB2, T-2 toxin, HT-2 toxin, T-2 triol, diacetoxyscirpenol (DAS), 15-monoacetoxyscirpenol (MAS), (DON), 3-acetyldeoxyNIV (3-AcDON), 15-acetyldeoxyNIV(15-AcDON), deepoxy-DON (DOM-1) and AFM1	LC_MS /MS Liquid chromatographic/tandem mass spectrometric methods using pneumatically assisted electrospray ionisation (LC–ESI-MS/MS)	Milk	Sørensen and Elbæk	Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry	J Chromatogr B, 820 (2005) 183–96	2005

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Fusarium mycotoxins NIV, DON, fusarenon-X, 3-acetyldeoxynivalenol, 3-acetyl-DONand 15-acetyl-DON, diacetoxy-scirpenol, HT-2 toxin, T-2 toxin, ZEN	RP-LC with atmospheric pressure chemical ionization triple quadrupole mass spectrometry (LC–APCI–MS/MS). LC–MS/MS analysis was performed	Maize	Berthiller and others	Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high-performance liquid chromatography–tandem mass spectrometry	Journal of Chromatography A, 1062 (2005) 209–16	2005
Co-occurrence of ochratoxin A and aflatoxin B1 one by one		Dried figs	Senyuva and others	Survey for co-occurrence of ochratoxin A and aflatoxin B1 in dried figs in Turkey using a single laboratory-validated alkaline extraction method for ochratoxin A.	J Food Prot 2005 Jul; 68(7):1512–5	2005
Aflatoxin M1	Enzyme immunoassay compared with a reference high-performance liquid chromatography method with a fluorescent detector.	Milk	Magliulo and others	Development and validation of an ultrasensitive chemiluminescent enzyme immunoassay for aflatoxin M1 in milk	J Agric Food Chem 2005, 53, 3300–05	2005
DON, Aflatoxins, Ochratoxin A, Zearalenone and Fumonisin	HPLC and postcolumn derivatization column: MYCOTOX^{TM} reversed-phase C18, 4.6 $\times$ 250 mm	Aliquot of the beverage	Ofitserova and others	Multiresidue mycotoxin analysis single run analysis of deoxynivalenol, aflatoxins, ochratoxin a, zearalenone and fumonisin by HPLC and postcolumn derivatization	www.pickeringlabs.com	2005
Aflatoxin M1, aflatoxin B1, and ochratoxin A.	HPLC pump Model 2248 together with a Low Pressure Mixer Fluorescence detection:.	Analyze 123 samples of 24-h diets	Sizoo and Van Egmond	Analysis of duplicate 24-h diet samples for aflatoxin B1, aflatoxin M1 and ochratoxin A	Food Additives and Contaminants, February 2005; 22(2):163–72	2005
Aflatoxins B_1, B_2, G_1 and G_2 (AFB1, AFB2, AFG1, AFG2) and ochratoxin A (OTA) one by one	high-performance liquid chromatography (HPLC) fluorescence detector	Spice ground red pepper, 6 black pepper, 5 white pepper, 5 spice mix, and 5 chili	Fazekas and others	Aflatoxin and ochratoxin A content of spices in Hungary	Food Additives & Contaminants, Volume 22, Issue 9 September 2005, pages 856–63	2005

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Simultaneous determination of T-2 and HT-2 toxins	HPLC- fluorescence quantified by reversed-phase HPLC with fluorometric detection (excitation wavelength 381 nm, emission wavelength 470 nm) after derivatization with 1-AN.	Cereal grains	Lattanzio and others	Analysis of T-2 and HT-2 toxins in cereal grains by immunoaffinity cleanup and liquid chromatography with fluorescence detection	Journal of Chromatography A, 1075 (2005) 151–58	2005
Ochratoxin A (OTA)	ELIZA	Roasted coffee	Lobeau and others	Development of a new cleanup tandem assay column for the detection of ochratoxin A in roasted coffee	Analytica Chimica Acta Volume 538, Issues 1-2, 4 May 2005, Pages 57–61	2005
Simultaneously aflatoxins B1, B2, G1, G2 and M1, ochratoxin A, mycophenolic acid, penicillic acid and roquefortine C simultaneously	HPLC_MS	Blue and white mold cheeses	Kokkonen and others	Determination of selected mycotoxins in mould cheeses with liquid chromatography coupled to tandem with mass spectrometry	Food Additives and Contaminants, May 2005; 22(5):449–56	2005
Ochratoxin A (OTA) analysis	(LC) with fluorescence detection (FD), (ELISA) kits, using anti-OTA antibodies (electrochemical immunosensors, fluorescence polarisation, lateral flow devices, enzyme-based flow through membranes, and surface plasmon resonance biosensors) Liquid chromatography-mass spectrometry represents an adequate alternative to LC-FD	Cereals, coffee, wine, and beer.	Visconti and De girolamo	Fitness for purpose–ochratoxin A analytical developments	Food Additives and Contaminants, Supplement 1 2005:37–44	2005
Aflatoxin M_1 (AFM_1) and ochratoxin A (OA)	Quantification by high-performance liquid chromatography (HPLC) with fluorescence detection.	Human Milk Bank	Navas and others	Aflatoxin M_1 and ochratoxin A in a human milk bank in the city of São Paulo, Brazil	Food Additives & Contaminants, Volume 22, Issue 5 May 2005, pages 457–62	2005
Ochratoxin A (OTA)	LC–MS system	Alcoholic beverages, wine and beer	Bacaloni and others	Automated online 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, 5518–25	2005

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins in a number of commodities, ochratoxin A (OTA) in wheat, deoxynivalenol (DON) in maize and wheat, and ZEA in maize	Review article	Aflatoxins in a number of commodities, ochratoxin A (OTA) in wheat, deoxynivalenol (DON) in maize and wheat, and ZEA in maize	Krska and others	Advances in the analysis of mycotoxins and its quality assurance	Food Additives and Contaminants, Volume 22, Number 4, April 2005, pp. 345–53(9)	2005
Aflatoxin (AF) or ochratoxin A (OTA) one by one	HPLC for AF OTA by (ELISA)	Seed-, pulses-, and cereal-flours and starches	Baydar and others	Aflatoxin and ochratoxin in various types of commonly consumed retail ground samples in Ankara, Turkey	Ann Agric Environ Med. 2005;12(2):193–7	2005
Aflatoxin, ochratoxin A,	Analyzed for the mycotoxins by RHM Technology. using high-performance liquid chromatography (HPLC).	Spices		Survey of spices for aflatoxins and ochratoxin A	Food Survey Information Sheets on the WWW:http://www.food.gov.uk/science/surveillance	2005
Fumonisin B1 and B2, T2 toxin, DON one by one	HPLC with fluorescence detector for fumonisins and with variable wavelength UV detector for T2 toxin and DON	161 cereal and cereal products 115 medicinal and herbal tea specimens 112 cereal and pulse products	Omurtag and others	A review on fumonisin and trichothecene mycotoxins in foods consumed in Turkey	ARI The Bulletin of the Ista nbul Technical University Volume 54, Number 4	2005
Fumonisin B1, fumonisin B2, zearalenone and ochratoxin A one by one	HPLCsystem (Varian, USA) with fluorescence detection.	Maize	Domijan and others	Fumonisin B1, fumonisin B2, zearalenone and ochratoxin A contamination of maize in Croatia	Food Additives and Contaminants, July 2005; 22(7):677–80	2005
Ochratoxin A (OTA) and aflatoxins B1, B2, G1 and G2	TLC and confirmation by HPLC extracts by HPLC with fluorescent detection.	Bee pollen	Gonzalez and others	Occurrence of mycotoxin producing fungi in bee pollen	Int J Food Microbiol 105 (2005) 1–9	2005
Fusarium mycotoxins (trichothecenes Type A and B, zearalenone) simultaneously	Liquid chromatography with tandem mass spectrometry (LC-ESI-MS/MS). HPLC	Cereals and cereal-based samples	Biselli and Hummert	Development of a multicomponent method for Fusarium toxins using LC–MS/MS and its application during a survey for the content of T-2toxin and deoxynivalenol in various feed and food samples	Food Additives and Contaminants, August 2005; 22(8): 752–60	2005

