




BIOTOXIN










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Biotoxin



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Biotoxin










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Identification of Biotoxin



- A biotoxin is a poisonous substance that is a specific product of the metabolic activities of a living organism (plant, animal, fungus, bacteria).
- Unlike most other biohazards, biotoxins do not replicate and, in some senses, are more analogous to chemical toxins.

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Food Safety Effects



- Biotoxins are extremely common, and they can grow on a wide range of substrates under a wide range of environmental conditions.
- Biotoxins can enter the food chain in the field, during storage, or at later points.
- Ranks mycotoxins as the most important chronic dietary risk factor, higher than synthetic contaminants, food additives, or pesticide residues.

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Human health effects



- Biotoxins have the potential for both acute and chronic health effects via ingestion, skin contact, and inhalation.
- These toxins can enter the blood stream and lymphatic system; they inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lung, and increase sensitivity to bacterial endotoxin.
- Depending on specific substances and concentration, they are cancerogenic, mutagenic, teratogenic and immunosuppressive.

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Classification of Biotoxin

Microbial toxins

- Phytotoxin
- Animal toxins
- Marine toxins



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Microbial toxins

- Microbial toxins are toxins produced by micro-organisms, including bacteria and fungi.
- Microbial toxins promote infection and disease by directly damaging host tissues and by disabling the immune system.

- Mycotoxin
- Bacterial toxin

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Mycotoxin

- Mycotoxins are secondary metabolites produced by microfungi that are capable of causing disease and death in humans and other animals.
- With regard to the widespread distribution of fungi in the environment, mycotoxins are considered to be one of the most important natural contaminants in foods and feeds.
- According to an estimate of FAO, roughly 25 % of the world's food production contains mycotoxins. The main source for mycotoxins entering the food chain are cereals, but many other food items such as fruits and nuts may be contaminated with mycotoxins as well.
- Currently, more than 500 different mycotoxins are known; however, sufficient knowledge has been collected only for a limited number of them.

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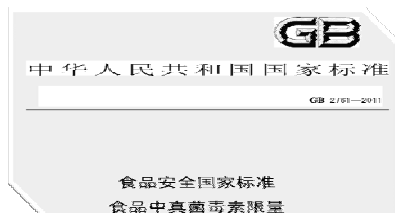
Types of Mycotoxins

- Aflatoxins (produced by *Aspergillus*) - includes Aflatoxin B1, B2, G1, G2, M1 and M2
- Ochratoxin - includes Ochratoxin A, B, and C
- Trichothecene (produced by *Stachybotrys*) - includes Satratoxin-H, Vomitoxin and T-2 mycotoxins
- Fumonisin - includes Fumonisin B1 and B2
- Zearalenone
- Citrinin
- Ergot Alkaloids
- Patulin
- Fusarium toxins

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MRL of mycotoxins in food

- GB 2761—2011 MRL of mycotoxins in food



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MRL of mycotoxins in food

Mycotoxins	Main producers/origin	Food commodity	Maximum level (EC 1831/2003 amended by EC 1126/2007)
Fumonisin			
Fumonisin A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i> , <i>F. moniliforme</i> , <i>F. dimerum</i> , <i>F. napiforme</i> , <i>F. oxysporum</i> , <i>Alternaria alternata</i>	Maize, maize-based products, sorghum, sorghum, asparagus, rice	Sum of fumonisins B1 and B2: 200 4,000 µg/kg (infant foods, processed maize-based foods, unprocessed maize)
Hydrolyzed and partially hydrolyzed fumonisins	Product of food processing		

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MRL of mycotoxins in food



Mycotoxins	Main producers/origin	Food commodity	Maximum level (EC 1881/2006 amended by EC 1126/2007)
<i>Trichothecenes</i> Type A trichothecenes: T-2 toxin, HT-2 toxin, diacetoxyscirpenol, neosolaniol, verrucarol	<i>Fusarium sporotrichioides</i> , <i>F. poae</i> , <i>F. culmorum</i> , <i>F. equiseti</i> , <i>F. graminearum</i> , <i>F. moniliforme</i> , <i>Cephalosporium</i> sp., <i>Myriophorum</i> sp., <i>Trichothecium</i> sp., <i>Phoma</i> sp., <i>Nectria</i> sp., <i>Verticillium</i> sp.	Cereals, cereal based products	In discussion for T-2 and HT-2 toxin

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MRL of mycotoxins in food



Mycotoxins	Main producers/origin	Food commodity	Maximum level (EC 1881/2006 amended by EC 1126/2007)
Type B trichothecenes: nivalenol, deoxynivalenol, 3-acetylDON, 15-acetylDON, fusarenon-X Deoxymetol-3-glucoside	<i>Fusarium graminearum</i> , <i>F. culmorum</i> , <i>F. sporotrichioides</i> , <i>F. cerealis</i> , <i>F. lanthoporum</i> Metabolic of deoxynivalenol	Cereals, cereal based products	Deoxynivalenol: 2000 1,750 µg/kg (in food, processed cereal-based foods, unprocessed cereals)

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MRL of mycotoxins in food



Mycotoxins	Main producers/origin	Food commodity	Maximum level (EC 1881/2006 amended by EC 1126/2007)
<i>Zearalenones</i> Zearalenone α- and β-zearalenol, α- and β-zearalanol	<i>F. graminearum</i> , <i>F. culmorum</i> , <i>F. crochodensis</i> , <i>F. equiseti</i> , <i>F. sporotrichioides</i> Metabolites of zearalenone	Barley, oats, wheat, rice, sorghum, sesame, soy beans, cereal based products, maize based foods, unprocessed maize, refined maize oil	20-400 µg/kg (maize based infant food, processed cereal-based and maize based foods, unprocessed maize, refined maize oil)

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MRL of mycotoxins in food



Mycotoxins	Main producers/origin	Food commodity	Maximum level (EC 1881/2006 amended by EC 1126/2007)
<i>Ochratoxins</i> Ochratoxins A, B, C Ochratoxin a	<i>Aspergillus ochraceus</i> , <i>A. niger</i> , <i>A. nidulans</i> , <i>A. alutaceus</i> , <i>A. flavus</i> , <i>A. terrestris</i> , <i>A. niger</i> , <i>Neurospora</i> sp., <i>Penicillium</i> sp., <i>Trichothecium</i> , <i>P. verrucosum</i> , <i>P. cyclopium</i> , <i>P. carbonatum</i> Metabolite of ochratoxin A	Cereals, dried fruit, raisins, wine, coffee, oils, spices, rye	Ochratoxin A: 0.5 10 µg/kg (infant food, processed cereal-based foods, unprocessed cereals, dried vine fruits and instant coffee)

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MRL of mycotoxins in food



Mycotoxins	Main producers/origin	Food commodity	Maximum level (EC 1881/2006 amended by EC 1126/2007)
<i>Aflatoxins</i> Aflatoxins B1, G1, B2, G2 Aflatoxins M1 and M2	<i>Aspergillus flavus</i> , <i>A. nomius</i> , <i>A. parasiticus</i> , <i>A. ochraceus</i> , <i>Aspergillus</i> sp., <i>E. nidulans</i> , <i>E. terrestris</i> Metabolites of aflatoxin B1 and B2	Maize, wheat, rice, quinoa, almonds, oilseeds, dried fruits, cheese, fruits, ground nuts, dried fruits, cereals, maize Milk, eggs, meat	Sum of aflatoxins B1, B2, G1 and G2: 4-15 µg/kg, aflatoxin B1: 0.1 8 µg/kg; (nuts, ground nuts, dried fruits, cereals, maize) Aflatoxin M1: 0.025-0.05 µg/kg (infant and dietary foods, milk)

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MRL of mycotoxins in food



Mycotoxins	Main producers/origin	Food commodity	Maximum level (EC 1881/2006 amended by EC 1126/2007)
<i>Ergot alkaloids</i> Ergosterine/amine, ergonovine/amine, ergocryptine/amine, ergosine/amine, ergosamine/amine	<i>Claviceps purpurea</i> , <i>C. africana</i> , <i>C. fusiformis</i> , <i>C. fusiformis</i> , <i>C. paspali</i> , <i>Neurospora</i> sp., <i>Aspergillus</i> sp.	Wheat, rye, barley, millet, oats, sorghum, triticale	

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MRL of mycotoxins in food

			Maximum level (EC 1831/2006 as amended by EC 1126/2007)
Mycotoxins	Main producers/origin	Food commodity	
<i>Alternaria tenuis</i>			
Alternaria	<i>A. alternata</i> , <i>A. flavus</i>	Wheat, rice, rye, olive,	
alternaria	<i>A. cacumata</i> , <i>A. solani</i>	sorghum, tobacco,	
alternaria monomelic	<i>A. tenuissima</i> , <i>A. citri</i>	apples, peppers,	
alternaria olivacea		sunflower seeds,	
alternaria I		oilseed rape, pecan	
alternaria II		nuts, tomatoes,	
alternaria III		mandarins	
trichothecic acid			

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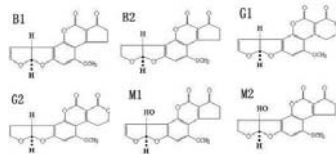
MRL of mycotoxins in food

Maximum level (EC 1881/2006 as amended by EC 1126/2007)		
Mycotoxins	Main producers/origin	Food commodity
<i>Ensatines</i>		
Ensatins A, enstatins A ₁ , enstatins B, enstatins B ₁	<i>Aspergillus Asenacorum</i> , <i>F. solani</i> , some <i>Alternaria</i> , <i>Helicoverpa</i> , <i>Verticillium</i> ssp.	Wheat, corn, barley, broad mill, oat Rice, rice
Fatallins	<i>F. verticillium</i> ssp., <i>A. fumigatus</i> , <i>A. solani</i> , <i>P. expansum</i> , <i>Penicillium</i> <i>griseofulvum</i> , <i>Rhizoglyphus</i> sp.	Apples, apple juice, cherries, cereals grains, grapes, pears, berries etc
		10-50 µg/kg (infants mostly apple juice, solid apple, spirit drinks derived from apples or containing apple juice, fruit juice)
Ensatoverlins	<i>F. solani</i> , <i>F. dimeris</i> , <i>F. lateris</i> , <i>F. phaeocephalum</i> , <i>F. griseofulvum</i> , <i>F. solani</i>	Wheat, corn, barley, broad mill, oat Rice, rice
Fusaricoproductins	<i>F. solani</i> , <i>Aspergillus fumigatus</i> , <i>P. solani</i> , <i>P. amstelredamum</i> , <i>F. roseum</i> , <i>F. fujikuroi</i> , <i>F. verticillium</i> , <i>F. solani</i> , <i>F. griseofulvum</i>	Wheat, corn, barley, broad mill, oat Rice, rice

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Aflatoxins

- The four major aflatoxins are called B1, B2, G1, and G2.



