DOI 10.1002/mnfr.200800145 Mol. Nutr. Food Res. 2009, *53*, 508 – 524

Review

Review on sample preparation strategies and methods used for the analysis of aflatoxins in food and feed

Elisabeth Reiter[1, 2], Jürgen Zentek[1, 2] and Ebrahim Razzazi[1, 3]

[1] Veterinärmed. Univ. Wien – Institut für Ernährung, Wien, Austria
[2] Freie Universität Berlin – Institut für Tierernährung, Berlin, Germany
[3] Veterinärmed. Univ. Wien – VetOMICS Technologie Plattform für Forschung, Wien, Austria

Mycotoxins are of toxicological relevance and consequently important contaminants in food and feed. The most common mycotoxins with the highest toxicity are the aflatoxins (AFs), which cause liver cirrhosis or primary liver carcinomas and have been shown to be the immunosuppressive. The European Union has set the maximum levels as low as reasonably achievable to protect consumers. To perform appropriate risk assessment for AFs robust analytical methods are required to provide reliable results. Different steps in the analysis of AFs in food and feed are necessary such as sampling, extraction, sample purification and detection. Throughout the analysis chain, methods have to be precise and reproducible. The sensitivity of an analysis, especially if the detection is not selective enough depends strongly on the sample clean up. However, techniques in sample preparation and analytical methods are improving continuously. This manuscript gives an overview on different analytical methods with emphasize on sample preparation strategies used in the analysis of AFs in food and feed.

Keywords: Aflatoxins / Extraction / HPLC / Methods / Sample preparation

Received: April 8, 2008; revised: September 3, 2008; accepted: October 31, 2008

1 Introduction

Mycotoxins are secondary metabolites produced by various fungi, which were found in various commodities. About 300 of mycotoxins have been detected, however only a few of these metabolites are responsible for significant food safety challenges [1]. Mycotoxins are of high relevance in human food and animal feed safety whereas the most harmful mycotoxins are the aflatoxins (AFs). Toxic effects in animals have been reported often having acute or chronic disease [2]. To reduce consumer's health risks worldwide maximum levels were set. The most common and most toxic AF is aflatoxin B_1 (AFB_1), which has historically been associated with the Turkey-X-Disease in the 1960s and can result in toxic hepatitis liver fibroses [3]. The biosynthesis depends on various factors like temperature, water activity, time of harvesting and storage [4]. According to the FAO the contamination of mycotoxins in food and feed is a real worldwide problem. The United Nation's Food and Agriculture Organisation has estimated that about 25% of the world's food is significantly contaminated (http://www.ifst.org/uploadedfiles/cms/store/ATTACHMENTS/Mycotoxins.pdf). AFs are difuranocumarin derivates, which consist of 5 heterocycles. Currently about 20 AFs are known. The most common AFs are B_1, B_2, G_1, G_2 (AFB_1 AFB_2 AFG_1 AFG_2), and those in milk-occurring metabolites M_1 (AFM_1) and M_2 (AFM_2) as shown in Fig. 1. The main producers of the AFs are the fungi *Aspergillus flavus* and *A. parasiticus*. AFs are further produced by phenotypically similar species of *A. flavus* such as *A. nomius*, *A. zhaoqingensis* and *A bombycis* and similar species of *A. parasiticus* namely *A. toxicarius* or *A parvisclerotigenus*. Moreover other AF producing species like *A. pseudotamarii, A. ochraceoroseus, A. rambellii, A. toxicarius, Emericella astellata, E. olivicola, E. venezuelensis* have been registered [5]. *A. flavus* produces only AFB_1 and AFB_2 yet *A.*

Correspondence: Professor Ebrahim Razzazi, Veterinärmed. Univ. Wien – Institut für Ernährung, Veterinaerplatz 1, Vienna A-1210, Austria
E-mail: Ebrahim.Razzazi@vu-wien.ac.at
Fax: +0043-1-250773290

Abbreviations: AF, aflatoxin; **AFB_1,** aflatoxin B_1; **AOAC,** Association of Official Analytical Chemist; **APCI,** atmospheric pressure chemical ionisation; **IAC,** immunoaffinity column; **MSPD,** matrix solid phase dispersion; **OPLC,** overpressured-layer chromatography; **PBPB,** pyridinium hydrobromide perbromide bromination; **PHRED,** photochemical reactor for enhanced detection; **SFE,** supercritical fluid extraction; **UV-LED,** UV-light emitting diode

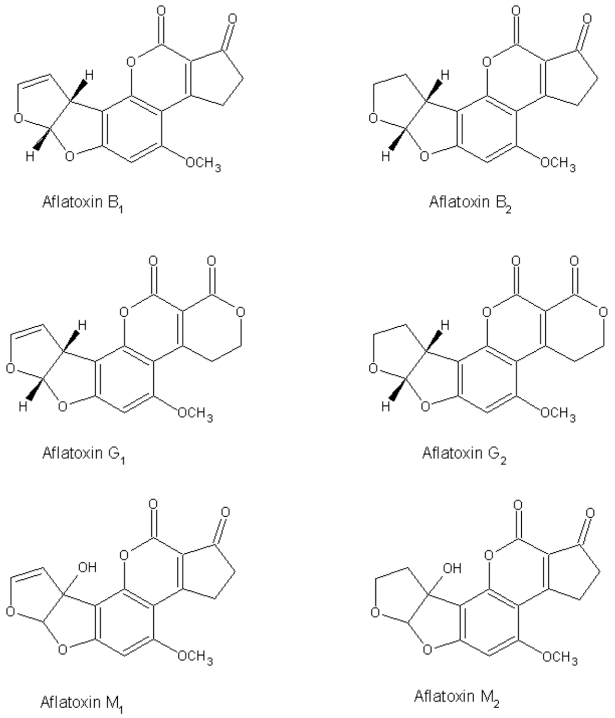

Figure 1. Chemical structure of major aflatoxins.

parasiticus is able to form all major AFs. *A. parasiticus* and *A. flavus* prefer to grow in temperatures between 22 – 35°C and a water activity of 0.98 – 0.95 a_w thereof AFs can be formed mainly in tropical and subtropical climate [3]. The AFs are named due to their properties under UV-irradiation, where AFB_1 and AFB_2 emit blue fluorescence (350 nm), AFG_1 and AFG_2 green fluorescence (350 nm). These important features can be used for rapid identification and detection [3].

AFs are generally found in fat containing food and feed like ground nuts and derived products, almonds, pistachios, Brazil nuts, maize, rice, figs, cotton seed and spices. The metabolites M_1 and M_2 have been found in all kinds of milk and baby food [6 – 28]. The occurrence ratio between AFB_1 and the other AFs (sum of $B_2 + G_1 + G_2$) is about 1:0.8 and the ratio of AFB_1 to AFB_2 has been described to be 4:1, respectively [29]. In the analysis of contaminants in food and feed performance criteria are important for obtaining reliable results. The evaluation of the validation parameters should be based on the intended use of the analytical method [30]. In general different parameters such as specificity, selectivity, precision, accuracy, linearity, range, LOD, LOQ, robustness as well as ruggedness are recommended and be determined [30]. Methods that are used in routine analyses should be tested in appropriate frequency using quality control material or certified reference material to ensure the reliability of analytical results. Furthermore every method has to be revalidated if any parameter in the analysis is changed [30]. In general one of the characteristics of analytical procedures is the recovery, which gives an evidence of the performance of the method and is summarized in Table 1. Most published papers nowadays give at least some information regarding above mentioned validation parameters, which will be discussed in each chapter.

1.1 Toxicity and metabolism

High amounts of AFs over a long duration are associated with liver cirrhosis or primary liver carcinomas and the suppressive effect of the immune system [3]. Carriers of the Hepatitis B Virus have, in combination with the exposure of AFs, an increasing risk on the appearance of hepatocellular carcinomas. And the virus has been shown to have a synergistic effect to AFB_1 in the induction of liver tumors [3]. In exposed animals liver cell damages were also found [31]. The AF-metabolite M_1 obviously shows similar mechanism to induce liver cancer [32]. The IARC concluded AFB_1 to be carcinogenic to humans (group1) and AFM_1 as possible carcinogenic to humans (group 2b) [33].

1.2 Legislations

To minimize human health risks maximum levels were set in food and feed for many years [29]. Regulatory limits exist in Europe for AFB_1 and other major AFs (B_1+B_2 + G_1 + G_2) [34]. Since AFB_1, has been considered to be genotoxic and carcinogenic, the European Union decided that a no-effect concentration limit cannot be established for genotoxic compounds and any dose will have a proportional effect. Thus, neither ADI nor TDI have been established for AFB_1, but it is recommended that its level in food should be as low as reasonably achievable (ALARA). In the US, the MERCOSUR states and many other countries maximum levels are set only for the sum of all AFs. In some African and Asian countries until now no maximum levels are set [29]. However, there are some attempts to harmonize the maximums levels [35]. Table 2 gives an overview of the maximum levels in food set in the European Union compared to the US limits.

2 Extraction and clean up techniques

Due to their occurrence AFs are generally analysed in nuts and derived products, cereals, maize and spices and metabolites in milk and derived products [6 – 28]. However, comparatively more seldom AFs have been analysed in feed [36 – 40]. Since AFs are inhomogeneous distributed in food and feed, high-contaminated hotspots can occur. Thus, sampling is an important step in the analysis of contaminated food and feed the aflatoxin concentration of lots cannot be determined with 100% certainty. According to Whitaker the sources of variability in the aflatoxin analysis are depending on each step within the aflatoxin analysis namely

Table 1. Matrix depended performance of HPLC-FLD

Matrix	LOD/LOQ (μg/kg)	Recoveries (%)	Ref.
Cereals			
Infant cereals	0.002–0.004	B1: 89.6 ± 8 B2: 87.9 ± 6.8 G1: 85.1 ± 12.3 G2: 68.8 ± 10	[107]
Entrails			
Animal liver	0.002	78.2	[61]
Pig liver	<1	B1: 73.6–88.4 M1: 68.3–81.4	[106]
Feed			
Feed	1 (LOQ)	B1: 80	[38]
Pet food	3–7	78–90	[108]
Pig feed	10	B1: 80.1 G1:79.6 B2: 67.0 G2:67.9	[37]
Poultry feed	B1; G1: <0.01 B2; G2: <0.02	>65–70	[39]
Food & fruits			
Figs	0.1	B1: 83 ± 15 B2: 77 ± 14 G1: 83 ± 11 G2: 76 ± 14	[109]
Sesame	<0.1	86	[27]
Herbs and medical products			
Botanical roots	n.a.	80	[110]
Herbal medical products	0.04	60.3–111.9	[111]
Herbs Medical plants	0.2–0.5	Herbs: 50–71.3 Med plants: 76.1–78.1	[21]
Medical herbs	0.19-0.6	98–103	[112]
Medical herbs and plant extracts	B1, G1: 0.2 B2, G2: 0.15	93–97	[113]
Milk and derived products			
Milk	0.006	M1: 91 ± 15	[114]
Nuts			
Hazelnut paste	0.15	86–89	[26]
Nuts	0.08–1.25	83.4–102.1	[14]
Peanut butter	<0.1	82	[27]
Peanut meal	<0.2	74.6–109.6	[28]
Pistachios	0.1-0.4	B1: 97.3 ± 10.2 B2: 95.1 ± 9.9 G1: 97.8 ± 10.1 G2: 61.8 ± 21.6	[22]
Spices			
Paprika	n.a.	B1: 78.3 B2: 84.8 G1: 77.9 G2: 74.6	[115]
Red paprika	B1: 0.01 B2: 0.01 G1: 0.15 G2: 0.02	B1: 74.7–95.3 B2: 84.9–98.7 G1: 81.7–103.9 G2: 68.1–88.5	[11]
Spices	B1: 0.10 (LOQ) B2: 0.02 (LOQ) G1: 0.09 (LOQ) G2: 0.21 (LOQ)	70–86	[10]
Spices	0.02	B1: 90.8 ± 6	[116]

the sampling, sample preparation and analytical analysis [41, 42]. The author described two types of mistakes that are related to sampling, where either good lots (lot has overall content lower than the legislation limit) will fail and will be rejected (false positive) or bad lots will be tested negative and accepted (false negative). Appropriate sampling procedures will reduce the variability of the results and the number of misclassified lots [41, 42]. Sampling procedures and its statistic have been reviewed in many papers [43–45]. Within the mycotoxin test procedure sampling has been shown to be the largest source of variability. Thus, the probability of errors due to sampling is higher, than those of analytical procedure.

