A comprehensive overview on methods for aflatoxin and other mycotoxins analysis with a focus on rapid methods

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1 Challenges of aflatoxins and other mycotoxins control

1.1 Aflatoxins and health

Aflatoxins remain an important global food safety issue. Aflatoxins are one form of many hundreds of toxic natural secondary fungal metabolites that are collectively called mycotoxins and have been observed in foodstuffs and feedstuffs worldwide. FAO has estimated that about 25% of global crops are contaminated by molts and thus affected by mycotoxins. The economic losses are estimated to be billions of dollars. Of the many different mycotoxins only a few specific mycotoxins (or groups) present considerably food safety concerns. These agricultural and health-important mycotoxins are aflatoxins, fumonisins, deoxynivalenol (DON), ochtratoxin A (OTA) and zearalenone (ZEA) (Moss, 1991; Steyn, 1995). Among them aflatoxins have a dominant role in terms of incidence in contaminated material.

Mycotoxins occurring depends on improperly condition with high humidity and temperature after harvest and storage. Therefore mycotoxin contamination is a major concern in tropical regions. Because the compounds are chemically stable under conditions usually present during food and feed processing, they are found in raw material as well as processed feedstuffs and foodstuffs. Due their stability they are also resistant to high temperature and long-term storage. The common occurrence of mycotoxins in foodstuff and feedstuff poses an extensive hazard for human and animal health (Hussein and Brasel, 2001; Rai, 2012; Wild, 2010). The great variability of mycotoxins in structure explains the great variation in clinical symptoms in man and animal. Mycotoxins toxicity vary from one form to another but generally they are acutely toxic, mutagenic, carcinogenic, teratogenic estrogenic, and immune suppressants (Table 1).

Table 1: Toxic effect of important mycotoxins

	Toxicity
Aflatoxin	pulmonary carcinogen, acute aflatoxicosis (Wild, 2010), liver cancerogen
Deoxynivalenol (DON)	abdominal stress, increased salivation, malaise, diarrhea, emesis (Pestka, 2005)
Fumonisin	toxicosis in swine (Haschek et al., 2001), equine leukoencephalopathy (Marasas et al., 2001)
Ochratoxin (OTA)	nephrotoxic, hepatotoxic, teratogenic in rats (Abdel-Wahhab M A, 2005)
T-2 and HT-2	nausea, emesis, abdominal pain, diarrhea, dermal necrosis (Omurtag and D., 2001)
Zearalenone (ZON)	change in the reproductive system of animals such as mice and rats, genotoxic and/or carcinogenic (Scientific Committee on Food, 2000)

Source: Rai , 2012

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While in the developed world mycotoxin exposure has greatly been under control, the potential health implication of mycotoxins are still considerably high in developing countries. Reasons for this are the wide spread occurrence of the mycotoxin at frequently high levels and food consumption patterns that can result in large intake in a single cereal such as corn. Additional negative influences on health impact are concomitant poverty and malnutrition (Shephard, 2008b).

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1.2 Prevention and reduction of aflatoxins and other mycotoxins contamination

Prevention in cultivation and harvest is a possible way to handle to reduce the challagges associated with mycotoxin contamination of crops. In this context biocontrol techniques were investigated to prevent the spreading of toxigenic fungi in corn. Such products are aflaguard® (www.syngenta-us.com) and aflasafe™ (www.aflasafe.com). Basically, atoxigenic strains of *Aspergillus flavus* will be applicated to the field and replace the toxigenic strains. Aflaguard includes the *A. flavus* AF36 which not produces aflatoxins. A single application reduces the toxigenic strains in crops by over 80% and leads to a reduction of aflatoxins (Cotty, 2006). Further investigations in the area of such exclusion techniques exist. Probst et al. investigated different isolated strains of *A. flavus*. Atoxigenic strains (LOD aflatoxin B_1 < 0,5 ppb) were co-inoculated with high toxic strains (Probst et al., 2011). Additionally, aflasafe™ (figure 1) was developed from a partnership of the International Institute of Tropical Agriculture (IITA) in Nigeria, the Agricultural Research Service (ARS) of USDA and the Universities of Bonn (Germany) and Ibadan (Nigeria). It includes four native atoxigenic strains adapted to various African countries or agroecosystems. Aflasafe™ will be injected to the crop 2-4 weeks before flowering in the field e.g. a rate of 10 kg/ha will be tossed per hand through small fields. Field testing in Nigeria and Senegal exhibits a reduction of Aflatoxins in products of maize and groundnuts by 80-90% (Bandyopadhyay and Cotty, 2013). In relationship with the researches about aflasafe the "Deutsche Gesellschaft für Internationale Zusammenarbeit" (GIZ) performed a project in Nigeria in which the efficiency of Aflatoxins biocontrol technology in chili peppers was tested (GIZ, 2012-2013).

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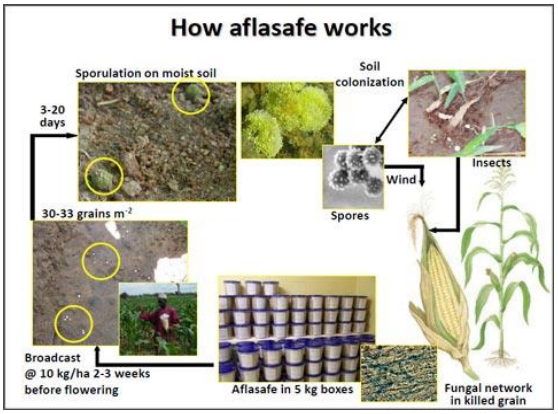

Figure 1: Application of aflasafe™ in the field from www.aflasafe.com.

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Important for the prevention of contaminations with mycotoxin is the fundamental knowledge in genomics, proteomics and metabolomics- so called "omics" - about the fungus and the mycotoxins. The knowledge enables the development of extensive prevention methods (Bhatnagar et al., 2008a; Bhatnagar et al., 2008b). The genomic includes the research in the complete set of genes of an organism. Special methods e.g. the microarray- or the Expressed Sequence Tags (EST) technology could help to understand the life cycle and the metabolism of the fungus which produces certain mycotoxins (Bhatnagar et al., 2008b).

The study of the whole proteins of a cell - their structure and their functions in the physiological pathways of cells is termed as proteomics. Analytical methods to elucidate the proteom are the two-dimensional polyacrylamide electrophoresis (2D-PAGE) followed by a protein cleavage and the identification by mass spectrometry particularly the matrix-assisted laser desorption-ionisation time-of-flight (MALDI-TOF) mass spectrometry. Promising are the resistance associated proteins (RAPs) e.g. the glyoxylase I which is a stress related aflatoxin resistance protein (Bhatnagar et al., 2008b).

Metabolomic studies complete the understanding of the fungus and the genesis of mycotoxins pre- and postharvest. Investigations contain metabolites as the results of specific cellular processes in biological organisms. With the "omics" it is possible to get important information about the contamination of plants and crops with fungus on special environmental conditions, to understand their metabolism and the biosynthesis of mycotoxins. In long-term orientation the investigations could eliminate the mycotoxins contamination problem (Bhatnagar et al., 2008b).

1.3 Maximum regulatory limits for aflatoxins and other mycotoxins

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Ideally, official methods for detection and quantification of contaminants (such as aflatoxins) in food are set for contaminants for which there are accepted maximum limits(MLs).

Different regions and countries have set MLs for for mycotoxins in food. In Europe limits of 2ppb (for aflatoxin B1) and 4ppb (for total aflatoxins (B1+B2+G1+G2)) for cereals and cereal products (including maize and maize products) for direct human consumption are in place. Likewise, MLs of 5ppb for

Aflatoxin B1 and 10ppb for total aflatoxins are set for maize to be sorted or otherwise processed physically before human consumption. The commission further set a method for sampling of cereals and cereals products in view of the prescribed limts. The regulated limits of mycotoxins in the European region are defined in the regulation of the European Community EG-VO 1881/2006. Limits for selected mycotoxins are summarized in Table 39. An abstract of the mycotoxin regulations in food and feed in the United States is shown in Table 40.

Codex Alimentarius Commission is repsonsible for setting maximum limits for mycotoxins in Food and feed. The Codex Commission has already adopted MLs for my cotoxins as shown below:

1. A maximum level of 10ppb for total aflatoxins in treenuts (almonds, hazelnuts, pistachios and shelled Brazil nuts) "ready to-eat" of 10 µg/kg.
2. ML of 15ppb for total aflatoxins in peanuts and treanuts destined for further processing,
3. ML of 200o ppb for fumonisins in maize and maize flour for direct human consumption
4. ML of 4000 ppb for fumonisins in maize for further processing
5. ML of 2000 ppb for Deoxynivalenol in raw cereal grains (wheat, maize and barley)
6. ML of 1000ppb for Deoxynivalenol in flour, semolina, meal and flakes derived from wheat, maize and barley
7. ML of 200 ppb for cereal-based foods for infants and young children

Sampling and analysis methods for these MLs are also prescribed by the Commission.

Countries in Africa who have set MLs for aflatoxins in food prescribe 5ppb for aflatoxin B1 and 10 ppb for total aflatoxins. In the East Africa region Limits are set for aflatoxins and fumonisins. These are 5ppb for aflatoxins B1 and 10ppb for total aflatoxins and 2000ppb for fumonisins in maize grain, maize flour, wheat grain, wheat flour, Milled rice Macaroni, spaghetti and vermicelli Durum wheat semolina Finger millet flour Maize gluten, Groundnuts (peanuts), Sorghum flour, Pearl millet/bulbrush flour, Dry beans, Dry soybeans, Cassava wheat composite Flour, Composite flour, Pearl millet grains, Green grams, Sorghum grains, Finger millet grains, Faba beans, Rough (paddy) Rice, Brown Rice, Soya protein products and Textured soya protein products

Table 39: Important EU-maximum limits for various mycotoxins

Compound	Commodity	maximum level [ppb]		
		B1	total	M1
Aflatoxins	groundnuts, nuts, dried fruit and processed products there of intended for direct human consumption or use as an ingredient in foodstuffs	2	4	-
	cereals (including buckwheat) and processed	2	4	-

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The legislations of mycotoxins in the European region are e.g. defined in the regulation of the European Community EG-VO 1881/2006. Important maximum and guidance levels are listed in Table 39.¶

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	products there of intended for direct human consumption or as an ingredient in foodstuffs			
	rice, including brown rice (intended for direct human consumption)	2	4	-
	milk (raw milk, milk for the manufacturer of milk based products and heat treated milk)	-	-	0,05
	baby foods and processed cereal, cereal based foods for infants and young children	0,1	-	-
	in feed: all feed materials	20	-	-
Deoxynivalenol	unprocessed cereals (excluding durum wheat, oats and maize)		1.250	
	unprocessed maize		1.750	
	in feed: cereals and cereal products with the exception of maize by-products		8.000*	
	in feed: maize by-products		12.000*	
Fumonisins	unprocessed maize		4.000	
	maize and maize based foods intended for direct human consumption		1.000	
	in feed: maize and maize based products		60.000*	
Ochratoxin A	unprocessed cereals		5	
	dried vine fruit (currants, raisins and sultanas)		10	
	roasted coffee beans and ground roasted coffee.		5	
	wine and fruit wine		2	
	baby foods and processed cereal based foods for infants and young children		0,5	
	in feed: cereal and cereal products		250*	
T-2/ HT-2	*unprocessed cereals*			
	- barley (including malting barley) and maize		200*	
	cereal grains for direct human consumption			
	- oats		200*	
	- maize		100*	
	breakfast cereals including formed cereal flakes		75*	
Zearalenone	unprocessed cereals other than maize		100	
	unprocessed maize		350	
	in feed: cereal and cereal products with the exception of maize by-products		2.000*	
	in feed: maize by-products		3.000*	

*guidance level

Table 40: Important US-Maximum Limits for various mycotoxins

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compound	Commodity	maximum level [ppb]		
		B1	total	M1
Aflatoxins	all food except milk	-	20	-
	Milk	-	-	0,5
	in feed: corn, corn products, cottonseed meal, and other animal feeds and feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known	-	20	-
Deoxynivalenol	finished wheat products for consumption by humans		1.000*	
	in feed: grains and grain by-products destined for ruminating beef and feedlot cattle older than 4 months and for chickens		10.000*	
Fumonisins	degermed dry milled corn products (e.g. flaking grits, corn meal, corn flour with fat content of <2.25%, dry weight basis)		2.000*	
	in feed: corn and corn by-products intended for equids and rabbits		5.000*	
	in feed: corn and corn by-products intended for swine and catfish		20.000*	
	in feed: corn and corn by-products intended for breeding ruminants, breeding poultry and breeding mink (includes lactating dairy cattle and hens laying eggs for human consumption)		30.000*	

*guidance level

2 Mycotoxin Analysis

The conventional methods for quantification of mycotoxins (aflatoxins inclusive) are based on chromatographic or immunochemical techniques. Reference methods are basically chromatographic systems with different detection applications such as HPLC-UV/DAD or LC-MS. They produce high resolution, sensitive and reproducible results. The disadvantages of these methods are that they are time consuming, high in costs, sophisticated in equipment and with the need of expert scientific knowledge. In general, they also are in need of very extensive pre-analytic clean-up steps prior to analytics. Instrumental methods are usually employed to confirm positive sampling results from screening methods. The basic principal and different applications have been widely published and summarized (Chiavaro et al., 2001; Chu, 1992; Di Stefano et al., 2012; Holcomb et al., 1992; Jansen et al., 1987; Kok, 1994; O'Mahony et al., 2013; Wilcox et al., 2015).