- Aflatoxin B1 is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains..

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Aflatoxins

- Many substrates support growth and aflatoxin production by aflatoxigenic molds. Natural contamination of cereals, figs, oilseeds, nuts, tobacco, and other commodities.
- Crops-in the field before harvest
- Crops-in storage-moisture content
- Animals –use grains as an animal feed
- Milk products –When cows consume aflatoxin-contaminated feeds, they metabolically biotransform aflatoxin B1 into a hydroxylated form called aflatoxin M1.



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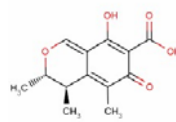
Aflatoxins

- Aflatoxin is associated with both toxicity and carcinogenicity in human and animal populations.
- Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other “slow” pathological conditions.

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Citrinin

- Citrinin was identified in over a dozen species of *Penicillium* and several species of *Aspergillus*.
- More recently, citrinin has also been isolated from *Monascus ruber*, industrial species used to produce red pigments.



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Citrinin



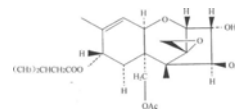
- Citrinin acts as a nephrotoxin in all animal species tested, but its acute toxicity varies in different species. The 50% lethal dose for ducks is 57 mg/kg; for chickens it is 95 mg/kg; and for rabbits it is 134 mg/kg.
- Citrinin can act synergistically with ochratoxin A to depress RNA synthesis in murine kidneys.
- Wheat, oats, rye, corn, barley, and rice have all been reported to contain citrinin.

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Fumonisin



- Fumonisin were first described and characterized in 1988. The most abundantly produced member of the family is fumonisin B1.
- The major species of economic importance is *Fusarium verticillioides*



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Fumonisin



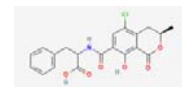
- Fumonisin affect animals in different ways by interfering with sphingolipid metabolism. They cause leukoencephalomalacia in equines and rabbits; pulmonary edema and hydrothorax in swine; and hepatotoxic and carcinogenic effects and apoptosis in the liver of rats.
- In humans, there is a probable link with esophageal cancer.
- The occurrence of fumonisin B1 is correlated with the occurrence of a higher incidence of esophageal cancer in regions of Transkei (South Africa), China, and northeast Italy.

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Ochratoxin



- Ochratoxin A was discovered as a metabolite of *Aspergillus ochraceus* in 1965.
- Members of the ochratoxin family have been found as metabolites of many different species of *Aspergillus*, including *Aspergillus alliaceus*, *Aspergillus auricomus*, *Aspergillus carbonarius*, *Aspergillus glaucus*, *Aspergillus melleus*, and *Aspergillus*.



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Ochratoxin



- Ochratoxin A is a nephrotoxin to all animal species studied to date and is most likely toxic to humans, who have the longest half-life for its elimination of any of the species examined.
- In addition to being a nephrotoxin, animal studies indicate that ochratoxin A is a liver toxin, an immune suppressant, a potent teratogen, and a carcinogen.

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Ochratoxin

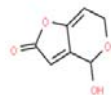


- Ochratoxin has been detected in blood and other animal tissues and in milk, including human milk. It is frequently found in pork intended for human consumption.
- Ochratoxin is associated with disease and death in poultry.

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Patulin

- Patulin, 4-hydroxy-4H-furo[3,2c]pyran-2(6H)-one, is produced by many different molds but was first isolated as an antimicrobial active principle during the 1940s from *Penicillium patulum* (later called *Penicillium urticae*, now *Penicillium griseofulvum*).



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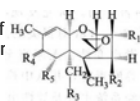
Patulin

- Patulin is regularly found in unfermented apple juice.
- Patulin is toxic at high concentration in laboratory settings, but evidence for natural poisoning is indirect and inconclusive.
- A provisional maximum tolerable daily intake for patulin of 0.4 mg/kg of body weight per day.

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Trichothecenes

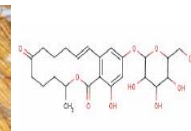
- The trichothecenes constitute a family of more than sixty sesquiterpenoid metabolites produced by a number of fungal genera, including *Fusarium*, *Myrothecium*, *Phomopsis*, *Stachybotrys*, *Trichoderma*, *Trichothecium*, and others.
- They are commonly found as food and feed contaminants, and consumption of these mycotoxins can result in alimentary hemorrhage and vomiting; direct contact causes dermatitis



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Zearalenone

- Zearalenone (6-[10-hydroxy-6-oxo-*trans*-1-undecenyl]-B-resorcylic acid lactone), a secondary metabolite from *Fusarium graminearum* (teleomorph *Gibberella zeae*).



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Bacterial toxin

- Bacterial toxins are by-products produced by pathogenic microbes that have taken up residence in the body.
- Bacteria generate toxins which can be classified as either exotoxins or endotoxins.
- Some bacterial toxins can be used in the treatment of tumors.

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Bacterial toxin

- Most cases of food poisoning are infections caused by bacteria such as *Salmonella* and *Campylobacter*.
- Only three bacterial species are considered important causes of the intoxication type of food poisoning.
 - Bacillus cereus*
 - Clostridium botulinum*
 - Staphylococcus aureus*

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Bacillus cereus

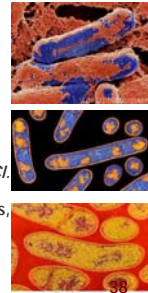
- *Bacillus cereus* is a Gram-positive spore-forming bacterium, which can produce two different types of toxin.
- Foods involved in *B. cereus* emetic food poisoning cases are usually starchy, such as boiled or fried rice, potatoes, pasta and noodles. The toxin is extremely heat-stable and will withstand cooking.



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Clostridium botulinum

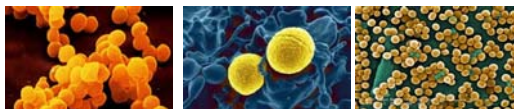
- The Gram-positive anaerobic bacterial species *Clostridium botulinum* is the causative organism for the very severe illness, botulism.
- Botulism is caused by highly potent neurotoxins, which can be pre-formed in food during growth of *Cl. botulinum* cells.
- There are at least seven different types of *Cl. botulinum* (A – G), each forming a different toxin. These can be divided into four groups, but only two, Groups I and II, are important in food safety.



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Staphylococcus aureus

- *Staphylococcus* spp. are Gram-positive, non-sporeforming cocci. The most important toxigenic species in food microbiology is *Staph. Aureus*.
- Staphylococcal enterotoxins are heat-stable proteins and pre-formed in foods. Ingestion of food containing at least 0.1-1 µg of toxin can cause a mild form of food poisoning with a rapid onset of symptoms.



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Staphylococcus aureus

- Foods associated with staphylococcal food poisoning include dairy-based products, such as cream and custard, cured and cooked meats and cheeses. Outbreaks have also been linked to pasta, sandwiches and sausages. Staphylococcal enterotoxins are heat resistant and will withstand cooking.



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Web sites for biotoxin information

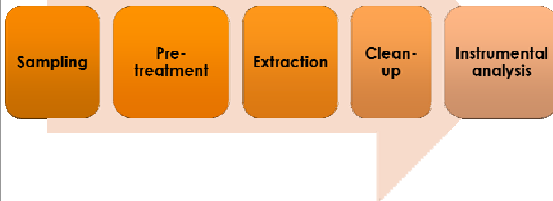
- www.cast-science.org
- www.mycotoxology.org
- www.mycotoxin.de
- www.aocs.org
- www.fao.org
- www.iupac.org
- www.chujo-u.ac.jp/myco/index.html
- www.fda.gov

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Analysis of Biotoxin



Analysis procedure



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Methods for Mycotoxins Analysis



- Sampling
- Extraction of analytes from the matrix (usually with mixtures of water and polar organic solvents) possibly followed by an extract purification
- Final detection and quantitative determination.

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Sampling



- Sampling - Your first step towards correct analytical results
- Sampling is the largest source of variability associated with the mycotoxins analysis procedure, and the most crucial step in obtaining reliable results.
- The European Commission issued the Commission Regulation (EC) 401/2006 laying out the sampling methods.
- Generally, it is possible to recommend that the most effective way to reduce the overall variability of results is to increase the size of the laboratory sample, ensure the proper milling, and homogenization.



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Sampling



- 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).
- Increasing the size of a sample decreases both the buyers' and sellers' risks but it will be very expensive.

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Extraction Method



- Extraction can be performed by liquid-liquid extraction (LLE) or solid phase extraction (SPE) using a solid and a liquid phase.
- liquid-liquid extraction (LLE)
 - Polar analytes favor polar solvents and pH plays a key role during extraction.
- solid phase extraction (SPE)
 - One of the most significant recent improvements in the purification step is the use of SPE. Test extracts are cleaned up before instrumental analysis to remove co-extracted materials that often interfere with the determination of target analytes.
- In some cases, multiple extractions are necessary for the analysis of mycotoxins.