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin-fumonisins Deoxynivalenol Zearalenone one by one.	HPLC and LCMS for AF Fumonisins ELISA for AF and Fumonisins	45 commercial corn (maize) hybrids	Abbas and others	Aflatoxin and fumonisin contamination of commercial corn (*Zea mays*) hybrids in Mississippi	J Agric Food Chem 2002, 50, 5246–54	2005
Ochratoxin A (OTA)	Competitive direct enzyme-linked immunosorbent assay (cdELISA) and a competitive indirect ELISA (ciELISA) were used efficacy of cdELISA was also confirmed by the highperformance liquid chromatography method	Soybean samples	Yu and others	Development of a sensitive enzyme-linked immunosorbent assay for the determination of ochratoxin A	J Agric Food Chem 2005, 53, 6947–53	2005
AFM1	HPLC-fluorescence, AFM1 quantified by HPLCtandem mass spectrometry with negative electrospray ionization.	Whole milk, low fat milk, milk powder	Chen and others	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, 8474–80	2005
(DON), (ZEN), fumonisin B_1 (FB_1) and moniliformin (MON)	Cell cultures sensitive cell lines for preliminary screening of DON, ZEN, and MON contaminated feed and food extracts	Chinese hamster ovary cells (CHO-K1) most sensitive for DON and FB_1 with IC_{50} values	Cetin and Bullerman	Cytotoxicity of *Fusarium* mycotoxins to mammalian cell cultures as determined by the MTT bioassay	Food and Chemical ToxicologyVolume 43, Issue 5, May 2005, Pages 755–64	2005
OTA	Liquid chromatography coupled with a fluorescence detector and confirmed by methyl ester derivatization.	Rice samples	Juan and others	ASE of ochratoxin A from rice samples	J Agric Food Chem 2005, 53, 9348–51	2005
A and B type trichothecenes, namely 4,15-diacetoxy-scirpenol, T2-toxin, deoxynivalenol (DON) and nivalenol (NIV). After derivatization with N,N-dimethyl-trimethylsilyl-carbamate	Gas chromatography with flame ionization (GC-FID) or mass selective detection (GC–MSD)	Semolina and corn grits	Eke and others	Simultaneous detection of A and B trichothecenes by gas chromatography with flame ionization or mass selective detection	Microchem J 78 (2004) 211–6	2004

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
	A significant decrease in the relative weight of the bursa of Fabricius was noted at the highest concentration of AF (2 mg/kg) and combinations of 1 and 2 mg/kg AF and 2 and 4 mg/kg OA	Broilers	Verma and others	Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers	Br Poult Sci 2004 Aug; 45(4):512–8	2004
Ochratoxin A (OTA)	Using RP-HPLC with a fluorescence detection	Cereals, red wine, raisins and green coffee	Buttinger and others	Performance of new cleanup column for the determination of ochratoxin A in cereals and foodstu.s by HPLC-FLD	Food Additives and Contaminants, Vol. 21, No. 11 (November 2004), pp. 1107–14	2004
Fusarium-toxin-deoxynivalenol and zearalenone one by one	HPLC DON in wheat and diet analysed by (HPLC) with diode array detection (DAD)	Wheat for duck diet	Danicke and others	Effects of graded levels of Fusarium-toxin-contaminated wheat in Pekin duck diets on performance, health and metabolism of deoxynivalenol and zearalenone	British Poultry Science Volume 45, Number 2 (April 2004), pp. 264–72	2004
Three type A (diacetoxyscirpenol, T-2 toxin, HT-2 toxin) and five typeBtrichothecenes [deoxynivalenol (DON), nivalenol, fusarenon-X, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol].	Simultaneous LC–fluorescence detection (FLD) determination	Finely ground cereals	Dall'Asta and others	Simultaneous liquid chromatography–fluorescence analysis of type A and type B trichothecenes as fluorescent derivatives via reaction with coumarin-3-carbonyl chloride	J Chromatogr A, 1047 (2004) 241–7	2004
Ochratoxin A (OTA) and/or citrinin (CIT) and/or aflatoxin B (AFB)	HPLC coupled to a fluorescence detector	Olive	El Adlouni and others	Preliminary data on the presence of mycotoxins (ochratoxin A, citrinin, and aflatoxin B1) in black table olives "Greek style" of Moroccan origin	Food Control Volume 15, Issue 7, October 2004, Pages 543–48	2004
Aflatoxin, ochratoxin, zearalenone and deoxynivalenone one by one wiyh different mobil phase	HPLC Waters 600 pump with diode array and a fluorescence detector linked with IBM computer was used	Various food products: cereal and cereal products, nuts and nut products, spices, dry fruits and beverages	Abdulkadar and others	Mycotoxins in food products available in Qatar	Food Control Volume 15, Issue 7, October 2004, Pages 543–8	2004
Ochratoxin A and aflatoxins B1, B2, G1, and G2	HPLC analytical method and fluorescence detection	Air samples workplaces of a coffee factory	Tarín, and others	Use of high-performance liquid chromatography to assess airborne mycotoxins. Aflatoxins and ochratoxin A.	J Chromatogr A 2004 Aug 27; 1047(2):235–40	2004

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
			Bueno and Oliver	Determination of aflatoxins and zearalenone in different culture media	Methods Mol Biol 2004:133–7	2004
Fumonisin B_1 (FB_1) and B_2 (FB_2)	Flow-through enzyme immunoassay results compared with validated HPLC method	Maize	Paepens and others	A flow-through enzyme immunoassay for the screening of fumonisins in maize	Analytica Chimica Acta Volume 523, Issue 2, 11 October 2004, Pages 229–35	2004
Ochratoxin A	Liquid chromatography with fluorescence detection	Bee pollen for control purposes corn, wheat and rice grains, and eleven liquid media	Medina and others	Bee pollen, a substrate that stimulates ochratoxin a production by *Aspergillus Ochraceus* wilh.	System. Appl. Microbiol. 27, 261–67 (2004)	2004
Aflatoxins B_1, B_2, G_1, G_2 and ochratoxin A (OTA)	HPLC conditions in gradient elution and a wavelength program for the separation fluorimetric quantitation	Bee pollen	Garcia-Villanova and others	Simultaneous immunoaffinity column cleanup and HPLC analysis of aflatoxins and ochratoxin a in spanish bee pollen	J Agric Food Chem, 52 (24), 7235–7239, 2004	2004
Aflatoxins (AfB1, AfB2, AfG1, AfG2) and Zearalenone (ZEN) OTA, FB1, FB2, Trichotecenes: one by one	TLC for AF and ZEN and Trichotecenes: HPLC for OTA And FB1 FB2	Medicinal herbs,	Rizzo and others	Assessment of toxigenic fungi on Argentinean medicinal herbs	Microbiological Research 159 (2004) 113–20	2004
AFB1	noninstrumental immunofiltration-based assay device consists of membranestrips, with antibody-immobilized zones, attached to a polyethylene card	Groundnut, corn, wheat, cheese, and chili	Pal and Dhar	An analytical device for on-site immunoassay. demonstration of its applicability in semiquantitative detection of afb1 in a batch of samples with ultrahigh sensitivity	Anal. Chem.2004, 76,98–104	2004
Aflatoxin and ochratoxin one by one	HPLC	Raw ingredients, finished bakery products, equipment surface swabs, workers' gloves, water used in processing lines and air	Al-Zenki[1] and others	Evaluation of microbial hazards associated with bread manufacturing in the State of Kuwait	2004 IFT Annual Meeting, July 12–16 - Las Vegas, NV	2004
Simultaneously Aflatoxin, Ochratoxin A and Zearalenone	Liquid chromatography with fluorescence detection	Rye and rice	Gobel and Lusky	Simultaneous determination of aflatoxin, ochratoxin A and zearalenone in grains by new imunoaffinity column/ liquid chromatography	J of AOAC Int 87(2) 411–6 Mar-apr	2004