2.1 State-of-the-art methods

As the most sensitive and reliable method to analyse mycotoxins in different commodities High-Performance-Liquid-Chromatography (HPLC) is denoted as the state-of-the-art analysis. Various HPLC methods have been developed for almost all major mycotoxins in grains, cereals and other agricultural food and feed products. Different validated HPLC-methods for the different mycotoxins are reviewed (Gilbert and Anklam, 2002). The focus of this study will be on rapid test methods rather than laboratory based reference methods.

The Association of Analytical Communities (AOAC) released a formal method for aflatoxin determination. After preparation the samples will be clean-up with immunoaffinity chromatography column (IAC column) followed by reversed phase - HPLC with post column bromiation (LOD: Aflatoxin B1 1ng/g, total Aflatoxin 2,4 ng/g) (Anklam et al., 2002; Stroka et al., 2000). Improvements in the detection tools of chromatographic techniques lead to the more popular determination of mycotoxins by HPLC-MS/MS, e.g. compared to HPLC-FLD or GC-MS no sample derivatization is required. Adapted to the EU and national legislation HPLC-MS/MS is sensitive, indicates no cross-reactivity and gives the possibility of multiple analyse.

However, under less controlled conditions measurement procedures must be reliable to control the legislation but also easy and just-in-time for use in the field. So in the last years investigations in rapid and simple techniques never become less important. Several more sensitive, specific and simple methods for mycotoxin detection are commercial available and are so called screening methods. They include enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD), fluorescence polarization immunoassay (FPI) or thin layer chromatography (TLC). The different methodological approaches have been widely reviewed (Anfossi et al., 2016; Contreras-Medina et al., 2013; Espinosa-Calderón et al., 2011; Hajslova et al., 2011; Manetta, 2011; Rai, 2012; Shephard, 2016; Yazdanpanah, 2011).

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2.2 General steps in the analysis of mycotoxins

Fast, cheap, portable and reliable determination methods are required for the analysis of mycotoxins in food and feed – not only for the developing countries but also for farmers and the processing industry in developed countries. Techniques should be reproducible, the methods sensitive and simple for non-scientist persons. The most methods go ahead with time consuming preparation, clean-up and purification and this is more or less independent from the method later used for analytic and quantification such as more instrumental methods or screening methods (Anfossi et al., 2010; Burger et al., 2014; Reiter et al., 2009; Shephard, 2008a, 2009; Whitaker, 2003; Whitaker et al., 2005)

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In general the different steps to be considered for the evaluation of test systems for mycotooxins in food and feed commodities is a multiple step process (Figure 2). It consist of a pre-analytical step in which the complex sample matrix is homogenized and the mycotoxins are solubilized. Thereafter, in this context samples are extracted, purified and/or enriched. Different analytical approaches are used for the detection of individual mycotoxins or mixtures of mycotoxins. In a final post-analysis step results are validated, stored or exchanged.

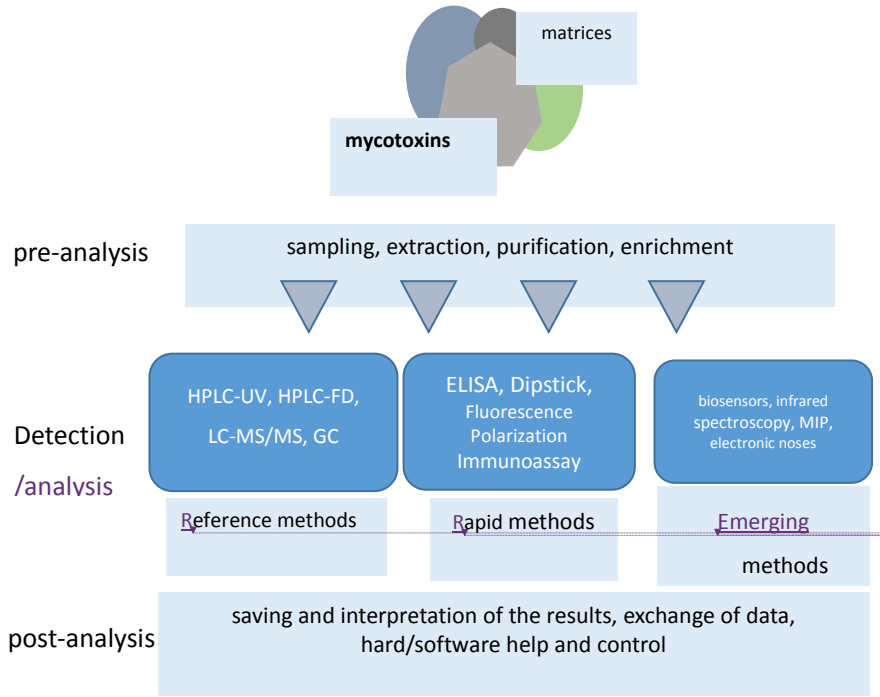

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Figure 2: Common sequence of mycotoxin analysis.

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2.3 Pre-analysis

2.3.1 Sampling and sample preparation

The heterogeneous distribution of mycotoxins in cereals, nuts, grains and other considerable products takes an important role in the analysis of mycotoxins in food and feed. A number of wheat kernels can be low contaminated with mycotoxins but a single kernel can be highly contaminated. Studies in corn and peanuts suggest that in one lot only 0.1% of the kernels are contaminated with e.g. aflatoxin and the concentration of these kernels can be extremely high. Because of this extreme concentration range in mycotoxins among a few contaminated kernels in a lot, variability in replicated sampling can be high (Biselli, 2006; Schatzki, 2000; Turner et al., 2009; Whitaker, 2003; Whitaker et al., 2005). Adequate sampling and sample-preparation is needed to get a homogenous and representative sample as the fundament for precise determination of mycotoxins.

A tool that provides support in analysing performance of sampling plans, and determining the most appropriate plan to meet user´s defined objectives. The Mycotoxin Sampling Tool (V 1.1) is available from the FAO website http://www.fstools.org/mycotoxins

Solid products, such as kernels and nuts must be ground to powder form with a selected size to enlarge the surface area and make them accessible for extraction solvents. The equipment includes mills, grinder, sieves and filters. Liquids and paste-like solutions must gentle mixed before extraction. Subsamples were taken for extraction and analysis (AOAC, 2000; Beuchat, 1987).

2.3.2 Extraction, clean-up and purification

Based on the fact that the concentrations of mycotoxins in samples are very low and the determination must be very sensitive a sample extraction, cleaning and purification are needed. Final cleaned-up extracts can be concentrated by evaporating the solvents (e.g. under a nitrogen stream). Techniques in use for extraction include Liquid-liquid extraction and Liquid-solid extraction.

2.3.2.1 Liquid-liquid extraction

Liquid–liquid extraction (LLE) utilizes the solubility or insolubility of mycotoxins in aqueous phase and organic phase (hexane, cyclohexane). Non-polar substances such as lipids and cholesterol can be removed by this approach from the sample solution.

Table 2: Strength and weakness of liquid-liquid extraction (LLE).

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Strength	purification, decrease interfering substances
weakness	- time consuming - matrix and mycotoxin depending - loss of sample because of adsorption to the glassware

There are different extraction methods for mycotoxins in addition to the matrix and the type of mycotoxin. High lipophilic or pigmented samples of food and feed require a more complex extraction which includes both clean-up and purification. Based on their chemical property aflatoxins will be extracted with mixtures of organic solvents (acetone, hexane, chloroform, methanol). It is also described that a combination of organic solvents with a small amount of water improves the extraction of aflatoxins. Other mycotoxins like the fumonisins are polar metabolites. The extraction of them must be basically with water adding organic solvents (Holcomb et al., 1992). An overview about the solvent of the choice for some mycotoxins is shown in Table 3.

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Table 3: The most effective extraction solvents and alternatives for a number of mycotoxins – reviewed and modified by Biselli (Biselli, 2006).

mycotoxin		**Alternative**
Aflatoxin	methanol-water (80:20 v/v)	acetonitrile-water, acetone-water
Ochratoxin A	methanol-water	methyl *tert*-butyl ether (MTBE), acetonitrile-water
Fumonisin	methanol-acetonitrile-water	acetonitrile-water, methanol-water (75:15 v/v)
Zearalenone	methanol-water	ethyl acetate, acetonitrile-water (86:14 v/v)

The official extraction method for the analysis of aflatoxins in peanuts and peanut products, oilseeds and food grains is the CB-method (Contaminants Bureau) (AOAC, 2000). Briefly - in a glass-stoppered flask there water, diatomaceous earth and chloroform are added to a powdered sample. After shaking the solution for 30 min it will be filtered and 50 ml of an extract will be placed on a special prepared column. After loading the column with the extract it will be washed with hexane and anhydrous ether. This fraction will be discarded. Aflatoxin will be eluted with a mixture of methanol-chloroform (3/97 v/v) and evaporated to dryness under a gentle stream of nitrogen or in a water bath. The residue will be used for the subsequent analysis.

Another method is the BF-method (Best Food) (AOAC, 2000). The aflatoxin will be extracted with a mixture of methanol-water-hexane, separated and extracted three times into chloroform. After evaporate to dryness it will be dissolved in a solution of benzene-acetonitrile. Now it can be used in TLC or HPTLC (Jaimez et al., 2000; Richard et al., 1993).

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2.3.2.2 *Liquid-solid extraction*

The liquid-solid extractions of mycotoxins is usually based on the principal of a chromatographic technique. They are unspecific extraction columns such as solid phase extraction (SPE) or specific extraction such as immunoaffinity columns (IAC).

SPE uses small disposable cartridges packed with different stationary phases (e.g. silica gel, octadecylsilane). A sample extract is loaded onto the cartridge and rinsed under reduced pressure. Contaminants or the analyte will be removed with selected solvents. The method is also known as multifunctional column preparation and used as preparation prior to HPLC-analysis (Figure 3).

The specific IAC-extraction is the state-of-the-art method of mycotoxin purification. It enables efficient mycotoxin and specific extraction from different kinds of commodities. The packing material of the column includes antibodies which specific bind to the analyte and passed the interfering substances. The elution of the analyte is achieved with an antibody denaturing solution.

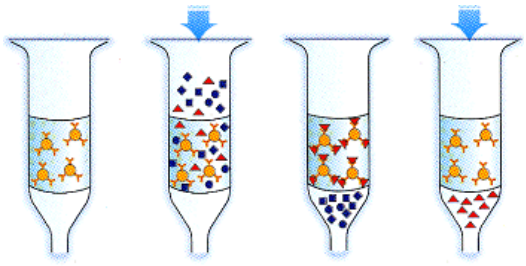

Figure 3: Model for immunoaffinity column (IAC) from European Mycotoxins Awareness Network (European Mycotoxins Awareness).

