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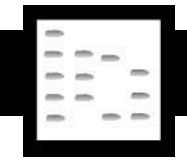
AFLATOXINS AND MYCOTOXINS



Chromatography

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Introduction

Mycotoxins have been defined as 'fungal metabolites which, when ingested, inhaled or absorbed through the skin, cause lowered performance, sickness or death in man or animals, including birds'.

Exposure to mycotoxins can produce both acute and chronic toxic effects ranging from death to deleterious effects on the central nervous, cardiovascular and pulmonary systems, and on the alimentary tract. Mycotoxins may be carcinogenic, mutagenic, teratogenic and immunosuppressive. The ability of some mycotoxins to compromise the immune system and, consequently, to reduce resistance to infectious disease, is now widely considered to be their most important effect.

The mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and both domestic and international trade. It has been estimated, for example, that annual losses in the USA and Canada arising from the impact of mycotoxins on the feed and livestock industries are in the order of US$5 billion. In developing countries where the food staples (e.g. maize and groundnuts) are susceptible to contamination, significant additional losses amongst the human population are likely, because of morbidity and premature death associated with the consumption of mycotoxins.

It is likely that mycotoxins have plagued mankind since the beginning of organized crop production. Ergotism (St Anthony's Fire), for example, which is caused by the consumption of rye contaminated with the 'ergot alkaloids', is discussed in the Old

Testament, and reached epidemic proportions in many parts of Europe in the tenth century.

Mycotoxins of Worldwide Importance

An 'important' mycotoxin will have demonstrated its capacity to have a significant economic impact on the exposed human and/or animal population. Those moulds and mycotoxins that are currently considered to be of worldwide importance are shown in **Table 1**, and the chemical structures of the mycotoxins in **Figure 1**.

Aflatoxins

The term 'aflatoxins' was coined in the early 1960s when the deaths of thousands of turkeys ('Turkey X' disease), ducklings and other domestic animals were attributed to the presence of *Aspergillus flavus* toxins in groundnut meal imported from South America. The acute and chronic effects of the aflatoxins on a wide variety of livestock are now well documented, and include death, decreased productivity, and increased susceptibility to disease. Aflatoxin B_1 is a human carcinogen and one of the most potent hepatocarcinogens known. Human fatalities have resulted from the consumption of heavily aflatoxin-contaminated foods, frequently when wholesome food is in short supply. Aflatoxin M_1 occurs in milk, and is produced by the bovine metabolism of aflatoxin B_1 when contaminated feed is ingested by dairy cows.

Trichothecenes

T-2 toxin, deoxynivalenol (and nivalenol) belong to a large group of structurally related sesquiterpenes known as the 'trichothecenes', which occur primarily in cereals. T-2 toxin is the probable cause of 'alimentary toxic aleukia' (ATA), a disease that affected thousands of people in Siberia during the Second World War, and led to the elimination of entire villages. The symptoms of ATA include fever, vomiting, acute inflammation of the alimentary tract and a variety of blood abnormalities. The same toxin is also associated with outbreaks of haemorrhagic disease in animals and with neurotoxic effects in poultry. An important effect of T-2 toxin (and other trichothecenes) is the immunosuppressive activity which has been clearly demonstrated in experimental animals.

Deoxynivalenol (DON) is probably the most widely occurring *Fusarium* mycotoxin. (The trivial name of 'vomitoxin' has also been accorded to DON because of outbreaks of emetic (and feed refusal) syndromes, amongst livestock, caused by this toxin.) The ingestion of DON has caused acute human mycotoxicoses in India, China and rural Japan. The Chinese outbreak, in 1984–85, was caused by mouldy maize and wheat. Symptoms occurred within 5 to 30 min and included nausea, vomiting, abdominal pain, diarrhoea, dizziness and headache.

Zearalenone

Zearalenone is an oestrogenic mycotoxin that is coproduced with DON, and which has been implicated, with DON, in outbreaks of acute human mycotoxicoses. In livestock, exposure to zearalenone-contaminated maize has caused hyperoestrogenism, especially in pigs, characterized by vulvar and mammary swelling and infertility.

Fumonisins

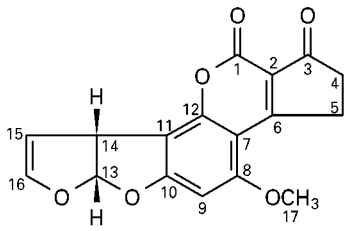
Fumonisin B_1 (FB_1) occurs in maize produced in a variety of agroclimatic zones. Two animal species, horses and pigs, are particularly targetted by FB_1. Exposure to FB_1 causes leukoencephalomalacia (LEM) in horses and pulmonary oedema in pigs. The presence of fumonisins in maize has been linked with human oesophageal cancer in the Transkei (South Africa) and China.

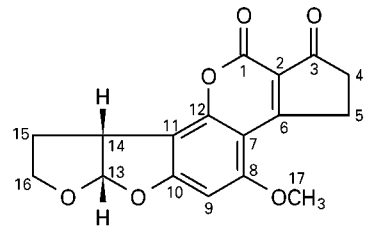
Ochratoxin A

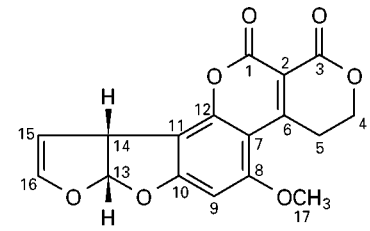
Ochratoxin A (OA) causes nephropathy and immunosuppression in several animal species, and is carcinogenic in experimental animals. OA has been linked to the human disease Balkan endemic

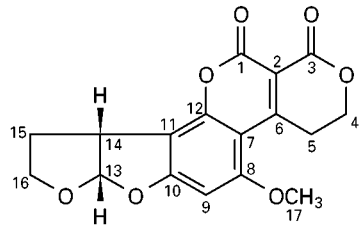
Table 1 Moulds and mycotoxins of worldwide importance

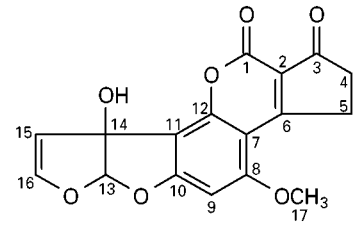
Mould species	*Mycotoxins produced*	*Main sources*
Aspergillus parasiticus	Aflatoxins B_1, B_2, G_1, G_2	Edible nuts, oilseeds and cereals
A. flavus	Aflatoxins B_1, B_2	
Fusarium sporotrichioides	T-2 toxin	Wheat and Maize
F. graminearum	Deoxynivalenol (or nivalenol in some areas) zearalenone	Wheat and Maize
F. moniliforme	Fumonisin B_1	Maize
Penicillium verrucosum and *A. ochraceus*	Ochratoxin A	Wheat, barley, coffee beans, vine fruits