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Extraction Method



- 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.

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Clean-up methods

In mycotoxins analysis, purification of extracts is important, especially in case of their determination at trace levels.

- Solid-phase extraction (SPE)
- Immuno- affinity columns (IACs)
- Multifunctional columns

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QuEChERS Approach

- QuEChERS approach (Quick, Easy, Cheap, Effective, Rugged, and Safe)
- Due to the acidic nature of some mycotoxins (e.g. fumonisins) and the risk of their binding on the sorbent, this approach is **not recommended**.



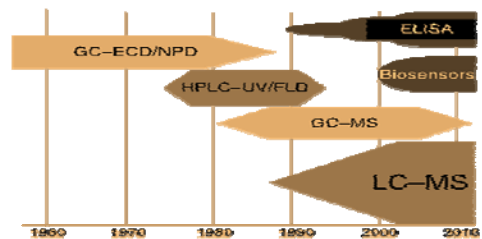
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Instrumental analysis

- Thin layer chromatography (TLC)
- Gas chromatographic
- HPLC-systems incl. fluorescence and UV-detection
- HPLC-MS/MS systems
- Immunoaffinity Chromatography
- Enzyme-linked immunosorbent assays (ELISA)
- Biosensors

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Instrumental analytical procedures



Trends in the analysis of mycotoxins from the time perspective years 1960-2016.

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Methods of Biotoxin Analysis—TLC

- Thin Layer Chromatography (TLC)
- Thin-layer chromatography (TLC) is a technique that can be used for the separation, purity assessment, and identification of organic compounds.
- Aflatoxin B1, B2, G1, G2, T-2 Toxin, Ochratoxin A, Zearalenone



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Methods of Biotoxin Analysis—GC

- In the past, methods based on GC approach were routinely used for determination of trichothecenes, zearalenone, ochratoxin A, patulin, and citrinin.
- Draw-back: need to carry out derivatization of analytes prior to sample analysis.

- GC-MS
- GC-ECD
- GC×GC-TOFMS



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- Fluorescence detector (FLD)
- UV detector
- Diode-array detector (DAD)
- Photodiode array detector (PDA).



Overview of latest analytical methods for mycotoxins determination employing liquid chromatography coupled with conventional detectors

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- without the need of derivatization
- low detection limits
- the ability to generate structural information of the analytes
- the minimal requirement of sample treatment
- mass spectrometers are general detectors that are not so dependent on chemical characteristics.

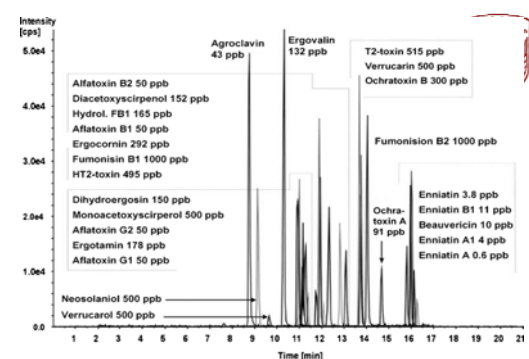


Fig. 3. The LC-ESI(+)-MS/MS total ion current chromatogram (sum of all MRM transitions) of a mixture of mycotoxins. The diluted wheat extract was spiked with a multi-mycotoxin standard and injected directly (reproduced from (73) with permission from Springer).

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Overview of recent LC-MS based methods for multi-mycotoxin analysis with none of minimal sample clean-up

Reference	(14)	(16)	(9)	(17)		(27)	(7)	(78)	(28)	(48)	(15)
Markers, which is method validated for	Wheat, maize, <i>Ustilago tritici</i>	Maize, wheat, eggs, milk, honey, horse feed	Peanut slurry, potato slurry, wheat slurry, maize slurry, dry-sinker cornflakes, rumin slurry, fig slurry	Wheat, maize	Wheat, maize	Wheat, barley, corn	Maize, vulture, beans, breakfast cereals	Maize, soy, induramide, garlic, buck rambai, St. John's wort, ginkgo biloba	Wheat, maize	Beer	Beer
Example of LOD	Break	Horse feed	Maize slurry	Wheat	Wheat	Maize	Maize	Wheat	Beer?	Beer?	Beer?
Example of LOD for pesticide analysis/ matrix combination?	Break	Horse feed	Maize slurry	Wheat	Wheat	Maize	Maize	Wheat	Beer?	Beer?	Beer?
PDON	20	>250	50	10	2,000	35	1.1	6	25	0.14	3
HT2	20	10	20	5	100	100	1	1	12.3	0.06	4
T2	2	20	25	1	20	15	0.1	3	5	0.07	2
ZEA	0.4	250	10	4	100	20	1.5	6	5	0.1	1
FBI	8	50	100	35	80	20	0.1	1	10	0.07	n.d.
FBI2	7	20	100	30	80	15	0.2	0.3	5	0.09	n.d.
OTA	1	50	1	4	12	30	0.1	1	n.d.	0.02	60
AFB1	0.8	10	0.5	0.5	20	10	0.02	6	n.d.	0.04	2
AFB2	0.7	10	1.0	30	20	30	0.1	6	n.d.	0.05	0.5
AFG1	0.5	10	1.0	1	20	10	0.2	6	n.d.	0.08	2
AFG2	1	20	0.5	10	20	20	0.2	6	n.d.	0.08	2
PAT	100	n.d.	n.d.	800	2,000	15	n.d.	n.d.	n.d.	n.d.	n.d.

Fracture solution (pHification)	MeCN: water: acetic acid	MeCN: water: formic acid	MeCN: water	MeCN: water: acetic acid	MeCN: water: acetic acid	MeCN: water	MeCN: water	EtOH: acetic: formic acid	MeCN: water: acetic acid (NaCl, 3Mg/l)	Succinate (c.a. 12 mg/l)	Succinate (MeCN precipitation)
Total number of trapped ions/spot	87	23	33	30	32	31	12	23	11	12	32
Run time (min)	21 min/2	22 min/2	35 min/1	33 min/2	33 min/1	35 min/2	8.5 min/1	25 min/1	18 min/2	6.5 min/1	18 min/1
Time (min) of chromatographic run necessary for MS detection											
Matrix component per 1 µl of injected sample; matrix equivalent in the injected volume	0.125/ 0.00625 g	0.125/ 0.00625 g	0.0625/ 0.00125 g	0.25/ 0.00125 g	0.25/ 0.0025 g	5/0.05 g	8.5/ 0.0025 g	5/0.1 g	0.2/0.001 g	5/0.025 µl	1/0.005 µl
Number of steps within the sample preparation and/or preparation of sample	2/98 min	1/100 min	2/130 min	1/93 min	1/93 min	3/49 min	1/18 min	5/185 min	3/18 min	2/45 min	3/18 min
Type of MS detection	ESI-MS/MS (2-step)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)	ESI-MS/MS (single quadrupole)
MS detector	Qtrap 4000 (Applied Biosystems)	Quattro Premier (Waters)	Quattro Ultima (Waters)	TSQ Quantum Ultra (Thermo Scientific)	LITQ (Thermo Scientific)	Microscan Micro (Waters)	Microscan TQ2 (Waters)	Microscan Quattro (Waters)	LCT Premier XE (Waters)	Aquity (Waters)	Excuteo (Thermo Scientific)

Abbreviations of analyses: DON deoxynivalenol, HT2 HT-2 toxin, T2 T-2 toxin, ZEA zearalenone, FB1 fumonisin B1, FB2 fumonisin B2, OTA ochratoxin A, AFB1 aflatoxin B1, AFB2 aflatoxin B2, AFG1 aflatoxin G1, AFG2 aflatoxin G2, PAT patulin *n.d.* not determined.

^aExamples of LODs (limits of detection) for selected mycotoxins (mostly regulated, maximum limits established by EC) No 1831/2006 implemented by EC) No 1126/2007 in selected matrices: LOD in beer is $\mu\text{g/L}$.

^bLOD in the sample standard due to the lack of blank.

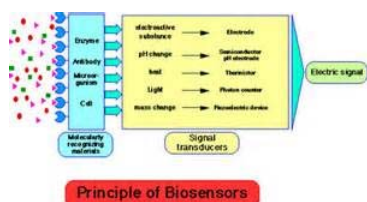
^cOperations considered as the sample preparation step: extraction, dilution, coextraction, liquid-liquid extraction, solid phase extraction, and partitioning.

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Methods of Biotoxin Analysis——Biosensor



- A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component..



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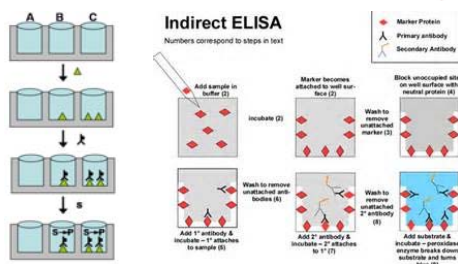
Methods of Biotoxin Analysis——ELISA



- **Enzyme-Linked Immunosorbent Assay (ELISA) Procedure**
- Nowadays, ELISA have also become widespread in biotoxin determination.
- ELISA techniques are based on a coupling reaction between a specific mycotoxin and antibodies specific for those biotoxins.
- ELISA tests can be performed in shorter time periods and provides relatively accurate screening results.
- Aflatoxins, Fumonisin, Ochratoxin, T-2 Toxin, Vomitoxin, Zearelenone (F-2 Toxin)

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Methods of Biotoxin Analysis——ELISA



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Recommendations



Following recommendations should always be considered within multi-mycotoxin analysis:

1. Blank matrix

- In mycotoxins analysis, matrix-matched standards should be used whenever possible.
- Higher background mycotoxin concentration tends to increase the analytical bias of the results.