Other special forms are SPME (solid phase micro extraction), Mycosep™ columns and molecular imprinted polymers (MIPs). Mycosep™ columns keep back the impurities of a sample but allows the mycotoxins to pass (Egmond, 1986; Pittet, 2005; Turner et al., 2009). MIPs results in the application of imprinting techniques. At first monomers will be co-polymerized with a presented target analyt (imprint molecule). The removal of the imprint molecule reveals a molecular memory for the analyte (in shape, size). The special synthetic cavity can be used to separate and to extract analytes from a sample. This extraction method is adapted to the pre-analysis of some mycotoxins (ochratoxin A (Baggiani et al., 2002; Jodlbauer et al., 2002; Turner et al., 2004; Zhou et al., 2004), DON and ZON (Weiss et al., 2003)). It has promising advantages (stability, easy preparation, low cost) but has not been applied widely even on academic level.

Table 4: Strength and weakness of solid phase extraction (SPE).

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Strength	- easy handling, low expenditure at time, no scientific stuff is needed - IAC: specific interactions between mycotoxins and antibodies, only denaturing solvent is needed, robust, large volumes
weakness	- consistent method for all mycotoxins not available - MIPs: inconsistent molecular recognition, limited number of re-use, sensibility of the polymers - IAC: costs, cross-reactivity, influence antibody activity, use only once

2.4

Moved down [4]: Post-analysis ¶
The companies of commercial available rapid screening assays provide portable reader, fluorometer or fluorescence polarization reading instruments as a function of their test systems. Most of the readers allowing primary analysis with the possibility to exchanging data or they are combined with PC and special software.¶
A new way of post-analysis is the using of smart phones for quantification of aflatoxins in the field. An app measures aflatoxins using a phone image of a color-changing strip test. The provider indicates the measuring is more accurate than immunoassay tests. The results will be uploaded to the internet. Geo-tagging, secure data storage, information management, compliance reporting are possible [www.mobileassay.com].¶
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Figure 4: Quantification of aflatoxin in the field with a smart phone. From www.mobileassay.com, Romer Labs® Division Holding GmbH and www.diachemix.com.¶
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2.5 Conventional methods for rapid detection of mycotoxins

Deleted: Basic rapid analyse test systems

Conventional systems which are commercially available for rapid detection of mycotoxins are enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD) and fluorescence polarization immunoassay (FPI). In some cases basic fluorometric measurements are used to detect and quantify mycotoxins in food or feed.

Deleted: Basic rapid screening tests

The strength and the weakness of these tests are shown in Table 5 giving a first overview.

Table 5: Strength and weakness of enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD) or fluorescence polarization immunoassay (FPI).

	ELISA	LFD	FPI
Strength	-easy handling -low expenditure at time -sensitive -multiple analysis	-easy handling -rapid -portable -no special equipment	easy handling -low expenditure at time -sensitive - portable -quantitative and qualitative
weakness	-cross-reactivity -false-positive because of matrix disruptions -costs	-not qualitative -costs	- high costs - currently only for certain mycotoxins available - in research

2.5.1 Enzyme-linked immunosorbent assay (ELISA)

This assay enables the qualitative, semi-quantitative and quantitative rapid determination of mycotoxins in food and feed. The principle is bases on the use of antibodies and specific color changes. Different forms of ELISA kits are commercially available (e.g. single disposable membrane-based test, microtitre plate and tube assays).

The basic ELISAs are competitive assays. Here a conjugate of an enzyme-coupled mycotoxin or a primary antibody specific for the toxin analyte is used (Figure 5).

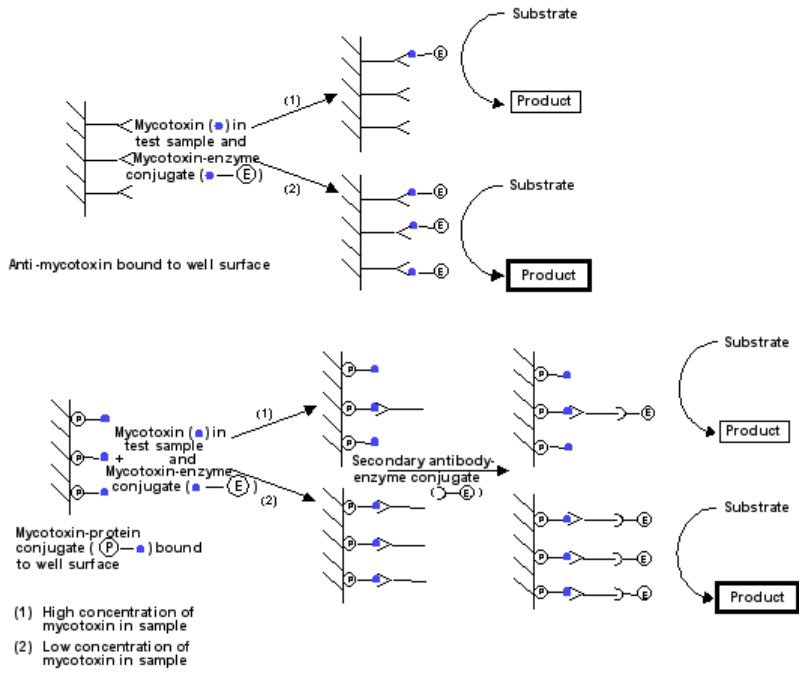

Figure 5: Principle of a competitive ELISA to screen mycotoxins (European Mycotoxins Awareness).

The experiment set-up uses a microtiter plate (Figure 6) which is coated with a mycotoxin-specific-immobilized antibody. In the first step a mycotoxin linked with an enzyme will be added to a sample. This mixture will be applied on the microtiter plate. The amount of the mycotoxin-linked enzyme that binds to the antibody on the plate depends on the amount of mycotoxins in the sample (e.g. the higher the amount of mycotoxins in a sample, the lower the amount of the mycotoxin enzyme conjugate). In the final step the substrate of the enzyme is added which leads to a chromogenic detectable signal. The concentration of this signal is inversely proportional to the concentration of the mycotoxin in the sample.

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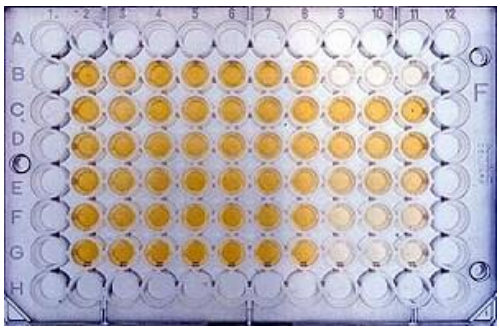

Figure 6: ELISA-microtiter plate after adding the substrate which leads to changes in colors (here: to yellow) (European Mycotoxins Awareness).

2.5.2 Lateral flow detection (LFD)

The lateral flow detection is a form of an immunoassay on a strip to detect the presence or absence of the analyte in a sample. Often they are called “dipstick”-tests. At first a pre-conditioned strip will be wetted. Now the extracted sample will be applied and after running the strip will be detected visualy or with a special reader (Figure 7).

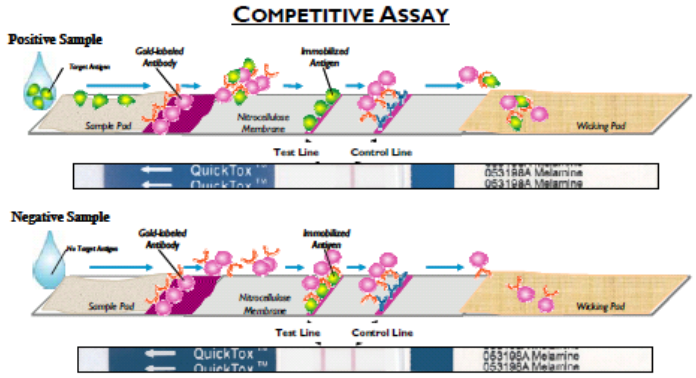

Figure 7: Model of the lateral flow detection-dipstick from Envirologix http://www.mycotoxins-rapid-tests.eu/

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2.5.3 Fluorescence polarization immunoassay (FPI)

This is a newly developed immunoassay based on the indirect measuring the changes of molecule rotation in a solution (Figure 8). There are only two suppliers of FPI to determinate a limited number of mycotoxins.

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Basically a fluorochrome labelled mycotoxin with a low molecular weight acts as the antigen. The aggregation with the anti-mycotoxin antibody results in the formation of an immune complex, gaining the weight and slowing the rotation rate of the molecule. That causes an increase in polarization of emitted light which can be detected by fluorescence polarization reading instruments (e.g. the portable Sentry200 from Ellie LLC/ Diachemix).

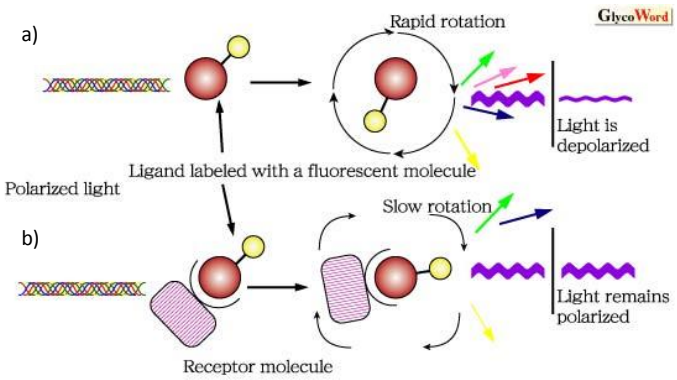

Figure 8: Principle of fluorescence polarization immunoassay (FPI). a) the rapid rotation of the fluorescence labelled ligand leads to a depolarization of light, b) binding the receptor molecule increases the weight of the fluorescence labelled ligand and slowing its rotation which generates a polarization of light (Glycoforum).

The deficiency of such assays is the problem of cross-reactivity which is not completely deleted and so further researches are needed to evaluate this influence. Cross-reactivity is a general problem of immune methods that clean-up or determine mycotoxins. Antigen-antibody reactions with metabolites or derivates of mycotoxins could not quite be eliminated in all immune methods. In addition to that further tests of different commercially available immunoaffinity columns for mycotoxins are needed. Scientists studied IACs to clean-up deoxynivalenol (DON) and zearalenone (ZON) in regard to the cross-reactivity of antibodies for conjugated mycotoxin forms e.g. glucosids, acetylated forms (Tangni et al., 2010; Versilovskis et al., 2011)

2.5.4 Thin Layer Chromatography (TLC)

A very first and well established method for separation and to quantify mycotoxins is the Thin Layer Chromatography (TLC). TLC provides a cheaper alternative to LC-based methods. Especially in developing countries it has an important role for surveillance proposes and control of regulatory limits (Gilbert and Anklam, 2002). Extensive investigations in the field of TLC lead to a high sensitive and well separating method with relatively little technical and methodological efforts (developing tank, coated plate, UV-detector). In summary, after preparation of the sample it is spotted along with standards onto a silica gel plate. It is then separated in a tank with mobile phase e.g. chloroform:acetyl (9:1, v:v) or diethyl ether:methanol:water (96:3:1, v:v:v). After developing the determination can be achieved with long-wave UV-light (Holcomb et al., 1992). Various applications of TLC are described (Turner et al., 2009). Both types - uni-dimensional and two-dimensional TLC - are used for quantitative and semi-quantitative determination of mycotoxins (Lin et al., 1998). Despite its ease and simplicity, the method needs a well-controlled laboratory environment and skilled laboratory personal.

Table 6: Detection limits for TLC-methods from www.eurofinsus.com.