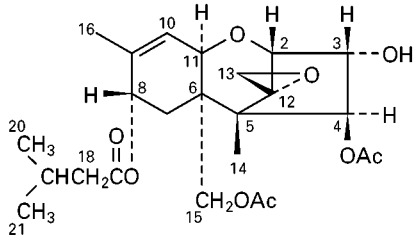

Aflatoxin B_1

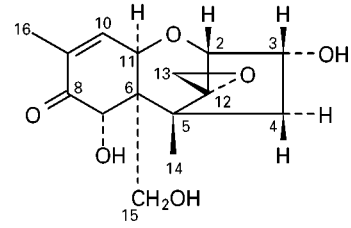

Aflatoxin B_2


Aflatoxin G_1


Aflatoxin G_2


Aflatoxin M_1


T-2 toxin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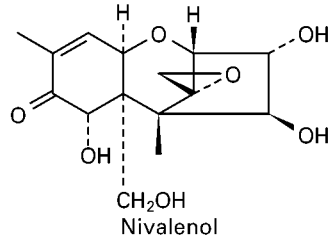
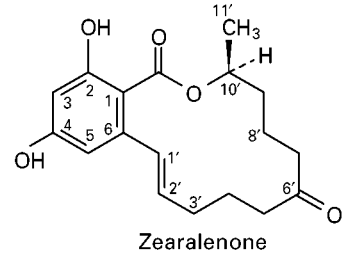
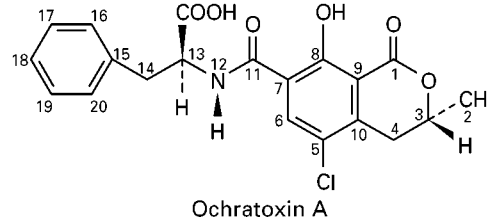
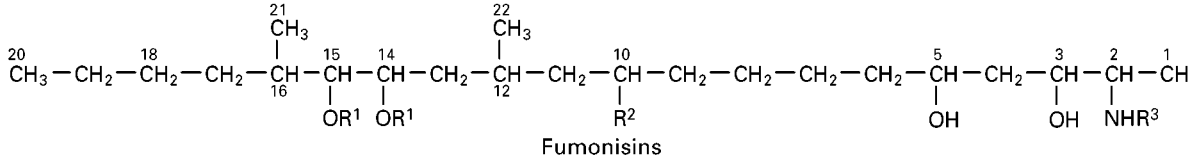

Nivalenol


Zearalenone


Ochratoxin A


Fumonisins

A_1: R^1=$COCH_2CH(CO_2H)CH_2CO_2H$; R^2=OH; R^3=$C^{23}OC^{24}H_3$

A_2: R^1=$COCH_2CH(CO_2H)CH_2CO_2H$; R^2=H; R^3=$COCH_3$

B_1: R^1=$COCH_2CH(CO_2H)CH_2CO_2H$; R^2=OH; R^3=H

B_2: R^1=$COCH_2CH(CO_2H)CH_2CO_2H$; R^2=R^3=H

Figure 1 Mycotoxins of worldwide importance.

nephropathy, a fatal, chronic renal disease occurring in limited areas of Bulgaria, the former Yugoslavia and Romania. It has been suggested that pork products are significant human dietary sources of OA.

Control of Mycotoxins

The control of mycotoxins is summarized in **Figure 2**. The interventions that may be employed for the control of mycotoxins are prevention of contamination, identification and segregation of contaminated material (quality control, monitoring and legislation), and detoxification.

Preventative measures that militate against the onset of biodeterioration and, subsequently, the production of moulds and mycotoxins, may be introduced throughout the commodity system. However, the preharvest control of biodeterioration is somewhat

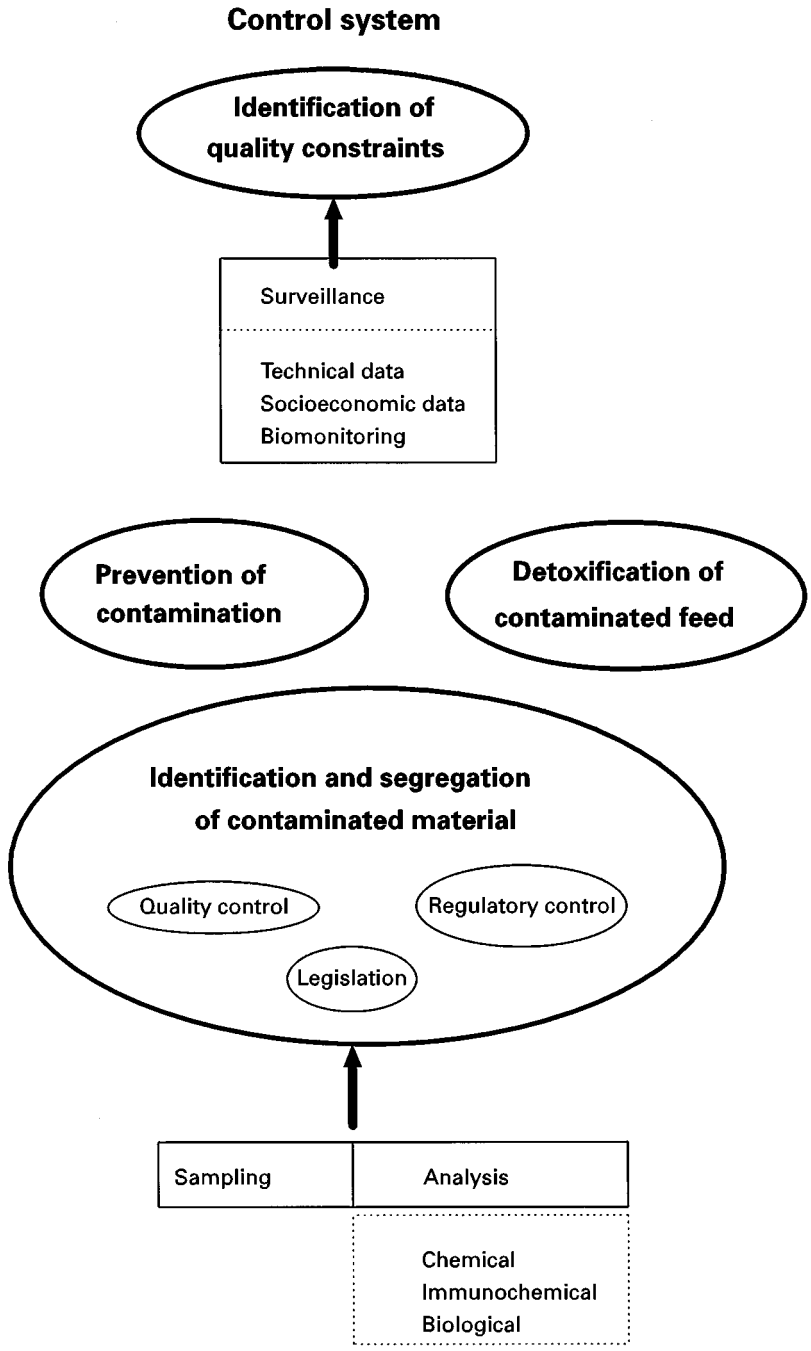


Figure 2 The mycotoxin control system.

compromised by our inability to control the climate! Attempts have been made to prevent preharvest contamination by breeding for resistance to moulds and by 'biocontrol' methods, involving the introduction, to the field, of atoxigenic strains of competing fungi. After harvest, it is important that the crop is dried to a 'safe' moisture level (which will not support the growth of moulds and mycotoxins) as quickly as possible.

The identification and segregation of mycotoxin-contaminated material may be pursued through quality control and regulatory procedures. More than 50 countries currently impose legal limits on the occurrence of mycotoxins (especially the aflatoxins) in foods and feeds.

Commercial detoxification plants, for the treatment of aflatoxin-contaminated groundnut meal, are currently operating in Senegal, France and the UK. The chemical detoxification reagent that is most widely used is ammonia, both as an anhydrous vapour and an aqueous solution.

If the package of control procedures described above is to be successfully implemented, it is essential that it is underpinned by an integrated package of sampling, sample preparation and analytical procedures.

Analysis of Mycotoxins

Worldwide, 5 parts per billion ($\mu g\,kg^{-1}$) is the most common maximum level of total aflatoxins permitted in foods. Similarly, aflatoxin M_1 is regulated in at least 14 countries, the permitted levels typically falling within the range 0.05 to 0.5 parts per billion. Consequently, it is essential that the analytical methods used for quality control and monitoring (regulatory control) purposes are accurate and precise at these extremely low concentrations.