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Recommendations



2. Internal standards

- As a general rule, internal standard employed for mycotoxins analysis must not be present in the sample, and should combine physiochemical properties chromatographically similar to those of target mycotoxins.

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Recommendations



3. Clean-up

- When immunoaffinity columns are used for purification of sample extract and/or pre-concentration of analytes, exceeding of the column capacity has to be avoided. **Breakthrough** of analytes may occur when antibodies binding sites are saturated.

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Recommendations



4. LC determinative steps

- For checking the signal stability during the sequence, running of **analytical standards** at the beginning and the end of each (longer) sequence is recommended.
- Analyses have to be performed **within the linear range**.
- In case of highly contaminated samples possibly exceeding the calibration range, they have to be **diluted** before the analysis.

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Recommendations



5. Instrument's maintenance

- When a significant decrease in signal of analytes is observed, instrument's maintenance including **cleaning of the ion source and ion optic** is required.
- Replacing of a **pre-column** or the LC column is recommended.
- Filtration of the final extract by a **syringe filter** (0.22 or 0.45 mm for U-HPLC or HPLC, respectively).

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Analysis Method



- AOAC Official Method 993.06: Staphylococcal Enterotoxins in Selected Foods

Analyte(s)	Agent Category	CAS RN / Description
Staphylococcal enterotoxins (SEA, SEC)	Protein	37337-57-8 (SEA) 39424-54-9 (SEC) Monomeric proteins of ~ 27-27.5 kDa
Staphylococcal enterotoxins (SEB)	Protein	39424-53-8 Monomeric protein of ~ 28 kDa

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Analysis Method



- Literature Reference for Microcystins (Analyst. 1994. 119(7): 1525—1530)

Analyte(s)	Agent Category	CAS RN
Microcystins (Principal isoforms: LA, LR, LW, RR, YR)	Small Molecule	96180-79-9 (LA), 101043-37-2 (LR), 157622-02-1 (LW), 111755-37-4 (RR), 101064-48-6 (YR)

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Analysis Method



Analyte(s)	Agent Category	CAS RN / Description
Abrin	Protein	1393-62-0 (Abrin) 526-31-8 (Abrine) Abrin: Glycoprotein consisting of a deadenyase (25-32 kDa A chain) and lectin (35 kDa B chain); an agglutinin (A2B2) may be present in crude preparations Abrine: Small molecule, abrin marker
Ricin (Ricinine)	Protein	9009-86-3 (Ricin) 5254-40-3 (Ricinine) Ricin: 60 kDa glycoprotein consisting of a deadenyase (~32 kDa A chain) and lectin (~34 kDa B chain); an agglutinin of MW 120 kDa may be present in crude preparations Ricinine: Small molecule, ricin marker

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Analysis Method



- Analysis Purpose: Presumptive and confirmatory Analytical Technique: Immunoassay (column) and HPLC-FL
- Method Developed for: Aflatoxins (Type B1) in corn, raw peanuts and peanut butter Method Selected for: SAM lists this method for presumptive and confirmatory analyses in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types. See [Bioterror Methods Query](#) for additional methods that should be used for this analysis.
- Method Description: This method is for the detection of aflatoxins in agricultural products. The sample is extracted with methanol-water (7 + 3), filtered, diluted with water, and applied to an affinity column containing mAbs specific for aflatoxins B₁, B₂ (CAS RN 22040-96-0), G₁ (CAS RN 1385-95-1), and G₂ (CAS RN 7241-99-7). Antibody-bound aflatoxins are removed from the column with methanol. For detection and quantitation of total aflatoxins, fluorescence measurement after reaction with bromine solution is performed. For individual aflatoxins, fluorescence detection and postcolumn iodine derivatization are performed and quantitation is by LC. Method performance was characterized using various commodities (e.g., corn) at aflatoxin levels over a range of 10 to 20 mg/g. This method was originally designed for the analysis of aflatoxins (B₁, B₂, G₁, and G₂) in samples where cleanup was necessary to remove food components, such as fats and proteins. The Cleanup procedure may not be necessary for analysis of water samples.
- Special Considerations: AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts, (AOAC International. 1998. Official Methods of Analysis of AOAC International, 16th Edition, 4th Revision, Vol. II. <http://www.aocac.org/>) may be used as a complementary HPLC-FL method in order to provide more flexibility for analysis.
- Source: AOAC International. 1994. "Method 991.31: Aflatoxins in Corn, Raw Peanuts, and Peanut Butter." Official Methods of Analysis of AOAC International, 16th Edition, 4th Revision, Vol. II. <http://www.aocac.org/>.

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Analysis Method



- Analysis Purpose: Confirmatory Analytical Technique: HPLC-PDA
- Method Developed for: Microcystins-LA, -LR, -LW, -RR, -YR in raw and treated waters Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water. See [Biotoxin Methods Query for additional methods that should be used for this analyte](#).
- Method Description: Procedures are discussed to test the presence of microcystin-LR, -LY, -LW, -LF (CAS RN 154097-70-4), and -RR in treated and untreated water samples. Cyanobacterial cells are separated from the water by filtration through 110-mm glass fiber grade C (GF/C) discs. The cellular components collected on the discs are extracted three times with methanol; the collected extraction fluids are combined and dried. The residue is resuspended in methanol and analyzed by HPLC-PDA. The liquid portion of the filtered water sample is subjected to trace enrichment using a C₁₈ SPE cartridge, followed by identification and determination by HPLC-PDA. This procedure can detect microcystin concentrations as low as 250 ng/L and is the basis of the World Health Organization (WHO) method for the detection of microcystins.
- Source: Lawton, L.A., Edwards, C. and Codd, G.A. 1994. "Extraction and High-Performance Liquid Chromatographic Method for the Determination of Microcystins in Raw and Untreated Waters." *Analyst*, 119(7): 1525–1530. <http://www.rsc.org/Publishing/Journals/AN/article.asp?doi=AN9941901525>

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Analysis Method



- Analysis Purpose: Confirmatory Analytical Technique: HPLC-FL (precolum derivatization)
- Method Developed for: Anatoxin-a in potable water Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water. See [Biotoxin Methods Query for additional methods that should be used for this analyte](#).
- Method Description: Procedures are described for HPLC analysis with fluorimetric detection of anatoxin-a in water samples after derivatization with 7-fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F). Samples are extracted at pH 7 with SPE using a weak cation exchanger. The toxin is eluted with methanol containing 0.2% trifluoroacetic acid (TFA). Samples are evaporated, reconstituted with acetonitrile, and re-evaporated prior to derivatization. This procedure detects anatoxin-a at concentrations of 0.1 µg/L with a good linear calibration.
- Source: James, K.J. and Sherlock, I.R. 1996. "Determination of the Cyanobacterial Neurotoxin, Anatoxin-a, by Derivatisation Using 7-Fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F) and HPLC Analysis With Fluorimetric Detection." *Biomedical Chromatography*, 10(1): 46–47. <http://www3.interscience.wiley.com/journal/18562/abstract>

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Analysis Method



- Analysis Purpose: Presumptive Analytical Technique: Immunoassay (ELISA)
- Method Developed for: Cylindrospermopsin in ground water, surface water and well water Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water. See [Biotoxin Methods Query for additional methods that should be used for this analyte](#).
- Method Description: Cylindrospermopsin is detected using a colorimetric immunoassay (competitive ELISA) procedure. A sample (0.05 mL), enzyme conjugate (cylindrospermopsin-HRP), and an antibody solution containing rabbit anti-cylindrospermopsin antibodies are added to plate wells containing immobilized sheep anti-rabbit antibodies. Both the cylindrospermopsin (if present) in the sample and cylindrospermopsin-HRP conjugate compete in solution to bind to the rabbit anti-cylindrospermopsin antibodies in proportion to their respective concentrations. The anti-cylindrospermopsin antibody-target complexes are then bound to the immobilized sheep anti-rabbit antibodies on the plate. After incubation, the unbound molecules are washed and decanted. A specific substrate is then added which is converted from a colorless to a blue solution by the HRP enzyme conjugate solution. The reaction is terminated with the addition of a dilute acid. The concentration of cylindrospermopsin in the sample is determined photometrically by comparing sample absorbance to the absorbance of the calibrators (standards) at a specific wavelength (450 nm). The applicable concentration range is 0.4 - 2.0 µg/L, with a minimum detection level of 0.4 µg/L.
- Source: NEMI. 2006. http://info.nie.eh.usgs.gov/pls/apex/f?p=119:38:7526698938332159:::P38_METHOD_ID-9507

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Analysis Method



- Analysis Purpose: Confirmatory Analytical Technique: High performance liquid chromatography - Photodiode array detector (HPLC-PDA)
- Method Developed for: Cylindrospermopsin in eutrophic waters Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water. See [Biotoxin Methods Query for additional methods that should be used for this analyte](#).
- Method Description: Cylindrospermopsin is detected using HPLC with photodiode array detector (PDA) in environmental waters. The suggested solvent range for cylindrospermopsin is below 50% methanol and 30% acetonitrile. Complex samples (culture medium) are purified on a C₁₈ column with a linear gradient of 1 to 12% (v/v) methanol/water over 24 minutes at 40° C, with monitoring at 262 nm. The use of C₁₈ columns for environmental waters is suggested for removal of the large number of organic compounds that may be present. This method detects and recovers cylindrospermopsin from spiked environmental water samples at 1 µg/L.
- Source: Metcalf, J.S., Beattie, K.A., Saker, M.L. and Codd, G.A. 2002. "Effects of Organic Solvents on the High Performance Liquid Chromatographic Analysis of the Cyanobacterial Toxin Cylindrospermopsin and Its Recovery From Environmental Eutrophic Waters by Solid Phase Extraction." *FEMS Microbiology Letters*, 216(2): 159–164. <http://cal.inist.fr/7aModel=afficheN&cpsid=14002569>