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Compound	limit of detection [ppb]
aflatoxin B_1, B_2, G_1, G_2	2
ochratoxin A	200
T-2	10
Zearalenone	100

Different conditions affect the result of the TLC analysis (Karunyavanij, 1991). For example there are different coatings and binders for the plates depending on the analyte. The plate itself could be glass, aluminum or plastic. Other factors are the pureness of the standards, the manner of spotting the plate and the developing of samples as a chromatogram. The determination of the results can be visual or with densitometry. Different spraying after developing the plate can enhance the visual effects. For example it has been shown that a complexation of sterigmatocystin with projected aluminum chloride on the plate increase the fluorescence intensity 100-fold (Stack and Rodricks, 1971) . Other author reviewed color reaction with iodine starch or Fast Corinth V to get more sensitive results in the analysis of e.g. ZON (Turner et al., 2009). Lin et al. summarized different detection techniques after TLC: UV-light of long and short wavelength, fluorescence quencher, autoradiography, vaporing of iodine or ammonium, exposing to X-ray (Lin et al., 1998).

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Table 7: Strength and weakness of Thin Layer Chromatography (TLC).

	TLC
strength	- multiple analysis(Lin et al., 1998; Turner et al., 2009) -cost efficiency(Espinosa-Calderón et al., 2011; Turner et al., 2009) - LOD: EU and US ✓ - rapid (Espinosa-Calderón et al., 2011; Lin et al., 1998) - repeatable(Espinosa-Calderón et al., 2011)

	- little or no clean-up[Pittet, 2005] - no interfering of the mobile phase[Espinosa-Calderón et al., 2011]
weakness	- solutions, reagents for colouring or enhancing the fluorescence - increase of the costs when IAC is used for purification of the samples

2.5.5 Fluorescence methods

2.5.5.1 Non-specific fluorescence methods

Basic rapid and easy handling determination methods of mycotoxins commonly use the physicochemical natures of mycotoxins e.g. by fluorescence-stimulating. Presumptive tests like the "black light" test (so called Bright Greenish Yellow Fluorescent (BGYF) - test) shows rapid, indirectly, with less equipment and cost but non-specific mycotoxin producing fungi-infections in samples under UV-light (365 nm). Such sorting tests can be execute automatically with optical sorters e.g. SORTEX from Bühler GmbH (Germany) (www.buhlergroup.com). On the basis of color or other optical properties, contaminated kernels and foreign materials are identified and separated from the stream of seeds (Figure 9). The company Bühler GmbH provides different SORTEX applications addicted to the sample e.g. SORTEX Z+ for rice, grain and beans https://www.buhlergroup.com/global/en/downloads/SR_Grain_Brochure_2014_EN.pdf

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There is also the Detox aflatoxin laser sorter from Best Optical and Laser Sorting Equipment. It makes it possible to detect aflatoxins in various grains and combines various lasers for detection.

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Figure 9: Picture of seeds in the "black light" test. A Bright-Green-Yellow Fluorescence (BGYF) will be reflected from *Aspergillus flavus*- infected seeds. The fluorescence is the result of the reaction of kojic acid (a fungal metabolite) and a host peroxidase. From http://cals.arizona.edu/research/cottylab/research/epidemiology.html

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Pearson et al. investigated in a high-speed dual-wavelength Sorting to reduce the aflatoxins and fumonisins contamination in yellow corn. The study is based on a sorting with near-infrared (NIR) reflectance spectra (500-1.700 nm). The scientist ascertained a reduction of aflatoxins with an average of 82% (level of aflatoxins in corn > 10 ppb) respectively with an average of 38% (level of Aflatoxins in corn < 10 ppb) by high-speed sorting (filters at 750 nm and 1.200 nm) (Pearson et al., 2004). Further investigations in optical sorting with NIR shows the classification accuracies in the

detection and removal of aflatoxins and fumonisins contaminated maize kernel post-harvest (Wicklow and Pearson, 2006). Another rapid and visual method was described by Atas and coworkers. They used hyperspectral imaging with UV and halogen excitation to differentiate between aflatoxin contaminated and non-contaminated chili peppers (Atas et al., 2012).

The application of these optical based techniques is still limited to screening purposes due to high matrix dependence and the lack of appropriate calibration materials.

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2.5.5.2 Fluorescence and column separation

Previous reviews described the so called Romer minicolumn method in reference to the AOAC International (AOAC, 2000). Here a special packed column is used.

Table 8: Strength and weakness of Romer minicolumn method.

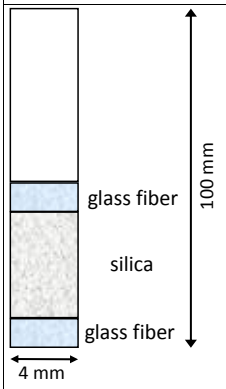
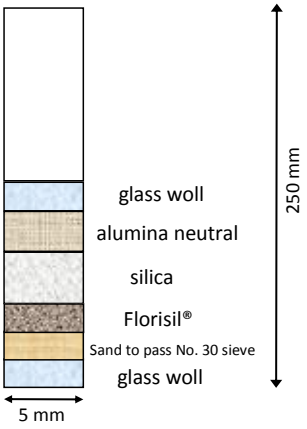
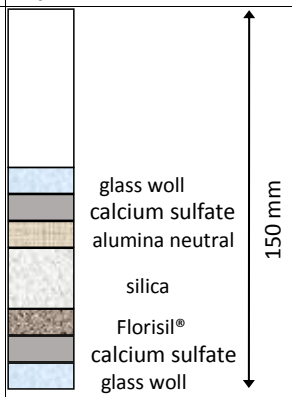
	Romer minicolumn method
strength	rapid, little equipment and amounts of solutions, easy handling, no special scientific knowledge
weakness	sample preparation, less sensitive, less selective, only semi-quantitative, high LOD (limit of detection)

In the early stages scientists introduced a minicolumn technique to detect aflatoxin in peanuts related to Thin Layer Chromatography (TLC) (Holaday, 1981). Contrary to TLC this method was faster and easier to use. A glass column (Table 9: Holaday "Dip" column) was packed with glass fiber plug to hold the packing material, silica gel and another glass fiber plug and placed in a beaker containing a developing solvent with the sample filtrate. After developing the minicolumn was removed and determination be effected under UV-light (e.g. aflatoxins - blue or bluish - green color). The detection limit was 10 µg/ kg.

Further developments leads to the official technique the Romer minicolumn (Table 9) which is packed with Florisil®. Florisil® is a magnesium silicate (MgO – SiOH 15:85) with a particle size of 150 to 250 µm (Nagl). It is widely used in analysis of feed and food. Magnesium silicate is also used as filler material and parting agent by the industry (E 553a).

In principle, a small glass column will be packed with various layers but basically including the magnesium silicate. For example, for sterigmatocystin analysis the minicolumn will be stuffed with glass wool and stacked with anhydrous sodium sulphate, neutral alumina, Florisil® and again anhydrous sodium sulphate (Ramakrishna and Bhat, 1990). Now the column will be prepared by purging with different organic solvents (e.g. dichlormethan, hexane) under gravity. In the following steps a methanol-sample solution rinsing through the minicolumn. The mycotoxin adsorbs to the layer in the column and can be detected under UV-light. The determination will be carried out by comparing the column with a column of a standard. They are also called "go-no-go" methods because of their semi-quantitative or quantitative but less sensitive determination (Egmond, 1986).

Table 9: Investigations in minicolumn methods to analyse mycotoxins. (AOAC, 2000; Egmond, 1986; Holaday, 1981; Shotwell, 1983).

Holaday "Dip" column (1968)	Velasco column (1972)	Romer minicolumn (official method 975.36 AACC-AOAC method)
LOD 10 µg/kg	LOD 5 µg/kg	LOD 5-15 µg/kg
25 min	> 15 min	n.d.
		
- extraction with chloroform/acetone (97:3 v/v)	- clean-up with ferric chloride solution (pH 4,6) - extraction with acetone/water (85:15 v/v)	-extraction with acetone/water (85:15 v/v) and filtering - purification with sodium hydroxide, ferric chloride and chloroform -sample in chloroform/acetone (9:1 v/v) drain by gravity through MC
- blue band 10 mm from the lower and of the MC under UV	- blue band in the interface of Florisil®-silica under UV	- blue band at the top of Florisil® under UV

2.5.5.3 *Fluorescence in solution*

Another semiquantitative fluorometric method to analyse mycotoxins is solution fluorometry. After extraction and clean- up with IAC or SPE the elute will be filled in a cuvette, derivatized with e.g. bromine and then measured with a fluorometer (Jansen et al., 1987). In the investigations from (Chiavaro et al., (2002) a sodium bicarbonate solution-methanol mixture was used for the extraction followed by IAC. Determination was performed using a xenon-lamp fluorometer from VICAM. The results were according to a reference RP-HPLC-method.

Deleted: Chiavaro et al.

Table 10: Strength and weakness of solution fluorometry.

	Fluorometry
Strength	rapid, easy handling, inexpensive, sensitive, multiple analysis
Weakness	derivatization, fresh derivatization solution every day, equipment, calibration of the system

Malone et al. describes a similar method for the quantification of aflatoxins in grains and raw peanuts in comparison to LC-analyse (Malone et al., 2000). The results were in a good agreement. In this method the fluorescence of the mycotoxins was enhanced by bromine-derivatization.

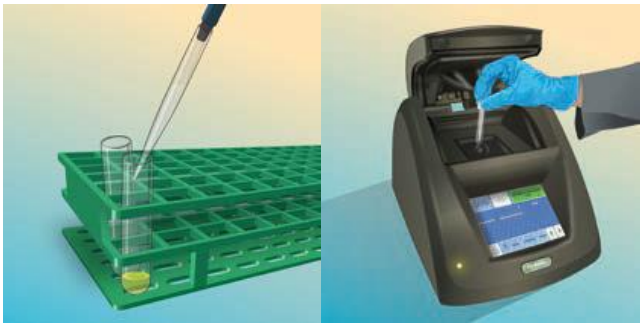


Figure 10: Transferring of the extracted sample in a tube and analyzing of it with the fluorometric test FluoroQuant® from Romer Labs Division Holding GmbH, 2013.

2.5.6 Labelling and derivatization

Combining enhancement of fluorescence and better separation of mycotoxins with add-on substances is widely used in toxin analysis to receive more sensitive results. Methodological approaches using different labelling or derivatization strategies are summarized in Table 10.

Table 10: Labeling or derivatization-methods to improve the results of mycotoxin analysis.

labeling/ derivatization	**Contact point**	**Compound**	**Reference**
1,2-diamino-4,5-dichlorbenze (DDB)	pre-column	Monoliformin	(Filek and Lindner, 1996)
Iodine	post-column	Aflatoxins	(Jansen et al., 1987; Lemke et al., 1988; Shotwell, 1983)
Fluorescein		T-2/ HT-2	(Lippolis et al., 2011; Maragos and Thompson, 1999; Thompson and Maragos, 1996)
Bromine	post-column	Aflatoxin	(Espinosa-Calderón et al., 2011; Stroka et al., 2000; Yuan, 2011)
Cyclodextrin		e.g. Aflatoxins, DON, ZON	(Espinosa-Calderón et al., 2011; Francis et al., 1988; Galaverna et al., 2008)
aluminium chloride spraying and heating	on TLC-plate	DON, Sterigmatocystin	(Egmond, 1986)
trifluoroacetic acid	pre-column	Aflatoxins	(Egmond, 1986; Espinosa-Calderón et al., 2011)
o-phthalaldehyde	pre-column	Fumonisin	(Shephard et al., 1990)

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2.5.6.1 Derivatization with fluorescine, trifluoroacetic-acid, iodine, bromine

The tracer fluorescein can be used in a fluorescence polarization immunoassay for the determination of T-2 and HT-2-toxins in wheat. The labelling not only increases the sensitivity of the test system but also shortens clean-up procedures and incubation-time (Lippolis et al., 2011). Other described

techniques are the pre- and post-column derivatization with trifluoroacetic-acid, iodine or bromine (Espinosa-Calderón et al., 2011). Especially in the case of bromine derivatization electrochemical cells can be used. Here the strong oxidizer bromine will be induced by the so called KOBRA® cell. Jansen et al. show a 20-fold increase of the fluorescence intensity of aflatoxin B1 and G1 with post-column iodine derivatization (Jansen et al., 1987).

