## **Integrated methodologies for the risk assessment of mycotoxin mixtures in food & feed**

**Analytical methods to evaluate co-occurrence and mycotoxin mixtures**

**Introduction**

Mycotoxins are low molecular weight compounds produced as secondary metabolites by different genera of fungi. Initially, the crops of most concern from mycotoxin contamination were those believed to be most susceptible to fungal contamination such as nuts, fruits, cereals and cereal based foods. Of course these are highly significant commodities in human nutrition and food security but also play an important role in the global economy. Due to the increase in the global population and worldwide trade the increasing demand for these products provides a challenging task of providing food free from all contaminants but especially mycotoxins. As such then these toxins can have serious consequences in terms of both human and animal health as well as huge economic impact (1). Although, identified in the scientific literature since the early 1960s researchers from multiple disciplines are only now understanding the complexity and full spectrum of mycotoxins produced by different fungal species and their physicochemical and toxicological properties due to the advanced methodologies and techniques currently used in research. However, the information at this time still remains inconclusive as to why these toxin compounds are produced and to their co-occurrence in different commodities but also in the full evaluation of current and emerging analytical and mitigation tools to ensure future food safety. To date greater than 350 different mycotoxins have been identified with different chemical structures and properties produced by a range of different fungal species. Humans and animals can be equally exposed through food and feed to different mycotoxins and those known to potentially cause health concerns and economic losses have either been regulated with maximum permitted levels or recommendations of safety levels provided in different regions. These include aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisins, T2/HT2, patulin and ergot alkaloids. As such contamination of food and feed commodities with these mycotoxins is of greatest concern due to the known consequences whereby these are monitored globally to a greater extent than other mycotoxins. Currently there are approximately 100 countries who have established limits on the presence of major mycotoxins in food and feed (Lee et al 2017 and Moretti et al 2017). Table 1 shows some of the commonly contaminated food commodities along with the mycotoxin and fungal species responsible and the EU regulatory limits of the toxin in food and feed. However, with the increasing awareness of different mycotoxins in the food chain other commodities are frequently being examined for contamination to increase the knowledge base in occurrence data and to understand other routes of contamination detrimental to human and animal health. Though the safety levels consider individual toxins and not mixtures or co-occurring toxins whereby synergistic or antagonistic toxicological effects may present. Due to the variety of structures of these toxins (Fig 1), it may be challenging for all laboratories to be able to apply only one standard method of analysis and/or detection. The need for a specialist laboratory and efficient turnaround alongside requirements of highly sensitive analysis can create problems for daily routine analysis for the detection of all governed mycotoxins. Therefore, a broad range of techniques used for practical analysis and detection of mycotoxins are available in an attempt to meet regulators and end-users needs (Nelis et al 2019). There are several different types of physiochemical methods available for mycotoxin analysis which include different types of chromatographic methods combined with different detectors. Other techniques include bioanalytical methods which such as ELISA techniques, lateral flow devices and biosensors may be used for detection and screening for mycotoxins. The aim of this review was to systematically evaluate the current and emerging methods of analysis applied for mycotoxin analysis and particularly for the simultaneous determination of multiple mycotoxins to risk rank their effectiveness to ensure future food safety.

*Search Strategy*

In the past 10 years, several review articles have been published relating to the occurrence and determination of mycotoxins (2-6). Furthermore, new developments, updates and emerging methods in this area are published yearly by the World Mycotoxin Journal (7-10). A systematic search of the literature was undertaken for the past 10 years to find articles investigating different types of methods of analysis used for determining mycotoxins in food and feed. Journal articles were identified using the following electronic databases;

1. Web of Science – core collection
2. KCI – Korean journal database
3. Medline
4. Russian Science Citation Index
5. Sci ELO Citation Index

Journals were searched for each year from 2010 to 2018. Articles published in the specified year and having met all the keyword search terms were eligible for inclusion. A search was conducted using the terms ‘Mycotoxin AND ‘specified method of analysis’ in the web of science database. Figure 2 summarises the findings from that search conducted in 2018.