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Simultaneous detection of aflatoxins (AF) and ochratoxin A (OA)	Automated HPLC method aqueous extracts were then transferred to an ASPEC HPLC system for automated cleanup using AflaOchra immunoaffinity columns. OA and AF were quantified using HPLC with fluorescence detection, with a run time of approximately 40 min.	Maize cereal products and peanut butter	Chan and others	Simultaneous determination of aflatoxins and ochratoxin A in food using a fully automated immunoaffinity column cleanup and liquid chromatography-fluorescence detection.	J Chromatogr A 2004 Dec 3; 1059(1–2):13–6	2004
Simultaneous detection of the Fusarium mycotoxins fusaproliferin and seven trichothecenes	(GC–MS)	Grains	Jestoi and others	Analysis of the *Fusarium* Mycotoxins fusaproliferin and trichothecenes in grains using gas chromatography-mass spectrometry	J Agric Food Chem 2004, 52, 1464–9	2004
Ochratoxin A (OTA) and fumonisin B1 (FB1)	three different cell-lines, C6 glioma cells, Caco-2 cells and Vero cells		Creppy and others	Synergistic effects of fumonisin B1 and ochratoxin A: are *in vitro* cytotoxicity data predictive of in vivo acute toxicity?	Toxicology 201 (2004) 115–23	2004
Trichothecenes DON, NIV, 3-acetyl DON and Fusarenone X	LC–MS methods TLC GC LC ELISA Molecular imprinted polymers (MIP)	Creal, coffee		Trichothecenes with a special focus on DON	Summary Report of a Workshop held in September 2003 ILSI Europe Report Series	2004
Cyclopiazonic acid, mycophenolic acid, tenuazonic acid, and ochratoxin A	HPLC	Cornflake	Aresta and others	Simultaneous determination of ochratoxin A and cyclopiazonic, mycophenolic, and tenuazonic acids in cornflakes by solid phase microextraction coupled to HPLC	J Agric Food Chem 2003, 51, 5232–7	2003
Simultaneous determination of B-trichothecenes and the major metabolites of NIV, (DON), (15-AcDON, (3-AcDON), fusarenon X (Fus-X) and de-epoxydeoxynivalenol (DOM-1)	High-performance liquid chromatography (HPLC), combined with atmospheric pressure chemical ionisation (APCI), mass spectrometry (MS), Dexamethasone (Dex) was used as internal standard	Pig urine and maize samples	Razzazi-Fazeli and others	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, 796 (2003) 21–33	2003

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin B1 (AfB1)	Radioimmunoassaay (RIA) Antiaflatoxin B1 serum was raised in-house using AfB1-bovine serum albumin conjugate as immunogen	Agricultural commodities Rice Wheat Soy bean	Korde and others	Development of a radioimmunoassay procedure for aflatoxin B1 measurement	J Agric Food Chem 2003, 51:843–6	2003
Ochratoxin A	HPLC MS analyzed on a narrow-bore reversed-phase C18 HPLC column with acetonitrile/ water (0.1% formic acid) (40:60) as mobile phase and quantified with a fluorescence detector OTA was confirmed by single-quadruple MS using an electrospray ionization source	Green and roasted coffee	Ventora and others	Analysis of ochratoxin A in coffee by solid phase cleanup and narrow-bore liquid chromatography-fluorescence detector-mass spectrometry	J Agric Food Chem 2003, 51:7564–67	2003
AFB1, ZEN, OTA, DON, and fumunosin B1	Immobilisation on the CM5 sensor surface took place using a modi.cation of the standard EDC–NHS reaction A diamine spacer was coupled to a EDC–NHS activated CM5-sensorchip after which EDC–NHS activated a.atoxin or zearalenone were injected. GC, HPLC		Gaag and others	Biosensors and multiple mycotoxin analysis	Food Control 14 (2003) 251–54	2003
Fumonisins	Bioassays review	Fungal cultures, cereals	Gutleb and others	Cytotoxicity assays for mycotoxins produced by *Fusarium* strains: a review	Environmental Toxicology and Pharmacology Volume 11, Issues 3-4, July 2002, Pages 309–20	2002
Main type A-trichothecenes such as T-2 Toxin, HT-2 Toxin, acetyl T-2 Toxin, diacetoxyscirpenol, monoacetoxyscir-penol (15-acetoxyscirpenol) and neosolaniol	HPLC liquid chromatography and atmospheric pressure chemical ionization mass spectrometry coupling of LC–MS	Oats, maize, barley, and wheat samples	Razzazi-Fazeli and others	Simultaneous quantification of A-trichothecene mycotoxins in grains using liquid chromatography–atmospheric pressure chemical ionization mass spectrometry	968 (2002) 129–42 J Chromatogr A,	2002

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Ochratoxin A and zearalenone	HPLC fluorescence detection.	Wheat, rye, barley, and oat	Eskola and others	Application of manual and automated systems for purification of ochratoxin A and zearalenone in cereals with immunoaffinity columns	J Agric Food Chem 2002, 50:41–7	2002
Ochratoxin A (OA) and T-2 toxin	Membrane-based flow-through enzyme immunoassay results confirmed by HPLC-fluorescence and by GC_MS	Cereals (wheat, rye, maize, barley)	De Saeger and others	A collaborative study to validate novel field immunoassay kits for rapid mycotoxin detection	Int J Food Microbiol Vol.75, Issues 1–2, Pages 135–42	2002
Aflatoxin	Fully automated tandem MS, with autosampler and injection at atmospheric pressure, was available.	Peanut	Schatzki and Haddon	Rapid, nondestructive selection of peanuts for high aflatoxin content by soaking and tandem mass spectrometry	J Agric Food Chem 2002, 50, 3062–69	2002
Aflatoxins	HPLC, fluorometry, ELISA	Tahini, a sesame butter	Nilufer and Boyacioglu	Comparative Study of Three Different Methods for the Determination of Aflatoxins in Tahini	J Agric Food Chem 2002, 50:3375–79	2002
Aflatoxins and ochratoxin A	Thin-layer chromatography, and then quantified with fluorescence.	Peanuts and its products, nuts, maize, oat and/or wheat products, rice and beans	Caldas and others	Aflatoxins and ochratoxin A in food and risks to human health	Rev Saude Publica. 2002 Jun;36(3):319–23	2002
Aflatoxin	Rapid fluorescence polarization (FP) assay compared with HPLC results.	Grains corn, sorghum, peanut butter, and peanut paste	Nasir and Jolley	Development of a fluorescence polarization assay for the determination of aflatoxins in grains	J Agric Food Chem 2002, 50, 3116–21	2002
Ochratoxin A (OA)	Membrane-based flow-through enzyme immunoassay flow-through enzyme immunoassay and HPLC methods.	Roasted coffee	Sibanda and others	Development of a solid phase cleanup and portable rapid flow-through enzyme immunoassay for the detection of ochratoxin A in roasted coffee	J Agric Food Chem 2002, 50:6964–67	2002
Aflatoxins B1, B2, G1 and G2, zearalenone, citrinin, deoxynivalenol, and ochratoxin A	Multi-mycotoxin thin-layer chromatography screening method and toxins were quanti®ed by high-performance liquid chromatography.	Brewed alcoholic beverages, beer	Odhav and Naicker	Mycotoxins in South African traditionally brewed beers	Food Additives and Contaminants, 2002, Vol. 19, No. 1, 55–61	2002

Continued

Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
OTA	Thin-layer chromatographic (TLC) screening method detection by visual estimation of fluorescence intensity under a UV lamp at 366 nm. Confirm by by a quantitative immunoaffinity/HPLC method.	Green coffee	Pittet and Royer	Rapid, low cost thin-layer chromatographic screening method for the detection of ochratoxin a in green coffee at a control level of 10 μg/kg	J Agric Food Chem 2002, 50, 243–47	2002
AFM1	ELISA High-titer rabbit polyclonal antibodies to aflatoxin M1 were produced by utilizing AFM1- bovine serum albumin (BSA) conjugate as an immunogen.	Milk	Devi and others	Development and application of an indirect competitive enzyme-linked immunoassay for aflatoxin M1 in milk and milk-based confectionery	J Agric Food Chem 2002, 50:933–7	2002
				Validation of analytical methods for determining mycotoxins in foodstuffs	trends in analytical chemistry, vol. 21, no. 6 + 7, 2002	2002
Aflatoxins and *Fusarium* toxins one by one	Aflatoxins by HPLC (a Jasco PU-980 pump with a Jasco AS-950 autosampler) with a Shimadzu RF-10 XLspectrofluorometer with excitation/emission wavelengths 253 nm/415 nm after derivatization with trifluoroacetic acid. Shimadzu GC/MS-QP 5000 with selected ion monitoring for Trichothecenes. Zearalenone was analyzed by HPLC fluorescence detector	Corn	Qin Li and others	Aflatoxins and Fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China	J Agric Food Chem 2001, 49:4122–26	2001
Aflatoxin B1 and ochratoxin A	RIA method Radio Immiunochemical method	Wheat and barely	Sedmikova and others	Potential hazard of simultaneous occurrence of aflatoxin B1 and ochratoxin A	Vet Med – Czech 46, 2001 (6):169–74	2001
AFB1		Maize animal	Lemke and others	Development of a multitiered approach to the *in vitro* prescreening of clay-based enterosorbents	Animal Feed Science and Technology Volume 93, Issues 1–2, 17 Pages 17–29	2001