It is important to remove interfering substances as much as possible in the clean up step, while extraction must be quantitative for the analyte. Different extraction procedures were used such as the extraction with chlorinated solvents that have been currently banned [46, 47]. Nowadays, extraction of the AFs can be carried out by using a mixture of water and organic solvent, *e.g.* ACN, methanol (MeOH) or acetone.

The liquid–liquid extraction (partitioning) is based on the distribution of analytes in two nonmiscible phases. The major drawback of this method is the high amount of applied organic and chlorinated solvents. As a result this method is being replaced either by SPE or immunoaffinity extraction. Nevertheless a few applications were published recently [46–48]. An application using liquid–liquid partitioning was carried out by Sizoo *et al.* [47] by analysing duplicate diets. The sample was extracted in chloroform and 0.1 M phosphoric acid using a shaking machine. After a second clean up using immunoaffinity columns (IACs) the determination was carried out by means of HPLC-FLD. Recoveries ranged from 68 to 74% for AFM_1 and from 95 to 97% for AFB_1, respectively, the LOD was determined at 0.0015–0.007 μg/kg. In this study the replicate diets of 123 participants were analysed. Although high background interferences have been removed the liquid–liquid partitioning this method is very time consuming.

A further liquid–liquid-partitioning technique was carried out in the multitoxin analysis in cheese using nonchlorinated solvents by Kokkonen *et al.* [48]. ACN (containing 0.1% formic acid) and hexane were added to the samples and homogenized prior a centrifugation step.

Table 2. Maximum levels for aflatoxins in foodstuffs set in the European Union (EC no. 1881/2006) and the US [34, 117, 118]

Foodstuff	EUROPE Maximum Level μg/kg			US Maximum Level μg/kg	
	B_1	Sum of $B_1+B_2+G_1+G_2$	M_1	Sum of $B_1+B_2+G_1+G_2$	M_1
Human food				20	–
Whole milk, low fat milk, skim milk				–	0.5
Groundnuts to be subjected to sorting, or other physical treatment, before human consumption or us as an ingredient in foodstuff	8	15	–		
Maize, nuts and dried fruit to be subjected to sorting, or other physical treatment before human consumption or use as an ingredient in foodstuff	5	10	–		
Following species of spices: – *Capsicum spp.* (dried fruits thereof, whole or ground, including chilies, chilli powder, cayenne and paprika) – *Piper spp.* (fruits thereof, including white and black pepper) – Myristica fragrans (nutmeg) – Zingiber officinale (ginger) – Curcuma longa (turmeric)	5	10	–		
Groundnuts nuts and dried fruit and processed products thereof, intended for direct human consumption or us as an ingredient in foodstuffs	2	4	–		
Raw milk, heat-treated mil and milk for the manufacture of milk-based products	–	–	0.05		
Infant formulae and follow-on formulae, including infant milk and follow-on milk	–	–	0.025		
Processed cereal-based foods and baby foods for infants and young children	0.1	–	–		
Dietary food for special medical purposes intended specifically for infants	0.1	–	0.025		

Afterwards the samples were filtered and a portion of the ACN-phase was evaporated to dryness under a stream of nitrogen prior to the LC-MS/MS determination. The performance of the method showed LOD's ranging 0.3 – 0.7 μg/kg and recoveries from 96 to 129% for the AFs. In all analysed samples no AFs were found [48].

Bourais *et al*. [46] recently reported a two-phase clean up system for AFs in food samples. In the first step, the samples were extracted with an organic solvent (MeOH) and then the solvent was evaporated and redissolved in toluene. The toluene phase was shaken with an aqueous phase like PBS/MeOH (35:65) in a shaker and stirred for 30 min. The phases were separated in a separatory funnel; then the aqueous phase was collected. The quantification of the AFs was carried out by direct fluorescence measurement at 350 nm without chromatographic separation. A LOD of 0.3 μg/kg and recoveries ranging 86 – 104% for AFB_1 and 95 – 100% for AFM_1 were obtained [46]. The authors have mentioned that using chloroform for initial extraction obtained good recoveries. With exception of barley matrix satisfactory results were obtained when the original extraction was done with methanol [46].

Möller and Nyberg [28] evaluated different solvents for the extraction of AFs from peanut meal. Mixtures of chloroform-water (100 + 10), ACN-water (60 + 40), acetone-water (85 + 15) and MeOH-water (80 + 20) were used. The extraction using four different solvents showed recoveries ranging from 74.6 to 109.6% for AFB_1 [28]. The best results were obtained with the extraction solvents chloroform-water and ACN-water, which have shown good analytical results, after determination using HPLC-FLD and TFA pre-column derivatization [28]. The analysis of a certified reference material provided results near the certified value using ACN-water and chloroform-water, whereas the other two extraction procedures only achieved 58 and 79% respectively of the certified values [28].

Stroka *et al*. [49] evaluated the interactions of different solvents used for extraction of AFs in food samples. The authors have further performed the analysis of possible water adsorption by matrix. The samples were extracted with different combinations of organic solvents and water. After dilution with PBS, the samples were cleaned up by IACs prior to HPLC-FLD. The authors reported a higher loss of water in the extractant, which has been absorbed by the sample, using ACN-water or acetone-water than MeOH-water as extraction solvent. The highest loss appeared *via* ACN-water contents (60:40 and 70:40), where up to 24% water loss was registered, which can effect in

false positive results. In contrast extraction techniques with solvents of MeOH: water have provided reliable results [49].

Some authors have investigated if the extraction should take place dry or with slurry mixing to increase the recoveries of the sample intend for analysis [50, 51]. Normally the slurry mixing is not used in the daily analysis. Both authors reported that slurry mixing of the whole sample with water has obtained a better homogenization and a smaller particle size of the sample.

Prior Schatzki and Toyofuku [51] applied the slurry mixing on the sample preparation of pistachios. After mixing the sample (1 kg) with water (sample/water 1:2) the subsamples were extracted with MeOH. After IAC clean up, the samples were determined with HPLC either using postcolumn bromination or precolumn derivatization using TFA. Analysing AF contaminated samples result in an increase in AFs of 32%, when applying slurry mixing [51].

Additionally Spanjer *et al*. [50] reported that the slurry mixing can handle samples up to 10 kg in such a way that it leads to the lowest possible CV's and reveals the best estimation of the mycotoxin content of a lot. In this way, subsampling errors as well as chances on false-positive or false-negative values are reduced to a minimum. According to these results slurry mixing of lot samples to improve the extraction should be respected.

Quite recently Bacaloni *et al*. [12] published three different extraction methods applied on four different nut samples including hazelnuts. Three different procedures namely, homogenization, ultrasonic extraction and matrix solid phase dispersion (MSPD) have been tested. The homogenization was carried out using a Polytron homogenizer. In the MSPD, the sample together with C_{18} sorbent and sand was blended using a mortar and pestle. Subsequent the mixture was put into a glass column and eluted. For all different extraction procedures a clean up step with Carbograph-4 SPE columns was followed. After detection with LC-MS/MS results showed that the ultrasonic extraction and homogenization are comparable extraction methods. For homogenization and ultrasound extractions the LOQs of the method were the same. Values ranged from 0.04 to 0.07 μg/kg, method identification limits ranged from 0.02 to 0.05 μg/kg for AFG_2. Recoveries for homogenization and ultrasonic extraction ranged between 92 and 101% For MSPD extraction, these levels were from 70 to 83%. The authors suggested the use of ultrasonic extraction as a further possibility to improve the extraction [12].

Blesa *et al*. also applied the MSPD extraction on peanut samples prior to HPLC-FLD detection. Recoveries for each AF spiked to peanut samples ranged between 78 and 86% with RSDs ranging from 4 to 7% [52]. Additionally, Cavaliere *et al*. [53] have applied MSPD extraction on oil samples before LC-MS. The authors noted recoveries ranging from 92 to 107% for oil samples spiked at levels from 0.5 to 5 μg/kg [53]. An advantage of the MSPD extraction is the cost saving clean up of large numbers of samples. On the other hand MSPD has featured low recoveries in contrast to other clean up procedures.

Supercritical fluid extraction (SFE) was applied by Liau *et al*. to extract AFs from Zizyphi Fructus a traditional plant in Chinese medicine. CO_2 was used first for dynamic as well as for static extraction plus collected in ethyl acetate, evaporated to dryness and redissolved in MeOH which was injected directly [54]. The determination was performed using LC/MS either with ESI or with atmospheric pressure chemical ionisation (APCI). The recoveries ranged depending on the extraction solvents from 28 to 105% for AFB1 and the LODs ranged from 0.17 to 0.32 μg/kg [54].

Moreover the SPE or the clean up with IACs have been frequently applied in the analysis of mycotoxins. Solid phase cartridges based either on a silica matrix or on polymeric phases. Due to the interactions of the analytes with the matrix they are absorbed to the solid phase and after a wash step the analyte is eluted commonly with organic solvents whereas the matrix compounds will not bind to the columns. The most common used SPE columns and cartridges can either contain silica gel, C18, phenyl or aminopropyl bonded phases [55]. Mycosep and Multisep columns can also be used in a one-step clean up and are able to remove impurities but are less selective in sample preparation (http://www.romerlabs.com/romer.htm). In the clean up of AF contaminated samples SPE techniques are of less significance and nowadays applied seldom [56–59].

Sobolev and Dorner described the use of aluminium oxide columns for the clean up of AF contaminated commodities like almonds, Brazil nuts or pistachios [60]. The samples were extracted with MeOH-water. Fatty extracts were diluted with a 0.2% silver nitrate solution in ACN to break the suspension; the extracts were put onto the columns. For the quantification, HPLC with postcolumn derivatization (PHRED, the photochemical reactor for enhanced detection), which is necessary to enhance the signals of the AFB_1 and AFG_1, was used. Recoveries for AFB_1 ranged from 73.9 to 90.3% and a LOD <1 μg/kg [60]. Characteristics for these methods were the low cost minicolumns and the solvent and material saving technique. Further, it was possible to clean up the extracts in one step and inject directly into HPLC.