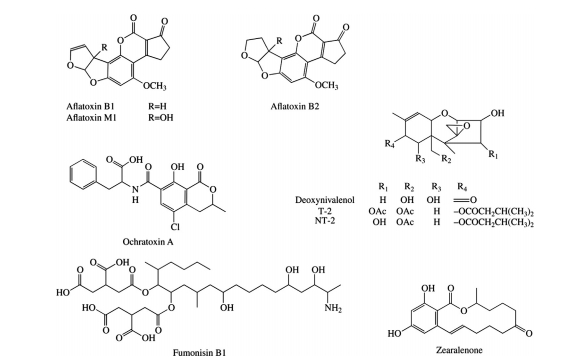


Figure 1 Chemical structures of important mycotoxins (11)

*Table 1 European Regulations Limits for major Mycotoxins in foods for human consumption (12)*

|  |  |  |  |
| --- | --- | --- | --- |
| Mycotoxin | Fungal species | Food commodity | EU limits (µg/kg) |
| Aflatoxin B1, B2, G1, G2 | *Aspergillus flavus, Aspergillus parasiticus* | Maize, wheat, rice, peanut, pistachio, almond, ground nuts, tree nuts, figs, spices | 2-12 for B1  4-15 for total |
| Aflatoxin M1 | Metabolite of AFB1 | Milk, Diary products | 0.05 milk  0.025 in infant formula/milk |
| Ochratoxin A | *Aspergillus ochraceus*  *Penicillium verrucosum*  *Aspergillus carbonarius* | Cereals, dried vine fruit, wine, grapes, coffee | 2-10 |
| Fumonisins B1, B2, B3 | *Fusarium verticillioides*  *Fusarium proliferatum* | Maize | 200-1000 |
| Zearalenone | *Fusarium graminearum*  *Fusarium culmorum* | Cereals, maize, wheat, barley | 20-100 |
| Deoxynivalenol | *Fusarium graminearum*  *Fusarium culmorum* | Cereals | 200-50 |
| Patulin | *Penicillum expansum* | Apples, Apple juice and concentrate | 10-50 |

Figure 2 Schematic showing the results from a Web of Science database search using the identified terms

Mycotoxins + ELISA

699 hits

23 reviews

‘Mycotoxins + lateral flow devices’

30 hits

7 reviews

‘Mycotoxins + High pressure liquid chromatography’

864 hits

21 reviews

‘Mycotoxins + Mass spectrometry’

2,205 hits

177 reviews

‘Mycotoxins + Capillary electrophoresis’

53 hits

11 reviews

**Web of science, All databases include:**

* ***Web of science core collection***
* ***MEDLINE, sciELO citation index***
* ***KCI-Korean journal database***
* ***Russian science citation index***

**Mycotoxins 2010-2018**

***Web of Science, all databases***

**11,029 hits 1,028 reviews**

‘Mycotoxins + Thin layer chromatography’

206 hits

26 reviews

‘Mycotoxins + Gas chromatography’

318 hits

32 reviews

Mycotoxins + biosensors

210 hits

33 reviews

Mycotoxins + Electrochemical biosensor

72 hits

Mycotoxins + electrochemical

205 hits

41 reviews

‘Mycotoxins + Electrochemical immunosensor’

95 hits

14 reviews

*Literature search methods*

Literature searching occurred in 2018. The web of science was used as the main search engine and included searches carried out in 5 different databases (listed above). The following word terms were searched for in the TOPIC field. The TOPIC field in web of science covers searching for the terms or keywords in the following fields within a record;

* Title
* Abstract
* Author keywords
* Keywords plus

When searching for exact phrases, quotation marks were used. Wildcards (such as \*) were used to find plural of inflected forms of words and these wildcards represent unknown characters so were useful when the Authors initials were not known. The main wildcard used in this search was the asterisk \* to represent any word or group of characters that follow a specific term, for example, lateral flow\* so phrases such as lateral flow device, lateral flow sensor or lateral flow immunosensor could all be included in the search. Search operators such as AND, NOT, OR where used to prescribe a relationship between terms such as equivalence, exclusion and proximity. The main operator used in this search was AND – searched for: TOPIC: (mycotoxins)

Refined by: TOPIC: (patulin) AND TOPIC: (liquid chromatography) AND TOPIC: (tandem mass spectrometry) AND TOPIC: (UPLC) Timespan: (selected year). The references of relevant papers were downloaded into an endnote library and were examined for suitability. Papers that were written in a language other than English were discarded. All papers and abstracts reporting the specified method of analysis, mycotoxin and related to food stuffs or feed were selected for full text review. Focus was placed on relevant studies published between 2010 and 2018. Any further articles of relevance were also considered in the study from 2018 to July 2019

***Introduction into how the methods of analysis for Mycotoxin detection work***

Determination of mycotoxin levels in food and feed is accomplished by methods that include certain common steps usually explained in all articles relating to this topic; sampling, homogenisation, extraction and clean up and detection and quantification performed by many of the methods of analysis discussed in the next section. This is represented by Figure 3.