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Simultaneous acetyl T-2 toxin, T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, neosolaniol, iso-neosolaniol, scirpentriol, 4,15-diacetoxyscirpenol, 15-acetoxyscirpenol, 4-acetoxyscirpentriol, NIV, fusarenon-X, DON, 15-acetyl-DON and 3-acetyl-DON	GC simultaneous full scan and tandem mass spectrometric detection negative ion chemical ionisation (NICI) GC–MS for molecular mass determination verification	Fungal cultures	Nielsen and Thrane	Fast methods for screening of trichothecenes in fungal cultures using gas chromatography–tandem mass spectrometry	Journal of Chromatography A Volume 929, Issues 1–2, 21 September 2001, Pages 75–87	2001
Simultaneous determination of aflatoxins (G1, G2, B1, B2) citrinine and ochratoxin A	Reversed-phase HPLC separation with tandem mass spectrometric identification and quantification using electrospray ionisation on a quadrupole ion trap mass analyser (ESI-MS-MS). The HPLC separation was run online with the ESI-MS-MS detection		Tuomi and others	Detection of aflatoxins (G(1-2), B(1-2)), sterigmatocystin, citrinine, and ochratoxin A in samples contaminated by microbes	Analyst 2001 Sep; 126(9):1545–50	2001
Aflatoxins B1, B2, G1, G2, zearalenone, and fumonisin B1	In-house validated methods	Unprocessed corn samples	Vargas and others	Co-occurrence of aflatoxins B1, B2, G1, G2, zearalenone and fumonisin B1 in Brazilian corn	Food Addit Contam. 2001 Nov;18(11):981–6	2001
Aflatoxin M1 and ochratoxin A (OTA)	Determination by HPLC fluorescence detector	Milk		Survey of milk for mycotoxins (nr 17/01)	Food Survey Information Sheet Friday 14 September 2001	2001
Ochratoxin A (OTA)	HPLC on a C18 column with gradient elution and quantitation at 333 nm by means of a photodiode array detector. second method utilized gas chromatography with mass selective detection monitoring eight specific ions	Wines and beers,	Soleas and others	Assay of ochratoxin A in wine and beer by high-pressure liquid chromatography photodiode array and gas chromatography mass selective detection	J Agric Food Chem 2001, 49:2733–40	2001

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin B1 (AFTB1), and fumonisin B1 (FB1) one by one	AFTB1 TLC, and FB1 levels by a Bond-Elut SAX cartridge and HPLC. TLC	White and yellow corn.	Martýńez and Martýńez	Mold occurrence and aflatoxin B1 and fumonisin B1 determination in corn samples in Venezuela	J Agric Food Chem 2000, 48:2833–36	2000
Ochratoxin A (OTA)	Confirmed by HPLC with fluorescence detection	Microbial media	Stander and others	Screening of commercial hydrolases for the degradation of ochratoxin a	J Agric Food Chem 2000, 48:5736–39	2000
T-2 toxin	Membranebased flow-through enzyme immunoassay Immunodyne ABC membrane was coated with 2 ÌL of goat anti-horseradish peroxidase (HRP) rabbit anti-mouse (test spot) (undiluted) immunoglobulins, and the free binding sites were blocked. In addition to the antibody-coated Immunodyne ABC membrane	Cereals	Sibanda and others	Detection of T-2 toxin in different cereals by flow-through enzyme immunoassay with a simultaneous internal reference	J Agric Food Chem 2000, 48:5864–67	2000
P. citrinum (25 isolates) citrinin and tanzawaic acid A, *P. steckii* (18 isolates) isochroman toxins (except 2) tanzawaic acid E, *P. sizovae* tanzawaic acid A, *P. corylophilum* (10 isolates) citreoisocoumarinol, *P. sumatrense* (15 isolates) curvularin	Carboxylic acids and the benzopyran were identified on the basis of mass spectrometry, and one and two dimensional NMR spectroscopic techniques			Secondary metabolites characteristic of *Penicillium citrinum*, *Penicillium steckii*, and related species	Phytochemistry Volume 54, Issue 3, 1 June 2000, Pages 301–9	2000
Aflatoxins B1, B2, G1 and G2, ochratoxin A, zearalenone and fumonisins B1, B2 and B3	Fully validated analytical HPLC methods	Raw maize as received at ports or at major maize mills	Scudamore and Patel	Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize imported into the United Kingdom.	Food Addit Contam. 2000 May;17(5):407–16	2000
Ochratoxin A (OA)	Enzyme-linked immunosorbent assay (ELISA), and detected OA concentrations up to 0.1 ng/mL.	Chilies,	Thirumala-Devi and others	Production of polyclonal antibodies against ochratoxin A and its detection in chilies by ELISA	J Agric Food Chem 2000, 48:5079–82	2000

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins B1, B2, and G1 and the cholera toxin A-subunit	Capillary electrokinetic chromatography with multiphoton-excited fluorescence		Wei and others	Determination of biological toxins using capillary electrokinetic chromatography with multiphoton-excited fluorescence	Anal Chem 2000, 72:1360–63	2000
Aflatoxin and fumonisin one by one	Aflatoxins B1, B2, G1, and G2 by (TLC), FB1, FB2 by HPLC fluorescence	Sorghum	da Silva and others	Mycoflora and occurrence of aflatoxin B1 and fumonisin B1 during storage of Brazilian sorghum	J Agric Food Chem 2000, 48:4352–56	2000
Ochratoxin A and citrinin	Enzyme immunoassays (EIA), Ochratoxin A-positive results were confirmed by HPLC after immunoaffinity chromatography	Cereal samples Sampling included foods (wheat, corn) and feeds (barley, oats, wheat bran)	Vrabcheva and others	Co-occurrence of ochratoxin a and citrinin in cereals from Bulgarian villages with a history of Balkan endemic nephropathy	J Agric Food Chem 2000, 48:2483–88	2000
8-ketotrichothecenes, (ZEA), and fumonisins, including FB1, FB2, FB3,DON), 15-acetyldeoxy NIV(15-ADON), 3-acetyldeoxyNIV,(3-ADON), nivalenol (NIV), and 4-acetylNIV (4-ANIV),		Corn	Sohn and others	Co-occurrence of Fusarium mycotoxins in mouldy and healthy corn from Korea	Food Addit Contam. 1999 Apr;16(4):153–8	1999
Ochratoxin A (OTA) and aflatoxins (AFs)	Reversed-phase liquid chromatography (LC) with fluorescence detection	Japanese beer samples	Nakajima and others	A survey of ochratoxin A and aflatoxins in domestic and imported beers in Japan by immunoaffinity and liquid chromatography.	J AOAC Int 1999 Jul-Aug; 82(4):897–902.	1999
Zearalenone, deoxynivalenol and aflatoxins, fumonisins one by one	Zearalenone, deoxynivalenol and aflatoxins by TLC and fumonisins (FB1, FB2, and FB3) by HPLC	Corn-based foods	Solovey and others	A survey of fumonisins, deoxynivalenol, zearalenone and aflatoxins contamination in corn-based food products in Argentina		1999
Aflatoxins fumonisins trichothecenes and zearalenone (ZEA)	HPLC, GC_MS	Corn samples	Ali and others	Natural co-occurrence of aflatoxins and Fusarium mycotoxins (fumonisins, deoxynivalenol, nivalenol and zearalenone) in corn from Indonesia.	Food Addit Contam. 1998 May-Jun;15(4):377–84	1998