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Analysis Method



- Analysis Purpose: Complementary presumptive for abrin Analytical Technique: LC-MS-MS
- Method Developed for: Abrin in beverages Method Selected for: SAM lists these procedures for complementary presumptive analysis of abrin by abrin detection in aerosol, solid, particulate, liquid and water samples. Abrin, an alkaloid present in equal concentrations with abrin in many plants (*Abura procumbens* L.) is found in crude preparations of abrin and may be an indicator of abrin contamination. Further research is needed to adapt and verify the procedures for environmental sample types.
- Method Description: Procedures are described for sample extraction by SPE or liquid-liquid extraction, followed by tandem mass spectrometry. The method was verified in beverages (bottled water, cola, juice drink, 1% low fat milk, bottled spa) spiked with abrin at either 0.5 µg/mL or 0.05 µg/mL. These samples were prepared for LC-MS-MS by either an optimized SPE procedure or a liquid-liquid extraction procedure. For SPE, optimal abrin recoveries were achieved with sample pH adjusted to 2–4 with formic acid, inclusion of a water/methanol (95/5, v/v) washing step prior to elution, and use of a Strata-X SPE cartridge. Liquid-liquid extraction was with an equal volume (2 mL) of acetonitrile/water (75/25, v/v). Differences in recovery between the two extraction methods were determined using the two-sided Student's t test, assuming equal variance. At 0.5 µg/mL, recovery of abrin by SPE was significantly higher ($P < 0.05$) for water and juice drink as compared to liquid-liquid extraction, but no significant differences were observed for cola and tea. At 0.05 µg/mL, the differences in recovery of abrin in water, tea, cola, and juice drink were highly statistically different ($P < 0.001$), with better recoveries for the optimized SPE method. The method had a LOD of 0.025 µg/mL and LOQ of 0.05 µg/mL. Storage stability was also tested for abrin at 10 µg/mL in a water/methanol stock solution (90/10, v/v) at three temperatures (0° C, 10° C, and 25° C). Aliquots were analyzed in triplicate at 0, 1, 7 and 21 days after sample preparation. There was no statistically significant difference between abrin standards stored at the three temperatures at 21 days and no loss of abrin concentration.
- Special Considerations: The biotoxin methods points of contact listed in Section 4.0 of SAM should be consulted for additional information regarding water and drinking water analysis.
- Source: Owens, J. and Koester, C. 2008. "Quantitation of Abrin, an Indole Alkaloid Marker of the Toxic Glycoproteins Abrin, by Liquid Chromatography/Tandem Mass Spectrometry When Spiked into Various Beverages." *Journal of Agriculture and Food Chemistry*, 56(25): 11139–11145. <http://dx.doi.org/10.1021/jf082614x>

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Analysis Method



- Analysis Purpose: Presumptive Analytical Technique: Immunoassay (ELISA)
- Method Developed for: 4-Amanitin, ricin and 1-2 mycotoxin in food and beverages Method Selected for: SAM lists these procedures for presumptive analysis of 4-amanitin and 1-2 toxin in aerosol, solid, particulate, liquid and water samples and for confirmatory analysis of ricin in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types. See [Biotoxin Methods Query for additional methods that should be used for this analyte](#).
- Method Description: Commercially available ELISAs are described and assessed for detection of ricin, amanitin and 1-2 toxin at levels below those described as a health concern in food samples. Solid food samples are prepared by washing the sample with sodium phosphate buffer followed by dilution with phosphate-buffered saline. Liquid beverage samples are prepared by dilution in sodium phosphate buffer. Amanitin samples are similarly prepared using water instead of buffer, and 1-2 toxin samples are similarly prepared using 35% methanol in water instead of buffer. The prepared samples are used with commercially obtained ELISA kits according to the manufacturer's directions, except for the incorporation of an eight-point calibration curve and reading the plates at both 405 and 650 nm after 24 minutes of incubation at 37° C. This assay detects ricin in food products at 0.01 µg/mL with acceptable background levels. Amanitin can be detected in food products at 1 µg/g with the ELISA. Background responses occurred, but at less than the equivalent of 0.5 ppm for amanitin. The ELISA kit will successfully detect 1-2 toxin at targeted levels of 0.2 µg/g. The ELISA kit successfully detects 1-2 toxin at targeted levels of 0.2 µg/g; the immunoassay for 1-2 toxin, however, shows significant background responses and varies up to 0.1 ppm.
- Source: Garber, E.A.E., Topley, R.M., Slack, M.E., McLaughlin, M.A. and Park, D.L. 2005. "Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and 1-2 Toxin in Food." *Journal of Food Protection*, 68(6): 1294–1301. <http://www.ingeniaconnect.com/content/iafp/jfp/2005/00000068/00000006/iaf000027>

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Analysis Method



- Analysis Purpose: Presumptive Analytical Technique: Immunoassay (ELISA, ECL-based)
- Method Developed for: Atrin in food Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water. See [Biotoxin Methods Query for additional methods that should be used for this analyte](#).
- Method Description: Procedures are described for using mouse monoclonal antibodies (mAbs) and rabbit-derived polyclonal antibodies prepared against a mixture of atrin isomers for three separate ELISA and ECL-based assays in food products. The three assays vary by use of antibody combination (e.g., assay configuration): (1) polyclonal (capture)/polyclonal (detection) ELISA, (2) polyclonal/monoclonal ELISA and (3) polyclonal/monoclonal ECL assay. The LODs, with purified Atrin C and various atrin extracts in buffer are between 0.1 and 0.5 ng/ml for all three assays. The LOD for atrin spiked into food products ranged from 0.1 to 0.5 ng/ml, using the ECL assay. The LOD for atrin spiked into food products for the ELISA assays ranged between 0.5 and 10 ng/ml depending on the antibody combination. In all cases, the LODs were less than the concentration at which atrin may pose a health concern.
- Special Considerations: Crude preparations of atrin may also contain agglutinins that are unique to rosy peas and that can cross-react in the immunoassays. Addition of non-fat milk powder to the sample buffer may eliminate false-positive results (Dayan-Kengsberg, J., Bertocchi, A. and Garber, E.A.E. 2008. "Rapid Detection of Ricin in Cosmetics and Elimination of Artifacts Associated With Wheat Lectin." Journal of Immunological Methods. 334(2): 231–254). <http://www.sciencedirect.com/science/journal/00221759>
- Source: Garber, E.A.E., Walker, J.L. and O'Brien, T.W. 2008. "Detection of Atrin in Food Using Enzyme-Linked Immunosorbent Assay and Electrochemiluminescence Technologies." Journal of Food Protection. 71(9): 1868–1874. <http://www.inqetacsmc.com/content/10.1008/0000071700000097.art0015>

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Analysis Method



- Analysis Purpose: Confirmatory Analytical Technique: LC/APCI-MS
- Method Developed for: DAS and T-2 mycotoxin in food Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types. See [Biotoxin Methods Query for additional methods that should be used for this analyte](#).
- Method Description: A LC/APCI-MS procedure based on TOF-MS, with a real-time reference mass correction, is used for simultaneous determination of *Fusarium* mycotoxins (to include DAS and T-2 mycotoxin) in foodstuffs. Mycotoxin samples are extracted with acetonitrile/water (85:15) and centrifuged, and the supernatant is applied to a column for cleanup. Prepared samples are separated by liquid chromatography with an aqueous mobile phase of ammonium acetate and methanol detection is provided in exact mass chromatograms with a mass window of 0.03 Th. The limits of detection range from 0.1 to 6.1 ng/g in analyzed foodstuffs.
- Source: Tanaka, H., Takino, M., Sugita-Konishi, Y. and Tanaka, T. 2006. "Development of Liquid Chromatography/Time-of-Flight Mass Spectrometric Method for the Simultaneous Determination of Trichothecenes, Zearalenone, and Aflatoxins in Foodstuffs." Rapid Communications in Mass Spectrometry. 20(9): 1422–1428. <http://cat.inist.fr/?aModele=affiche&cpsid=17607074>

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Analysis Method



- Analysis Purpose: Confirmatory Analytical Technique: HPLC-MS-MS
- Method Developed for: Brevetoxins in shellfish Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types. See [Biotoxin Methods Query for additional methods that should be used for this analyte](#).
- Method Description: Shellfish sample homogenates are extracted with acetone, and centrifuged. The supernatants are combined, evaporated, and re-solubilized in 80% methanol. Following a wash with 95% n-hexane, the methanolic layer is evaporated, and the residue re-solubilized in 25% methanol and applied to a C₁₈ SPE column. Analytes are eluted with 100% methanol, evaporated, and re-solubilized in methanol for analysis. Analysis of prepared samples is performed using HPLC-MS-MS with a mobile phase of water and acetonitrile with acetic acid. Analytes are detected by an MS with ES3 interface. Brevetoxins are extensively metabolized, with many sub-forms. This method describes multiple liquid chromatography/electrospray ionization mass spectrometry (LC-ESI-MS) profiles for metabolites of brevetoxins from oysters.
- Source: Wang, Z., Plakas, S.M., El Said, K.R., Jester, E.L., Granade, H.R. and Dickey, R.W. 2004. "LC/MS Analysis of Brevetoxin Metabolites in the Eastern Oyster (*Crassostrea virginica*)." Toxicon. 43(4): 455–465. <http://cat.inist.fr/?aModele=affiche&cpsid=15648117>

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Procedures for the Analysis of Biotoxins