Analytical Sequence

The analysis of mycotoxins may be considered in terms of a sequence of four operations: extraction, clean-up, quantification and confirmation. Some of the more commonly used procedures associated with these operations are illustrated in **Table 2**.

The mycotoxin(s) under investigation must first be extracted from the complex and variable chemical milieu of the food or feed under investigation, using an appropriate extraction solvent. Commonly used solvent systems include acetone, acetonitrile, methanol, ethyl acetate, chloroform and water, either singly or as mixtures of two or more solvents. The extraction is performed either by shaking the mixture of sample and solvent for 30–45 min or by blending at high speed for approximately 3 min. The choice of solvent can significantly affect the extractability of the mycotoxin. The extraction of the aflatoxins from corn, for example, is significantly enhanced if the aqueous extraction solvent contains acetone as opposed to methanol. Supercritical fluid extraction is an emerging alternative to liquid extraction, and has been successfully applied to the extraction of aflatoxin B_1 from corn.

The crude extract, obtained after filtration of the shaken or blended mixture, is cleaned-up in order to remove as much non-mycotoxin material as possible, since the presence of extraneous compounds can seriously diminish the efficiency of the analysis. Clean-up procedures include liquid–solid extraction (defatting), liquid–liquid partitioning, chemical adsorption and chromatographic methods.

Table 2 The analysis of mycotoxins

Operation	*Commonly used procedure*
Extraction	Sample extracted by shaking or blending with chloroform, or mixtures of water/methanol, water/acetonitrile or water/acetone
Clean-up	Liquid–liquid partitioning or liquid–solid extraction
	Chemical adsorption
	Solid-phase extraction (SPE)
	Multifunctional clean-up column
	Chromatography
	Immunosorbent columns
Quantification	Thin layer chromatography (TLC)
	High performance thin layer chromatography (HPTLC)
	High performance liquid chromatography (HPLC)
	Gas chromatography (GC)
	Fluorimetry
Confirmation	Cochromatography
	Visual observation of colour change after derivatization
	Spectroscopy (with or without derivatization)
	Mass spectrometry

Solid-phase extraction (SPE) and immunosorbent columns are examples of recently introduced clean-up procedures that are now frequently used. SPE cartridges are available with a wide variety of polar, nonpolar and ion exchange bonded phases.

A 'multifunctional clean-up column' (MFC), composed of lipophilic, dipolar and anion exchange sites, reportedly affords the efficient clean-up of acetonitrile/water extracts within 10 s. MFC high performance liquid chromatography (HPLC) analysis methods have been applied to at least 10 mycotoxins.

The chromatographic quantification techniques used for the determination of mycotoxins in cleaned-up extracts include thin-layer chromatography (TLC), high performance TLC (HPTLC), high performance liquid chromatography (HPLC), and gas chromatography (GC). Worldwide, TLC is the most common method employed for the estimation of mycotoxins.

No assay can be considered as complete until the presence of the presumptive mycotoxin has been confirmed. This is especially important when an unusual commodity is under investigation. The ultimate confirmation involves the comparison of the physicochemical characteristics of the presumptive mycotoxin with those of a standard compound. Such a course of action is not normally utilized as a routine procedure. Confirmatory techniques used in conjunction with HPLC include mass spectrometry and ultraviolet spectroscopy. When TLC or HPTLC are used for quantification, the formation of derivatives with characteristic chromatographic and fluorescence properties is commonly employed to confirm the presence of the presumptive mycotoxin(s).

Analytical Accuracy

The overall accuracy of the determination of mycotoxins will be governed by the combined effects of the sampling, sample preparation and analytical components of the analytical process. Undoubtedly, the sampling component is currently the greatest source of analytical error. Until effective sampling (and sample preparation) procedures have been developed for a variety of mycotoxin/commodity combinations, the accuracy and precision of methods for the determination of mycotoxins will be severely compromised.

The reliability of an analytical procedure may be expressed in terms of the accuracy, precision and limit of detection of the method. Interlaboratory precision is determined by the implementation of check-sample and collaborative studies. The level of interlaboratory precision for the determination of mycotoxins is still disappointing. A review of the reliability of mycotoxin assays, conducted in 1993, indicated that little or no improvement in interlaboratory precision had occurred over the previous 20 years. The precision of TLC and HPLC methods were reportedly similar, whereas the precision of enzyme-linked immunosorbent assay (ELISA) methods was somewhat poorer. A series of proficiency testing exercises were carried out during 1993 and 1994 involving those European laboratories who contribute analytical data on food contamination to the World Health Organization (WHO) Global Environmental Monitoring Scheme (GEMS). The tests were performed according to the International Organization for Standardization/International Union of Pure and Applied Chemistry/Association of Official Analytical Chemists (ISO/IUPAC/AOAC) International Harmonized Protocol, and laboratories were awarded '*z* scores' that signified their analytical capability. Eighty eight per cent of the laboratories obtained results of acceptable accuracy for the determination of the aflatoxins, whereas only 53% of the laboratories demonstrated acceptable accuracy for patulin (a mycotoxin produced by *Penicillium expansum* and other moulds.)

Simple Methods

Methods of quantification employing HPTLC, HPLC and GC require expensive equipment and skilled personnel. However, such procedures are not normally available in the basic analytical laboratories that exist in, for example, exporting developing countries and in some food and feed manufacturing plants.

Basic laboratory environments require simple, robust, low-cost methods that can afford reliable results in the hands of semiskilled operators. Methods that have been developed with such an application in mind include minicolumn and immunodiagnostic procedures. The minicolumn approach utilizes small glass columns packed either with selected chromatographic adsorbents or with other inorganic adsorbing materials. Minicolumns are used either to clean up the crude extract before quantification; or the mycotoxin under test is adsorbed onto the column, as a band, which is normally visually determined under ultraviolet (UV) light. Immunodiagnostic procedures take the form either of immunoaffinity columns or of solid-phase ELISA methods. Immunoaffinity columns are used to effect the sample clean-up before the mycotoxin is quantified, either by adsorption onto a Florisil 'tip' or by elution into a simple fluorimeter.

Solid-phase ELISA methods have been developed where the mycotoxin antibody is immobilized, for example, onto a card (about the size of a credit card), a plastic cup (the 'immunodot' approach) or a plastic probe. The presence of the mycotoxin, above a

predetermined level, is indicated by a visually observed colour change within small indentations within the card, cup or probe.

Chromatography of Selected Mycotoxins

The methods used for the chromatographic analysis of mycotoxins will now be further illustrated by describing the determination of the 'important' mycotoxins listed in Table 1. In each case, 'official' methods that have been approved by an appropriate internationally recognized body will be described, together with a selection of recently developed procedures.

Aflatoxins

The chromatographic methods employed for the determination of the aflatoxins (B_1,B_2,G_1,G_2,M_1,M_2) include TLC, HPTLC and HPLC, usually in combination with fluorescence detection. The aflatoxins exhibit an intense fluorescence when subjected to UV irradiation.

For TLC and HPTLC the intensity of fluoresence may be estimated either visually (using, for example, the 'comparison of standards' procedure) or densitometrically.

When HPLC methods are employed, the intensity of the fluorescence and the position of the excitation/emission maxima vary with the composition of the mobile phase. For example, the aflatoxins B_1 and G_1 are much less intense than aflatoxins B_2 and G_2 in aqueous or alcoholic solutions. The fluorescence excitation maximum for B_1 occurs at 355 and 363 nm in acetonitrile and water, respectively, whereas the emission maximum varies from 415 (in chloroform) to 450 nm (in water). In aqueous solutions, the sensitivity of the fluorescence detection system may be enhanced by the pre-column treatment of the aflatoxins B_1 and G_1 with trifluoracetic acid (TFA), or by post-column treatment with either iodine or bromine solutions.