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin	Available AOAC Methodswith slight modificationsfor the recovery of aflatoxins Using the HPLC and postcolumn derivatization procedure	Date palm	Ahmed and Robinson	Selection of a suitable method for analysis of aflatoxins in date fruits	J Agric Food Chem 1998, 46:580–4	1998
(DON), 3-acetylDON, 15- acetylDON, fusarenone X (FX), T-2 Toxin (T-2), diacetoxyscirpenol (DAS), (ZEA), fB1, AFB1,OTA, and citrinin (CT)	Analyzed by enzyme immunoassays 3,15-diacetylDON was detected in some samples by HPLC-EIA analysis	Wheat maize as feed	Curtui and others	A survey on the occurrence of mycotoxins in wheat and maize from western Romania.	Mycopathologia 1998; 143(2):97–103	1998
Aflatoxins, fumonisin B1, and zearalenone one by one		Sorghum,maize, peanuts, peanut butter, pulses (cowpeas beans)	Siame and others	Occurrence of aflatoxins, fumonisin B1, and zearalenone in foods and feeds in Botswana.	J Food Prot. 1998 Dec;61(12):1670–3	1998
AF B1,B2, G1,G2,OTA, ZEA, T2- toxin, Penicilic acid	TLC with pre-coated glass plates (20 × 20 cm of silica gel D.G.60 Merck, Darmstadt)	Medicinal plant samples and spices	Aziz and others	Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins	Aziz et al. ó Medicinal plant, spices, Bot Bull Acad Sin (1998) 39:279–85	1998
Aflatoxins B1, B2, G1 and G2, ochratoxins A and B, citrinin, cyclopiazonic acid, zea, sterigmatocystin, DON, nivl together with seven related richothecene mycotoxins, fumonisins B1 and B2 moniliformin	Analytical methods	Maize gluten and other maize products used in the animal feed industry	Scudamore and others	Mycotoxins in ingredients of animal feeding stuffs: II. Determination of mycotoxins in maize and maize products.	Food Addit Contam. 1998 Jan;15(1):30–55	1998
Aflatoxins, ochratoxin A, and fumonisins. zearalenone, and deoxynivalenol.	Further separation can be performed with IAC, followed by liquid chromatographic (LC) quantitation, either off-line or online in an automated system, or by fluorometry.	10 grains Regeneration of IACs for reuse in aflatoxin, ochratoxin A, fumonisin, and zearalenone analyses has been investigated	Scott and Trucksess	Application of immunoaffinity columns to mycotoxin analysis	J AOAC Int 1997 Sep–Oct; 80(5):941–9	1997
Fumonisin B1 and aflatoxin B1.one by one	FB1: (HPLC) on a reverse-phase column AFB1 : derivatization with trifluoroacetic acid. The derivative was detected by HPLC analysis B1	Rain-affected sorghum, rain-affected maize, normal maize, normal sorghum, and poultry feed	Shetty and Bhat	Natural occurrence of fumonisin B1 and its co-occurrence with aflatoxin B1 in indian sorghum, maize, and poultry feeds	J Agric Food Chem 1997, 45:2170–173	1997

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin B1	Capillary electrophoresis (CE) CE method was compared to an established HPLC method	Corn	Maragos* and Greer	Analysis of aflatoxin B1 in corn using capillary electrophoresis with laser-induced fluorescence detection	J Agric Food Chem 1997, 45:4337–41	1997
Aflatoxins B1, B2, G1 and G2 and ochratoxin A, fumonisins B1 and B2 simultaneous determination of aflatoxins and ochratoxin A	HPLC	Dry cereal-based pet foods and wild bird food	Scudamore and others	Determination of mycotoxins in pet foods sold for domestic pets and wild birds using linked-column immunoassay cleanup and HPLC	Food Addit Contam. 1997 Feb-Mar;14(2):175–86	1997
Ochratoxin A (OA) and aflatoxins	Analysis was performed by high-performance liquid chromatography (HPLC), with fluorescence detection and acetonitrile/water/acetic acid mobile phase (based on method by Sharman et al. 1992)	Cereals and a variety of retail products		Survey of aflatoxins and ochratoxin a in cereals and retail products	Food surveillance information sheet No.130 November 1997	1997
Fumonisins		Corn liver and kidney of rat	Norred and others	Time- and dose-response effects of the mycotoxin, fumonisin B_1 on sphingoid base elevations in precision-cut rat liver and kidney slices	Toxicology in Vitro Volume 10, Issue 3, June 1996, Pages 349–58	1996
Aflatoxins and ochratoxin A		Maize-based gruels	Oyelami and others	Aflatoxins and ochratoxin A in the weaning food of Nigerian children.	Ann Trop Paediatr. 1996 Jun;16(2):137–40	1996
Ochratoxin A (OA)	Competitive enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody HPLC fluorescence detector to confirm	Different cereals.	Barna-Vetro and others	Sensitive ELISA test for determination of ochratoxin A	J Agric Food Chem 1996, 44:4071–74	1996
Aflatoxins	Scientifically operated charge-coupled device (CCD) high-performance thin-layer chromatographic (HPTLC) Fluorescence excitation of the aflatoxins was accomplished with an ultraviolet transilluminator, which caused the analytes of interest to emit in the bluegreen portion of the visible spectrum	Peanut butter samples	Liang and others	Quantitative analysis of aflatoxins by high-performance thin-layer chromatography utilizing a scientifically operated charge-coupled device detector	Anal Chem 1996, 68:3885–91	1996

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Ochratoxin A (OTA)	Immunoaffinity column/HPLC procedure Model 420 dual piston pump, a Model 460 autosampler, a SFM 25 fluorescence detector,	Green coffee beans, roasted coffee beans, and soluble (instant) coffee	Pittet and others	Liquid chromatographic determination of ochratoxin a in pure and adulterated soluble coffee using an immunoaffinity column cleanup procedure	J Agric Food Chem 1996, 44:3564–69	1996
Fumonisins and aflatoxins	HPLC	Corn samples	Yoshizawa and others	Occurrence of fumonisins and aflatoxins in corn from Thailand.	Food Addit Contam. 1996 Feb–Mar;13(2):163–8	1996
		Amaranth grains on dichloran-chlor-amphenicol-peptone agar (DCPA) and dichloran-18% glycerol agar (DG18).		Mycotoxin-producing potential of fungi isolated from amaranth seeds in Argentina	Int J Food Microbiol Volume 25, Issue 1, March 1995, Pages 101–8	1995
Aflatoxins B1, B2, G1, and G2, ochratoxin A, zearalenone and sterigmatocystin	Thin-layer chromatography	36 sample of stored maize	Hennigen and others	Incidence and abundance of mycotoxins in maize in Rio Grande do Sul, Brazil.	Food Addit Contam 1995 Sep–Oct;12(5):677–81	1995
Trichothecenes and fumonisins) and aflatoxin B1 (AFB1) one by one	trichothecenes (NIV), (DON), and T-2 toxin (T-2) (GC/MS); fumonisins B1 (FB1), B2 (FB2), and B3 (FB3) by (HPLC) with a flourescence detector; and AFB1 by and ELISA kit based on a monoclonal antibody	Corn	Wang and others	Natural co-occurrence of Fusarium toxins and aflatoxin B1 in corn for feed in north Vietnam.	Nat Toxins 1995;3(6):445–9	1995
Aflatoxins B1, B2, G1 and G2, ochratoxin A and zearalenone	The liquid chromatographic method developed for the separation of the six mycotoxins involves gradient elution with a reversed-phase C18 column and fluorescence detection	Animal feed ingredients	Dunne and others	Multi-mycotoxin detection and cleanup method for aflatoxins, ochratoxin and zearalenone in animal feed ingredients using high-performance liquid chromatography and gel permeation chromatography.	J Chromatogr 1993 Jan 22; 629(2):229–35	1993
AF DON	Using biological species review	Animal feeds	Panigrahi	Bioassay of mycotoxins using terrestrial and aquatic, animal and plant species	Food and Chemical Toxicology Volume 31, Issue 10, Pages 767–90	1993

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins B1, B2, G1, G2 nor sterigmatocystin, zearalenone, or ochratoxin B		Grain samples of oats, wheat, rye, barley, and maize	Juszkiewicz and Piskorska-Pliszczyńska	Occurrence of mycotoxins in animal feeds. (Poland)	J Environ Pathol Toxicol Oncol. 1992 Jul–Aug;11(4):211–5	1992
Ochratoxin A, aflatoxin B1 and T-2 toxin	Monoclonal antibodies specific for	Cereal grain	Lacey and others	Immunoassay of ochratoxin and other mycotoxins from a single extract of cereal grains utilizing monoclonal antibodies.	IARC Sci Publ 1991; (115):97–103	1991
Aflatoxins B1, B2, G1, and G, ochratoxin A, citrinin, zearalenone and vomitoxin one by one	Thin-layer chromatography (TLC)	Feedstuffs, maize, wheat, wheat bran, beans, rice germ, rice germ cake, broilers feed, egg feed, milk feed; yellow maize soya beans, wheat soya meal, rice crack, seed cake, fish meal	Abdelhamid	Occurrence of some mycotoxins (aflatoxin, ochratoxin A, citrinin, zearalenone and vomitoxin) in various Egyptian feeds.	Arch Tierernahr. 1990 Jul;40(7):647–64	1990
Simultaneous determination of aflatoxins, ochratoxin A, sterigmatocystin, and zearalenone	TLC	Rice, beans, dried beans, corn products, cassava flour	Soares and others	Survey of aflatoxins, ochratoxin A, zearalenone, and sterigmatocystin in some Brazilian foods using multitoxin thin-layer chromatographic method	J Assoc Off Anal Chem. 1989 Jan-Feb;72(1):22–6	1989
Detection of a wide range of trichothecenes, including the most polar one scirpentriol, nivalenol and 15-monoacetoxyscirpendiol	Gas chromatography-mass spectrometry with selected ion monitoring, or gas chromatography with electron-capture detection	Sorghum	Black and others	Detection of trace levels of trichothecene mycotoxins in environmental residues and foodstuffs using gas chromatography with mass spectrometric or electron-capture detection.	J Chromatogr 1987 Feb 13; 388(2):365–78	1987
afB1, B2, G1,G2, OTA, citrinin, penicillin acid, viomellein, penitrem A, patulin, sterigmatocystin, alternariol,,	High-performance liquid chromatography (HPLC) with an alkylphenone retention index and photodiode-array detection combined with thin-layer chromatography (TLC) in two different eluents		Frisvad and Thrane	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 Aug 28; 404(1):195–214	1987
Products of *Penicillium* species	Standardized thin-layer chromatographic (TLC)		Paterson	Standardized one- and two-dimensional thin-layer chromatographic methods for the identification of secondary metabolites in *penicillium* and other fungi	Journal of Chromatography A Volume 368, 1986, Pages 249–64	1986