IACs in combination with HPLC are increasingly used nowadays as reference method and allow due to their high selectivity a sufficient elimination of matrix interferences. The immunoaffinity is based on the binding of the immobilized specific antibodies on the surface of a column. IACs are nowadays the most common clean up method used in analysis [6, 7, 11, 22, 39, 61–65]. After the extraction procedure Ab–Ag complexes are formed and the matrix compounds are eluted. Following a wash-step the Ab–Ag complexes will be destroyed using an organic solvent. The selective binding of antigens within the formation of Ab–Ag complexes allows a selective sample preparation and

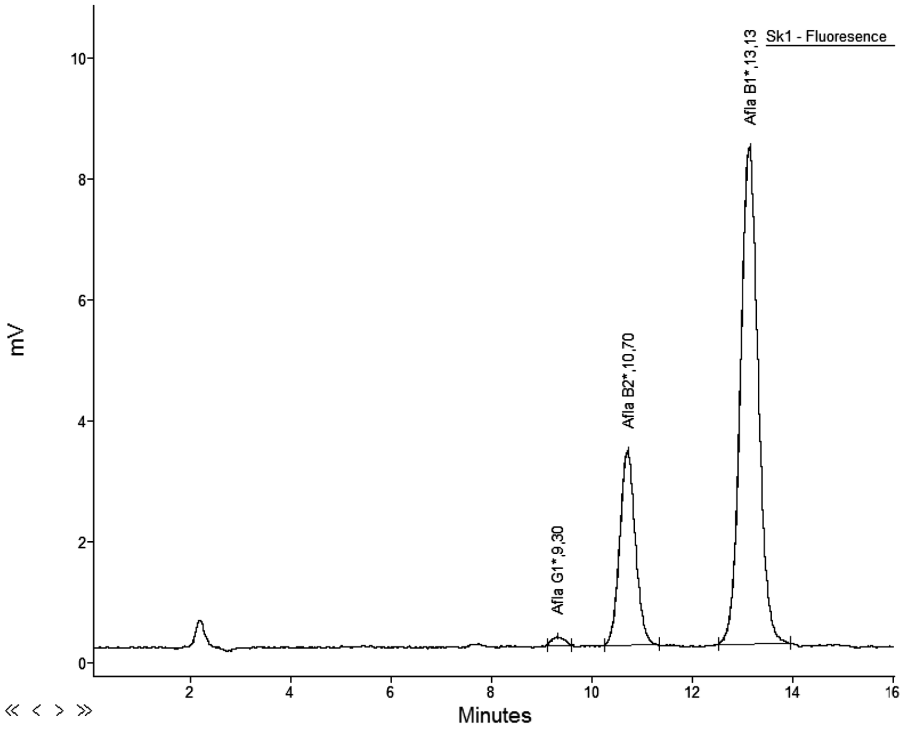

Figure 2. Chromatogram of a naturally contaminated positive peanut butter sample after clean up using IAC and HPLC-FLD detection (c = AFG_1: 0.38 μg/kg, AFB_2: 4.27 μg/kg, AFB_1: 15.1 μg/kg).

very good chromatograms with HPLC-FLD determination. In Fig. 2 the chromatogram of a naturally contaminated peanut butter sample after IAC clean up is shown.

Nowadays an increasing number of IACs is commercially available (http://www.romerlabs.com/romer.htm; http://www.vicam.com; http://www.r-biopharm.com; http://www.lctech.de). In an interlaboratory study composed of 14 different laboratories; Senyuva *et al*. [26] evaluated the clean up with IACs coupled with HPLC using post-column bromination. Samples were spiked with AFs at levels of 4 and 10 μg/kg. For the postcolumn derivatization the Kobra cell and in one case the pyridinium hydrobromide perbromide (PBPB) bromination were used. The average recoveries ranged from 75 to 104% for all laboratories. The LOD was reported to be 0.15 μg/kg with a S/N of 6:1 [26]. In this collaborative study, they evaluated MeOH/water (60:40) as most suitable for hazelnut paste extraction. Out of 14 laboratories only one produced outliers, this has been excluded. Some attempts to reuse IAC were also made by Scott and Trucksess and Toyoda *et al*. [66, 67]. IACs were reused up to 100 times after regeneration with PBS or water [66]. Toyoda *et al*. [67] reported the reusability of single IACs up to eight times, intended for the clean up of AFM_1 prior HPLC-FLD detection. In contrast to conventionally used solvents DMSO/phosphate buffer (1:1) was used as the eluent that did not alter the binding capacity of the antibody. IACs were able to be reused up to eight times. The recovery of AFM_1 ranged from 98.0 to 104.9% the LOD was determined at 0.05 μg/kg. The reuse of IACs is an important step to reduce costs in clean up and analysis. Recently new applications of reusable IACs based on the sol-gel-technology were published by Brenn-Struckhofova *et al*. In this paper anti-DON-antibodies were entrapped in columns and were reused up to 20 times for the clean up of DON contaminated food or feed samples [68]. In summary, IACs are being used increasingly in recent years because of their ability of the specific and selective clean up resulting in effective exclusion of matrix interferences.

Attallah *et al*. [69] published a gel permeation chromatography clean up followed by HPLC for the determination of AFs in spices. This published precolumn derivatization method using TFA achieved recoveries ranging from 80 to 120% for the AFs at spiking levels of 2 and 4 μg/kg. LODs were determined between 0.51 to 0.81 μg/kg for all AFs. The LOQ was determined to be <2 μg/kg [69]. Table 3 shows an overview of commonly used clean up methods for AF analysis. To save time, solvents and to minimize possible losses, the SPE and IAC clean up procedures have been often coupled online with the HPLC system [70, 71]. Additionally, SPE can be easily automated for high throughput applications and could be seen as a cheap alternative to IAC. Several approaches regarding the automatization of the clean up coupled to the HPLC analysis have been undertaken [72 – 74]. IAC or SPE columns were coupled online with HPLC, where an automated sample preparation system (ASPEC) was applied. This type of sample preparation has the advantages, that large number of samples can be purified automatically. A disadvantage of using IACs is the pressure instability in the applied system [72 – 74].

Table 3. Clean up methods for aflatoxins

Clean up method	Matrix	Producer	Detection	Range of detection (μg/kg)	LOD/LOQ μg/kg or μg/L	Recoveries ± RSD%	Ref.
IAC	Animal liver	R-Biopharm	HPLC – FLD (Kobra cell)	0.002 – 0.75	0.002	78.2	[61]
	Botanical roots	AflaTest, Vicam	HPLC – FLD (TFA, Kobra cell; PHRED)	>16	n.a.	80	[110]
	Chilli	Easy-extract (R-Biopharm)	HPLC – FLD (Kobra cell)	1 – 93	n.a.	n.a.	[119]
	Diary feed	R-Biopharm	HPLC – FLD (TFA)	n.a.	n.a.	n.a.	[16]
	Figs (dried)	AflaTest, Vicam	HPLC – FLD (Kobra cell)	1 – 472	0.1	B1: 83 ± 15 B2: 77 ± 14 G1: 83 ± 11 G2: 76 ± 14	[109]
	Hazelnut paste	R-Biopharm	HPLC – FLD (Kobra cell; PBPB)	<10	0.15	86 – 89	[26]
	Herbal medicinal products	Aflaprep, Rhone-Diagnostics	HPLC – FLD (Kobra cell)	1.7 – 14.3	0.04	60.3 – 111.9	[111]
	Herbs, herb teas, medical plants, spices	Easy-extract, Rhone-Diagnostics	HPLC – FLD (Kobra cell)	0.2 – 26.9	0.2-0.5	Spices: 81.7 – 99 Herbs: 50 – 71.3 Med plants: 76.1 – 78.1	[21]
	Infant cereals	AflaTest, Vicam	HPLC – FLD (PBPB)	0.05 – 0.35	0.002-0.004	B1: 89.6 ± 8 B2: 87.9 ± 6.8 G1: 85.1 ± 12.3 G2: 68.8 ± 10	[107]
	Maize	AOFZDT2, Vicam	LC/ESI-MS/MS		B1: 1.1 B2: 0.6 G1: 0.3 G2: 0.4	B1: 104 ± 10 B2: 98 ± 6 G1: 102 ± 6 G2: 95 ± 7	[120]
	Medical herbs	Easy-extract (R-Biopharm)	HPLC – FLD (Kobra cell, PBPB, PHRED)	0.2 – 8	0.19-0.6	98 – 103	[112]
	Medical herbs and plant extracts	AflaTest, Vicam	HPLC – FLD (PBPB)	<4.27	B1, G1: 0.2 B2, G2: 0.15	93 – 97	[113]
	Milk	Rida (R-Biopharm)	HPLC – FLD	<2	0.006	M1: 91 ± 15	[114]
	Paprika	AflaTest, Vicam	HPLC – FLD (Kobra cell)	<163.8	n.a.	B1: 78.3 B2: 84.8 G1: 77.9 G2: 74.6	[115]
	Peanut butter	AflaTest, Vicam	HPLC – FLD (TFA)	B1: 2.06 – 63.72 G1: 1.66 – 32.78	<0.1	82	[27]
	Peanut meal	R-Biopharm	HPLC – FLD (TFA)	B1: 0.7 – 206	<0.2	74.6 – 109.6	[28]
	Pet food	Easy-extract (Biocode)	HPLC – FLD (TFA)	0.5 – 46	3 – 7	78 – 90	[108]
	Pig liver	AflaTest, Vicam	Fluorometer	0 – 22	<1	B_1: 73.6 – 88.4 M_1: 68.3 – 81.4	[106]
	Pistachio nuts	AflaTest, Vicam	HPLC – FLD (Kobra cell)	1 – 500	0.1 – 0.4	B1: 97.3 ± 10.2 B2: 95.1 ± 9.9 G1: 97.8 ± 10.1 G2: 61.8 ± 21.6	[22]
	Poultry feed	AflaTest, Vicam	HPLC – FLD (TFA)	0.23 – 11.2	B1; G1: <0.01 B2; G2: <0.02	>65 – 70%	[39]
	Sesame	AflaTest, Vicam	HPLC – FLD (TFA)	G1: 0.06 – 2.04	<0.1	86	[27]