HPTLC, involving semiautomated sample application and fluorescence densitometry, is sufficiently robust to have been successfully exploited in laboratories in developing countries.

Official methods Those methods that have been approved by the AOAC and other international bodies are described in **Table 3**. Methods 968.22 and 971.24 have also been adopted by the International Union of Pure and Applied Chemistry (IUPAC); methods 975.36 and 972.26 by the American Association of Cereal Chemists (AACC); and methods 970.45 and 971.24 by the American Oil Chemists Society (AOCS). It is evident from Table 3 that many of the official methods are based upon analytical procedures that were developed many years ago, using a combination of silica gel column chromatography clean-up and normal phase silica gel TLC.

Recent developments Reversed-phase HPLC, with post-column derivatization and fluorescence detection, is now widely used in the developed world for the analysis of the aflatoxins. Post-column iodination is performed within a heated reaction coil, where the column eluent is mixed with iodine-saturated water. Post-column bromination can be performed where bromide ion in the mobile phase is converted to bromine using a commercially available electrochemical cell. Sample clean-up is frequently performed using proprietary immunoaffinity or SPE columns. The AOAC Official Method 991.31, for example, utilizes the Aflatest immunoaffinity column in combination with reversed-phase C_{18} HPLC for the determination of the aflatoxins. A similar approach was reported in 1995 for the determination of aflatoxin M_1 in cheeses. Briefly, the dichloromethane extract is evaporated to dryness in a rotary evaporator, redissolved in a mixture of methanol/water/hexane (1 : 30 : 50 v/v), and subjected to liquid partitioning. The aqueous phases are then cleaned up using an immunoaffinity column containing monoclonal antibodies against aflatoxin M_1.

Reversed-phase (C_{18}) HPLC quantification, in combination with fluorescence detection, affords an approximately 75% recovery of aflatoxin M_1, and a limit of quantification of 0.02 μg kg^{-1}. The fluorimetric excitation and emission wavelengths are 360 and 435 nm. In the EC method (92/95/EEC), the sample clean-up is performed using a combination of Florisil™ and C_{18} SPE columns. The combination of C_{18} SPE column clean-up and HPLC quantification, with fluorescence detection, is frequently used for the determination of the aflatoxins in a variety of substrates.

HPTLC, in combination with phenyl bonded phase SPE and fluorescence densitometry, has been successfully applied to the determination of aflatoxins in a variety of commodities including corn, cottonseed, sorghum, peanut butter and palm kernels. Typically, aluminium-backed silica gel HPTLC plates are subjected to bidirectional chromatography using anhydrous diethyl ether and chloroform/xylene/acetone (6 : 3 : 1 v/v) in the first and second directions, respectively. Interfering components may be removed by carefully cutting away the upper part of the plate after the first development, before rotating the plate through 180° prior to the second development. The estimation of aflatoxin B_1, by bidirectional HPTLC, in a variety of commodities is illustrated in **Table 4**. HPTLC has also been recently used for the

Table 3 Official methods for the analysis of aflatoxins; these are AOAC methods unless stated otherwise

Method no.	*Date method developed*	*Aflatoxin*	*Commodity*	*Extraction solvent*	*Development solvent/mobile phase*	*Stationary phase*	*Clean-up method*	*[a]Detection limit, LOD* ($\mu g\ kg^{-1}$)*/ Additional information*
HPLC (with fluorescence detection)								
[b]980.20	1980	B_1, B_2, G_1, G_2	Cottonseed products	Acetone/H_2O	H_2O saturated $CHCl_3$/(cyclohexane) CH_3CN (25:7.5:1) + 1.5% abs. ethanol or 2.0% isopropanol	Silica gel	Chemical adsorption and silica gel column	LOD not specified
986.16	1986	M_1, M_2	Liquid milk	C_{18} SPE	H_2O/isopropanol/ CH_3CN (80:12:8)	C_{18} column (pre-column derivatization)	Small silica gel column	LOD not specified
990.33	1990	B_1, B_2, G_1, G_2	Corn and peanut butter	CH_3OH/ 0.1M HCl	H_2O/CH_3CN/ CH_3OH (700:170:170)	C_{18} column (pre-column derivatization)	Silica gel column	5.0 10.0, total (AOAC/IUPAC method)
991.31	1991	B_1, B_2, G_1, G_2	Corn, raw groundnuts and peanut butter	CH_3OH/H_2O	H_2O/CH_3CN/ CH_3OH (3:1:1)	C_{18} column (post-column derivatization)	Aflatest Immunoaffinity column	10.0, total (AOAC/IUPAC method)
[c]92/95/ EEC	1991	B_1	Animal feeds	$CHCl_3$	H_2O/CH_3OH/ CH_3CN (130:70:40)	C_{18} column (post-column derivatization)	Florisil and C_{18} SPE	1.0
TLC								
[b]968.22	1968	B_1, B_2, G_1, G_2	Groundnuts and their products	$CHCl_3/H_2O$	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Silca gel column	LOD not specified (IUPAC/AOAC method; CB[d] method)
970.45	1970	B_1, B_2, G_1, G_2	Groundnuts and their products	CH_3OH/H_2O/ hexane	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Centrifugation and liquid partitioning	LOD not specified (AOCS/AOAC method; BF[d] method)
971.23	1969	B_1, B_2, G_1, G_2	Cocoa beans	$CHCl_3/AgNO_3$ solution	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Defatting and silica gel column	LOD not specified (IUPAC/AOAC method; modified CB method)
971.24	1971	B_1, B_2, G_1, G_2	Coconut, copra, copra meal	$CHCl_3$/NaCl solution	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Silca gel column	LOD not specified (IUPAC/AOCS/ AOAC method)
972.26	1972	B_1, B_2, G_1, G_2	Corn	$CHCl_3/H_2O$	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Silca gel column	LOD not specified (AACC/AOAC method; based upon CB method)
972.27	1972	B_1, B_2, G_1, G_2	Soya beans	$CHCl_3/H_2O$	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Silca gel column	LOD not specified (based upon CB method)
974.16 (Method 1)	1974	B_1, B_2, G_1, G_2	Pistachio nuts	$CHCl_3/H_2O$	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Silca gel column	LOD not specified (based upon CB method)
(Method 2)				CH_3OH/H_2O/ hexane	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Centrifugation and liquid partitioning	LOD not specified (based upon BF method)
978.15	1977	B_1	Eggs	Acetone/H_2O/ saturated NaCl solution	2D TLC: (a) anhydrous diethyl ether/CH_3OH/H_2O (96:3:1) (b) Acetone/$CHCl_3$ (1:9)	Silica gel	Chemical adsorption, liquid partitioning and silica gel column	LOD not specified