- Determination of aflatoxins B1, B2, G1 and G2 in food and feed. pREN 14123, modified, 2007-05
- Determination of aflatoxins B1, B2, G1 and G2 in infant and dietary food AOAC 84, No. 4, 2001, modified, 2005-03
- Determination of aflatoxins M1 and M2 in milk and dairy products by HPLC ISO 14501, modified, 1998-11
- Determination of ochratoxin A in cereal products and coffee DIN EN 14132, modified, 2003-09
- Determination of ochratoxin A in beverages DIN EN 14133, modified, 2003-10
- Determination of ochratoxin A in baby food, AOAC 84, No. 5, 2001, modified, 2004-03
- Determination of ochratoxin A in dried fruits, AOAC 86, No. 6, 2003, modified, 2004-02
- Multitoxin methods: determination of about 60 different mycotoxins by LC-MS/MS, Food Addit. Contam. 2005, 22, 752-760
- Determination of fumonisins B1 and B2 in food and feed by HPLC, E DIN EN 14352, modified, 2002-02
- Determination of patulin in apple juice and other fruit preparations, Eurofins Method 2005-10
- Determination of ergot alkaloids in cereal products by HPLC-MS/MS, Eurofins Method 2009-06

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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 spectrometry	Sweet pepper	Montoliu and others	Development of a multi-mycotoxin liquid chromatography-tandem mass spectrometry method for sweet pepper analysis	Rapid Commun Mass Spectrom 23(13):1–11	2009
Aflatoxin B1	Indirect competitive ELISA	Rice	Reidy and others	Detection of Aflatoxin B1 and aflatoxin B1 in rice in India	Food Microbiology 26:27–31	2009
Fumonisin	Liquid chromatography (LC) with fluorescence (FD) and mass spectrometry (MS) detectors	Corn-based food	Sika 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	Izumi and others	Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages	Food Microbiology 30:62–70	2009
Aflatoxin B1	Flow through quartz crystal microbalance (QCM) immunosensor	–	Wang and Gan	Biomolecule-functionalized magnetic nanoparticles for flow-through quartz crystal microbalance immunosensor of aflatoxin B1	Biosensors Bioelect Eng 33(1):108–116	2009
Aflatoxins B1, B2, G1, and G2, alternaria toxins, ochratoxin, patulin, trichothecenes, ZEA	Review paper on sampling and analysis of mycotoxins	–	Shepherd and others	Development in mycotoxin analysis: an update for 2002–2008	World Mycotoxin Journal 2(1):9–21	2009
OTA	HPLC/MS/MS	Cheese	Zhang and others	Direct monitoring of ochratoxin A in cheese with self-heating microextraction coupled to liquid chromatography-tandem mass spectrometry	J Chromatography A in press	2009
Aflatoxins and OTA	Reverse-phase liquid chromatography	Dietary supplements	Tuckness 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 Cambodia	Journal of agricultural and food chemistry 57(2):321–325	2009

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Continued

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	Offenberg and others	Multiresidue Mycotoxin Analysis in Corn Grain by Column High-Performance Liquid Chromatography with Postcolumn Photochemical and Chemical Derivatization	J AOAC Int 82(1): 15–25	2009
OTA	LC-4D LC-MS/MS	Dry pasta	NG and others	Survey of Dry Pasta for Ochratoxin A in Canada	J Food Prot 72(4): 890C	2000
Aflatoxins B1, B2, G1, and G2	LC-MS/MS	Peanut butter sesame paste	Li and others	Natural Occurrence of Aflatoxins in Chinese Peanut Butter and Sesame Paste	J Agric Food Chem, 57(9):2614–2624	2009
Aflatoxins B1, B2, G1, and G2	Liquid chromatography-mass spectrometry	Nuts, cereals, dried fruits, and spices	Noroka and others	Determination of aflatoxins in food samples by automated on-line multi-toxic-phase microextraction coupled with liquid chromatography-mass spectrometry	Journal of Chromatography A 1216(2): 4416–4422	2009
Aflatoxins B1, B2, G1, and G2, ochratoxin, ZEA DON, fumonisins, T-2, HF-2	Ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry (UHPLC/MS/MS)	Different cereal food	Sedran 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	Sol-phase microextraction (SPME)-LC-4D	Green coffee	Vidotto and others	Determination of ochratoxin A in green coffee beans by solid-phase microextraction and liquid chromatography with fluorescence detection	J Chromatography 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	Tuckness and others	Determination of aflatoxins B1, B2, G1, and G2 and ochratoxin A in ginseng and ginger by multi-toxin immunoaffinity column clean-up and liquid chromatographic quantitation: Collaborative study	Journal of AOAC International 91(3):311–323	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	Thongkummal and others	Monoclonal-based immunosorbent assay for the detection of zearalenone in cereals	Food Addit Contam 25(8):991–1006	2008
OTA	HPLC-ED	Wine	Talari 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-ED	Grapes, dried vine fruits, and winery byproducts	Salazar and others	Determination of ochratoxin A in grapes, dried vine fruits, and winery byproducts by high-performance liquid chromatography with fluorescence detection (HPLC-ED) and immunoenzymatic assay (LC-MS/MS)	Agri Food Chem 56(23):1108–16	2008
33 mycotoxins include 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, rusk, etc.	Spencer and others	LC-MS/MS method for the determination of 33 mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, rusk, and figs	Food Addit Contam 25(4):473–89	2008
Fusarium B1, H2 toxin, patulin, and ZEA	Liquid chromatography coupled with time-of-flight mass spectrometry (LC-TOF-MS)	Dried figs	Sanyova and Gilbert	Identification of fusarium B1, H2 toxin, patulin, and zearalenone in dried figs by liquid chromatography/mass spectrometry	J Food Prot 71(7):1330–4	2008
Macrocytic lactone mycotoxins (zearalenone, DON, alpha-zearalenol, alpha-ZOL, and beta-zearalenol, beta-ZOL)	Supercritical fluid extraction (SFE) and detection by Filtered adsorption cartridge before Chromatography	Maize flour	Zouaghi and Rios	Supercritical fluid extraction of macrocytic lactone mycotoxins in maize flour samples for rapid amperometric sensing and alternative liquid chromatographic method for confirmation	J Chromatogr A 1177(1):35–7	2008
T-2 and HT-2 toxins	LC-ED	Cereals	Trebst and others	Determination of T-2 and HT-2 toxins in cereals including cleanup by liquid chromatography and fluorescence detection	J Agric Food Chem 56(13):4665–4675	2008

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Table 1 – Continued.						
Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Fusarins	LC-MS/MS	Maize	Zimmer and others	A single extraction method for the analysis by liquid chromatography/mass spectrometry of fusarins and trichothecenes of dehydrated sphagnum peat in tissues of maize seedlings	Anal Bioanal Chem 391:2021–31	2008
Aflatoxins (B1, B2, G1, and G2), OTA, ZEA,	HPLC-ED	Poultry house	Wang and others	Simultaneous detection of aflatoxins, ochratoxin, and zearalenone in poultry house by immunoenzymatic assay and high-performance liquid chromatography	Environ Res 107(2):129–44	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-mycotoxins analysis using ACQUITY UPLC and Quattro Premier XE	Waters Applications Note 2007 Volume Page 5 pp	2007
Aflatoxins, ochratoxin, fumonins, trichothecenes		Tropical cereals	Magan and Abad	Fluorescent control analogs: Minimizing mycotoxins in the food chain	Int J Food Microbiol 2007 Jul 31	2007
Aflatoxins, ochratoxin A, fumonins, deoxynivalenol and zearalenone		Cereal grains	Stamen and Bianchi	Stability of mycotoxins during food processing	Int J Food Microbiol 2007 Jul 31	2007
Aflatoxins	HPLC columns were qualified by HPLC coupled with a C18 column, a photochemical reactor, and a fluorescence detector	Agricultural commodities ground sample	Sokolov	Simple, rapid, and inexpensive device method for quantification of aflatoxin in impure agricultural products by HPLC	J Agric Food Chem 55(2):138–41	2007
Ochratoxin A (OTA) and 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):1016–9	2007
Simultaneous	Membrane-based enrichment consisting of a membrane with immobilized anti-B1 (anti-B1) and anti-OTA antibodies and a filter paper attached to a polystyrene card below the membrane	Chili samples	Saha and others	Simultaneous enzyme immunoassay for the screening of aflatoxin B1 and ochratoxin A in chili samples	Anal Chim Acta 2007 584(2):343–9	2007
		Cereal	Berthel 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 (B1, B2, G1), ochratoxin A, fumonins (B1, B2), deoxynivalenol, zearalenone, T-2 and H2 toxins	Liquid chromatography/tandem mass spectrometry (LC-MS/MS) liquid chromatography coupled with electrospray ionization triple quadrupole mass spectrometry (LC-ESI/MS/MS) using an chromatographic mode	Maize	Lattanzio and others	Simultaneous determination of aflatoxins, ochratoxin A, and fumonins in maize by liquid chromatography/tandem mass spectrometry after multistage immunoenzymatic cleanup	Rapid Commun Mass Spectrom 2007 Sep 10; 21(20):3535–41	2007
Aflatoxins aflatoxins (B1, B2, G1, and G2)	Enzyme-linked immunosorbent assay (ELISA)	Rice artificially contaminated with brown, polished, and polished white kernels	Castell and others	Distribution of total aflatoxins in milled fractions of hulled rice	J Agric Food Chem 2007; 55:2765–4	2007
Simultaneously aflatoxins, type A trichothecenes, type B trichothecenes, OTA, zearalenone, fumonins, and patulin	Comprehensive LC/MS/MS in a single run	Analysis of corn flake extracts	Rudolfshalle and others	Multicomponent mycotoxin analysis by LC/MS/MS	The 10th annual meeting of the Israel Analytical Chemistry Society Conference & Exhibition, January 2–4	2007
Simultaneously measure mycotoxins (NDV, DON, AFG1, AFG2, AFB1, AFB2, FB1, FB2, Deoxynivalenol (DON), T-2 toxin, Ochratoxin A, and ZEN	LC-MS/MS method HPLC (Thermo Scientific, San Jose, Calif.)	Cattle Forages and Food Materials	Hubs and others	Analysis of mycotoxins in various cattle forages and food materials with the TSQ Quantum Discovery MAX	30 Mass spectrometry advancing supplement the application notebook March 2007	2007
Reduced up to 88% aflatoxin B1, 44% zearalenone, and 29% for fumonins ochratoxin. Standard OTE was ineffective in reducing DON uptake			Averagato and others	Assessment of the reducing potential of a carbon/activated carbon-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
AI B1, B2, G1, G2, M1, trichothecenes, DON, deoxynivalenol, fumonins (B1, B2, G1, and G2) one by one	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
Aflatoxins and ochratoxin	HPLC system consisted of a P1000R pump HPLC-MS/MS system.	Corn silage	Niderhorn and others	Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol	Food Addit Contam, April 2007; 24(4):409–15	2007
Fumonins (FB1 and FB2) and also used for aflatoxins (B1, B2, G1, and G2) one by one	FB1 FB2 HPLC/fluorescence following negative-ion 3,3'-disubstituted (NDA) derivatization A1 on C18 plate under UV light	Different corn-based food products	Calbas and Silva	Mycotoxins in corn-based food products consumed in Brazil: an exposure assessment for fumonins	J Agric Food Chem 2007; 55(19):391–40	2007
Aflatoxin B1	Optical density/fluorescence spectroscopy (OWS) technique in competitive and in direct	Bulley and wheat flour	Adani and others	Development of immunosensor based on OWS technique for determining aflatoxin B1 and ochratoxin A	Biosens Bioelectron 2007; 22:797–802	2007
Extended multi-mycotoxin method, for 25 contaminants	HPLC CM device coupled through a switching valve to a reversed-phase column, namely Chromatoderm Performance RP-HPLC, fully automated HPLC fluorescence detection		Calder and others	Development and integration of an immunosorbent membrane disk for the online solid phase extraction and HPLC determination with fluorescence detection of aflatoxins B1 in aqueous solutions	J Pharma Biomed Anal 2007; 44:398–403	2007
Aflatoxin B1 (AFB1)	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
	HPLC isocratic reverse-phase HPLC using a C18 column 150 mm x 4.6 mm i.d., EonPac (Merck, Portugal), with post column derivatization with TAC	Cattle feed collected from 7 dairy cow's farms from Portugal	Martins and others	Occurrence of aflatoxin B1 in dairy cows' feed over 10 y in Portugal (1995 to 2004)	Rev Bras Med 2007; 64(3):416–21	2007