Table 3. Continued

Clean up method	Matrix	Producer	Detection	Range of detection (μg/kg)	LOD/LOQ μg/kg or μg/L	Recoveries ± RSD%	Ref.
LLE	Cereals and grains (Brazilian pet food	–	TLC	15 ± 374	B1: 8 B2: 6 G1: 7 G2: 5	B1: 92.4 – 98.9 B2: 93.2 – 96.6 G1: 96 – 98.1 G2: 95.6 – 98.6	[121]
	Nuts	n.a.	ELISA HPLC – FLD LC-MS	<28.2	0.08 – 1.25	85 – 130	[14]
	Spices	Aflaprep, R-Biopharm	HPLC – FLD (PBPB)	n.a.	0.02	B1: 90.8 ± 6	[116]
SPE	Corn silage	Oasis-HLB	LC-ESI-MS	5 – 500	n.a.	n.a.	[122]
	Diary feed	Mycosep® #226 AflaZon; Romer Labs	ELISA	n.a.	n.a.	n.a.	[16]
	Feed	Mycosep® #226 AflaZon; Romer Labs	HPLC – FLD (Kobra cell)	1 – 18.99	1 (LOQ)	B_1: 80	[38]
	Hazelnuts	Carbograph-4 Oasis-HLB	LC/ESI-MS/MS	n.a.	n.a.	93 – 101	[12]
	Milk	Mycosep #226; Romer Labs	LC/ESI-MS/MS	<8	0.009 – 0.014	31 – 33.5	[58]
	Peanut and agricultural products	Florisil	HPLC-FLD (PHRED) LC/ESI-MS/MS	0.5 – 5	B1: 0.05 (LOQ) B2: 0.017 (LOQ)	B1: 89.5 ± 2.2 B2: 94.7 ± 2.5 G1: 90.4 ± 1.0 G2: 98.2 ± 1.1	[123]
Aluminium oxide	Paprika	Florisil	OPLC – FLD	1 – 74.8	0.5	B1: 78.5 – 105.3	[13]
CIM	Aqueous solution		Immunoaffinity monolithic disk – HPLC – FLD (Cyclodextrin)		0.05	83.7 – 103.55	[70]
Gel permeation chromatograpy	Spices		HPLC – FLD (TFA)	<4	B1: 0.51 B2: 0.54 G1: 0.81 G2: 0.63	80 – 120	[69]
LLE-SPE	Barley	–	Fluorimeter (350 nm)	0.3 – 31.4	0.3	86 – 104	[46]
MSPD	High pigment samples		HPLC – FLD (Kobra cell)	<2.5	B1; G1: 0.25 B2; G2:0.1	B1: 92 – 95 B2: 88 – 91 G1: 90 – 92 G2: 89 – 93	[124]
SFE	Zizyphi fructus		LC-ESI/APCI/MS	n.a.	0.17 – 0.32	86 – 105	[54]
Without clean up	Baby food, Corn and peanut		ELISA Ridascreen® (R-Biopharm)	0 – 400	<1.7	n.a.	[95]
			ELISA Aflaplate® (R-Biopharm)		<2		
	Milk		ELISA Ridascreen® (R-Biopharm)	<0.11	0.05	M1: 96.3	[15]
	Moldy food	Centrifugation, dilution	LC-ESI-MS	1 – 100	0.5 – 1.5	97 – 100	[125]

In developing countries an important aspect against the use of IACs is the cost of analysis. Therefore, in the developing countries the use of SPE, due to its lower costs, compared to IAC can be taken into consideration.

3 Quantification techniques

Currently 41 methods for sampling and determination of AFs in different food and feed have been published by the AOAC (Association of Official Analytical Chemists). The CEN (European Committee for Standardization) has reported six methods for AF detection until now (http://www.cen.eu/cenorm/homepage.htm; http://www.eoma.aoac.org). Different analytical methods such as TLC, HPLC and ELISA have been used for determination of AFs in feed and food [75]. The methods can be submitted together with the submission fee to the AOAC either first through a government agency or organization. Secondly, a community may be formed of stakeholders from government, industry, and/or academia who need validated methods. Thirdly a company or organization that has an economic interest that their products/methods are approved by the AOAC (http://www.aoac.org). Nowadays official methods of the AOAC for determinations of AFs, such as ELISA and HPLC-FLD, have been established in various food and feed. Immunobased methods have often replaced conventional methods; though HPLC has been widely used as a reference method.

3.1 TLC

The TLC was used widely in the past, however nowadays more labs use HPLC. Recently a TLC method for the AFs (B_1, B_2, G_1, G_2) in various food matrices such as corn, paprika, peanuts and pistachios was performed by Stroka *et al.* [76]. The authors used IAC clean up prior to TLC. The identification and quantification of the separated AFs was performed by comparison with AF standards using densitometry. The LOD's ranged from 0.1 to 0.2 ng/g for all AFs, while the LOQ's were from 0.2 to 0.3 ng/g and the recoveries ranged from 76 to 87% [76]. The method is able to detect the maximum levels which are set in the European Union. In this application no chlorinated solvent was used and for this the method can be used as an alternative of highly instrumented methods [76]. A further application of TLC was carried out by Stroka and Anklam [77] using a miniaturized and low power consuming detector cell (SeBaDeC) for the densitometric measurement of AFs. A UV-light emitting diode (UV-LED; emission wavelength of 370 nm) was used for fluorescence excitation, while a photodiode (440 nm) in combination with a cut-off filter (>418 nm) was applied for detecting the fluorescence intensity. The samples were measured in an angle of 90°. The densitometer was able to detect concentrations at 1 ng AFB_1 per spot. For simultaneous determination of the AFs, the LODs were determined at 1.2, 1.7, 1.7 and 4.8 ng for AFB_1, AFB_2, AFG_1 and AFG_2, respectively. For comparison, fortified test samples of paprika powder and pistachios were analysed by TLC and the SeBaDeC. The results of both methods were comparable in a range from 1 to 4 ng. This densitometer was able to determine AF levels at the European legislation limit and is an inexpensive alternative to the commonly used scanner. Yet the lifetime of the lamp (UV-LED) is higher than the common used mercury or gas lamps. Quite recent applications using TLC were published by Nakai *et al*. in stored peanuts and Var *et al*. in traditional Turkish food [78, 79]. An advantage of the TLC is the qualitative and also quantitative analysis of mycotoxin contaminated samples without the use of cost intensive instruments which reduces the costs of analysis in developing countries.

3.2 HPLC-FLD

The most widely used method for the determination of AFs in food and feed is the use of HPLC coupled with fluorescence detection. However AFB_1 and AFG_1 possess less natural fluorescence. To improve the signals during analysis various pre or postcolumn derivatization methods are used for signal enhancement [80–82]. The detection is commonly carried out by FLD using nm λ-Excitation 365 nm λ-Emission 435–440 nm [81]. Different strategies to improve the sensitivity are carried out nowadays. AFs are derivatized either with TFA, iodine or bromine. In the precolumn derivatization step using TFA AFB_1 and AFG_1 are converted, due to acid hydrolysis, into their high fluorescent hemiacetals AFB_{2a} and AFG_{2a}. A disadvantage of this method is the reaction time, which takes about 30 min at 50°C. The iodination and bromination are postcolumn derivatization steps. The iodination has the disadvantages of being corrosive, the solution has to be prepared daily and an additional pump is needed. Further the day to day reproducibility is inadequate [81]. An additional strategy to enhance the florescence of AFG_1 and AFB_1 is the use of pyridinium bromide perbromide (PBPB) as published by Garner *et al*. [83]. Within this method PBPB is added postcolumn to the mobile phase and allows the reaction prior fluorescence detection. Garner *et al*. reported that all AFs especially AFB_1 obtained higher signals than using TFA. An additional benefit of the PBPB postcolumn derivatization method is that high temperatures are not necessary [83]. In the electrochemical cell, iodine and bromine is generated electrochemically due to the IBr or KBr added to the mobile phase (Fig. 3). Iodine or bromine bind to the 8,9 double bond of the AFs and lead an enhancement of the fluorescence (Fig. 4) [81]. In Fig. 5 chromatograms of a standard solution containing all AFs with and without using Kobra cell are shown. Further methods for the signal enhancement are the use of Cyclodextrin or also the use of the PHRED [84, 85]. Cyclodextrins are mainly used as fluorescence enhancers, for instance as post column addi-

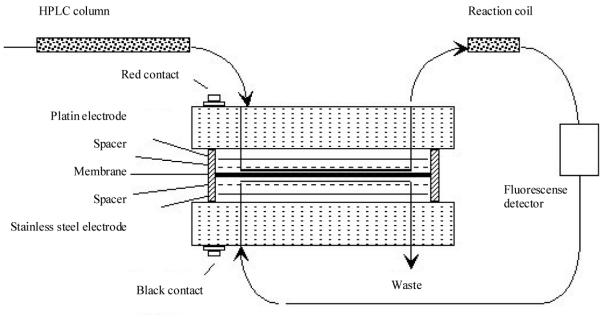

Figure 3. Schematic structure of a postcolumn derivatization cell for aflatoxins (produced by Coring GmbH, Germany).

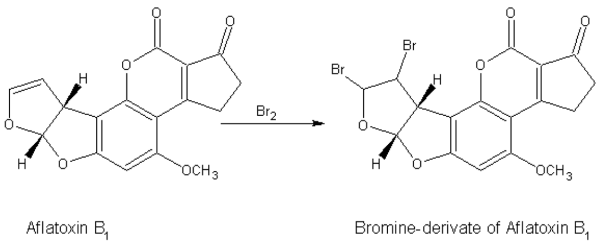

Figure 4. Derivatization of AFB_1 using electrochemically generated bromine.

tives, they can be further added to the mobile phase as described by Chiavaro *et al*. [84]. Cyclodextrins are responsible for the formation of inclusion complexes. The enhancement may result through the interactions of the cyclodextrin and the AFs or the possible interactions of water and AFs in the presence of the cyclodextrin. For the analysis of AFs an increasing fluorescence of the AFs with an unsaturated furancycle (B_1 and G_1) facilitates the detection [86]. Chiavaro evaluated the three cyclodextrins succynil-β-cyclodextrin (β-CD-Su), dimethyl-β-cyclodextrin (DIMEB) and β-cyclodextrin (β-CD) for enhancing the AF signals. After addition of 6×10^{-3} M of β-CD and β-CD-Su an appropriate improvement with a LOD < 0.3 μg/kg for all AFs could be obtained. The new developed method achieved recoveries for all AFs ranging from 73.2 to 76.5%. This technique of signal improvement is a further reliable tool for signal enhancement [84].

Papadopoulou-Bouraoui *et al*. compared the Kobra cell (PCD_{EC}) with photochemical fluorescence enhancement (PCD_{UV}) for the analysis of AFs in food (hazelnuts, corn, and citrus pulp containing animal feed) [87]. The photochemical derivatization devices were purchased from two different producers and possessed reaction coils of 20 and 25 m, respectively. Within the photolysis AFB_1 and AFG_1 are converted into their hemiacetals AFB_{2a} and AFG_{2a}. Prior to determination, the sample clean up was performed using IACs. In a comparison of the two postcolumn detection techniques, matrix effects and quenching phenomena have been investigated. The signal ratios between postcolumn derivatization using Kobra cell and photochemical fluorescence enhancement were determined for all AFs (PCD_{UV}/PCD_{EC} 0.60 – 0.96) [87]. The authors reported that both methods are suitable for the postcolumn derivatization of AFs, however lower signals were obtained using the photochemical derivatization technique.