Table 3 *Continued*

Method no.	*Date method developed*	*Aflatoxin*	*Commodity*	*Extraction solvent*	*Development solvent/mobile phase*	*Stationary phase*	*Clean-up method*	*[a]Detection limit, LOD* ($\mu g\ kg^{-1}$)/ *Additional information*
980.20	1980	B_1, B_2, G_1, G_2	Cottonseed products	Acetone/H_2O	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	Chemical adsorption and silica gel column	LOD not specified
980.21	1978	M_1	Milk, cheese	$CHCl_3$/NaCl solution	For milk: $CHCl_3$/acetone/ isopropanol (87:10:3) For cheese: 2D TLC: (a) diethyl ether/CH_3OH/H_2O (95:4:1) (b) $CHCl_3$/acetone/ isopropanol (87:10:3)	Silica gel	Silica gel column	LOD not specified
982.24	1981	B_1, M_1	Liver	CH_2Cl_2/citric acid solution	2D TLC: (a) diethyl ether/CH_3OH/H_2O (95:4:1) (b) $CHCl_3$/acetone/ isopropanol (87:10:3)	Silica gel	Silica gel column	LOD not specified
993.17	1994	B_1, B_2, G_1, G_2	Corn and groundnuts	CH_3OH/H_2O	$CHCl_3$/acetone (9:1)	Silica gel	Silica gel column	5.0, densitometrically 10.0, visually
Minicolumn								
975.36	1975	B_1, B_2, G_1, G_2	Food and feeds	Acetone/H_2O	$CHCl_3$/acetone (9:1)	$CaSO_4$, Florisil, silica gel, neutral alumina	Chemical adsorption	5.0, total; almonds 10.0, total: corn, groundnuts, peanut butter, pistachio nuts, groundnut and cottonseed meals 15, total, mixed feeds Romer method (AACC/AOAC method)
979.18	1979	B_1, B_2, G_1, G_2	Corn, groundnuts	$CHCl_3$/H_2O	$CHCl_3$/acetone (9:1)	$CaSO_4$, Florisil, silica gel, neutral alumina	Liquid partitioning	10.0 (Holaday-Velasco method)

[a]The minimum contamination level to which the method is applicable: applies to aflatoxin B_1, unless otherwise stated.
[b]AOAC classification. [c]EC Directive. [d]Scott (1998).

determination of aflatoxin M_1 in milk. The samples were extracted with chloroform contained within a hydrated dialysis tube, before subjecting the concentrated extract to HPTLC on silica gel plates. This method gave a recovery of 96% and fluorescence densitometry gave a detection limit of 0.002 $\mu g\ L^{-1}$. The excitation wavelength was 350 nm, with an emission cut-off of below 400 nm.

A recently reported novel approach to the determination of aflatoxins in corn utilizes silica or immunoaffinity column clean-up in combination with capillary electrophoresis, with laser-induced

Table 4 Estimation of aflatoxin B_1 by bidirectional HPTLC

Commodity	*Extraction solvent*	*Clean-up method*	*Limit of detection* (B_1, $\mu g\,kg^{-1}$)
Peanut butter	Acetone/H_2O	Phenyl SPE	2.0
Corn	Acetone/H_2O	Phenyl SPE	1.7
Cottonseed	Acetone/0.1N HCl	Phenyl SPE	2.7
Sorghum	$CHCl_3$/H_2O	Florisil column	1.3

fluorescence detection. The reported limit of detection is 0.5 $\mu g\,kg^{-1}$ aflatoxin B_1, with an average recovery of 85% over the range 1 to 50 $\mu g\,kg^{-1}$.

Ochratoxins

Official methods Official AOAC methods exist for the determination of the ochratoxin A in barley, corn and green coffee. These procedures are summarized in **Table 5**. It is evident from Table 5 that both the TLC methods are rather old, whereas the HPLC procedure is reasonably modern. Each of the official methods utilizes the native fluorescence of ochratoxin A for detection purposes. On a silica gel TLC plate, ochratoxin A fluoresces most intensely under 365 nm UV light. If the plate is sprayed with alcoholic $NaHCO_3$ solution the fluorescence increases in intensity, and changes from greenish blue to blue in colour. If the TLC plate is quantified densitometrically, the optimum excitation and emission wavelengths are 310–340 and 440–475 nm, respectively. When employing HPLC, the recommended fluorescence detection wavelengths are 333 (excitation) and 460 nm (emission).

The AOAC Method 991.44 has been subjected to an interlaboratory study involving 12 European laboratories, under the auspices of the AOAC/IUPAC/NMKL (Nordic Committee on Food Analysis). The results of the intercomparison are given in **Table 6** for contamination levels, in wheat bran, rye and barley, of between 2 and 9 $\mu g\,kg^{-1}$ ochratoxin A. The mean recoveries varied from 64 to 72%. The method has been accepted as an official NMKL procedure.

Recent developments Recently developed HPLC methods for the determination of ochratoxin A employ silica gel SPE and immunoaffinity clean-up followed by reversed-phase C_8, C_{18} and C_{22} HPLC columns, in combination with fluorescence detection. The ionization of the phenolic group in the underivatized toxin is suppressed by the presence of phosphoric or acetic acids in the mobile phase.

An HPLC method (Method 1, **Table 7**) for the determination of ochratoxin A in roast and ground coffee uses a combination of silica gel SPE and immunoaffinity clean-up in order to ensure a good recovery (87%) of toxin. (Very low recoveries were obtained when immunoaffinity clean-up alone was used.) Fluoresence detection with excitation and emission wavelengths of 333 and 470 nm

Table 5 Official methods for the analysis of ochratoxin A; these are AOAC methods unless stated otherwise

Method no.	*Date method developed*	*Commodity*	*Extraction solvent*	*Development solvent/Mobile phase*	*Stationary phase*	*Clean-up method*	*Detection limit, LOD* ($\mu g\,kg^{-1}$)/*Additional information*
TLC							
973.37	1973	Barley	$CHCl_3$/ 0.1 mol L^{-1} H_3PO_4 soln	Acetone/$CHCl_3$ (5:95 to 15:85)	Silica gel	$NaHCO_3$/ diatomaceous earth column	LOD not specified (IUPAC/AOAC method)
975.38	1975	Green coffee	$CHCl_3$	Toluene/ethyl acetate/ formic acid (5:4:1) or benzene/CH_3OH/acetic acid (18:1:1, two sequential developments)	Silica gel	$NaHCO_3$/ diatomaceous earth column	LOD not specified
HPLC							
991.44	1992	Corn and barley	$CHCl_3$/ 0.1 mol L^{-1} H_3PO_4 soln	H_2O/CH_3CN/acetic acid (99:99:2)	C_{18} column	C_{18} SPE	10.0 (IUPAC/ [a]NMKL method)

[a]NMKL, Nordic Committee on Food Analysis.

Table 6 Interlaboratory study of the official NMKL HPLC method for the analysis of ochratoxin A

Commodity	*Coefficient of variation* (%)	
	Intralaboratory	*Interlaboratory*
Wheat bran	21	23–28
Rye	17	20–28
Barley	12	18–31

was employed. The presence of ochratoxin A was confirmed by the HPLC determination of its methyl ester.

HPLC quantification has also been used to determine the ochratoxin A content of milk (Method 2, Table 7). The emulsion produced during the chloroform/methanol extraction was broken by refrigerated centrifugation. After clean-up, the purified extract was dissolved in methanol, by ultrasonic treatment, before application to the HPLC column. The emission and excitation wavelengths of the fluorescence detector were set at 330 and 460 nm. The presence of ochratoxin A, in the range 0.01 to 0.03 $\mu g\,L^{-1}$, was confirmed by ELISA.

An HPTLC method (Method 3, Table 7) has recently been developed for the determination of ochratoxin A in parboiled rice. Extraction was performed with chloroform and phosphoric acid; the clean-up involved a combination of partitioning into sodium bicarbonate solution and phenyl bonded-phase SPE. Bidirectional HPTLC using aluminium-backed silica gel plates was employed, using diethyl ether/methanol (98 : 2 v/v) and toluene/ethyl acetate/formic acid (5 : 4 : 1 v/v) in the first and second directions, respectively. After removing the bottom portion of the plate, a third development was performed, in the same direction, with *n*-hexane/ethyl acetate/acetic acid (18 : 3 : 1 v/v). Flurodensitometric detection (excitation at 365 nm) afforded a mean intralaboratory precision of 5.4% over the concentration range 10 to 200 $\mu g\,kg^{-1}$ ochratoxin A. The mean recovery and limit of detection were 83% and 11.6 $\mu g\,kg^{-1}$, respectively.