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Table 1 – Continued.						
Mycotoxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins B1, ochratoxin, deoxynivalenol, fumonins (B1, B2), ochratoxin A, and zearalenone	HPLC-MS, Zorbax SB-C18 column (Agilent Technologies, Palo Alto, USA) with a 1 min OptiGuard C18 precolumn. Mass spectrometry was performed on a quadrupole analyzer equipped with electron spray ionization (ESI).	Corn silage	Richard and others	Toxicogen fungi and mycotoxins in mature corn silage	Available online 22 June 2007	2007
Ochratoxin A (OTA)	Immunosensor	High-colored matrices (pepper, ginger, nutmeg, black pepper, white pepper, Capsicum spp, spices)	Goryachova and others	Rapid alkali-one three-step immunosensor for non-instrumental detection of ochratoxin A in high-colored herbs and spices	Talanta Volume 72, Issue 3, 15 May 2007, Pages 1239–44	2007
Fusarium toxins (fumonins (FB1, B2), ochratoxin A, and zearalenone)	HPLC or GC in combination with a variety of detectors. Screening mycotoxins is performed by (TLC) ELISA	Feeds	Kiska and others	Analysis of Fusarium toxins in feed	Animal Feed Science and Technology 137(3–4):241–44	2007
Aflatoxins B1, ochratoxin, deoxynivalenol, fumonins (B1, B2), ochratoxin A, and zearalenone	High-performance liquid chromatography coupled to mass spectrometry (HPLC-MS)	Corn silage	Toxicogen fungi and mycotoxins in mature corn silage			2007
Aflatoxins, ochratoxins, fumonins, deoxynivalenol, zearalenone	Analyzed by HPLC ochratoxin A and aflatoxin B1 was performed using a reversed phase Symmetry C18 column (15 mm x 4.6 mm, 5 μm particles) preceded by a phenyl gel 50 μm filter. The fluorescence detection for ochratoxin A emission for fluorescence	A blend of naturally contaminated grains	Averagato and others	Assessment of the multi-mycotoxin-binding efficacy of a carbon/activated carbon-based product in an in vitro gastrointestinal model	J Agric Food Chem 2007; 55:4810–9	2007
Fusarium toxins (DON, ZEA, FB1, ...)	Liquid chromatography/mass spectrometry (LC-MS/MS)	Maize meal	Cavaliere and others	Mycotoxins produced by Fusarium species: determination by screening and confirmatory methods based on liquid chromatography/mass spectrometry	Food Chem 105(2):70–10	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 alkali-one 3-step immunosensor for non-instrumental detection of ochratoxin A in high-colored herbs and spices	Talanta Vol. 72, Issue 3, 15 May 2007; 152–4	2007
Simultaneous detection of aflatoxin B1 and ochratoxin A	Tandem immunoassay 1 mL column with 1 cleanup layer and two detection immunolayers ELISA	Spices (ginger, pepper, chili)	Goryachova and others	Simultaneous noninstrumental detection of aflatoxin B1 and ochratoxin A using a cleanup tandem immunoassay column	Anal Chim Acta 2007; 590:119–24	2007
Aflatoxin B1 (AFB1), ochratoxin A (OTA)	HPLC with fluorescence detection equipped with an injector 20 μL loop, a C18 reversed-phase column (3 μm, 150 × 4.6 mm i.d., EonPac (Merck, Portugal), with post column derivatization with TAC	Rice	Nguyen and others	Occurrence of aflatoxin B1, ochratoxin A, and zearalenone in rice in the province of the central region of Vietnam	Food Chem 2007; 103:45–7	2007
Aflatoxin M1 (AFM1), ochratoxin A (OTA)	Simultaneous determination of aflatoxins (AFB1, B2, G1, and G2) and ochratoxin A (OTA) by HPLC-MS/MS	Raw bulk milk	Abdel	Carcinogenic food contaminants	Cancer Invest 2007 Apr–May; 25(3):189–96	2007
Simultaneous determination of trichothecenes (HT-2, DON, F-2, T-2, etc., etc.)	HPLC coupled to UV and mass spectrometry (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; 73:231–7	2007
Aflatoxins, ochratoxins, fumonins, deoxynivalenol, zearalenone	High-performance liquid chromatography coupled with mass spectrometry (HPLC/MS) LC analysis by Ventan system, 2 pump, polar modified RP-18 column	Maize	Adejumo and others	Survey of maize from south-western Nigeria for zearalenone, α- and β-zearalenols, fumonins B1 and ochratoxin A produced by Fusarium species	Food Addit Contam, September 2007; 24(9):954–1000	2007

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Table 1 – Continued.						
Myco toxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins and ochratoxin A	were determined by TLC and HPLC methods	Poultry feeds	Frage and others	Potential Aflatoxin and Ochratoxin A production by <i>Aspergillus</i> species in poultry feed processing	Vol Res Commun Volume 31, Number 3:943-953 Dec: 2006	2007
Simultaneous determination of aflatoxin B1 (AFB1) and ochratoxin A (OTA)	HPLC column was Bio-el C18 LK 90x5 (5 mm, 4.6 x 102 mm) 200 rpm with a corresponding limit of detection	Olive oil	Ferracane and others	Simultaneous determination of Aflatoxin B1 and ochratoxin A and their natural occurrence in Moroccan virgin olive oil	Food Addit Contam, Vol. 24, 2:173-80	2007
Simultaneous, aflatoxins (AF1, i.e., B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2), and ochratoxin A (OTA)	AF reversed-phase liquid chromatography (HPLC) with fluorescence detection after postcolumn UV photochemical derivatization. OTA was separated and determined by HPLC with fluorescence detection. Revers	Ginseng and ginger	Trudassess and others	Use of multibore immunoreactivity columns for determination of aflatoxins and ochratoxin A in ginseng and ginger.	J AOAC Int 2007 Jul to Aug; 90(4):108-9	2007
Aflatoxins or ochratoxins	Flow	Tree nuts (almonds, pistachios, and walnuts)	Malpoux and others	Myco toxins in edible tree nuts	Int J Food Microbiol 2007 Jul 31	2007
Aflatoxins, deoxynivalenol, fumonins, zearalenone, T2 toxin, ochratoxin and certain ergot alkaloids	Flow	Crop plants	Richard	Some major myco toxins and their mycotoxins: an overview.	Int J Food Microbiol 2007 Jul 31	2007
Aflatoxin B1, deoxynivalenol, fumonisin B1, zearalenone, ochratoxin A and zearalenone	High-performance liquid chromatography coupled to mass spectrometry (HPLC-MS)	Corn silage myco toxin on nutrient agar	Richard and others	Toxicity of food and mycotoxins in mature corn silage	Food Chem Toxicol 2007 Jun 22	2007
13 trichothecenes, aflatoxins, fumonins, zearalenone, ochratoxin A, and zearalenone	Gas chromatography/mass spectrometry, zearalenone (ZEA), α - and β -zearalenol (α -ZEA and β -ZEA) by high-performance liquid chromatography (HPLC) with fluorescence and UV-detection.	Whole beans, roasted soy nuts, flour and flours, textured soy protein, tofu, protein isolate including infant formulae and fermented products (soy sauce)	Schellberger and others	Natural occurrence of Fusarium toxins in soy food marketed in Germany	Int J Food Microbiol 2007; 113:143-6	2007