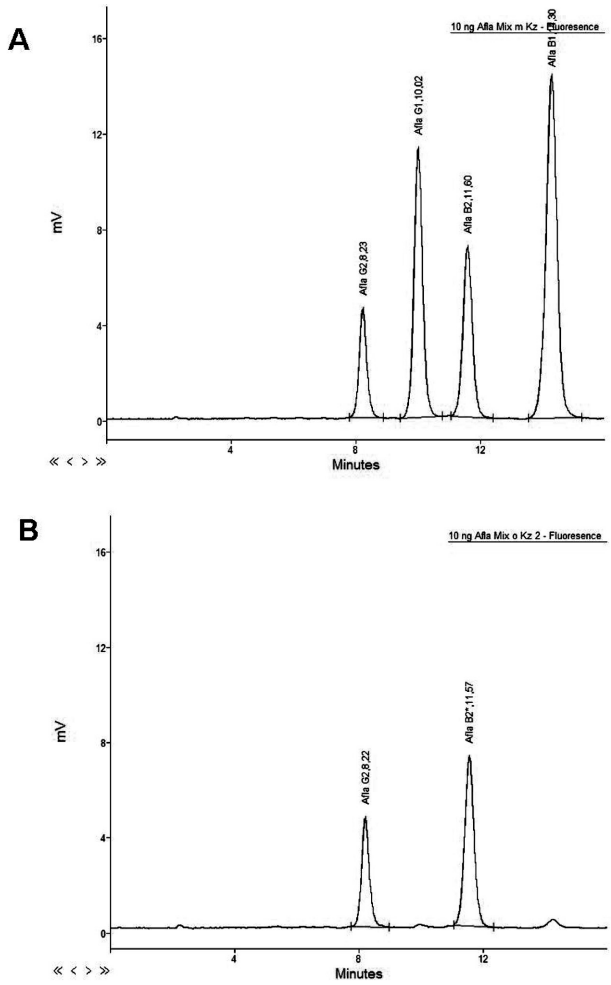

Figure 5. Chromatograms of an aflatoxin standard solution with (A) and without (B) Kobra cell (c: AFB_1: 8.93 μg/L, AFB_2: 3.04 μg/L, AFG_1: 10.46 μg/L AFG_2: 3.34 μg/L) where 100 μL were injected.

3.3 OPLC

A further technique, the overpressured-layer chromatography (OPLC), for the analysis of AFs in food or feed was applied by Otta *et al*. [88]. The authors used SPE or liquid–liquid partitioning, after evaporation the samples were redissolved in dichloromethane-acetone and pored onto the chromatographic plate [88]. After concentration of the extracts the AFs were separated by use of an OPLC System where an external pressure is applied on the chromatographic plate, a pump is used for the admission of the

mobile phase [13, 88]. The quantification was carried out using a densitometer. According to the authors, OPLC technique combines the advantages of the HPLC and HPTLC which allows high sample throughput and low costs [88]. Using this method, AF contents in corn, fish and wheat samples were determined having recoveries greater than 73%. With the method 2 μg/kg of each AF can be detected, which seems to be the LOD [88]. Quite recently Moricz *et al*. applied the OPLC technique for the determination of AFs in spices [13]. Depending on the used mobile phase recoveries of 78.5% for AFB_1 and 81.8% for total AFs and 105.3% for AFB_1 and 97.4% for total AFs, respectively were obtained by comparison with HPLC [13]. Samples contaminated higher than 2 μg/kg can be detected [13].

3.4 MALDI-TOF

Matrix assisted laser desorption ionization MS coupled with TOF (MALDI-TOF) was used as a screening method for AFs by Catharino *et al*.[89]. The LOD was determined at a level of 50 fmol which were calculated to be 16 pg. Peanut samples contaminated with aflatoxigenic fungi were mixed with MeOH, KCl, diatomaceous earth, $CuSO_4$ and water. Finally the mixture was extracted twice with chloroform. In this method all AFs showed similar abundances (equimolar mixture). The use of MALDI-TOF is rapid and allows a high throughput [89].

3.5 LC-MS and the possibility of multitoxin analysis

Methods based on the LC-MS technology are of increasing interest and allows the analysis of more than one metabolite of interest in a single run. It has often replaced commonly used techniques. Lately various reviews were published [90–93]. The LC-MS is coupled to various ionization techniques such as ESI, APCI and APPI (atmospheric pressure photoionization) and is commonly used as a confirmatory technique. Since various reviews were published recently this technique will not be discussed in detail. Several advantages of LC-MS technique were discussed. The most important advantage of this hyphenated technique is its high selectivity due to unambiguous analyte identification. Some authors proposed the analysis of mycotoxin contaminated samples even without clean up. Furthermore, LC-MS provides the possibility of high throughput analysis and simultaneous analysis of several mycotoxins and their metabolites within one run. Drawbacks of LC-MS methods have been described as being the ion-suppression phenomena that is matrix depended and efforts a more sophisticated clean up. To provide good accuracy and precision either isotope labelled or internal standards are applied to eliminate matrix interferences [90–93].

The detection selectivity in the case of LC-MS would compensate the drawbacks of an intensive sample clean up.

4 Immunochemical tests

Different fast tests and a variety of ELISA test kits are available on the market from various manufacturers based on immunochemical reactions. Furthermore immunoassays and lateral flow dipsticks have been developed and commercialized. ELISA test kits have been used for quantitative determination as well as for screening purposes.

4.1 ELISA

Direct or indirect competitive ELISA has also been extensively used for quantification of AFs in different food and feed. In this case, antibodies against AFs were used in negative competitive ELISA. Besides commercialized ELISA tests different groups have developed their own ELISA tests. An advantage of ELISA-based tests is the low LOD, which is comparable with those obtained with the HPLC. Kolosova *et al*. [94] developed a direct competitive ELISA using monoclonal antibodies for the determination of AFB_1 in grain samples. After extraction the samples were applied directly onto the coating plates. Determined concentrations were ranging from 0.1 to 10 μg/kg. Spiked rice samples achieved a recovery ranging from 94 to 113% with a LOD of 0.05 μg/kg [94]. The ELISA test was also compared with HPLC by Razzazi *et al*. [95], where baby food, peanut and corn products were analysed with both methods. The extract was either applied directly onto the ELISA to determine AFB_1 or total AFs. The ELISA kits achieved LODs <1.7 μg/kg for total AFs and <2 μg/kg for AFB_1. Thirty contaminated samples were selected and confirmed using IAC prior HPLC-FLD and Kobra cell derivatization. Within this work a better correlation between HPLC-FLD and ELISA (0.9244 *vs*. 0.7590 for total AFs and 0.8805 *vs*. 0.7234 for AFB_1) was observed at lower concentration levels (0–80 μg/kg for total AFs, 0–120 μg/kg for AFB_1) than at higher levels (0–400 μg/kg) [95]. Trucksess *et al*. tested a modified commercially available ELISA screening method in 15 laboratories [96]. Samples were fortified at levels of 10, 20 and 30 μg/kg. The results showed a high true positive rate without clean up [96]. Only one false positive result was registered. The authors suggested that this test is a rapid screening technique at levels >20 μg/kg [96]. Chun *et al*. verified positive ELISA samples of nut products using HPLC (TFA) [14]. A total of 85 nuts and nut products were analysed. For AFB_1 the LOD was 0.08 μg/kg and the LOQ was 0.15 μg/kg, respectively. The recoveries ranged from 83.4 to 102% for all AFs. After ELISA screening the 31 positive samples, which gave results above 0.06 μg/kg, were quantified with HPLC, whereas nine samples were found to be contaminated up to 28 μg/kg [14]. Due to these results about two-thirds were false positive in ELISA. The major problem of ELISA is possible false positive results due to cross reactions of antibodies [14]. A great advantage of ELISA techniques is the high throughput, whereas new

qualitative/semiquantitative techniques allow a faster detection, which relate with the loss of accuracy. Therefore, ELISA based tests are indented for screening where positive tests will be confirmed with HPLC.

4.2 Immunofiltration

Pal *et al*. published some new developments based on immunofiltration technique. The immunofiltration method consists of a membrane coated with anti-AFB_1 antibodies and allows the determination of less diluted samples [97]. After application of the samples into the spots, washing and cleaning step to remove matrix interferences was used. The spots were visualized using AFB_1-HRP-conjugate. The average recoveries within a spiking range of 5 – 100 µg/kg were 99 – 105%. The LOD was determined at 5 pg per spot. The method was applied to ground nuts, corn, wheat, processed soybean, chilli, poultry feed [97]. Additionally, Pal and Dhar published an on-site immunoassay using a semiquantitative determination of AFB_1 in batch of samples [98]. This immunoassay method consists of a nitrocellulose membrane attached to a polyethylene card. The colour intensity of the extracted samples was compared with the intensity of standard solutions. The test allows the analysis of 12 samples within 10 min. The mean recoveries ranged between 91 and 104% with a LOD of 0.01 ng/mL. The authors reported a good correlation between the immunoassay and HPLC by comparison of infected samples ($R^2 = 0.99$) [98].

4.3 Lateral flow dipstick

Lately, a new type of immunoassay based on the formation of a Ag – Ab complexes, has been introduced. In lateral flow tests, the sample is put onto a pad, where the mycotoxins are bound to antibody gold particles. This Ab-gold particles and second (on the pad mobilized) antibody gold particles migrate to the test zone and control zone, which are containing a membrane. The mycotoxin antibody gold particle complex binds in the test zone to an AF protein conjugate, the second antibody gold complex binds in the control zone that allows the formation of a coloured line. If the AFs concentration is equal or greater than the defined cut-off level, a coloured line in the test zone will be visible. In the control zone always a line appears (in absence of AFs) after binding of the gold particle to the second antibody. The screening results can be obtained in short time within 3 – 5 min. A semiquantitative lateral flow-based rapid one step assay 'ROSA' which is produced by Charm Sciences' was tested in an interlaboratory study within 21 public health, state agriculture and industrial laboratories [99]. This study revealed 4.8% false negative results. The used test kit is a colloidal gold lateral flow immunoassay for analysis of AFM_1. Samples were spiked with different levels of AFM_1 (0 – 550 ppt). Levels below 400 ppt were registered as negative; samples fortified with higher levels were noted as positive. The lateral flow strips have to be incubated for 10 min at 45°C and then applied to the ROSA reader. A full analysis is able within 12 min [99]. Since lateral flow dipstick is a semi-quantitative assay the authors suggested that the results have to be confirmed with conventional methods [99]. This dipstick has not been compared with other detection techniques such as ELISA or HPLC.

In contrast Delmulle *et al*. developed a lateral flow dipstick for the analysis of AF in pig feed [100]. The visual LOD was determined at 5 µg/kg and results were obtained within 10 min. Using MeOH/Water (80:20) the highest recoveries were obtained (84.6 ± 12.8%). In an intralaboratory validation of 88 samples four false positive and no false negative results were obtained. Further the evaluation of certified reference material attained no false negative and false positive result [100]. Lateral flow dipsticks are also offered by different companies (http://www.neogen.com; http://envirologix.com/artman/publish/index.shtml).
Immunochemical methods for rapid detection were recently reviewed by Goryacheva *et al*. [101]. This type of technique is an important strategy in screening of contaminated food and feed. Nevertheless, positive results have to be confirmed with methods that are more sophisticated.

5 Biosensors

Recently an electrochemical immunosensor array for AFB_1 detection was published by Piermarini *et al*.[102]. A 96 well microtiterplate was coupled to a multichannel electrochemical detection. For the quantification, the microplate reader uses intermittent pulse amperometry (IPA). Millisecond pulses were applied onto each of the 96 sensing electrodes with a potential of +400 mV and a frequency of 50 Hz. For the application of this method corn samples were used. They achieved a LOD of 50 pg/mL a mean recovery was 103 ± 8% [102].

A rapid biosensor has been developed by Sapsford *et al*. [103]. AFs were bound to biotin in a two-step synthesis. The biotin-AFB_1 was immobilized on microscope slides with flow cells. After addition of a mixture of the extract of spiked food samples and labelled antibodies, the slides were washed, dried under nitrogen and detected. The excitation was measured at 635 nm using a diode laser. With increasing AF content in the solution, the signal is decreasing. The method performed a LOD ranging from 0.28 in buffer to 5.1 µg/L depending on the matrix compounds and was comparable to the LODs of ELISA tests. The authors concluded that within this assay, little or no sample pretreatment is necessary. Moreover, the results were obtained in 30 min after 2 h extraction. Further the simultaneous screening of various toxins is possible [103].