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Secondary metabolites of *Penicillium)* and other fungi identification of products of *Penicillium* species	Standardized thin-layer chromatographic (TLC)			methods for the identification of secondary metabolites in *penicillium* and other fungi	Journal of Chromatography A Volume 368, 1986, Pages 249–64	1986
Simultaneous detection of the 11 mycotoxins af (B1, B2, G1, G2), OTA, ZEA sterigmatocystin, citrinin, penicillic acid, T-2 toxin and rubratoxin B		Cereal species (rye, barley, wheat, oats, and corn)	Grabarkiewicz-Szczesna and others	Mycotoxins in cereal grain. Part XI. Simple multidetection procedure for determination of 11 mycotoxins in cereals.	Nahrung 1985; 29(3):229–40	1985
AFIs, sterigmatocystins, versicolorins, OTs, rubratoxins, patulin, penicillic acid, ...	Two-dimensional TLC, high-performance TLC (HPTLC), quantitation and preparative TLC (PLC). Special applications of TLC deal with multi-mycotoxin analyses		Betina	Thin-layer chromatography of mycotoxins.	J Chromatogr 1985 Nov 15; 334(3):211–76	1985
Aflatoxins, ochratoxin A, zearalenone, T-2 toxin	Mycotoxins were separated by bidimensional thin-layer chromatography	Foodstuff samples	Tapia	A quantitative thin-layer chromatography method for the analysis of aflatoxins, ochratoxin A, zearalenone, T-2 toxin and sterigmatocystin in foodstuffs	Rev Argent Microbiol. 1985;17(4):183–6	1985
Multi-mycotoxin method aflatoxins, ochratoxin A, zearalenone, secalonic acid D, and vomitoxin	Aflatoxins and ochratoxin were quantitated by fluorescence measurement on silica thin-layer chromatographic plates. The other mycotoxins were quantitated after cleanup by reverse-phase liquid chromatography and ultraviolet detection	Grain dust.	Ehrlich and Lee	Mycotoxins in grain dust: method for analysis of aflatoxins, ochratoxin A, zearalenone, vomitoxin, and secalonic acid D.	J Assoc Off Anal Chem 1984 Sep-Oct; 67(5):963–7	1984
Simultaneous determination of aflatoxin B1 and ochratoxin A	Thin-layer chromatography	Black olives	Le Tutour and others	Simultaneous thin-layer chromatographic determination of aflatoxin B1 and ochratoxin A in black olives	J Assoc Off Anal Chem 1984 May-Jun; 67(3):611–2	1984

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Table 1—Continued.

Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Deoxynivalenol, T-2 toxin, zearalenone, and aflatoxin. One by one	Zearalenone-TLC Deoxynivalenol-GC_MS Aflatoxin -TLC	Wheat	Hagler and others	Simultaneous occurrence of deoxynivalenol, zearalenone, and aflatoxin in 1982 scabby wheat from the midwestern United States	Applied and Environmental Microbiology, Jan. 1984,Vol. 47, No. 1 p. 151–4	1984
6 Mycotoxins Zearalenone, aflatoxins, ochratoxin A	Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) with fluorescence detection	Feed	Howell and Taylor	Determination of aflatoxins, ochratoxins A, and zearalenone in mixed feeds, with detection by TLC or HPLC	J Assoc Off Anal ChemVolume: 64(6) Page:1356–63	1981
			Lee and others	Simultaneous multimycotoxin determination by high-performance thin-layer chromatography	Anal Chem 1980 May;52(6):837–42	1980
Aflatoxin B1, ochratoxin A, and sterigmatocystin,T-2 toxin and zearalenone	2-Dimensional thin-layer chromatography and appropriate solvent systems	Animal feedstuffs	Patterson and Roberts	Mycotoxins in animal feedstuffs: sensitive thin-layer chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone, and T-2 toxin	J Assoc Off Anal Chem 1979 Nov;62(6):1265–7	1979
Aflatoxin B1 or G1, ochratoxin A or ethyl ester A; zearalenone, sterigmatocystin, diacetoxyscirpenol,; T-2 toxin, patulin, penitrem A penicillic acid	Thin-layer chromatography is used to separate the toxins; toxins are then quantitated by the limit detection method	In mixed feeds and other food products used in the manufacture of these feedstuffs	Gimeno	Thin-layer chromatographic determination of aflatoxins, ochratoxins, sterigmatocystin, zearalenone, citrinin, T-2 toxin, diacetoxyscirpenol, penicillic acid, patulin, and penitrem A.	J Assoc Off Anal Chem 1979 May;62(3):579–85	1979
Aflatoxins B1, B2, G1, and G2, citrinin, diacetoxyscirpenol, ochratoxin A, patulin, penitrem A, sterigmatocystin, T-2 toxin, and zearalenone		Most animal feedstuffs	Roberts and Patterson	Detection of 12 mycotoxins in mixed animal feedstuffs, using a novel membrane cleanup procedure.	J Assoc Off Anal Chem 1975 Nov;58(6):1178–81	1975

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 and others (1996). Recently, Toteja and others (2006) determined aflatoxin B1 of rice samples, using HPTLC after extraction with water/chloroform and silica gel column cleanup.

The overpressured-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) (Otta and others 2000; Papp and others 2002; Móricz and others 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 (Le-Tutor and Tantaoui Elaraki 1984; Liang and others 1996; Aziz and others 1998; Odhav and Naicker 2002; Rizzo and others 2004; Caldas and Silva 2007) and qualitative determination (Grabarkiewicz-Szczesna and others 1985; Liang and others 1996; Abrunhosa and others 2001; 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 and others 2007). Pre- and postcolumn derivatizations are used for improvement of sensitivity (Hu and others 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, and quantification 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 (Li and others 2001, 2006; Razzazi-Fazeli and others 2002, 2003; Eke and others 2004; Gobel and Lusky 2004; Klotzel and others 2005; Visconti and others 2005), fungal cultures (Delmulle and others 2006), cheese (Kokkonen and others 2005), milk (Sorensen and Elbaek 2005), bee pollen (Garcia-Villanova and others 2004), cereal products (Aresta and others 2003; Chan and others 2004; Biselli and Hummert 2005), beer and wine (Soleas and others 2001), and feeds (Vrabcheva and others 2000; Biselli and Hummert 2005; Martins and others 2007; Krska and others 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; Abdulkadar and others 2004; Saez and others 2004; Gonzalez and others 2005; Zinedine and others 2006; Calleri and others 2007; 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 and others (2007) determined aflatoxin B1 by anti-aflatoxin B1 immunoaffinity monolithic disk. Polyclonal anti-AFB1 was 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 and others 2007).

HPLC–UV technique. The reversed-phase HPLC–UV technique was an early method used for the determination of mycotoxins in grains (Cahill and others 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 Wilczyńska 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 and others 2004). Abdulkadar and others (2004) used HPLC–UV for the determination of DON in foods after extraction by acetonitrile : water (15:85). Also, Briones-Reyes and others (2007) developed and optimized an RP-HPLC-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 and others 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 (Holcomb and others 1992; 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 and others 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 and others 2004; Medina and others 2004; Hinojo and others 2006; Zinedine and others 2006), feeds (Charoenpornsook and Kavisarasai 2006), beverages (Abdulkadar and others 2004; Saez and others 2004; Varelis and others 2006), high-pigment-content samples such as chili powder, green bean, black sesame, and other spices (Fazekas and others 2005; Hu and others 2006), nuts (Abdulkadar and others 2004), coffee (Ventura and others 2003), ginseng and ginger (Trucksess and others 2008), and bee pollen (Gonzalez and others 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 time-resolved 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) (Sydenham and others 1990; Stroka and others 2002; Hinojo and others 2006), 4-fluoro-7-nitrobenzofurazan (NBD-F) (Scott and Lawrence 1994; Jimenez and Mateo 1997), 9-fluorenilmethyl chloroformate (Holcomb and others 1993), or naphthalene 2,3-dicarboaldehyde (Bennett and Richard 1994). Fumonisins (FB1 and FB2) were determined in different corn-based food products using HPLC-fl with quantification limit (LOQ) of 0.020 mg/kg (Caldas and Silva 2007). Trebstein and others (2008) 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 and others 2004) or mass spectrometry (LC–MS–MS) (Delmulle and others 2006; Tanaka and others 2006; Lattanzio and others 2007; Sulyok and others 2007) has also been reported. However, these 2 detection methods are less sensitive than FLD in some cases but can aid as confirmative tools (Saez and others 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 (Dick and others 1988; Zimmerli and others 1989), and decreased sensitivity and accuracy resulting from stability of citrinin in organic eluents, ion-pair, reagents, and acid environment.