2.5.6.2 Derivatization with cyclodextrin

A new and promising substance that combines the selective separation with the enhancement of native fluorescence of mycotoxins is cyclodextrin (CD). The cyclic oligosaccharides are formed by 6(α-CD), 7 (β-CD), 8 (γ-CD) glucose units linked by α-1,4-glycosidic bonds (Figure 11). They are cone-shaped. The pore diameter of α-CD, β-CD and γ-CD differs from 4,7 to 7,5 Å. Besides their good solubility in water and dipolar solvents they are able to form inclusion-complexes as host for a wide range of hydrophobic compounds (guest). The complexation affected the guests solubility, stability, physical and chemical properties. The inexpensive substance is widely used in pharmaceutic products (solubility, stability) and in the textile industry (masking tastes and smells) (Galaverna et al., 2008).

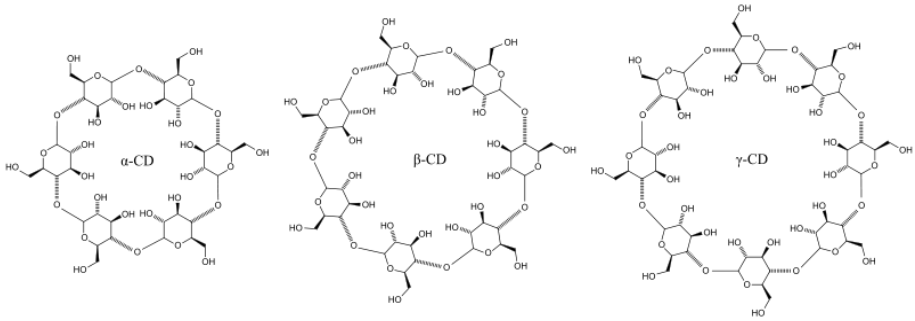

Figure 11: Chemical structure of different cyclodextrins with a pore diameter of α-CD = 4,7 Å, β-CD = 6,8 Å and γ-CD = 7,5 Å.

The forming of an inclusion complex between different mycotoxins and cyclodextrins leads to an enhancement of the native fluorescence of mycotoxins. This is described below as a result in the interaction of the coumarin structured mycotoxins and cyclodextrin. Otherwise the inclusion results in changes of the polarity and intermolecular rotation, but also in interaction with quenchers (Galaverna et al., 2008). Cucci et al. described a method to analyse aflatoxin M1 in milk with the use of β-CD. After cyclodextrin was added the detection limit of analysis were decreased from 25 ng/l to 5 ng/l. Also there was no need to clean-up the samples with IAC before analysis (Cucci et al., 2007).

Maragos et al. investigate a fluorometric method to detect the non-fluorescence T-2 in maize. They derivatized T-2 with pyrene-1-carbonyl cyanide (T-2-Pyr) and studied the enhancement of the fluorescence by adding different CD´s as buffer modifier in capillary electrophoresis laser-induced-fluorescence. The most effective CD was heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB) (Figure 12) (Maragos et al., 2008).

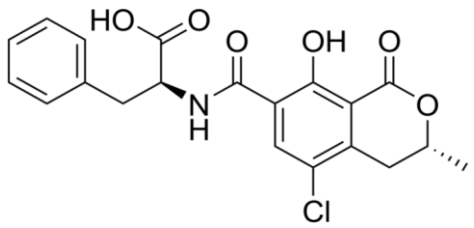

Figure 3: Structure of Ochratoxin A from http://commons.wikimedia.org/wiki/File:Ochratoxin_A_structure.png.

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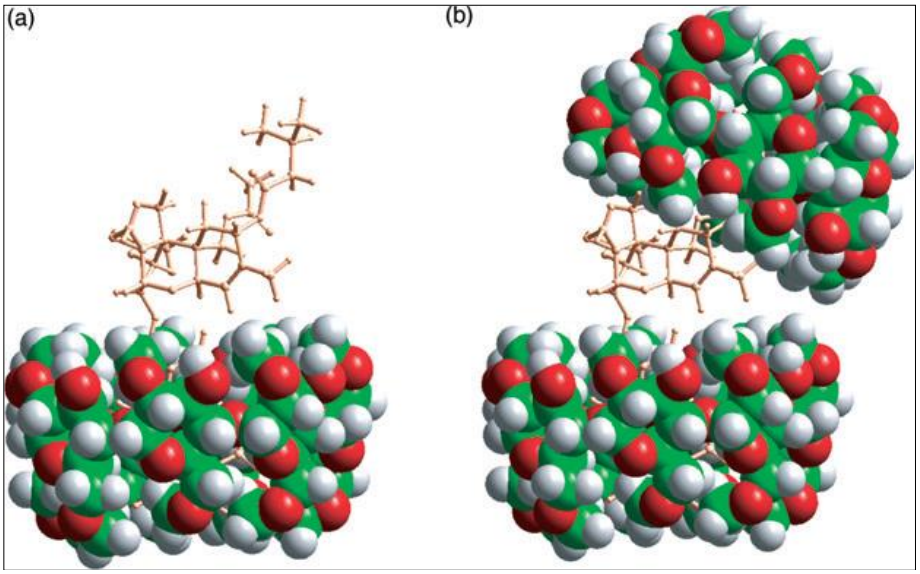

Figure 4: Molecular representation of possible interactions between T2-Pyr and DIMEB: (a) 1:1 ratio-, (b) 1:2 ratio. Modelling with HyperChem (Version 7.25) from Maragos et al. (Maragos et al., 2008).

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2.5.7 Specific fluorescence methods

Referring to the fluorescence methods to analyse mycotoxins the fluorescence polarization immunoassay (FPI) is mentioned above as a rapid screening test. This method based on the indirect measuring of the changes in molecule rotation of a solution as a function in size of molecules. It is one of the methods which are actually in research and shows great promises in the field of rapid, sensitive analysis of mycotoxins. Commercial available test kits are from Diachemix and Aokin AG but only for a limited number of mycotoxins and high in price. The problem of cross-reactivity is not completely deleted.

In principle - the fluorochrome labelled mycotoxin with a low molecular weight acts as the antigen. Aggregation with the anti-mycotoxin antibody results in the formation of an immune complex, gaining the weight and slowing the rotation rate of the molecule. This causes an increase in polarization of emitted light which can be detected by fluorescence polarization reading instruments (e.g. the portable Sentry200 from Ellie LLC/ diachemix).

Table 11: Strength and weakness of fluorescence polarization immunoassay (FPI).

	FPI
strength	- easy handling - portable
weakness	- in research - expensive equipment

2.6 Other methods in research use

2.6.1 Laser-Induced Fluorescence (LIF)

Espinosa-Calderón et al. reviewed researches concerning the Laser-Induced Fluorescence (LIF) as a fluorescence screening method for mycotoxins (Espinosa-Calderón et al., 2011). A method based on the detection of a mobile phase which includes the sample solution and passes through the detection window of the LIF-detector. Fluorescence which is excited by the laser will be detected. The method enables to analyse samples with very low concentrations. Because of the high costs for the LIF (laser, special dyes for labelling) this method is rarely used.

2.6.2 Near infrared spectroscopy (NIR)

Another method in practice and researches is the near infrared spectroscopy (NIR). Petterson et al. described the determination of deoxynivalenol in wheat kernel with a wavelength 570 – 1.100 nm.

The detection limit was 400 ppt (Pettersson and Åberg, 2003). While NIR can be used for the determination of aflatoxins at levels between 200-500 ppb in sample with solid or liquid physical conditions this method is not yet established for the detection of aflatoxin in human food at regulatory levels (Jagger et al., 2013).

2.6.3 Biosensor techniques

In the last decade different immunochemical assays and assays including biosensor techniques are investigated. Biosensors enables the detection of an analyte in a sample because of the interaction between the analyte and a biological sensitive element e.g. enzyme, tissues, nucleic acids or antibodies. The interaction results in a signal which can be detected by a transducer (e.g. optical or physicochemical detection) and will be transformed in an utilizable measured variable.

A biosensor method to determine mycotoxins is surface plasmon resonance (SPR) (Schnerr et al., 2002; Tudos et al., 2003; van der Gaag et al., 2003). Here the measured variable is the change in mass of mycotoxins which are immobilised at a surface of a sensor chip. The mass change results in the attaching of a specific antibody to the mycotoxins. The advantages get from studies are:

- results compares to LC-MS data

- re-using of the sensor ship without loss in acticity (500 times).

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Such SPR biosensor protocol described Puiu et al. for the direct measurement of albumin-bound AFB1 in blood samples (Puiu et al., 2012).

2.6.4 DNA-based and aptamer-based biosensors

Dinckaya et al. published a DNA biosensor-based method to analyze aflatoxin M1 in samples e.g. in milk. A thiol-modified single stranded DNA (ss-HSDNA) probe was immobilised on a monolayer of cysteamine and gold nanoparticles prepared on gold electrodes. The DNA biosensor particular bound Aflatoxin M1. The detection of the process is carried out with electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques. But there is no information if the method is assignable within the aflatoxin M1 limits of the national and international legislations (Dinckaya et al., 2011).

Another form to use DNA in biosensors are aptamer-based. Aptamers are peptide molecules or DNA, respectively, RNA duplex structures that can bind a special analyte. Chen et al. investigated a DNA duplex structure with an anti-ochratoxin A-aptamer including a fluorophore and a quencher. Binding ochratoxin A to this structure leads to an increase of the fluorescence. With this rapid and high selectively method (only 1 min as per measurment) OTA can be determined with a LOD of 0,8 µg/l (Chen et al., 2012). Similar to that a biosensor DNA-enzyme aptamer was described by Yang et al. The presence of OTA bound to the DNAzyme hairpin leads to open the hairpin structure and activates a horseradish peroxidase-mimicking DNAzyme. This process can be detected with colorimetric

measurement at 620 nm in microtiter wells (Yang et al., 2012). Other aptamer-senor-based assays are currently under investigation (Prabhakar et al., 2011; Wu et al., 2011).

2.6.5 Electronic nose

A new field on the basis of biosensors are electronic noses developed for the rapid non-destructive analysis of mycotoxins (Cheli et al., 2007; Dell'Orto et al., 2007; Olsson et al., 2002; Tognon et al., 2005). The electronic nose - an array of biosensors detects volatiles emanating- could dinstinguish between the presence and the abscence of the mycotoxins. The fungi growth and their producing of mycotoxins leads to biochemical changes rather to changes in the chemical composition of volatiles. Different volatile molecules act within the electronic nose and generate a special detectable electronic signal. Changes in the relative composition of the molecules lead to changes in the electronic signal. The investigations of Cheli et al. shows that electronic noses can differ between aflatoxins -positive and –negative samples but further quantitative analysis are needed. The model electronic nose needs continuing improvements and research to be a sensitive and reliable method in the mycotoxins analysis due basis of international legislations (Cheli et al., 2009).

2.7 Post-analysis

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The companies of commercial available rapid screening assays provide portable reader, fluorometer or fluorescence polarization reading instruments as a function of their test systems. Most of the readers allowing primary analysis with the possibility to exchanging data or they are combined with PC and special software.

A new way of post-analysis is the using of smart phones for quantification of aflatoxins in the field. An app measures aflatoxins using a phone image of a color-changing strip test. The provider indicates the measuring is more accurate than immunoassay tests. The results will be uploaded to the internet. Geo-tagging, secure data storage, information management, compliance reporting are possible [www.mobileassay.com].

Figure 4: Quantification of aflatoxin in the field with a smart phone. From www.mobileassay.com, Romer Labs® Division Holding GmbH and www.diachemix.com.