An optical waveguide lightmode spectroscopy (OWLS) technique was applied in competitive and in direct immuno-

assays by Adanyi *et al*. [104]. The resonance angle of polarized laser light which has been diffracted by a grating and incoupled into a thin waveguide is measured and is detected by photodiodes in the OWLS technique. Biomolecules can be coupled to the modified SiO_2-TiO_2 layer. After immobilizing the antibody or antigen conjugate on the surface, the sensor chip was used in a flow-injection analyser (FIA) system. The range of sensitive detection has been determined from 0.5 to 10 μg/kg. The new developed sensor has been further compared with ELISA. A competitive ELISA has been applied, where conjugated AF has been immobilized on microtitre wells. No information about the LOD and recoveries of the ELISA method was given [104].

6 Screening methods

The fluorimetric measurement has been used for semiquantitative determination of AFs in food and feed samples. For this type of measurements it is very important to remove interferences as much as possible, mostly done by IAC. Furthermore, the signal has to be enhanced using various derivatization techniques (http://www.romerlabs.com/romer.htm; http://www.vicam.com) [105, 106].

Cucci *et al*. [105] reported about the screening of AFM_1 using a portable fluorometer. The used fluorometer was based on a LED source and an extremely sensitive PMT detector, which is able to detect low concentrations of AFs (>50 ng/kg). Cyclodextrin was used for the signal enhancement and AF contaminations ranged between 0 and 125 ng/kg [105]. No information was mentioned regarding the used clean up method [105]. In this work, no correlation with the HPLC was evaluated. The authors complained the variability of quantitative results [105].

Chiavaro *et al*. [106] compared the direct fluorometric measurement with HPLC–FLD detection after immunoaffinity clean up. Contents of AFB_1 and AFM_1 were determined in 50 pig liver samples. The fluorometric method obtained a LOD <1 μg/kg for both AFs and the recoveries were between 73.6 and 88.4% for AFB_1 and 68.3 and 81.4% for AFM_1, respectively. The method using HPLC achieved lower LODs 0.005 μg/kg for AFB_1 and 0.04 μg/kg for AFM_1 [106]. Recoveries obtained with the HPLC method were ranging from 75.9 to 83.7% and 74.2 to 76.6% for AFB_1 and AFM_1, respectively. Between both methods, no significant differences were found. The authors reported the applicability of the method for the determination of samples, which were higher contaminated than 1 μg/kg [106].

Recently FluoroQuant® have been introduced into market by Romer Labs, where the samples are cleaned-up with a SPE column. After clean up, the samples are mixed with a developer and finally measured with the fluorometer. This method shows a recovery ranging between 84 and 115% with LODs of 0.6–1.4 μg/kg (http://www.romerlabs.com/romer.htm). A similar product is available from Vicam ($AflaM_1$ FL^+), where the IAC clean up is coupled with fluorometric measurement without HPLC separation (http://www.vicam.com).

7 Conclusion

Food safety is of special significance to public health worldwide. In the chain from raw material to food, consumer protection begins with assurance of the quality of agricultural material and feed as well as animal health. Among all mycotoxins AF contamination has been registered as a worldwide health-threatening problem due to the globalization of food and feed trade. Therefore, the detection of AFs in extremely low quantities in food and feed is of important relevance and requires sophisticated sampling, sample preparation, extraction and analytical techniques. The analysis of AFs can be carried out using different strategies.

In the sample clean up of AFs immunobased IAC have nearly replaced other methods such as liquid–liquid partitioning and SPE. Comparing the clean up methods IACs show the highest selectivity.

IAC allow for exclusion of almost all undesirable matrix interferences in the chromatograms. On the other hand, the HPLC technique allows accurate determination at very low levels for all AFs in food and feed. Therefore, IAC in combination with HPLC and fluorescence detection has become the reference method for the accurate analysis of AFs in food and feed. In contrast, TLC has less relevance nowadays in industrial countries. However, due to the low cost it is used as an alternative in developing countries.

Immunobased techniques such as ELISA have many advantages since no clean up is suggested. However, drawbacks of the ELISA are cross reactivities of the antibodies, which can effect in false positive results. Lateral flow dipstick is an appropriate tool for rapid and semi quantitative screening on field, however results need to be confirmed using other analytical techniques. However, drawbacks of IACs are cost intensive clean up due to the use of monoclonal antibodies and the single use of columns. Aims of further developments should result in an appropriate coupling of costless sample preparation techniques with the accurate determination using HPLC.

This work was supported by BIOTRACER, an integrated project within the European Union 6th Framework Programme.

The authors have declared no conflict of interest.

8 References

[1] Murphy, P. A., Hendrich, S., Landgren, C., Bryant, C. M., Food mycotoxins: An update, *J. Food Sci.* 2006, *71*, R51–R65.

[2] Bennett, J. W., Klich, M., Mycotoxins, *Clin. Microbiol. Rev.* 2003, *16*, 497 – 516.

[3] Mücke, W., Lemmen, C., *Schimmelpilze*, ecomed MEDIZIN, Landsberg am Lech 2004.

[4] Bhatnagar, D., Payne, G. A., Cleveland, T. E., Robens, J. F., in: Barug, D., Van Egmond, H., López-Garzía, R., van Osenbruggen, T., Visconti, A. (Eds.), *Meeting the mycotoxin menace*, Wageningen Academic Publishers, Wageningen 2004, pp. 17 – 47.

[5] Frisvad, J. C., Thrane, U., Samson, R. A., in: Dijksterhuis, J., Samson, R. A. (Eds.), *Food Mycology – A Multifaceted Approach to Fungi and Food*, CRC Press, Boca Raton 2007, pp. 135 – 159.

[6] Hussain, I., Anwar, J., A study on contamination of aflatoxin M_1 in raw milk in the Punjab province of Pakistan, *Food Control* 2008, *19*, 393 – 395.

[7] Tekinsen, K. K., Ucar, G., Aflatoxin, M_1 levels in butter and cream cheese consumed in Turkey, *Food Control* 2008, *19*, 27 – 30.

[8] Virdis, S., Corgiolu, G., Scarano, C., Pilo, A. L., De Santis, E. P. L., Occurrence of Aflatoxin M_1 in tank bulk goat milk and ripened goat cheese, *Food Control* 2008, *19*, 44 – 49.

[9] Torkar, K. G., Vengust, A., The presence of yeasts, moulds and aflatoxin M_1 in raw milk and cheese in Slovenia, *Food Control* 2008, *19*, 570 – 577.

[10] O'Riordan, M. J., Wilkinson, M. G., A survey of the incidence and level of aflatoxin contamination in a range of imported spice preparations on the Irish retail market, *Food Chem.* 2008, *107*, 1429 – 1435.

[11] Cho, S.-H., Lee, C.-H., Jang, M.-R., Son, Y.-W., *et al*., Aflatoxins contamination in spices and processed spice products commercialized in Korea, *Food Chem.* 2008, *107*, 1283 – 1288.

[12] Bacaloni, A., Cavaliere, C., Cucci, F., Foglia, P., *et al*., Determination of aflatoxins in hazelnuts by various sample preparation methods and liquid chromatography-tandem mass spectrometry, *J. Chromatogr. A* 2008, *1179*, 182 – 189.

[13] Moricz, A. M., Fater, Z., Otta, K. H., Tyihak, E., Mincsovics, E., Overpressured layer chromatographic determination of aflatoxin B1, B2, G1 and G2 in red paprika, *Microchem. J.* 2007, *85*, 140 – 144.

[14] Chun, H. S., Kim, H. J., Ok, H. E., Hwang, J.-B., Chung, D.-H., Determination of aflatoxin levels in nuts and their products consumed in South Korea, *Food Chem.* 2007, *102*, 385 – 391.

[15] Oveisi, M.-R., Jannat, B., Sadeghi, N., Hajimahmoodi, M., Nikzad, A., Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran, *Food Control* 2007, *18*, 1216 – 1218.

[16] Decastelli, L., Lai, J., Gramaglia, M., Monaco, A., *et al*., Aflatoxins occurrence in milk and feed in Northern Italy during 2004-2005, *Food Control* 2007, *18*, 1263 – 1266.

[17] Iamanaka, B. T., de, Menezes, H. C., Vicente, E., Leite, R. S. F., Taniwaki, M. H., Aflatoxigenic fungi and aflatoxins occurrence in sultanas and dried figs commercialized in Brazil, *Food Control* 2007, *18*, 454 – 457.

[18] Ayar, A., Sert, D., Con, A. H., A study on the occurrence of aflatoxin in raw milk due to feeds, *J. Food Saf.* 2007, *27*, 199 – 207.

[19] Ghiasian, S. A., Maghsood, A. H., Neyestani, T. R., Mirhendi, S. H., Occurrence of aflatoxin M_1 in raw milk during the summer and winter seasons in Hamedan, Iran, *J. Food Saf.* 2007, *27*, 188 – 198.

[20] Aydin, A., Emin Erkan, M., Baskaya, R., Ciftcioglu, G., Determination of Aflatoxin B1 levels in powdered red pepper, *Food Control* 2007, *18*, 1015 – 1018.

[21] Romagnoli, B., Menna, V., Gruppioni, N., Bergamini, C., Aflatoxins in spices, aromatic herbs, herb-teas and medicinal plants marketed in Italy, *Food Control* 2007, *18*, 697 – 701.

[22] Cheraghali, A. M., Yazdanpanah, H., Doraki, N., Abouhossain, G. *et al*., Incidence of aflatoxins in Iran pistachio nuts, *Food Chem. Toxicol.* 2007, *45*, 812 – 816.

[23] Molyneux, R. J., Mahoney, N., Kim, J. H., Campbell, B. C., Mycotoxins in edible tree nuts, *Int. J. Food Microbiol.* 2007, *119*, 72 – 78.

[24] Tajkarimi, M., Shojaee, Aliabadi, F., Salah Nejad, M., *et al*., Seasonal study of aflatoxin M_1 contamination in milk in five regions in Iran, *Int. J. Food Microbiol.* 2007, *116*, 346 – 349.

[25] Bandyopadhyay, R., Kumar, M., Leslie, J. F., Relative severity of aflatoxin contamination of cereal crops in West Africa, *Food Addit. Contam.* 2007, *24*, 1109 – 1114.

[26] Senyuva, H. Z., Gilbert, J., Arasiler, Z., Ayabakan, A., *et al*., Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxins in hazelnut paste: Interlaboratory study, *J. AOAC Int.* 2005, *88*, 526 – 535.

[27] Yentuer, G., Er, B., Guer Oezkan, M., Bayhan Oektem, A., Determination of aflatoxins in peanut butter and sesame samples using high-performance liquid chromatography method, *Eur. Food Res. Technol.* 2006, *224*, 167 – 170.

[28] Möller, T. E., Nyberg, M., Efficiency of different extraction solvent mixtures used in analyses of aflatoxins from a certified peanut meal reference material, *Food Addit. Contam.* 2004, *21*, 781 – 785.

[29] FAO, *Worldwide regulations for mycotoxins in food and feed in 2003*, Rome 2004.

[30] Huber L. (Ed.), *Validation and Qualification in Analytical Laboratories*. Informa Healthcare USA, New York 2007.