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Table 1 – Continued.						
Myco toxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxins, type A trichothecenes, type B trichothecenes, ochratoxin A, zearalenone, fumonins, and others	LC/MS/MS	Corn flake	Multicomponent myco toxin analysis by LC/MS/MS	The 10 th annual meeting of the International analytical chemistry society 2007	2007	2007
Myco toxins within 12 minutes (NFV, DON, AFB1, AFB2, AFB1, AFB2, FB1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12)	HPLC LC-MS/MS method for the determination of myco toxins	Various cattle forages.		Analysis of myco toxins in various cattle	Mass spectrometry	2007
Zearalenone, deoxynivalenol, fumonins, zearalenone, ochratoxin A, and zearalenone	LC coupled to tandem mass spectrometry (LC-MS/MS), analysis by HPLC/MS/MS, Agilent C18 column (100 Å, 4.6 mm, 3 mm)	Using the model plant <i>Setaria viridis</i> (L.) Gaertn.	Berthiller and others	Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) determination of phase B metabolites of the myco toxin zearalenone in the model plant <i>Setaria viridis</i>	Food Additives and Contaminants November 2006; 23(11):1194-1200	2006
Aflatoxin B1, fumonisin B1, zearalenone, ochratoxin A, and zearalenone	HPLC-fluorescence detection (FLD) with postcolumn electrochemical derivatization in a HPLC cell	Chili powder, green bean, and black sesame.	Hu and others	Determination of aflatoxin B1, fumonisin B1, zearalenone, and ochratoxin A in chili powder, green bean, and black sesame	J Agric Food Chem 2006; 54, 4:128-30	2006
Aflatoxin B1, fumonisin B1, zearalenone, ochratoxin A	ELISA HPLC AG positive samples were also analyzed and confirmed by HPLC.	Rice, maize and peanuts	Sargere-Tigori and others	Co-occurrence of aflatoxin B1, fumonisin B1, zearalenone, and ochratoxin A in cereals and peanuts from Côte d'Ivoire	Food Additives and Contaminants October 2006; 23(10):1004-1017	2006
Aflatoxins	ELISA HPLC AG positive samples were also analyzed and confirmed by HPLC.	Red scab, red and black pepper.	Celik and others	Determination of aflatoxin B1, fumonisin B1, zearalenone, and ochratoxin A in red scab, red and black pepper by ELISA and HPLC	Journal of Food and Drug Analysis Vol. 14, No. 3, 2006, Pages 258-69	2006
Trichothecenes, ochratoxins, fumonins, zearalenone, ochratoxin A, and zearalenone	Attoxin B1, fumonisin B1, zearalenone using immunosays, and ochratoxin A using a validated HPLC method with fluorescence detector	Red scab, red and black pepper.	Zhang and Mayersbach	Attoxin B1, fumonisin B1, zearalenone using immunosays, and ochratoxin A using a validated HPLC method with fluorescence detector	Journal of Food and Drug Analysis Vol. 14, No. 3, 2006, Pages 258-69	2006

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Table 1 – Continued.						
Myco toxins	Quantification method	Type of food	Author	Article	Reference	Year
Aflatoxin; Ochratoxin A; Patulin; Fusarium toxins	PCR review		Paterson	Identification and quantification of mycotoxins using PCR	Process Biochemistry Volume 41, Issue 7, July 2006, Pages 1467-74	2006
Trichothecenes, ochratoxins, zearalenone, fumonins, aflatoxins, emmatins, moniliforms	LC-AP/MS review			Trace myco toxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionization mass spectrometry	Journal of Chromatography A Volume 1136, Issue 2, Pages 153-69	2006
Aflatoxin M1 in milk and B1 in feed	ELISA immunoassay used as screening test, positive samples confirmed by HPLC	Milk and feed	Decastell and others	Aflatoxin M1 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	Gullien 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 3198-204	2006
Myco toxins OTA, DON, AFB1, and FB2 were detected simultaneously	ELISA	Food sample		Rapid detection of foodborne contaminants using an Array Biosensor	Sensors and Actuators B 111 (2006) 586-607	2006
AFB1 and FB2 were detected simultaneously	Review		Malir and others	Monitoring the myco toxins in food and their biomarkers in the Czech Republic	Mal Nutr Food Res 2006 Jun; 50(6):513-8	2006
AFB1 and ochratoxin A (OTA)	Liquid chromatographic separation, and fluorescence detection	Ginseng and other selected botanical roots	Trudassess and others	Determination of aflatoxins and ochratoxin A in ginseng and other botanical roots by immunoreactivity column cleanup and liquid chromatography with fluorescence detection	J AOAC Int 2006 Sep; 89(3):834-40	2006

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Table 1 – Continued.						
Myco toxins	Quantification method	Type of food	Author	Article	Reference	Year
Analysis of myco toxins analysis of three myco toxins	Aflatoxin G1 has been detected by liquid-liquid partitioning methods with HPLC detection as Fluorimetric (FB)	Maize	Castegnaro and others	Advantages and drawbacks of immunoreactivity columns in analysis of myco toxins in food	Mal Nutr Food Res 2006 May; 50(5):480-7	2006
Aflatoxin G1	Simultaneously: NV, DON, ZEN, deoxynivalenol, T2 toxin, verruculic acid, fumonins A, fumonins B, zearalenone, ochratoxin A, and zearalenone			Compounds interfering with the FB antibodies were also observed while analyzing breakfast cereals leading to underestimation of FB, Ochratoxin A (OTA)	Available online 27 October 2006	2006
Ochratoxin (OT) and aflatoxin (AF)	LC-AP/MS	Barley rootlets (BR)	Ribeiro and others	Influence of water activity, temperature, and time on myco toxins production on barley rootlets.	Let Appl Microbiol 2006 Feb; 42(2):175-84	2006
Trichothecenes, ochratoxins, zearalenone, fumonins, aflatoxins, emmatins, moniliforms and several other myco toxins	LC-AP/MS	Review		Trace myco toxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionization mass spectrometry	Journal of Chromatography A Volume 1136, Issue 2, 15 December 2006, Pages 123-69	2006
Simultaneously: NV, DON, ZEN, deoxynivalenol, T2 toxin, verruculic acid, fumonins A, fumonins B, zearalenone, ochratoxin A, and zearalenone	HPLC fluorescence detector, injector, gradient and data handling capability is required. The fluorescence detector settings, excitation 315 nm, emission 415 nm	Two fungal media were used as samples	Dehrault and others	Development of a liquid chromatography/tandem mass spectrometry method for the simultaneous determination of 16 myco toxins in cellulose fibers and in fungal cultures	Rapid Commun Mass Spectrom 2003; 17(1):1-4	2006
Aflatoxin B1, fumonisin B1, zearalenone, ochratoxin A, and zearalenone	ELISA HPLC AG positive samples were also analyzed and confirmed by HPLC.	Food stuff review		Identification and quantification of mycotoxins using PCR	Process Biochemistry Volume 41, Issue 7, July 2006, Pages 1467-74	2006

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Table 1 – Continued.						
Myco toxins	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	Vandis 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):1338-5	2006
Aflatoxins B1, B2, G1, and G2, patulin and ergonolone only by one	HPLC (Agilent, 1100 series, USA) equipped with a fluorescence detector (G1312A, Agilent, 1100 series, USA) after postcolumn bromination immunoaffinity column (Vcam, Watertown, MA, USA)	Dried figs	Karaca and Nas	Aflatoxins, patulin and ergonolone contents of dried figs in Turkey	Food Additives and Contaminants, May 2006; 23(5):550-60	2006
Trichothecenes, ochratoxins, zearalenone, fumonins, aflatoxins, emmatins, moniliforms and several other myco toxins	Application of LC-AP/MS atmospheric pressure ionization (API) techniques			Trace myco toxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionization mass spectrometry	Department of Clinical Pharmacology Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Wien, Austria	2006
AFB1	Thin-layer chromatography for determining AFB1, zearalenone, and high treated temperature (LHT)	Raw, pasteurized and ultra-high treated temperature (LHT) milk	Shundo and Sabino	Aflatoxin M1 in milk by immunoreactivity column cleanup with TLC/HPLC determination	Brazilian Journal of Microbiology (2006) 37:164-67	2006
Ochratoxin A (OTA) and aflatoxin B1 (AFB1) only by one	Medicinal herbs, it has a rectangular shape with 10.65x1.01 mm. OTA was detected and quantified by reversed-phase HPLC, autoinjector (Agilent 1100, G1312A, Agilent) and a fluorescence detector A selected RP-C18 column HPLC method for aflatoxin B1 analysis both fluorescence and UV detector	Wine-grapes in Lebanon on Czappe yeast extract agar (CYA), olive medium	El Khoury and others	Determination of Ochratoxin A and Aflatoxin B1 producing fungi in Lebanese grapes and ochratoxin A content in muffs and finished wines during 2004	J Agric Food Chem 2006; 54, 8:977-82	2006

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Table 1 – Continued.						
Myco toxins	Quantification method	Type of food	Author	Article	Reference	Year
	ELISA		Mahdoui and others	Generation and characterization of polyclonal antibodies against Myco toxins Application to immunosays and immunoaffinity sample preparation prior to analysis by liquid chromatography and UV detection	Talanta 70 (2006) 225-32	2006
Aflatoxin B1	TLC silica gel as an adsorbent and 7% methanol in chloroform as the developing solvent. And fluorescently using HPLC plates aluminum sheets, silicagel 60 F254 precoated, enzyme linked immunosorbent assay (ELISA), flow through membrane based immunoassays, chromatographic techniques: acidic acid amplification assays, bioassays, and fluorimetry for detection of muffs and myco toxins.	Rice	Touja and others	Aflatoxin B1 contamination of puffed rice samples collected from different states of India: a multicentre study	Food Additives and Contaminants April 2006; 23(4):411-14	2006
AFB1 and OTA only by one	Quadrant by HPLC using a fluorescence detector.	Black and green olives of Greek origin	Ghazalou and others	Study of aflatoxin B1 and ochratoxin A production by natural microflora and <i>Aspergillus parasiticus</i> in black and green olives of Greek origin	Food Microbiol 23 (2006) 512-21	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, 24:531-35	2006

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