2.8 Commercial available rapid analysis test systems

The following section summarizes some currently used screening test kits for the rapid quantitative analytic of mycotoxins. It is not entitled to be comprehensive. All prices are based on 2012 list prices and are entitled to serve as reference. A complete list of currently approved analytical methods are given in GIPSA (Performance Verified Test Kits – Effective 12/16/2016) https://www.gipsa.usda.gov/fgis/metheqp/GIPSA_Approved_Mycotoxin_Rapid_Test_Kits.pdf

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2.8.1 Aokin AG

Table 12: Summarized facts about the rapid analysis test systems from Aokin AG.

	http://www.aokin.de/
Principle	FPI
equipment	EUR 25.000 (FP-spectrometer, liquid handling workstation)
cost per analysis/ consumables	EUR 9 – 15
Portability	No
Laboratory	Preperation: yes - Analytics: yes

Table 13: Overview test systems for various mycotoxins from aokin AG.

Analyte	Matrix	LOD [µg/kg]	detection range [µg/kg]
DON	wheat, corn, oats, barley, rye, durum, cereals, flakes, pasta	5 (for wheat: 3)	50-5,000
ZON	wheat, corn, oats, barley, rye, durum, cereals, flakes, pasta	1 (for wheat: 10)	50-5,000

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- analysis:

- sample preparation: IAC, SPE (QuickClean)

- as order the analysis of DON, ZON, Aflatoxins, Ochratoxin A, Fumonisin, T-2/ HT-2

2.8.2 Beacon Analytical Systems Inc.

Table 1411: Summarized facts about the rapid analysis test systems from Beacon Analytical Systems Inc.

	http://www.beaconkits.com/welcome/category/mycotoxins
Principle	ELISA (plate or tube)
equipment	reader (costs not denoted)
cost per analysis/ consumables	EUR 18
Portability	No
Laboratory	Preperation: yes - Analytics: yes

Table 15: Overview test systems for various mycotoxins from Beacon Analytical Systems Inc..

Analyte	Matrix	detection range	method
Aflatoxin M1	Milk	0 – 100 ppt	ELISA
Aflatoxin	nuts, grain, grain products	0 – 100 ppb	ELISA, plate
Aflatoxin	corn, peanuts	0 – 100 ppb	ELISA, tube
DON	wheat, barley, malted barley, corn, oats	0 – 6 ppm	ELISA
Fumonisin	corn, corn meal, corn germ meal, corn gluten meal, corn/ soy blend	0 – 6 ppm	ELISA
Ochratoxin A	corn, corn meal, grain	0 – 25 ppb	ELISA
T2	corn, corn meal, corn germ meal, corn gluten meal, corn/ soy blend	0 – 500 ppb	ELISA
ZON	corn, corn meal, corn germ meal, corn gluten meal, corn/ soy blend	0 – 1.000 ppb	ELISA

2.8.3 Charm Sciences Inc.

Table 16: Summarized facts about the rapid analysis test systems from Charm Sciences Inc..

	http://www.charm.com
Principle	LFD
equipment	EUR 2.995 (ROSA® EZ-M system)
cost per analysis/ consumables	EUR 7-10
Portability	No
Laboratory	Preparation: yes - Analytics: yes

ROSA® Grain

-a variety of ROSA (Rapid One Step Assay) tests to detect mycotoxins (aflatoxin, DON/vomitoxin, fumonisin, ochratoxin, T-2/HT2, and zearalenone) in feed and grain

-lateral flow, quantitative results, all mycotoxins same equipment

Water Extraction Technology (WET®)

-eliminates the use of organic solvents, a non-hazardous powder (composition is not disclosed because of IP – patent has not been obtained) is added to the sample followed by water (e.g., bottled water), materials may be disposed as normal waste providing positive mycotoxin samples do not violate local regulations

-extraction method can be used for multiple commodities, a single extraction for the following mycotoxins: aflatoxin, DON, fumonisin, T2/H2, and zearalenone (ochratoxin requires a separate extraction powder)

e.g.:*ROSA WET® Aflatoxin Quantitative Test:* results in less than 5 min, disposed as normal waste providing positive mycotoxin samples do not violate local regulations, multiple samples can be run at the same time, uses existing equipment; ROSA ® EZ-M system (Figure 13) does not require end-user instrument calibration, standard curve built in reader

Figure 5: ROSA® EZ-M system from Charm Science which combines to incubate and to analyze lateral flow test strips.

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Table 17: Overview test systems for various mycotoxins from Charm Sciences Inc..

Analyte	Matrix	Method	extraction buffer	detection range	[EUR]/100 pcs.
Aflatoxins		3, 5, 10 min			
ROSA WET®Aflatoxin Quantitative Test (USDA(GIPSA)*-approved)	barley, corn, corn flour, corn germ meal, corn gluten meal, corn meal, corn/soy blend, Distiller's Dried Grain with Solubles (DDGS), hominy, oats, popcorn, rice bran (defatted), rough rice, sorghum, soybeans, wheat	LFD	Water	n.d.	765,00
FAST Aflatoxin Quantitative	Corn	LFD	70% methanol	0 – 150 ppb	

BEST Aflatoxin Qualitative	Corn	LFD	non-toxic solution called BEST (composition is a trade security)	10 ppb, 20 ppb	
Aflatoxin P/N Test Qualitative	Corn	LFD	50% Methanol or 70% Ethanol	10 ppb, 20 ppb	
DON		3, 5, 10 min			
ROSA DON P/N Test Quantitative	barley, corn, wheat	LFD	deionized or distilled water	0,5ppm, 1 ppm, 2 ppm, 5 ppm	
ROSA DON Quantitative Test	barley, brewer's rice, buckwheat, corn, corn bran, corn germ meal, corn gluten meal, DDGS, hominy, malted barley, milled rice, oats, palm kernel meal, rapeseed meal, rice bran, rough rice, rye, sorghum, soybean meal, triticale, wheat, wheat bran, wheat flour, wheat midds, wheat red dog	LFD	deionized or distilled water	0 – 6 ppm, 6 – 12 ppm, 12 – 24 ppm	
ROSA FAST5 DON Quantitative	barley, corn, DDGS, malted barley, milled rice, oats, rough rice, sorghum, wheat, wheat bran, wheat flour, wheat midds	LFD	deionized or distilled water	0 - 1,5 ppm, 1 – 6 ppm	830,00
Fumonisin		5, 10 min			

ROSA FAST5 Fumonisin Quantitative	barley, corn, flaking corn grits, millet, oats, rough rice, sorghum, wheat	LFD	70% Methanol	n.d.	
ROSA Fumonisin Quantitative	barley, corn, flaking corn grits, DDGS, millet, oats, rough rice, sorghum and wheat	LFD	70% Methanol	0 to 1 ppm, 0 to 6 ppm, and 6 to 60 ppm	
ROSA Fumonisin Quantitative	barley, corn, DDGS, hominy, oats, sorghum, soybean meal	LFD	50% Ethanol	0 to 1 ppm, 0 to 6 ppm, and 6 to 60 ppm	
Ochratoxin A		10 min			
ROSA Ochratoxin Quantitative Test	barley, corn, corn gluten meal, malted barley, oats, rye, sorghum, soybean meal, wheat, buckwheat, rice	LFD	70% Methanol	0 to 30 ppb, 0 to 150 ppb	950,00

*USDA(GIPSA)=United States Department of Agriculture (Grain Inspection, Packers and Stockyard Administration)

2.8.4 Diachemix Inc.

Table 18: Summarized facts about the rapid analysis test systems from Diachemix Inc..

	http://www.diachemix.com/en/
Principle	FPI
equipment	EUR 26.000
cost per analysis/ consumables	EUR 4-5
Portability	Yes
Laboratory	Preparation: yes - Analytics: no

Table 19: Overview test systems for various mycotoxins from Diachemix Inc..

Analyte	method	detection range	matrix
Aflatoxin			
Aflatoxin FPA qualitative test	FPA 2 min	≥ 20 ppb or ≥ 10 ppb	corn
Aflatoxin FPA quantitative test*	FPA 2 min	0 – 100 ppb	grain, nuts
DON Vomitoxin FPA qualitative test	FPA 2 min	≥ 1 ppm	wheat
DON Vomitoxin FPA quantitative test*	FPA 15 min	0 – 6 ppm (LOD 0,17 ppm)	wheat
Fumonisin FPA qualitative test**	FPA 1-2 min	≥ 1 ppm	corn

*<10 kits EUR 503,2; >10 kits EUR 407,00 ;**presently not available for sale

Figure 6: Portable fluorescence polarization reader Sentry® 100 for determination of various mycotoxins with assays from Diachemix Inc..

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2.8.5 EnviroLogix Inc.

Table 20: Summarized facts about the rapid analysis test systems from EnviroLogix Inc..

	http://envirologix.com/artman/publish/index.shtml for EU: http://www.mycotoxins-rapid-tests.eu/
principle	LFD
equipment	Not disclosed
cost per analysis/ consumables	Not disclosed
Portability	No
Laboratory	Preparation: yes - Analytics: yes

- Sample preparation:
  - (1) Extraction: matrix preparation (e.g. grinding), analyte extraction with water or 50% EtOH
  - (2) Strips wetting
  - (3) Running
  - (4) Detection
  - (5) Analysis (qualitative or quantitative)

- which mycotoxins: aflatoxins, fumonisins, ochratoxin A, deoxynivalenol (DON), zearalenon (ZON) > simultaneous detection of mycotoxins
- other possible analytes: genetically modified organism (*GMO; e.g. Soybean, corn*), plant disease, domestic molds
- results in less then 10 minutes, non-specialized operators
- equipment: test kit, QuickScan with software, standard PC-platform with a MS-Windows interface

Table 21: Overview test systems for various mycotoxins from EnviroLogix Inc..

Analyte	Matrix	detection range (ppb)	extraction buffer
Aflatoxin			
Aflatoxins B and G quantitative	corn, wheat	2,5 – 180	50% ethanol
aflatoxin residues quantitative	corn, wheat	3 – 180	Water
aflatoxin residues quantitative	dried distilled grains with soluble (DDGS)	10 – 450	50% ethanol
B1 and B2 *qualitative*	Corn	20	50% ethanol
DON			
quantitative	corn, wheat, oats	200 - 10.000	Water
	Barley	200 - 10.000	Water
	DDGS	200 - 10.000	Water
	corn, corn gluten meal, DDGS; wheat, wheat bran, wheat midds, whole-wheat flour, white wheat flour; barley, malted barley; milled rice, rough rice; oats	300 - 12.000	Water
qualitative	Corn	500 – 2.000	Water

ZON			
quantitative	Corn	50 – 520	50% ethanol
Fumonisin			
quantitative	corn and corn by-products in food and animal feeds	n.d.	50% ethanol
Ochratoxin A			
quantitative	Wheat	0 – 150 ppb	water based

2.8.6 EuroProxima B.V.

Table 22: Summarized facts about the rapid analysis test systems from Europroxima B.V..

	http://europroxima.com/
Principle	LFD, ELISA
equipment	reader (not denoted)
cost per analysis/ consumables	LFD: EUR 20, ELISA: EUR 500/plate
Portability	no
Laboratory	Preparation: yes - Analytics: yes

-LFD: Zearalenone (ZON) and Deoxynivalenol (DON) a colloidal gold based flow-through immunoassay is used, 10 pieces EUR 220-275 + charge (approx. EUR 20)

-Further informations about costs for preparation and clean-up: in the kit

Table 23: Overview Lateral Flow test systems for various mycotoxins from EuroProxima B.V..

Analyte	Matrix	detection range (ppb)
Aflatoxins		
B1	cereals, soy beans, nuts, derived products	2
Total	cereals, soy beans, nuts, derived products	4
DON	n.d.	n.d.
Ochratoxin A	cereals, wine, green coffee	4
Ochratoxin A in wine	red, rose, white wine	2
ZON	n.d.	n.d.

-ELISA (>60 min), 96 well plate EUR 498,50 + charge

Table 24: Overview ELISA test systems for various mycotoxins from Europroxima B.V.