Two intercomparison studies have recently been performed, within the European Commission, Measurements and Testing Programme, on the HPLC determination of ochratoxin A. The first study, using kidney naturally contaminated at 10 $\mu g\,kg^{-1}$ ochratoxin A, involved 20 European laboratories. A variety of extraction and clean-up procedures were used, and recoveries ranged from 43 to 128%. The second study, involving 26 European laboratories, used wheat naturally contaminated with approximately 7 $\mu g\,kg^{-1}$ ochratoxin A. Again, a variety of extraction and clean-up procedures were employed. Some laboratories compared their normal clean-up method with the use of immunoaffinity columns supplied from two different sources. The recoveries and interlaboratory precision obtained using the normal and immunoaffinity clean-up methods are compared

Table 7 Contemporary methods for the analysis of ochratoxin A

Method no.	*Commodity*	*Date method developed*	*Extraction solvent*	*Mobile phase/Developing solvent*	*Stationary phase*	*Clean-up method*	*Detection limit, LOD* ($\mu g\,kg^{-1}$)
HPLC							
1 (1)	Roast and ground coffee	1997	$CHCl_3$/ 0.1 mol L^{-1} H_3PO_4 soln	H_3PO_4/CH_3CN (1:1)	C_{18} column	Silica gel SPE + immunoaffinity	0.1
2 (2)	Milk	1996	$CHCl_3/CH_3OH$ (pH 1.6–2)	H_3PO_4 (0.008 mol L^{-1})/CH_3CN[a] (a) (60:40), (b) (90:10), (c) (60:40)	C_{18} column	Centrifugation (4°C) + silica gel SPE	0.01 $\mu g\,L^{-1}$ [b]0.03 $\mu g\,L^{-1}$
HPTLC							
3 (3)	Rice	1996	$CHCl_3$/ 0.1 mol L^{-1} H_3PO_4 soln	Bidirectional HPTLC: (a) diethyl ether/CH_3OH (98:2) (b) toluene/ethyl acetate/formic acid (5:4:1) (c) *n*-hexane/ethyl acetate/acetic acid (18:3:1)	Silica gel HPTLC plate	Liquid partitioning + phenyl SPE	11.6

[a]Successive mobile phases.
[b]Quantitation limit.

(1) Patel S, Hazel CM, Winterton AGM and Gleadle AE (1997) Survey of ochratoxin A in UK retail coffees. *Food Additives and Contaminants* 14: 217–222.
(2) Valenta H and Goll M (1996) Determination of ochratoxin A in regional samples of cows milk in Germany. *Food Additives and Contaminants* 13: 669–676.
(3) Dawlatana M, Coker RD, Nagler MJ and Blunden G (1996) A normal phase HPTLC method for the quantitative determination of ochratoxin A in rice. *Chromatrographia* 42: 25–28.

Table 8 Intercomparison of clean-up methods used for the HPLC determination of ochratoxin A in wheat

Clean-up method	*Coefficient of variation* (%) (*Interlaboratory*)	*Recovery* (%)
Normal	34	58–114
Immunoaffinity (first source)	34	58–114
Immunoaffinity (second source)	42	4–86

in **Table 8**. A recovery within the range 70 to 110% was considered to be acceptable. The interlaboratory coefficient of variation obtained using normal (and one immunoaffinity) clean-up methods were similar to, but slightly greater than, the values obtained by the intercomparison study of the official AOAC/IUPAC/NMKL procedure.

Deoxynivalenol

Official methods The two official AOAC methods for the determination of deoxynivalenol in wheat both date from 1986; these are outlined in **Table 9**. The TLC procedure (Method 986.17) involves extraction with acetonitrile/water followed by clean-up using a small column packed with a mixture of charcoal, alumina and Celite. The deoxynivalenol is observed as a blue fluorescent spot, under UV light, on the heated, aluminium chloride-treated plate. When subjected to a collaborative study the reported average recoveries were between 78 and 96%, with intra- and interlaboratory precisions (CV%) of 30–64 and 33–87% respectively.

The GC method includes extraction with water/chloroform/methanol, a silica gel column clean-up (under centrifugation) and derivatization with heptafluorobutyric acid anhydride (HFBAA). Chromatography is performed on a 3% OV-101 column (using/argon methane as the carrier gas) with a ^{63}Ni electron capture detector. A collaborative study of this procedure afforded an average recovery of 92% and intra- and interlaboratory precisions (CV%) of 31 and 47%, respectively, for naturally contaminated samples.

Recent developments In 1992, an intercomparison study was reported on the determination of deoxynivalenol in wheat and corn flours. Fifteen laboratories participated, using one- and two-dimensional TLC (five participants), GC (four) and HPLC (six) procedures. Ten of the laboratories used a charcoal-based clean-up method. A mixture of acetonitrile/water was widely used as an extraction solvent. HPLC quantification was performed using UV detection at 225 nm, whereas the GC determinations employed trimethylsilyl, trifluoroacetyl and heptafluorobutyryl derivatives. For all methods the recoveries varied between 68 and 116% for wheat and 53 and 100% for corn. There was no discernible difference in the efficacy of the various quantification procedures.

Typically, TLC methods for the anlaysis of trichothecenes involve extraction with acetonitrile or methanol followed by clean-up using liquid partitioning and column chromatography on silica gel or Florisil. Deoxynivalenol may be visualized on the TLC plate by spraying with, for example, aluminium chloride, 4-(*p*-nitrobenzyl)-pyridine, *p*-anisaldehyde or cerium sulfate.

Recently developed methods for the determination of deoxynivalenol, T-2 toxin (and zearalenone) are summarized in **Table 10**.

The HPLC analysis of trichothecenes is frequently performed using gradients of methanol/water or acetonitrile/water in conjunction with C_{18} (or occasionally C_8) columns and detection by UV absorption. Electrochemical detection has also been employed, together with a variety of derivatization techniques. The extraction/clean-up step in the HPLC procedure (Method 1) includes the precipitation of milk protein, with acetic acid, pH adjustment to 7–8, Extrelut™ column chromatography and a charcoal–alumina clean-up column. The recovery, for the concentration range 25–200 μg L^{-1} deoxynivalenol, was low (57%)

Table 9 Official methods for the analysis of deoxynivalenol; these are AOAC methods unless stated otherwise

Method no.	*Date method developed*	*Commodity*	*Extraction solvent*	*Development solvent or carrier gas*	*Stationary phase*	*Clean-up method*	*Detection limit, LOD* (μg kg^{-1})
TLC							
986.17	1986	Wheat	CH_3CN/H_2O	$CHCl_3$/acetone/isopropanol	Silica gel	Small column; mixture of charcoal, alumina and Celite	300
GC							
986.18	1986	Wheat	$H_2O/CHCl_3$/ethanol	CH_4/Ar (5:95)	3%OV-101 (on 80–100 mesh Chromosorb WHP)	Quick-Sep silica gel column	350

Table 10 Recently developed methods for the determination of deoxynivalenol, T-2 toxin and zearalenone