[31] EFSA, Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Aflatoxin B_1 as undesirable substance in animal feed (Request N° EFSA-Q-2003-035), *EFSA J.* 2004, *39*, 1 – 27.

[32] Joint FAO/WHO Expert Committee on Food Additives, *Evaluation of certain mycotoxins in food,* Geneva 2002.

[33] IARC, *Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins*, IARC Monographs on the evaluation of carcinogenic risks to humans, Lyon 1993.

[34] European Commission, Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuff, *Official J. Eur. Union* 2006, *L364*, 5 – 24.

[35] Codex Alimentarius Commission. JOINT FAO/WHO Food Standards Programme – CODEX ALIMENTARIUS COMMISSION 30th Session, *Report of the 1st Session of the Codex Committee on Contaminants in Foods*, Beijing, China, 16 – 20 April 2007.

[36] Fraga, M. E., Curvello, F., Gatti, M. J., Cavaglieri, L. R., *et al*., Potential aflatoxin and ochratoxin a production by Aspergillus species in poultry feed processing, *Vet. Res. Commun.* 2007, *31*, 343 – 353.

[37] Thieu, N. Q., Ogle, B., Pettersson, H., Screening of Aflatoxins and Zearalenone in feedstuffs and complete feeds for pigs in Southern Vietnam, *Trop. Anim. Health Prod.* 2008, *40*, 77 – 83.

[38] Binder, E. M., Tan, L. M., Chin, L., Handl, J., Richard, J., Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients, *Anim. Feed Sci. Technol.* 2007, *137*, 265 – 282.

[39] Zinedine, A., Juan, C., Soriano, J. M., Molto, J.C., *et al*., Limited survey for the occurrence of aflatoxins in cereals and poultry feeds from Rabat, Morocco, *Int. J. Food Microbiol.* 2007, *115*, 124 – 127.

[40] Leung, M. C. K., Diaz-Liano, G., Smith, T. K., Mycotoxins in pet food: A review on worldwide prevalence and preventative strategies, *J. Agric. Food Chem.* 2006, *54*, 9623 – 9635.

[41] Johansson, A. S., Whitaker, T. B., Giesbrecht, F. G., Winston, M. H., Jr., Young, J. H., Testing shelled corn for aflatoxin, part II: Modeling the observed distribution of aflatoxin test results, *J. AOAC Int.* 2000, *83*, 1270 – 1278.

[42] Whitaker, T. B., Sampling foods for mycotoxins, *Food Addit. Contam.* 2006, *23*, 50 – 61.

[43] Ozay, G., Seyhan, F., Yilmaz, A., Whitaker, T. B., *et al*., Sampling hazelnuts for aflatoxin: Uncertainty associated with sampling, sample preparation, and analysis, *J. AOAC Int.* 2006, *89*, 1004 – 1011.

[44] Whitaker, T. B., Slate, A. B., Hurley, J. M., Giesbrecht, F. G., Sampling almonds for aflatoxin, part II: Estimating risks associated with various sampling plan designs, *J. AOAC Int.* 2007, *90*, 778 – 785.

[45] Whitaker, T. B., Slate, A. B., Jacobs, M., Michael Hurley, J., *et al*., Sampling almonds for aflatoxin, part I: Estimation of uncertainty associated with sampling, sample preparation, and analysis, *J. AOAC Int.* 2006, *89*, 1027 – 1034.

[46] Bourais, I., Amine, A., Venanzi, M., Micheli, L., *et al*., Development and application of a two-phase clean-up system in food samples prior to fluorescence analysis of aflatoxins, *Microchim. Acta* 2006, *153*, 101 – 108.

[47] Sizoo, E. A., van Egmond, H. P., Analysis of duplicate 24-hour diet samples for aflatoxin B_1, aflatoxin M_1 and ochratoxin A, *Food Addit. Contam.* 2005, *22*, 163 – 172.

[48] Kokkonen, M., Jestoi, M., Rizzo, A., Determination of selected mycotoxins in mould cheeses with liquid chromatography coupled to tandem with mass spectrometry, *Food Addit. Contam.* 2005, *22*, 449 – 456.

[49] Stroka, J., Petz, M., Joerissen, U., Anklam, E., Investigation of various extractants for the analysis of aflatoxin B_1 in different food and feed matrices, *Food Addit. Contam.* 1999, *16*, 331 – 338.

[50] Spanjer, M. C., Scholten, J. M., Kastrup, S., Jorissen, U., *et al*., Sample comminution for mycotoxin analysis: Dry milling or slurry mixing? *Food Addit. Contam.* 2006, *23*, 73 – 83.

[51] Schatzki, T. F., Toyofuku, N., Sample Preparation and Presampling of Pistachios, *J. Agric. Food Chem*. 2003, *51*, 6068 – 6072.

[52] Blesa, J., Soriano, J. M., Molto, J. C., Marin, R., Manes, J., Determination of aflatoxins in peanuts by matrix solid-phase dispersion and liquid chromatography, *J. Chromatogr. A* 2003, *1011*, 49 – 54.

[53] Cavaliere, C., Foglia, P., Guarino, C., Nazzari, M., *et al*., Determination of aflatoxins in olive oil by liquid chromatography-tandem mass spectrometry, *Anal. Chim. Acta* 2007, *596*, 141 – 148.

[54] Liau, B.-C., Jong, T.-T., Lee, M.-R., Chang, C.-M. J., Supercritical fluid extraction and quantification of aflatoxins in Zizyphi Fructus by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 2007, *21*, 667 – 673.

[55] Scott, P. M., Recent developments in methods of analysis for mycotoxins in foodstuffs, *TrAC Trends Analyt. Chem.* 1993, *12*, 382 – 386.

[56] Cavaliere, C., Foglia, P., Guarino, C., Marzioni, F., *et al*., Aflatoxin M_1 determination in cheese by liquid chromatography-tandem mass spectrometry, *J. Chromatogr. A* 2006, *1135*, 135 – 141.

[57] Park, J. W., Kim, Y.-B., Effect of pressure cooking on aflatoxin B_1 in rice, *Environ. Sci. Technol.* 2006, *35*, 2401 – 2406.

[58] Chen, C.-Y., Li, W.-J., Peng, K.-Y., Determination of aflatoxin M_1 in milk and milk powder using high-flow solid-phase extraction and liquid chromatography-tandem mass spectrometry, *J. Agric. Food Chem.* 2005, *53*, 8474 – 8480.

[59] Cavaliere, C., Foglia, P., Pastorini, E., Samperi, R., Laganà, A., Liquid chromatography/tandem mass spectrometric confirmatory method for determining aflatoxin M_1 in cow milk – Comparison between electrospray and atmospheric pressure photoionization sources, *J. Chromatogr. A* 2005, *1101*, 69 – 78.

[60] Sobolev, V. S., Dorner, J. W., Cleanup procedure for determination of aflatoxins in major agricultural commodities by liquid chromatography, *J. AOAC Int.* 2002, *85*, 642 – 645.

[61] Tavcar-Kalcher, G., Vrtac, K., Pestevsek, U., Vengust, A., Validation of the procedure for the determination of aflatoxin B_1 in animal liver using immunoaffinity columns and liquid chromatography with postcolumn derivatisation and fluorescence detection, *Food Control* 2007, *18*, 333 – 337.

[62] Gurbay, A., Aydin, S., Girgin, G., Engin, A. B., Sahin, G., Assessment of aflatoxin M_1 levels in milk in Ankara, Turkey, *Food Control* 2006, *17*, 1 – 4.

[63] Ip, S.-P., Che, C.-T., Determination of aflatoxins in Chinese medicinal herbs by high-performance liquid chromatography using immunoaffinity column cleanup: Improvement of recovery, *J. Chromatogr. A* 2006, *1135*, 241 – 244.

[64] Diaz, G. J., Espitia, E., Occurrence of aflatoxin M_1 in retail milk samples from Bogotá, Colombia, *Food Addit. Contam.* 2006, *23*, 811 – 815.

[65] Majors, R. E., Immunosorbents for selective sample preparation of complex mixtures, *LC-GC* 2008, *21*, 10 – 19.

[66] Scott, P. M., Trucksess, M. W., Application of immunoaffinity columns to mycotoxin analysis, *J. AOAC Int.* 1997, *80*, 941 – 949.

[67] Toyoda, M., Saisho, K., Aoki, G., Kobayashi, A., *et al*., Repeated use of a single immunoaffinity column for sample clean-up in the HPLC determination of aflatoxin M_1 in powdered milk, *Int. Dairy J.* 1994, *4*, 369 – 375.

[68] Brenn-Struckhofova, Z., Cichna-Markl, M., Böhm, C., Razzazi-Fazeli, E., Selective sample cleanup by reusable sol-gel immunoaffinity columns for determination of deoxynivalenol in food and feed samples, *Anal. Chem.* 2007, *79*, 710 – 717.

[69] Attallah, E. R., Gad, S. A., Dogheim, S. M., Hashem, A. I., El-Sawi, E. A., Gel permeation chromatography clean-up prior to liquid chromatography for determination of aflatoxins in spices. *J. Food, Agric. Environ.* 2006, *4*, 134 – 136.

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

[71] Garms, P., Van Essche, G., Automatic sample prep using on-line immunoaffinity chromatography, *LC-GC* 2005, *23*, 40.

[72] Chan, D., MacDonald, S. J., Boughtflower, V., Brereton, P., Simultaneous determination of aflatoxins and ochratoxin A in food using a fully automated immunoaffinity column clean-up and liquid chromatography-fluorescence detection, *J. Chromatogr. A* 2004, *1059*, 13 – 16.

[73] Niedwetzki, G., Lach, G., Geschwill, K., Determination of aflatoxins in food by use of an automatic work station, *J. Chromatogr. A* 1994, *661*, 175 – 180.

[74] Sharman, M., Gilbert, J., Automated aflatoxin analysis of foods and animal feeds using immunoaffinity column clean-up and high-performance liquid chromatographic determination, *J. Chromatogr. A* 1991, *543*, 220 – 225.

[75] Association of Official Analytical Chemists, *Official Methods of Analysis of AOAC International*, AOAC International, Arlington 2000.

[76] Stroka, J., van Otterdijk, R., Anklam, E., Immunoaffinity column clean-up prior to thin-layer chromatography for the determination of aflatoxins in various food matrices, *J. Chromatogr. A* 2000, *904*, 251 – 256.

[77] Stroka, J., Anklam, E., Development of a simplified densitometer for the determination of aflatoxins by thin-layer chromatography, *J. Chromatogr. A* 2000, *904*, 263 – 268.

[78] Nakai, V. K., de Oliveira Rocha, L., Goncalez, E., Fonseca, H. *et al*., Distribution of fungi and aflatoxins in a stored peanut variety, *Food Chem.* 2008, *106*, 285 – 290.

[79] Var, I., Kabak, B., Gok, F., Survey of aflatoxin B_1 in helva, a traditional Turkish food, by TLC, *Food Control* 2007, *18*, 59 – 62.

[80] Traag, W. A., Van Trijp, J. M. P., Tuinstra, L. G. M. T., Kok, W. T., Sample clean-up and post-column derivatization for the determination of aflatoxin B_1 in feedstuffs by liquid chromatography, *J. Chromatogr. A* 1987, *396*, 389 – 394.