Analyte	Matrix	detection range (ppb)
Aflatoxins		
B1	n.d.	depends on the kind of sample: 0,3 – 2
M1	milk, milk powder, cheese, butter	< 0,006
Total	n.d.	< 0,5
DON	cereals, beer, silage	depends on the kind of sample: 30 or 50
Fumonisin	Maize	2,3
Ochratoxin A	cereals, food and feed	barley, soy: 1 oats: 0,5
ZON	Cereals	0,13
T 2	Cereals	20 - 50

2.8.7 Neogen Corporation

Table 25: Summarized facts about the rapid analysis test systems from Neogen Corporation.

	http://www.neogen.com/index.html
Principle	LFD, ELISA
equipment	reader: LFD: EUR 1.700, ELISA: EUR 4.500
cost per analysis/ consumables	LFD: EUR 6, ELISA: EUR 6-7
Portability	no
Laboratory	Preparation: yes - Analytics: yes

(detection: visual or reader, quantitative or qualitative)

-Multipurpose AccuScan Pro

- reads and records all Reveal Q+ products
- complete archival recording
- by recording the sample identification, results, time and date, the AccuScan Pro eliminates manual recording and assists with report generation
- export to AccuScan Pro Data Manager software
- provides additional reporting, tracking and trend capabilities

Table 26: Overview test systems for various mycotoxins from Neogen Corporation.

Analyte	Method	detection range	matrix
Aflatoxin			
Veratox® Aflatoxin total *quantitative*	ELISA 5 min	5 - 50 ppb	corn, cornmeal, corn gluten meal, corn/soy blend, wheat, rice, milo, soy, whole cottonseed, cottonseed meal, raw peanuts, peanut butter and mixed feeds
Veratox® for Aflatoxin HS (High Sensitive)	ELISA 20 min	1 – 8 ppb	corn, cornmeal, corn gluten meal, corn/soy blend, wheat, rice, milo, soy, whole cottonseed, cottonseed

Quantitative			meal, raw peanuts, peanut butter and mixed feeds
AgriScreen for Aflatoxin *qualitative*	ELISA 5 min	20 ppb	corn, cornmeal, corn gluten meal, corn/soy blend, wheat, rice, milo, soy, whole cottonseed, cottonseed meal, raw peanuts, peanut butter and mixed feeds
Reveal®Aflatoxin M1	LFD 5 min	500 ppt	milk
Reveal® Q+ Aflatoxin total *quantitative*	LFD 6 min	2 - 150 ppb	corn, corn products
Reveal® Aflatoxin total *qualitative*	LFD 3 min	20 ppb	corn, corn gluten meal, corn meal, corn/soy blend, cottonseed, cottonseed meal, hominy, milo, peanuts, popcorn, rice, soy meal and wheat green test kit: corn
DON			
Veratox ®DON 2/3 *quantitative*	ELISA 5 min	0,5 – 5 ppm	wheat, wheat flour, wheat midds, wheat bran, corn, cornmeal, corn screenings, barley, malted barley and oats
Veratox® DON 5/5 *quantitative*	ELISA 10 min	0,5 – 5 ppm	wheat, wheat flour, wheat midds, wheat bran, corn, cornmeal, corn screenings, barley, malted barley and oats
Veratox® DON HS *quantitative*	ELISA 20 min	25 – 250 ppb	wheat, wheat flour, wheat midds, wheat bran, corn, cornmeal, corn screenings, barley, malted barley and oats, processed cereal
Reveal® Q+ DON *quantitative*	LFD 3 min	0,3 – 6 ppm	corn, barley, DDGS(distillers dried grains with soluble), malted barley, oats and wheat products
Fumonisin			

Veratox® Fumonisin *quantitative*	ELISA 20 min	1 – 6 ppm	corn, barley, DDGS, milo, popcorn, rice, soybeans and wheat
Veratox® Fumonisin 5/10 *quantitative*	ELISA 15 min	0,5 – 6 ppm	corn, barley, DDGS, milo, popcorn, rice, soybeans and wheat
Veratox® Fumonisin HS *quantitative*	ELISA 15 min	50 - 600 ppb	corn, barley, DDGS, milo, popcorn, rice, soybeans and wheat
Reveal® Q+ Fumonisin *quantitative*	LFD 6 min	0,3 – 6 ppm	corn products
Ochratoxin			
Veratox® Ochratoxin *quantitative*	ELISA 20 min	2 – 25 ppb	corn, barley, wheat, green coffee, various dried fruit
Veratox® Ochratoxin Grain *quantitative*	ELISA 20 min	2 – 25 ppb	corn, barley, other grains
Reveal® Q+ Ochratoxin *quantitative*	LFD 9 min	2 – 20 ppb	grain and grain products
T-2/ HT-2			
Veratox® T-2/ HT-2 *quantitative*	ELISA 10 min	25 – 250 ppb	corn, barley, wheat, oats, rye
Reveal® Q+ T-2/ HT-2 *quantitative*	LFD 6 min	50 – 600 ppb	grain and grain products
ZON			
Veratox ® ZON *quantitative*	ELISA 10 min	25 – 500 ppb	corn, wheat, barley, DDGS
Reveal® Q+ ZON *quantitative*	LFD 6 min	50 – 1.200 ppb	corn and wheat products

2.8.8 R-Biopharm AG

Table 27: Summarized facts about the rapid analysis test systems from R-Biopharm AG.

	http://www.r-biopharm.com/products/food-feed-analysis/mycotoxins
Principle	LFD, ELISA
equipment	reader: EUR 1.200
cost per analysis/ consumables	LFD: EUR 10-20, ELISA: EUR 4-6
Portability	No
Laboratory	Preparation: yes - Analytics: yes

-RIDASCREEN®: ELISA format enables high-throughput testing of many commodities for most regulated mycotoxins

-RIDA®QUICK: quantitative lateral flow format enables rapid, on-site decisions about the mycotoxin contamination of commodities

-AFLACARD® / OCHRACARD®**:** specially designed to get a rapid, semi-quantitative result for more complex commodities

-Others:

- Immunoaffinity columns (EUR 10-20/ sample) enable efficient mycotoxin clean-up for all kinds of commodities prior to HPLC, LC- MSMS or ELISA

- PuriToxSR solid phase extraction columns (EUR 1-10/ sample) enable efficient mycotoxin clean-up of many commodities prior to TLC, HPLC, GC or LC-MSMS

- detection with StatFax Reader Model 303 Plus or RIDA®Quick SCAN Model ZG5005

- standard solution: EUR 45 per ml

Table 28: Overview test systems for various mycotoxins from R-Biopharm AG.

Analyte	method	detection range	matrix
aflatoxin			
AFLACARD®B1 *qualitative*	LFD/card 10 min	n.d.	a wide range of commidities
AFLACARD®total *qualitative*	LFD/card 10 min	n.d.	a wide range of commidities
RIDA®Quick Aflatoxin *quantitative*	LFD	4 – 20 ppb	grain, soy flour, nuts, pistachios, coconut flour, sunflower seeds, figs, dates and cashew nuts
Aflatoxin RQS *quantitative*	LFD	4 ppb	corn
RIDASCREEN®Aflatoxin M1 *quantitative*	ELISA 1 h 15 min	5 ppt, 50 ppt	milk, milk powder, cheese
RIDASCREEN®Aflatoxin B1 30/15 *quantitative*	ELISA 45 min	1 ppb	cereals, feed
RIDASCREEN®Aflatoxin total *quantitative*	ELISA 45 min	1,75 ppb	residues in cereals and feed
RIDASCREEN®FAST Aflatoxin *quantitative*	ELISA 15 min	1,75 ppb	cereals, feed
RIDASCREEN®FAST Aflatoxin M1 *quantitative*	ELISA 15 min	125 ppt	milk, milk powder
RIDASCREEN®FAST Aflatoxin SC *quantitative*	ELISA 15 min	2 ppb	cereals, feed
DON			
RIDASCREEN®FAST DON *quantitative*	ELISA 8 min	0,2 ppm	wheat, corn, barley, malted barley, and

			oats
RIDASCREEN®FAST DON SC *quantitative*	ELISA 8 min	0,074 ppm	cereals, malt and feed
RIDASCREEN® DON *quantitative*	ELISA 45 min	cereals, malt, feed: 18.5 ppb beer: 3.7 ppb wort: 3.7 ppb	cereals, malt, feed, beer and wort
RIDA®Quick DON *semi-quantitative orquantitative*	LFD 5 min	0,5 ppm, 1,25 ppm	wheat, triticale and corn
Fumonisin			
RIDASCREEN® Fumonisin *quantitative*	ELISA 45 min	25 ppb	corn, corn products
RIDASCREEN®FAST Fumonisin *quantitative*	ELISA 15 min	0,222 ppm	corn
RIDA®Quick Fumonisin *semi-quantitative*	LFD 5 min	0,8 ppm, 4 ppm	corn
RIDA®Quick Fumonisin RQS *quantitative*	LFD 5 min	0,8 ppm, 4 ppm	corn
Ochratoxin A			
OCHRACARD®	LFD/card 30 min	n.d.	a wide range of commodities
RIDASCREEN® Ochratoxin A 30/15 *quantitative*	ELISA 45 min	cereals and feed: 2.5 ppb cereals and feed: 1.25 ppb beer pig serum: approx. 50 ppt	cereals, feed, beer and pig serum
RIDASCREEN®FAST Ochratoxin A	ELISA 15 min	5 ppb	cereals and feed

Quantitative			
T-2			
RIDASCREEN®T-2 *quantitative*	ELISA 1 h 30 min	< 5 ppb	cereals and feed
RIDASCREEN®FAST T-2 *quantitative*	ELISA 15 min	20 ppb	cereals and feed
ZON			
RIDASCREEN®FAST ZON *quantitative*	ELISA 15 min	17 – 41 ppb	cereals and feed
RIDASCREEN®FAST ZON SC *quantitative*	ELISA 15 min	5 ppb	cereals
RIDA®Quick ZON RQS *quantitative*	LFD 5 min	75 ppb	corn

2.8.9 Romer Labs®

Table 29: Summarized facts about the rapid analysis test systems from Romer Labs®.

	www.romerlabs.com/en/products/mycotoxins/ in Germany: coring system diagnostix (www.coring.de/catpages.php?s=ae6d867f38f0a9574e3d9689c4754920&nav=2_4_0&catPage=4)
Principle	LFD, ELISA, fluorometry
equipment	reader: LFD: EUR not denoted , ELISA: EUR 3.000-4.000, fluorometer: EUR 6.000
cost per kit/ consumables (per sample not denoted)	LFD: EUR 245, ELISA: EUR 285-630, fluorometry: EUR 373-408
Portability	no
Laboratory	Preparation: yes - Analytics: yes

- Sample preparation for mycotoxin analyses with IAC (StarLine®): aflatoxin B1, B2, G1, G2, M1; ochratoxin A; DON, ZON; T2/HT2 extracted with PBS-buffer

- AgraQuant® ELISA: - quantitative, 10 – 20 min, different kinds of well-plates, detection with StatFax® 303 ELISA Reader or ChroMate® 4300 ELISA Reader

Table 30: Overview ELISA test systems for various mycotoxins from Romer Labs®.

Analyte	quantification range
total Aflatoxin	1 - 20 ppb, 4 - 40 ppb
AB1	2 - 50 ppb
AM1	100 - 2.000 ppt, 25 - 500 ppt
DON	250 – 5.000 ppb
Fumonisin	250 – 5.000 ppb
Ochratoxin A	2 – 40 ppb

ZON	25 – 1.000 ppb
T2	– 500 ppb

- AgraStrip® qualitative and quantitative test kits: - lateral flow detection, 5 min, different kinds of well-plates, detection with AgraVision™

Table 31: Overview Lateral Flow test systems for various mycotoxins from Romer Labs®.

Analyte	quantification range
total Aflatoxin	4 ppb, 10 ppb, 20 ppb, 5 – 100 ppb
AM1	0,1 – 0,6 ppb
DON	0,25 – 5 ppm
Fumonisin	0,5 – 5 ppm

- FluoroQuant®: -quantitative fluorometric test, for corn, wheat, milo, popcorn, soybeans, cornsoy blends, raw peanuts, rice, cotton seed™

Table 32: Overview fluorometric test systems for various mycotoxins from Romer Labs®.