Chromatography and mass spectrometry combination technique

LC–MS technique. Coupling of LC and MS provides a great opportunity for the analysis of mycotoxins. HPLC with MS detection eliminates the need for sample derivatization for fluorescence activity enhancement. Furthermore, the use of LC and tandem mass spectrometry enables a very selective and sensitive detection. Liquid chromatography tandem mass spectrometry (LC–MS/MS) is a useful technique for identification and quantification of chemicals such as mycotoxins. Before mass spectrometer, HPLC will separate the sample to chemical compounds. Then mass spectrometer will ionize molecules and sort, and identify them according to their mass-to-charge ratio (*m/z*).

The first LC–MS methods for the determination of trichothecenes were based on fast-atom bombardment (FAB), thermospray, and plasmaspray ionization (Kostiainen 1991). Later, soft-ionization techniques such as atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), and atmospheric pressure photo-ionization (APPI) were widely used, which are suitable for different molecular weights and polarity of compounds.

APCI is more practical for low to medium molecular mass and low to medium polar analytes, but ESI is more helpful for medium to high molecular mass and medium to high polar analytes. However, there are some reports in using both of these ionizers in mycotoxin detection literatures. For instance, APCI have been used in the determination of trichothecenes (Berger and others 1999; Razzazi-Fazeli and others 2002, 2003; Berthiller and others 2005) and Zollner and Mayer-Helm (2006) reviewed trace mycotoxin analysis using APCI. Also, ESI have been used by Berthiller and others (2006) for zearalenone and by Delmulle and others (2006) for simultaneous determination of 16 mycotoxins in fungal cultures and by Ventura and others (2006) for simultaneous determination of aflatoxins and OTA in beer. Lau and others (2000) have determined ochratoxin A (OTA) in coffee samples using 3 different quantitative approaches (standard method addition, internal standard, and external standard methods) by means of ESI-MS–MS detection. As well, LC–ESI-MS–MS determination in combination with C18 SPE has been reported as a valid alternative to liquid chromatography-fluorescence protocols for the detection of OTA in wine samples at trace levels (Leitner and others 2002). Due to its higher sensitivity in comparison with APCI, ESI-MS–MS detection was proposed for quantitative analysis at low levels (Biselli and others 2004; Klotzel and others 2005).

Only a few LC–MS methods for the simultaneous determination of both type A and B trichothecenes exist in the literature (Biselli and others 2004; Dall'Asta and others 2004). LC–tandem-MS has been also proposed as a valid technique for the determination of mycotoxins in contaminated foodstuffs and beverages (Lau and others 2000; Leitner and others 2002).

In addition, there are many types of mass analyzers such as quadrupole, time-of-flight, ion-trap, and Fourier transform-ion cyclotron resonance (FT-ICR); however, for mycotoxin analysis the most important mass analyzers are the triple quadrupole and the ion-trap and time of flight.

Triple quadrupole consists of 3 parts for 3 important functions. The first quadrupole acts as an ion filter. Then the mass separated ions pass into the collision cell and change to fragments. Finally, the selected fragment ions pass into the third quadrupole that is for detection. Lattanzio and others (2007) applied a double extraction approach, using a phosphate-buffered solution followed by methanol, for the simultaneous detection of 11 mycotoxins (aflatoxins (B1, B2, G1, G2), ochratoxin A, and fumonisins (B1, B2), DON, zearalenone, and T-2 and HT-2 toxins) having quite different polarities and chemical structures. A new multitoxin immunoaffinity column containing antibodies for all these mycotoxins was used to clean up the extract, and a liquid chromatography/tandem mass spectrometry method was developed. Reversed-phase liquid chromatography coupled with electrospray ionization triple quadrupole mass spectrometry (LC/ESI–MS/MS) using a linear gradient of methanol/water containing 0.5% acetic acid and 1 mM ammonium acetate, as chromatographic mobile phase, achieved recoveries higher than 79% in maize. Limits of detection in maize ranged from 0.3 to 4.2 μg/kg (Lattanzio and others 2007).

An extended multi-mycotoxin method for 25 contaminants in a variety of sample types has been carried out by separation and detection using tandem quadrupole mass spectrometry (Kok and others 2007). Furthermore, fully automated tandem MS, with injection at atmospheric pressure, has been applied to examine aflatoxins in peanuts (Schatzki and Haddon 2002). In addition, mycotoxins [NIV, DON, AFG1, AFG2, AFB1, AFB2, FB1, FB2, diacetoxyscripenol (DAS), T2-toxine, ochratoxin A, and ZEN] were simultaneously measured in cattle forages and food matrices by the LC–MS/MS method. The MS analysis was carried out on a TSQ Quantum Discovery MAX^{TM} triple-stage quadrupole mass spectrometer with a heated electrospray ionization (H-ESI) probe in the positive ionization mode. TSQ Quantum Discovery MAX offers the unique capability of highly selective reaction monitoring (H-SRM) (Huls and others 2007). Monbaliu and others (2009) have developed a multi-mycotoxin method using a liquid chromatography/tandem mass spectrometry method for the simultaneous determination of trichothecenes (nivalenol, DON, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, neosolaniol, fusarenon-X, diacetoxyscirpenol, HT-2 toxin, T-2 toxin), aflatoxins (aflatoxin-B1, aflatoxin-B2, aflatoxin-G1, and aflatoxin-G2), alternaria toxins (alternariol, alternariol methyl ether, and altenuene), fumonisins (fumonisin-B1, fumonisin-B2, and fumonisin-B3), ochratoxin A, zearalenone, beauvericin, and sterigmatocystin in sweet pepper.

In addition, simultaneous determination of aflatoxins, type A trichothecenes, type B trichothecenes, OTA, zearalenone, fumonisins, and patulin have been done on corn flake extracts with no sample cleanup in a single run by comprehensive LC/MS/MS (Rudrabhatla and others 2007). In another study, Niderkorn and others (2007) used an HPLC–MS/MS system for the determination of DON, ZEN, and fumonisins B1 and B2 (FB1, FB2) in corn silage after separation by C18 reversed-phase columns. On the other hand, HPLC–MS analysis was performed using a C18 column with a C18 precolumn for the detection of aflatoxin B1, citrinin, DON, fumonisin B1, gliotoxin, ochratoxin A, and zearalenone in corn silage. Mass spectrometry was performed on a quadrupole analyzer equipped with an electron-spray ionization (ESI) source and operating in positive and negative modes. The detection and quantification limits for aflatoxin B1, citrinin, fumonisin B1, and ochratoxin A were 1.5 and 5 ppb, and 6.5 and 20 ppb for DON, gliotoxin and zearalenone, respectively (Richard and others 2007).

Sulyok and others (2006) improved the LC–MS/MS method for the determination of 39 mycotoxins to allow the unambiguous detection and quantification of about 90 mycotoxins and some of their naturally occurring metabolites. MS conditions for all analyzed substances were optimized to gain multiple reaction monitoring (MRM) transitions. One MRM transition per analyte

was used for quantification, while another one was used as a qualifier (Sulyok and others 2006). Berthiller and others (2007) mentioned several of the published LC–MS/MS multi-mycotoxin methods and also introduced a new method, which allowed the concurrent detection and quantification of major mycotoxins and other secondary fungal metabolites in cereals.