[81] Kok, T. W., Van Neer, Th. C. H., Traag, W. A., Tuinstra, L. G. M. T., Determination of aflatoxins in cattle feed by liquid chromatography and post-column derivatization with electrochemically generated bromine, *J. Chromatogr.* 1986, *367*, 231 – 236.

[82] Stroka, J., Anklam, E., Joerissen, U., Gilbert, J., Determination of aflatoxin B_1 in baby food (infant formula) by immunoaffinity column cleanup liquid chromatography with post-column bromination: Collaborative study, *J. AOAC Int.* 2001, *84*, 1116 – 1123.

[83] Garner, R. C., Whattam, M. M., Taylor, P. J. L., Stow, M. W., Analysis of United Kingdom purchased spices for aflatoxins using an immunoaffinity column clean-up procedure followed by high-performance liquid chromatographic analysis and post-column derivatisation with pyridinium bromide perbromide, *J. Chromatogr. A* 1993, *648*, 485 – 490.

[84] Chiavaro, E., Dall'Asta, C., Galaverna, G., Biancardi, A., *et al*., New reversed-phase liquid chromatographic method to detect aflatoxins in food and feed with cyclodextrins as fluorescence enhancers added to the eluent, *J. Chromatogr. A* 2001, *937*, 31 – 40.

[85] Waltking, A. E., Wilson, D., Chan, D., Dunn, E., *et al*., Liquid chromatographic analysis of aflatoxin using post-column photochemical derivatisation: Collaborative study, *J. AOAC Int.* 2006, *89*, 678 – 692.

[86] Maragos, C. M., Appell, M., Lippolis, V., Visconti, A., *et al*., Use of cyclodextrins as modifiers of fluorescence in the detection of mycotoxins, *Food Addit. Contam.* 2008, *25*, 164 – 171.

[87] Papadopoulou-Bouraoui, A., Stroka, J., Anklam, E., Comparison of two post-column derivatization systems, ultraviolet irradiation and electrochemical determination, for the liquid chromatographic determination of aflatoxins in food, *J. AOAC Int.* 2002, *85*, 411 – 416.

[88] Otta, K. H., Papp, E., Bagocsi, B., Determination of aflatoxins in food by overpressured-layer chromatography, *J. Chromatogr. A* 2000, *882*, 11 – 16.

[89] Catharino, R. R., De Marques, L. A., Santos, L. S., Baptista, A. S. *et al*., Aflatoxin screening by MALDI-TOF mass spectrometry, *Anal. Chem.* 2005, *77*, 8155 – 8157.

[90] Razzazi-Fazeli, E., in: Niessen, W. (Ed.), *The Encyclopedia of Mass Spectrometry*, Elsevier, Amsterdam 2006, pp. 707 – 721.

[91] Sforza, S., Dall'Asta, C., Marchelli, R., Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic techniques/mass spectrometry, *Mass Spectrom. Rev.* 2006, *25*, 54 – 76.

[92] Zöllner, P., Mayer-Helm, B., Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionisation mass spectrometry, *J. Chromatogr. A* 2006, *1136*, 123 – 169.

[93] Songsermsakul, P., Razzazi-Fazeli, E., A review of recent trends in applications of liquid chromatography-mass spectrometry for determination of mycotoxins, *J. Liq. Chromatogr. Relat. Technol.* 2008, *31*, 1641 – 1686.

[94] Kolosova, A. Y., Shim, W.-B., Yang, Z.-Y., Eremin, S. A., Chung, D.-H., Direct competitive ELISA based on a monoclonal antibody for detection of aflatoxin B1, Stabilization of ELISA kit components and application to grain samples, *Anal. Bioanal. Chem.* 2006, *384*, 286 – 294.

[95] Razzazi-Fazeli, E., Noviandi, C. T., Porasuphatana, S., Agus, A., Böhm, J., A survey of aflatoxin B_1 and total aflatoxin contamination in baby food, peanut and corn products sold at retail in Indonesia analysed by ELISA and HPLC, *Mycot. Res.* 2004, *20*, 51 – 58.

[96] Trucksess, M. W., Stack, M. E., Enzyme-linked immunosorbent assay of total aflatoxins B_1, B_2, and G_1 in corn: Follow-up collaborative study, *J. AOAC Int.* 1994, *77*, 655 – 658.

[97] Pal, A., Acharya, D., Saha, D., Roy, D., Dhar, T. K., In situ sample cleanup during immunoassay: A simple method for rapid detection of aflatoxin B_1 in food samples, *J. Food Prot.* 2005, *68*, 2169 – 2177.

[98] Pal, A., Dhar, T. K., An analytical device for on-site immunoassay, Demonstration of its applicability in semiquantitative detection of aflatoxin B_1 in a batch of samples with ultrahigh sensitivity, *Anal. Chem.* 2004, *76*, 98 – 104.

[99] Salter, R., Douglas, D., Tess, M., Markovsky, B., *et al*., Interlaboratory study of the charm ROSA safe level aflatoxin M1 quantitative lateral flow test for raw bovine milk, *J. AOAC Int.* 2006, *89*, 1327 – 1334.

[100] Delmulle, B. S., DeSaeger, S. M. D. G., Sibanda, L., Barna-Vetro, I., Van Peteghem, C. H., Development of an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B_1 in pig feed, *J. Agric. Food Chem.* 2005, *53*, 3364 – 3368.

[101] Goryacheva, I. Y., De Saeger, S., Eremin, S. A., Van Peteghem, C., Immunochemical methods for rapid mycotoxin detection: Evolution from single to multiple analyte screening: A review, *Food Addit. Contam.* 2007, *24*, 1169 – 1183.

[102] Piermarini, S., Micheli, L., Ammida, N. H. S., Palleschi, G., Moscone, D., Electrochemical immunosensor array using a 96-well screen-printed microplate for aflatoxin B_1 detection, *Biosens. Bioelectron.* 2007, *22*, 1434 – 1440.

[103] Sapsford, K. E., Taitt, C. R., Fertig, S., Moore, M. H. *et al*., Indirect competitive immunoassay for detection of aflatoxin B_1 in corn and nut products using the array biosensor, *Biosens. Bioelectron.* 2006, *21*, 2298 – 2305.

[104] Adanyi, N., Levkovets, I. A., Rodriguez-Gil, S., Ronald, A. *et al*., Development of immunosensor based on OWLS technique for determining aflatoxin B_1 and ochratoxin A, *Biosens. Bioelectron.* 2007, *22*, 797 – 802.

[105] Cucci, C., Mignani, A. G., Dall'Asta, C., Pela, R., Dossena, A., A portable fluorometer for the rapid screening of M_1 aflatoxin, *Sens. Actuators B Chem.* 2007, *126*, 467 – 472.

[106] Chiavaro, E., Cacchioli, C., Berni, E., Spotti, E., Immunoaffinity clean-up and direct fluorescence measurement of aflatoxins B_1 and M_1 in pig liver: Comparison with high-performance liquid chromatography determination, *Food Addit. Contam.* 2005, *22*, 1154 – 1161.

[107] Tam, J., Mankotia, M., Mably, M., Pantazopoulos, P., *et al*., Survey of breakfast and infant cereals for aflatoxins B1, B2, G1 and G2, *Food Addit. Contam.* 2006, *23*, 693 – 699.

[108] Sharma, M., Marquez, C., Determination of aflatoxins in domestic pet foods (dog and cat) using immunoaffinity column and HPLC, *Anim. Feed Sci. Technol.* 2001, *93*, 109 – 114.

[109] Karaca, H., Nas, S., Aflatoxins, patulin and ergosterol contents of dried figs in Turkey. *Food Addit. Contam.* 2006, *23*, 502 – 508.

[110] Trucksess, M., Weaver, C., Oles, C., D'Ovidio, K., Rader, J., Determination of aflatoxins and ochratoxin a in ginseng and other botanical roots by immunoaffinity column cleanup and liquid chromatography with fluorescence detection, *J. AOAC Int.* 2006, *89*, 624 – 630.

[111] Tassaneeyakul, W., Razzazi-Fazeli, E., Porasuphatana, S., Böhm, J., Contamination of aflatoxins in herbal medicinal products in Thailand, *Mycopathologia* 2004, *158*, 239 – 244.

[112] Arranz, I., Sizoo, E., Van Egmond, H., Kroeger, K., *et al*., Determination of aflatoxin B_1 in medical herbs: Interlaboratory study, *J. AOAC Int.* 2006, *89*, 595 – 605.

[113] Zhang, X., Liu, H., Chen, J., Immunoaffinity column cleanup with liquid chromatography using post-column bromination for aflatoxins in medicinal herbs and plant extracts, *J. Chromatogr. Sci.* 2005, *43*, 47 – 51.

[114] Muscarella, M., Lo Magro, S., Palermo, C., Centonze, D., Validation according to European Commission Decision 2002/657/EC of a confirmatory method for aflatoxin M1 in milk based on immunoaffinity columns and high performance liquid chromatography with fluorescence detection, *Anal. Chim. Acta* 2007, *594*, 257 – 264.

[115] Ehlers, D., Czech, E., Quirin, K.-W., Weber, R., Distribution of aflatoxins between extract and extraction residue of paprika using supercritical carbon dioxide, *Phytochem. Anal.* 2006, *17*, 114 – 120.

[116] Zinedine, A., Brera, C., Elakhdari, S., Catano, C., *et al*., Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco, *Food Control* 2006, *17*, 868 – 874.

[117] FDA, Sec. 527.400 Whole Milk, Low Fat Milk, Skim Milk – Aflatoxin M1 (CPG 7106.10), *FDA/ORA Compliance Policy Guides* 2005.

[118] FDA, Sec. 555.400 Foods – Adulteration with Aflatoxin CPG 7120.26. *FDA/ORA Compliance Policy Guides* 2005.

[119] Paterson, R. R. M., Aflatoxins contamination in chilli samples from Pakistan. *Food Control* 2007, *18*, 817 – 820.

[120] Lattanzio, V. M. T., Solfrizzo, M., Powers, S., Visconti, A., Simultaneous determination of aflatoxins, ochratoxin A and Fusarium toxins in maize by liquid chromatography/tandem mass spectrometry after multitoxin immunoaffinity cleanup, *Rapid Commun. Mass Spectrom.* 2007, *21*, 3253 – 3261.

[121] Maia, P. P., Bastos de Siqueira, M. E. P., Occurrence of aflatoxins B_1, B_2, G_1 and G_2 in some Brazilian pet foods, *Food Addit. Contam.* 2002, *19*, 1180 – 1183.

[122] Garon, D., Richard, E., Sage, L., Bouchart, V., *et al*., Mycoflora and multimycotoxin detection in corn silage: Experimental study, *J. Agric. Food Chem*. 2006, *54*, 3479 – 3484.

[123] Sobolev, V. S., Simple, rapid, and inexpensive cleanup method for quantitation of aflatoxins in important agricultural products by HPLC, *J. Agric. Food Chem*. 2007, *55*, 2136 – 2141.

[124] Hu, Y.-Y., Zheng, P., Zhang, Z.-X., He, Y.-Z., Determination of aflatoxins in high-pigment content samples by matrix solid-phase dispersion and high-performance liquid chromatography, *J. Agric. Food Chem.* 2006, *54*, 4126 – 4130.

[125] Sulyok, M., Krska, R., Schuhmacher, R., A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples, *Anal. Bioanal. Chem.* 2007, *389*, 1505 – 1523.