Analyte	LOD [ppb]	extraction buffer
Aflatoxin		
FluoroQuant®Afla	3	n.d.
FluoroQuant®Afla Plus	1	acetonitrile
FluoroQuant®Afla Plus	1	methanol
FluoroQuant®Afla IAC	3	US Domestic
FluoroQuant®Afla IAC	3	international

2.8.10 Tecnalab s.r.l.

Table 3312: Summarized facts about the rapid analysis test systems from tecnalab.

	http://www.tecnalab.it/
Principle	ELISA
equipment	n.d.
cost per sample/ consumables	n.d.
Portability	no
Laboratory	Preperation: yes - Analytics: yes

-rapid quantitative screening with ELISA:

- **BZERO**: fast ELISA test kits with **no calibration curve** for aflatoxin B1 and deoxynivalenol

- **Celer**: fast ELISA test kits for total aflatoxins, aflatoxin B1, fumonisins, zearalenone, deoxynivalenol, T2/HT2 toxins

- **IScreen**: high sensitivity ELISA test kits for aflatoxins and ochratoxin A

2.8.11 ToxiMet Ltd

Table 34: Summarized facts about the rapid analysis test systems from ToxiMet Ltd.

	http://www.toximet.com/
Principle	Fluorometry
equipment	EUR 22.500 (ToxiQuant)
cost per sample/ consumables	EUR 23
Portability	No
Laboratory	Preperation: yes - Analytics: yes

- rapid testing sequence for aflatoxins and ochratoxin
- high sensitive, simultaneous measuring and identification of toxins
- analyte will be immobilised on a disposable cartridge, excited with UV
- fluorescence will be detected by a spectrometer which outputs quantity of the
- different mycotoxins (chemometrically analyse)
- available for: aflatoxins (B1, B2, G1, G2, total), ochratoxin A
- *costs:*- 1 test with cartridge ToxiSep (clean-up) and ToxiTrace (for analysis): EUR 15-23,

ToxiQuant EUR 7.000 – 22.500

- *analysis:*

- extract toxin from the matrices into a solvent e.g. methanol, filter, pass it through a ToxiSep clean up cartridge

- pass resultant liquid through the ToxiTrace detection cartridge, the cartridge is conditioned with a solvent and the toxin will be immobilized on the cartridge

- after washing place the cartridge in ToxiQuant, follow the symbol driven menu

- after five minutes read the results straight from the screen (no further analysis required)

Figure 7: ToxiQuant, an instrument from ToxiMet Ltd, which automatically scans and analyses mycotoxins of a sample in a cartridge.

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- advantages

- Single test simultaneous multi-mycotoxin analysis
- Accurate at the sub parts per billion level
- Cost effective single-test capability.
- non-scientists, No training or sophisticated laboratory required

- Environmentally friendly with very low solvent usage
- 5 min
- operates at temperatures up to 50°C
- low maintenance and low running costs
- LOD: EU: ✓ international: ✓

- disadvantages
    - not portable

2.8.12 VICAM

Table 35: Summarized facts about the rapid analysis test systems from VICAM.

	http://vicam.com/
Principle	LFD, fluorometry
equipment	EUR 6.000 (Series-4EX Fluorometer) (for LFD not denoted)
cost per sample/ consumables	fluorometry: EUR 10-15 (for LFD not denoted)
Portability	No
Laboratory	Preparation: yes - Analytics: yes

Fluorometer

- simple rapid quantitative method delivers parts-per-trillion (ppt) numerical results in as little time as 10 minutes (excluding preparation and extraction)—without costly HPLC or UPLC equipment or special training
- equipment: Series-4EX Fluorometer approx. EUR 6.000
- costs: basic packages with test + fluorometer approx.. EUR 7.950,00; upgrade packages EUR 18,50 – 675,45

Table 36: Overview fluorometric test systems for various mycotoxins from VICAM.

Analyte	detection range	extraction buffer	costs [EUR] per column
AflaTest (AFB1, AFB2, AFG1, AFG2 and AFM1) (feeds, food, grains, nuts, dairy product)	0,1 – 300 ppb	salt and methanol/water	9,97-10,40
AflaB (AFB1, AFB2, AFG1, AFG2 and AFM1)	1 – 300 ppb	salt and methanol/water	12,78
Afla M1FL+ (for milk)	12,5 - 200 ppt		12,78
FumoniTest™ (Fumonisin B1, B2 and B3)	0,016 – 10 ppm	salt and methanol/water	14,32
OchraTest™ (Ochratoxin A)	0,1 – 100 ppb	methanol/water	11,25
ZearalaTest	0,1 – 5 ppm	salt and methanol/water	12,27

Lateral Flow Reader

- results in or less than 5 minutes (excluding preparation and extraction), non-specialized operators

Figure 8: VertuTM Lateral Flow Reader from VICAM.

Deleted: 16

- equipment: test kit, Vertu™ Lateral Flow Reader (figure 10), standard PC to print or to download

Table 37: Overview Lateral Flow test systems for various mycotoxins from VICAM.

Analyte	Matrix	detection range	extraction buffer
Afla-V (aflatoxin B1, B2, G1, G2)	n.d.	0 -100 bbp	ethanol 70%
DON-V	grain, feed	0 – 5 bbm	Water
Fumo-V (Fumonisin)		0 – 5 bbm	ethanol 70%

Qualitative Strip Tests

- qualitative one-step test kit as first step in detection of contaminations
- visual results in less than 3 minutes (excluding preparation and extraction), non-specialized operators
- detection of aflatoxin (Afla-Check) with range of 10 or 20 ppb
- detection of DON (DON-Check) with range of 1 ppm
- extraction with water

2.9 Comparison between selected important methods

Table 38: Comparison between selected important methods.

Method	HPLC or LC-MS/MS [IGV GmbH, Map Milling 2007;2013]	ELISA [r-biopharm-RIDASCREEN®]	LFD/Dipstick [EuroProxima]	Fluorescence Polarization Immunoassay (FPI) [diachemix]
	quantitative	quantitative	qualitative or semi-quantitative	quantitative
principle	extraction, clean-up HPLC-UV or –FD with derivatization ***or*** LC-MS/MS	competitive immunoassay, detection about colour changes in the substrate	form of immunoassay, detect the presence (or absence) of target analyte in sample	based on the measurement of polarization (polarization ↑= mycotoxin ↑)
time	3 – 4 d	45 min	< 10 min	2 min
legislation	EU:✓ US:✓	EU:no US:✓	EU:✓ US:✓	EU:no US:✓
investment costs [EUR]	10.000 - 50.000	1.200	n.d.	26.000 – 27.000
costs/ sample [EUR]	50 – 180	15	22 – 27,50	40 - 50
strength	sensitive,	easy handling, low expenditure	easy handling, rapid, portable,	rapid, easy handling,

	reproducible	at time, sensitive, multiple analysis	no special equipment	portable
weakness	high costs and equipment, scientific stuff, time	cross-reactivity, false-positive because of matrix disruptions, costs	not qualitative, costs	in research, ex-pensive equipment

2.10

Moved up [5]: Legislation ¶
The legislations of mycotoxins in the European region are e.g. defined in the regulation of the European Community EG-VO 1881/2006. Important maximum and guidance levels are listed in Table 39.¶
¶
Table 39: Important EU-legislation for various mycotoxins from romerlabs (romerlabs).¶
Compound

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Deleted: (romerlabs)

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4 Helpful links and facts

Informations

http://www.mycotoxins.org/

http://www.mycotoxins.info/myco_info/qanda.html

http://www.mykotoxin.de/docs/public/tmplt_article.asp?CntID=16&PCat_ID=1&Lang=EN

http://www.mold-help.org/content/view/776/

http://www.fao.org/docrep/T1838E/T1838E00.htm

http://www.sigmaaldrich.com/life-science/cell-biology/cell-biology-products.html?TablePage=9619444

http://www.bfr.bund.de/de/presseinformation/2009/01/von_aflatoxin_bis_zearalenon___wissenschaft_macht_lebensmittel_sicher-27754.html

Legislation

http://www.romerlabs.com/de/knowledge/mycotoxin-regulations/regulations-europe/

http://www.romerlabs.com/de/knowledge/mycotoxin-regulations/regulations-usa/

http://europa.eu/legislation_summaries/food_safety/contamination_environmental_factors/index_en.htm

http://eur-lex.europa.eu/en/index.htm

http://ec.europa.eu/food/food/chemicalsafety/contaminants/legisl_en.htm

Food

http://www.codexalimentarius.org/

http://www.efsa.europa.eu/en/topics/topic/mycotoxins.htm

Organisations

http://www.aaccnet.org/Pages/default.aspx

http://www.acs.org/content/acs/en.html

http://www.aoac.org/

http://www.cen.eu/cen/Pages/default.aspx

http://www.fooddrinkeurope.eu/

http://www.citac.cc/

http://www.european-accreditation.org/home

http://www.eurachem.org/

http://www.euramet.org/index.php?id=homepage

http://www.eurolab.org/

http://www.iaf.nu/

http://www.foodprotection.org/

http://www.ilac.org/

http://www.iso.org/iso/home.htm

http://www.iupac.org/

http://www.measurement.gov.au/Pages/default.aspx

http://www.nmkl.org/

http://www.oecd.org/index.htm

http://www.usda.gov/wps/portal/usda/usdahome

http://www.wto.org/english/thewto_e/thewto_e.htm

http://www.lgcgroup.com/our-science/national-measurement-institute/#.WIe-DYWcHy9

EU-legislation

Links to EU-legislation concerning mycotoxins.

EU-legislation concerning mycotoxins	Compounds
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF	- maximum levels for DON, Afla, ZON, Fumonisin B1, - B2, Ochratoxin A, T2-HT2
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20100302:EN:PDF	- maximum levels for Afla

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF	- maximum levels for DON, ZON, Fumonisin, Ochratoxin A, T2-HT2
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:234:0035:0040:EN:PDF	- Fusarium toxins
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R0401:20100313:EN:PDF	- DON, Afla, ZON, Fumonisin B1, - B2, Ochratoxin A, T2-HT2

Reviewed wavelength of various mycotoxins.

mycotoxin	$\lambda_{excitation\ [nm]}$	$\lambda_{emmision\ [nm]}$
Aflatoxin (Huang and Elmashni, 2007)	365	455
Aflatoxin (Rasch et al., 2007)	360	427
Ochratoxin A (Rasch et al., 2007)	333/380	455/427
Ochratoxin B (Rasch et al., 2007)	320/367	460/422
Zearalenone (Rasch et al., 2007)	318	466
Aflatoxins (Jansen et al., 1987)	365	440

Spectrophotometric parameters for various mycotoxins from
http://www.fao.org/docrep/x5036e/x5036E0c.htm

mycotoxin	molecular weight	Solvent	absorbtivity	-max (nm)
Aflatoxin B.	312	Benzene:acetonitrile		
		(98:2 v/v)	19,800	353
Aflatoxin B1	312	Chloroform	22,300	353
Aflatoxin B2	314	Benzene:acetonitrile		
		(98:2 v/v)	20,900	355
Aflatoxin G1	328	Benzene:acetonitrile		
		(98:2 v/v)	17,100	355
Aflatoxin G2	330	Benzene:acetonitrile		
		(98:2 v/v)		357
Aflatoxin M1	328	Chloroform	19,950	357
Ochratoxin A	403	Benzene:acetic acid		
		(99:1 v/v)	5,550	333
Ochratoxin B	369	Benzene:acetic acid		
		(99:1 v/v)	6,000	320
Ochratoxin A	431	Benzene:acetic acid		
ethyl ester		(99:1 v/v)	6,200	333
Ochratoxin B	397	Benzene:acetic acid		
		(99:1 v/v)		320
Patulin	154	Absolute ethanol	14,540	276
Patulin	154	Methanol	12,880	275
Sterigmatocystin	324	Benzene	15,200	325

Citrinin	259	Chloroform	16,100	322
Zearalenone	318	Ethanol	29,700	236
Zearalenone	318	Ethanol	13,909	274
Zearalenone	318	Ethanol	6,020	316