The liquid chromatography/tandem mass spectrometry (LC–MS/MS) method includes relatively few and nonlaborious sample treatment steps, and it allows for a high throughput of samples (Delmulle and others 2006). Furthermore, according to Silva and others (2009), LC–MS/MS is the most precise, accurate, and sensitive method in comparison with LC-FL and LC–MS, at least for fumonisin determination. They reported that liquid chromatography coupled to tandem mass spectrometry provides higher sensitivity (12 μg/kg for fumonisins B1 and B2) when compared to mass spectrometry (40 μg/kg for both fumonisins) and fluorescence detection (20 μg/kg for fumonisin B1 and 15 μg/kg for B2) (Silva and others 2009).

Recently, an HPLC-ESI-MS/MS method was developed for simultaneous determination of 33 mycotoxins in various products such as peanuts, pistachios, wheat, maize, cornflakes, raisins, and figs. The mycotoxins were extracted with acetonitrile/water and then directly injected into a LC–MS/MS system without any cleanup. The limit of quantification for the aflatoxins and ochratoxin A was 1 mg/kg and for the other mycotoxins were in the range of 10 to 200 mg/ kg (Spanjer and others 2008).

An ion trap can be considered as a "3-dimensional quadrupole" in which the ions of all masses are trapped in a chamber. At first, targeted ions are selected (mass-to-charge ratio) by expelling all the others from the ion trap. Then, fragmentation of the selected ions will be done. At the end, the fragmented ions are analyzed by expelling those of a selected mass-to-charge ratio.

An HPLC–MS analysis method for determining mycotoxins was established, which included extraction, sample pretreatment, and reversed-phase HPLC separation with MS identification and quantification using electrospray ionization on a quadrupole ion trap mass analyzer (ESI–MS–MS) (Razzazi-Fazeli and others 2002; Klotzel and others 2005; Kokkonen and others 2005). Mass spectral analysis was performed on a Finnigan LCQ fitted with an electrospray ionization (ESI) probe in the positive ion mode. Aqueous methanol was used in the initial extraction, solvent partition, and solid phase extraction in the purification of samples. The HPLC separation was run online with the ESI-MS-MS detection. Recoveries of the sample pretreatment varied from 28% to 99%. The average accuracy and precision (RSD) were 21% and 113%, respectively. In addition, a standardized LC–UV–MS micro-scale method for the screening of fungal metabolites and mycotoxins in culture extracts was presented; the database of 474 mycotoxins including citrinin was established (Nielsen and Smedsgaard 2003).

In time-of-flight mass analyzer, the same electromagnetic force will be applied to push the ions accelerate down a flight tube. Lighter ions will flight faster and will be received faster by the detector. Their mass-to-charge ratio will be determined according to their receiving time. Tanaka and others (2006) developed a LC-TOF-MS method for the simultaneous determination of trichothecenes, zearalenone, and aflatoxins in foodstuffs. Also, Elosta and others (2007) reported the same method for the simultaneous determination of trichothecene mycotoxins in barley and malt extracts.

Although analytical methods might consist of different extraction, cleanup, and quantification steps, the results of the analyses by such methods should be similar. The confirmation techniques used can be either chemical derivatization or mass spectrometry (MS).

GC–MS technique. In history, gas chromatography (GC) was introduced in the field of mycotoxins in the early 1970s. If mycotoxins are sufficiently volatile at the column temperature, or can be converted into volatile derivatives, GC can be applied for their determination. Trichothecenes were extracted directly from sorghum by Clin Elut columns, and cleaned up on Florisil Sep-Pak cartridges. Simultaneous determination of trichothecenes and macrocyclics and neosolaniol were done after hydrolysis to verrucarol and T-2 tetraol, respectively, and determination by gas chromatography-mass spectrometry with selected ion monitoring, or gas chromatography with electron-capture detection. In one study, use of a magnetic sector instrument with electron-impact ionization gave comparable sensitivity for most trichothecenes, but was less useful for the simultaneous detection of verrucarol in the presence of other trichothecenes (Black and others 1987).

Aflatoxin B could be detected by fused silica capillary GC–MS with on-column injection. Aflatoxins B1, B2, G1, and G2 could be separated, and the limit of quantification was 1 ng for B1 and B2 and 2 ng for G1 and G2, when a flame ionization detector was used; however, the limit of detection by GC–MS was somewhat lower (Scott 1993). Ochratoxin A was converted to its O-methyl ochratoxin A, a methyl ester derivative that was identified by capillary GC–MS (Scott 1993). Also, Soleas and others (2001) utilized gas chromatography with mass-selective detection, monitoring 8 specific ions for ochratoxin A in wines and beers. Limits of detection (LOD) and quantification (LOQ) were 0.1 and 2 μg/L, respectively, whereas the recovery and imprecision were 69 to 75 and 9% to 11.1%, respectively. The GC method is not suitable for routine quantification but is potentially useful as a confirmatory tool for samples with OTA up to 0.1 μg/L (Soleas and others 2001). In another study, SPE cleanup with Florisil cartridge columns was used followed by a derivatizing procedure to prepare trimethylsilyl derivatives of 7 trichothecene mycotoxins (DON, 3-acetyldeoxynivalenol, diacetoxyscirpenol, fusarenon-X, nivalenol, neosolaniol, T-2 toxin) and zearalenone in cereals (Tanaka and others 2000). The possibility of using fungal volatile metabolites as indicators of ochratoxin A (OTA) and DON mycotoxins to determine grain quality has been investigated by Olsson and others (2002) using both electronic nose and gas chromatographic–mass spectrometric detection.

For a study on Fusarium, extracts were micro-scale extracted from crude Fusarium culture extracts and derivatized: acetyl T-2 toxin, T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, neosolaniol, iso-neosolaniol, scirpentriol, 4,15-diacetoxyscirpenol, 15-acetoxyscirpenol, 4-acetoxyscirpentriol, NIV, fusarenon-X, DON, 15-acetyl-DON and 3-acetyl-DON. For derivatization, pentafluoropropionic anhydride was used before determination by GC-tandem mass spectrometric detection. Negative ion chemical ionization (NICI) GC–MS was used for molecular mass determination verification (Nielsen and Thrane 2001). In the development of a multi-mycotoxin detection method by GC–MS, A and B type trichothecenes, namely 4,15-diacetoxyscirpenol, T2-toxin, DON, and nivalenol (NIV) of semolina and corn grits were derivatized by cleanup cartridge consisting of ammonium sulfate, celite, alumina, charcoal, and C18 with N,N-dimethyl-trimethylsilyl-carbamate, then they were determined by gas chromatography with flame ionization detector (GC-FID) or mass selective detector (GC–MSD). Limit of detection of the method was from 0.30 to 0.47 mg/kg for GC-FID and from 0.05 to 0.35 mg/kg for GC–MSD. Using this cartridge, no further sample cleanup steps are required thus making the developed method time- and cost-effective (Eke and others 2004). Recently, 13 trichothecenes were determined in whole beans, roasted soy, nuts, flour and flakes, textured soy protein, tofu, infant formulas, and fermented products (soy sauce) by gas

chromatography/mass spectrometry, and HPLC with fluorescence and UV-detection (Schollenberger and others 2007). Careri and others (2002) presented the applications of mass spectrometry (MS)-based techniques for the analysis of compounds in foods and discussed differences between LC–MS and GC–MS techniques.

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. Noncomplexed 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 and others 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 and others 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 $NaHCO_3$ 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 and others 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 and others (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 and others 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) and Gutleb and others (2002) 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 and others 2004) and aflatoxin M1 in milk and aflatoxin B1 in feed (Decastelli and others 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 and others 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 semiquantitative method for mycotoxins and commercial enzyme-linked immunosorbent assays (ELISAs) are widely used (Ruprich and Ostry 1995; Papadopoulou-Bouraoui and others 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 and others 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 and others 2006). Aflatoxin M1 in milk has been determined by ELISA by some researchers (Thirumala-Devi and others 2002; Magliulo and others 2005; 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 and others 1996). Later, Thirumala and others (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 enzyme-linked 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 the membrane. In an experiment on chili samples the limit of quantification obtained was 2 and 10 μg/kg for AFB1 and OA, respectively (Saha and others 2007). At the same time, aflatoxin and ochratoxin were determined in barley and wheat flour by Adanyi and others (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 and others 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 (Goryacheva and others 2007a).

Recently, Iacumin and others (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 and others (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 and matrix dependence, often resulting in overestimation (Schuhmacher and Magnuson 1997; Josephs and others 2001; Krska and Molinelli 2007). Besides the common ELISA procedures, there is an increasing demand for immunoassay techniques for field use, offering protocols for quick and reliable results. Multianalyte dipstick immunoassays for various mycotoxins have been developed, however, with limited sensitivity (Schneider and others 1995).

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 and others 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).

Conclusions

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 multitarget 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 as low 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 semiquantitative 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 semiquantitative 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.

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