Method no.	*Date method developed*	*Commodity*	*Extraction solvent*	*Clean-up method*	*Development solvent/mobile phase/carrier gas*	*Stationary phase*	*Derivatization method*	*Detector/detection limit, LOD* ($\mu g\ kg^{-1}$)
HPLC								
1 (4)	1994	Cow's milk	Extrelut column	Centrifugation and charcoal/alumina column	H_2O/CH_3CN (96:4)	Reversed-phase C_{18} column	N/A	UV absorption (220 nm) 25 $\mu g\ L^{-1}$ (Deoxynivalenol only)
GC								
2 (5)	1996	Barley, mixed feed, sweet corn	CH_3CN/H_2O	Celite mixed charcoal, alumina, Celite column and C_8SPE	Helium (1.5% argon added for GC/MI	Cross-linked methyl silicone capillary column	Trimethyl-silylation	NICI/MS LOD not reported (T-2 and deoxynivalenol, only)
3 (6)	1996	Barley, maize	CH_3CN/H_2O	Defatting (hexane) + Florisil column	Helium	Cross-linked methyl silicone capillary column	Trimethyl-silylation	EI/SIM/MS 5 $\mu g\ kg^{-1}$
HPTLC								
4 (7)	1998	Corn	$H_2O/CHCl_3$	Liquid partitioning	Toluene/ethyl acetate/formic acid (6:2:1)	Silica gel HPTLC plate	N/A	Fluorodensitometry 2.6 (Zearalenone, only)

(4) Vudathala DK, Prelusky DB and Trenholm HL (1994) Analysis of trace levels of deoxynivalenol in cow's milk by high pressure liquid chromatography. *Journal of Liquid Chromatography* 17: 673–683.

(5) Mossoba MM, Adams S and Roach JAG (1996) Analysis of trichothecene mycotoxins in contaminated grains by gas chromatography/matrix isolation/Fourier transform infrared spectroscopy and gas chromatography/mass spectrometry. *Journal of AOAC International* 79: 1116–1123.

(6) Ryu JC, Song YS, Kwon OS, Park J and Chang IM (1996) Survey of natural occurrence of trichothecene mycotoxins and zearalenone in Korean cereals harvested in 1992 using gas chromatography mass spectrometry. *Food Additives and Contaminants* 13: 333–341.

(7) Dawlatana M, Coker RD, Nagler MJ, Blunden G and Oliver GWO (1998) An HPTLC method for the quantitative determination of zearalenone in maize. *Chromatographia* 47: 215–218.

but consistent; the extensive clean-up probably contributed to the loss of toxin.

GC is widely employed for the determination of trichothecenes, including deoxynivalenol, notwithstanding the inconvenience of lengthy clean-up and derivatization steps prior to quantification. Typically, either the original trichothecene, or the alcohol produced by alkaline hydrolysis, is determined. The hydroxyl group(s) of trichothecenes are normally derivatized in order to attain the required volatility and sensitivity. Trimethylsilyl (TMS) derivatives are frequently utilized for the GC of trichothecenes; heptafluorobutyryl and pentafluoropropionyl derivatives are employed for electron capture detection (ECD) whereas trifluoroacetates are utilized for flame ionization (FID), ECD and mass spectrometric (MS) detection. GCMS methods have the advantage of high sensitivity together with the opportunity of using mass spectrometry for confirmation purposes. The specificity of MS detection affords the reliable detection of toxins in grains, biological fluids and other matrices. Generally, capillary GC is preferred to the use of packed columns since the efficiency of the latter can be compromised by interferences. Capillary GC has been used for the analysis of trichothecenes in a variety of commodities.

Both GC/matrix isolation (MI)/Fourier transform infrared (FTIR) spectroscopy and GCMS have been used to analyse mixtures of trichothecenes in a variety of commodities (Method 2, **Table 10**). Matrix isolation was performed by adding argon to the carrier gas and trapping the effluent on the outer ring of a slowly rotating gold disc, at low temperatures. The IR-transparent argon matrix, containing the isolated trichothecenes, was then analysed by IR spectroscopy, and the presence of individual toxins confirmed by observing the characteristic MI/FTIR bands. Negative ion chemical ionization (NICI) mass spectrometry was used to quantify the high levels (67–445 $mg\ kg^{-1}$) of deoxynivalenol found in naturally contaminated sweet corn. Seven *Fusarium* mycotoxins (including deoxynivalenol, T-2 toxin and zearalenone) in barley and maize have also been determined by GC/electron impact-selective ion monitoring MS (Method 3, **Table 10**). 5α-Cholestane was used as an internal

standard. The mean recovery for the seven mycotoxins was 92%.

T-2 Toxin

Official methods There are no official AOAC methods for the determination of T-2 toxin.

Recent developments Methods available for the determination of T-2 toxin include TLC, GC and HPLC.

T-2 toxin and other type A trichothecenes (characterized by a hydrogen atom or an hydroxyl group at the C8 position) are visualized on TLC plates by treatment with sulfuric acid or chromotropic acid (disodium 4,5-dihydroxynaphthalene-2,7-disulfonate dihydrate). Another approach involves the formation of the diphenylindenone sulfonyl (Dis) esters of trichothecenes and their visualization, as fluorescent spots under UV light, by spraying the TLC plate with sodium methoxide. Using this procedure 20–25 ng per spot of T-2 toxin can be detected.

The HPLC determination of T-2 toxin is compromised by the lack of the enone chromophore possessed by deoxynivalenol. The successful HPLC determination of T-2 and other Type A trichothecenes requires effective clean-up and derivatization procedures. A variety of post-column derivatization methods have been developed including those involving the UV detection of *p*-nitrobenzoate and diphenylindenone sulfonyl esters of T-2 toxin; the reported detection limits are approximately 10 and 30 ng T-2, respectively.

The capillary GC-ECD determination of T-2 toxin, and other Type A trichothecenes, afford detection limits of about 200 $\mu g\ kg^{-1}$ (with one chromatographic clean-up) and 50–100 $\mu g\ kg^{-1}$ (with two chromatographic clean-ups). A similar result has been reported using a capillary GC-FID method. T-2 toxin has also been detected in spiked wheat (in combination with deoxynivalenol), at levels of 1 $\mu g\ kg^{-1}$, by using a GC-NICI MS-MS method. A highly sensitive method for T-2 in urine employs capillary GCMS (EI and NICI) with a detection limit of 2–5 $\mu g\ L^{-1}$. Capillary GC-PICI MS was employed after clean-up of an acetonitrile extract on an XAD-2 column and derivatization with TFA.

Recently developed GC/NICI/MS and GC/EI/MS methods for the determination of T-2 toxin, and other trichothecenes, are outlined in Table 10 (Methods 2 and 3).

Zearalenone

Official methods There are two official AOAC methods (TLC and HPLC) for the determination of zearalenone in corn (**Table 11**). The TLC method (976.22) dates from 1976 and has also been adopted by the AACC. The HPLC method (985.18) dates from 1985 and can also be used for the determination of α-zearalenol. No limits of detection are given for these procedures.

The official TLC method for zearalenone involves extraction with chloroform/water, clean-up by silica gel column chromatography and liquid partitioning followed by TLC using either ethanol/chloroform or acetic acid/benzene. Zearalenone fluoresces greenish-blue under 254 nm UV light; and blue under 365 nm UV light after treatment with aluminium chloride.

The official HPLC method for zearalenone and α-zearalenol involves extraction with chloroform/water (in the presence of diatomaceous earth), clean-up by liquid partitioning and chromatography on a C_{18} column using water/acetonitrile/methanol as the mobile phase. Fluorescence detection is employed.

Recent developments A variety of HPLC methods have been developed for the analysis of zearalenone in corn together with methods for milk, blood, urine and animal tissue. Clean-up procedures include liquid partitioning and the use of silica gel cartridges. The mobile phases used for reversed-phase HPLC include

Table 11 Official methods for the analysis of zearalenone; these are AOAC methods unless otherwise stated

Method no.	*Date method developed*	*Commodity*	*Extraction solvent*	*Development solvent/mobile phase/carrier gas*	*Stationary phase*	*Clean-up method*	*Detection limit, LOD* ($\mu g\ kg^{-1}$) *Additional information*
TLC							
976.22	1976	Corn	$H_2O/CHCl_3$	Ethanol/$CHCl_3$ [a](5:95)	Silica gel	Liquid partioning	AACC/AOAC method LOD not specified
HPLC							
985.18	1985	Corn	$H_2O/CHCl_3$	$CH_3OH/CH_3CN/H_2O$ (1.0:1.6:2.0)	Reversed-phase C_{18} column	Liquid partitioning	LOD not specified

[a]Or ethanol/$CHCl_3$ (3.5:96.5), acetic acid/benzene (5:95 or 10:90).

Table 12 AOAC Official First Action HPLC method for the analysis of the fumonisins

Date method developed	*Commodity*	*Extraction solvent*	*Mobile phase*	*Stationary phase*	*Clean-up method*	*Detection limit, LOD* ($\mu g\ kg^{-1}$)
1990	Corn	H_2O/CH_3OH	Na_2HPO_4 (buffered to pH 3.3)/CH_3OH	C_{18} column	SPE SAX cartridge	10

acetonitrile/water, acetonitrile/water/acetic acid, methanol/acetonitrile/water and methanol/water. Water-saturated dichloromethane containing 2% 1-propanol has been used for normal-phase HPLC. Fluorescence detection is most commonly used; other methods include electrochemical, voltametric and UV spectroscopic detection.

An HPTLC method (Method 4, **Table 10**) for the determination of zearalenone in maize has recently been developed, based upon the AOAC HPLC procedure (985.15). The mean recovery is 75.3%, over the range 10 to 320 $\mu g\ kg^{-1}$ zearalenone.

Most of the numerous GC methods for the determination of zearalenone (and zearalenol) utilize trimethylsilyl derivatization. A recently developed GC method for the determination of zearalenone and other *Fusarium* toxins, in barley and corn, is shown in **Table 10** (Method 3).

Fumonisins

Official methods An HPLC method has received Official First Action status from the AOAC International (**Table 12**). The procedure uses methanol/water (3 : 1 v/v) as the extraction solvent followed by strong ion exchange (SAX) clean-up and pre-column derivatization with *o*-phthaldialdehyde (OPA). The mobile phase is sodium dihydrogen phosphate solution (buffered to pH 3.3)/methanol and fluorescence detection is employed.

Recent developments Typically, the fumonisins are determined by TLC, HPLC or GCMS, using ion exchange SPE clean-up and quantification, after derivatization of the primary amino group. HPLC is by far the most widely used quantification method. A worldwide survey of methods used for the analysis of the fumonisins was reported in 1996. Of the 32 laboratories included, 91% used HPLC. TLC and GC/MS methods were each used by 3% of the laboratories. (ELISA was utilized by the remaining 3%.)

HPLC methods that are broadly similar to the AOAC Official First Action method have also been developed using other clean-up procedures (e.g. C_{18} SPE and immunoaffinity columns) and mobile phases. The latter include mixtures of acetonitrile/methanol/acetic acid; acidified methanol; and sodium

Table 13 Recently developed methods for the analysis of the fumonisins

Method no.	*Date method developed*	*Commodity*	*Chromatography type*	*Clean-up method*	*Detection limit, LOD*	*Additional information*
HPLC						
1 (8)	1996	Corn and corn products	Ion-pair chromatography	SAX and C_{18} SPE	20 ng	Derivatization with OPA and *N*-acetyl-*L*-cysteine; fluorescence detection
2 (9)	1998	Corn-based feed	Reversed-phase	On-line immunoaffinity column	5 ng	Electrospray ionization MS
HPTLC						
3 (10)	1998	Rice	Silica-gel HPTLC	SAX SPE	[a]250 $\mu g\ kg^{-1}$	Derivatization by dipping plate into 0.17% *p*-anisaldehyde solution; fluorescence densitometry

[a]Limit of quantification.

(8) Miyahara M, Akiyama H, Toyoda M and Saito Y (1996) New procedure for fumonisins B_1 and B_2 in corn and corn products by ion pair chromatography with *o*-phthaldialdehyde post column derivatization and fluorometric detection. *Journal of Agricultural and Food Chemistry* 44: 842–847.

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hydrogen phosphate solution/methanol followed by acetonitrile/water. Although OPA is used as the derivatization reagent by the majority of laboratories, other reagents have been employed including naphthalendialdehyde, fluoronitrobenzofurazan and fluorescamine. The last reagent is unsatisfactory as it generates two peaks in the HPLC chromatogram for fumonisin B_1.

Three recently developed methods for the determination of the fumonisins in corn-based commodities are outlined in **Table 13**. Method 1 uses a combination of SAX and C_{18} SPE clean-up prior to ion-pair HPLC and fluorescence detection; on-line derivatization within a reaction coil is employed. The recovery of the fumonisins ranged from 54 to 110% at 40 and 80 $\mu g\,kg^{-1}$, respectively. Method 2 is an automated procedure using on-line immunoaffinity clean-up, reversed-phase HPLC and electrospray ionization MS detection. The protonated molecule signal (m/z 722) was used to achieve a limit of quantification of 250 pg.

An HPTLC method (Method 3, **Table 13**), for the determination of fumonisin B_1 in rice, has recently been reported. A novel derivatization step involved the brief immersion of the HPTLC plate in a 0.16% acidic solution of *p*-anisaldehyde, followed by quantification by scanning fluorodensitometry. The response was linear over the range 0 to 5 $mg\,kg^{-1}$ (ppm).

An intercomparison study on a variety of methods for the determination of the fumonisins in maize has recently been undertaken under the auspices of the European Commission, Measurements and Testing Programme. Twenty-four laboratories participated, using their normal routine procedure for the determination of fumonisins B_1 and B_2 in the range 0.5–3.0 and 0.2–1.5 $mg\,kg^{-1}$ (ppm), respectively. All laboratories used a similar method involving extraction with methanol/water, clean-up with an SAX SPE column and HPLC fluorescence quantification of the OPA derivative. The intra- and interlaboratory precisions were high (10 and 11%, respectively, for fumonisin B_1; and 11 and 13%, respectively, for fumonisin B_2). However, the recoveries were low ($70 \pm 14\%$ and $69 \pm 16\%$ for fumonisins B_1 and B_2, respectively). Interestingly, higher recoveries were associated with extraction by shaking ($85 \pm 12\%$ for fumonisin B_1) rather than by blending ($62 \pm 6\%$).

Conclusions

The continued use of a variety of chromatographic procedures for the determination of mycotoxins is envisaged. Although HPLC is the method of choice in the developed world for a wide range of applications, it is important that precise and accurate methods continue to be developed that are appropriate to the special needs of developing country laboratories.

See Colour Plate 53.

See also: **II/Affinity Separation:** Immunoaffinity Chromatography. **Chromatography: Gas:** Detectors: Mass Spectrometry. **Chromatography: Liquid:** Derivatization. **III/Aflatoxins and Mycotoxins:** Thin Layer (Planar) Chromatography. **Membrane Preparation:** Phase Inversion Membranes.

Further Reading

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Thin-Layer (Planar) Chromatography

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The aflatoxins are toxic and carcinogenic metabolites of the moulds *Aspergillus flavus* and *A. parasiticus*. They are often found as contaminants of peanuts, tree nuts, corn and cottonseed. They were discovered as a result of investigations into Turkey X disease in Britain, in which 100 000 turkeys and numerous other poultry died as a result of feeding on peanut meal which had been contaminated with mould.