**Sampling**

**(Representative sample selection)**

**Sampling preparation**

**Extraction of toxin**

**Filtration**

**Clean up (SPE or IAC)**

**Detection**

**Quantitative analysis**

**(LC/MS, GC/MS, ELISA)**

**Qualitative analysis**

**(TLC, LFD, rapid test strips)**

**Single toxin analysis**

**(HPLC, GC, ELISA**)

**Multiple toxin analysis**

**(ELISA, LC/MS/MS)**

Figure 3 Simple flow chart showing stages of determination of mycotoxins in food and feed

*Sampling methods*

Traditionally sampling methods for food stuffs are not usually suitable for mycotoxin analysis as the toxins are not homogenously spread throughout the commodity (Whitaker et al 2006 and Shepard et al 2016) and commodities such as cereals and animal feed are prone to hotspots. It is therefore very challenging to get a representative sample of the entire product (Ridgeway et al 2012). The only way to address this problem is by developing regulatory legislation to overcome the problems posed by sampling methods. Current sampling methods are laid out in a document by the EU under Regulation (EC) No. 401/2006 (Krska et al 2008 and Shephard et al 2016). Irrespective of the mycotoxin present these sampling protocols are to ensure representative sampling occurs.

*Sample Extraction*

Traditionally some form of extraction procedure is required before analysis for mycotoxins and the method depends upon whether the food is solid or liquid in nature. Solid food samples are extract into a liquid phase in the first step of sample preparation and possibly followed by a clean-up process thereafter to enhance the sensitivity of the method of analysis. The type of extraction process used on the food samples is governed by (i) the chemical nature of the mycotoxin, (ii) the nature of the food matrix and (iii) the method of analysis. Similarly, liquid food samples must also undergo some form of extraction procedure, possibly a liquid-liquid extraction to firstly separate the mycotoxins. According to Pereira et al 2014, further solid-liquid extraction may be required to separate toxins from grains or cereals. Extraction is performed through a variety of organic solvents and water. More recently, extraction for mycotoxin analysis has been carried out by instrumental automated solvent extraction techniques which include supercritical fluid extraction (SFE) (Perira et al 2014). Even though this technique may accelerate mycotoxin extraction with a requirement of smaller volumes of solvent, it is quite expensive. As shown in the flowchart above, after extraction 2 more important steps are required, filtration and clean-up. These important steps will remove further impurities that may interfere with the detection of the mycotoxin in question and improve accuracy and precision (Turner et al 2009). Over the years a variety of clean-up methods have been employed which include liquid-liquid partitioning, solid phase extraction (SPE), immunoaffinity columns (IAC), ion exchange columns and multifunctional clean up columns namely MYCOSEP (Periera et al). The most commonly used techniques for clean-up in mycotoxin analysis are IAC (immunoaffiniy columns) and solid phase extraction (SPE). A recent survey conducted by Periera et al 2014 showed that the most popular clean up technique was Immunoaffinity column clean up method which was swiftly followed by QuEChERS (quick, easy, cheap, effective, rugged, safe) sample preparation approach. Figure 4 shows the sample preparation/clean-up method for papers published between 2010-2014.

Figure 4 Graph showing sample preparation and clean up techniques for paper published between 2010-2014 (2)

Immunoaffinity columns are leading the way when it comes to clean up methods but more recently there has been an increase in the QuEChERS sample preparation approach. According to Rejczak et al (2015), due to the flexible nature of this method it should be thought of as more of a methodology than a specified method. Even though it is used in conjunction with LC/MS/MS to simultaneously analyse multiple mycotoxins and other analytes and produce high quality results, it may be thought of as an expensive methodology as it uses exclusively this kind of instrumentation. As an example of how this method is best described for mycotoxin analysis, Cunha, Fernandes et al 2010, developed and validated a method for the simultaneous analysis of 5 mycotoxins (zearalenone, deoxynivalenol, Fusarenon X, 15-acetyldeoxynivalenol and nivalenol) in breakfast cereals and flours by GC/MS. A total of 18 samples of breakfast cereals were randomly purchased in local supermarkets. The mycotoxins were extracted from the samples by QuEChERS methodology. In this work several different types of d-SPE clean up sorbents were tested namely, magnesium sulfate, C18, primary secondary amine (PSA; particle size 50 μm), Florisil (pore size 60–100 mesh) and magnesium sulfate mixed with C18. A visual representative of Cunha et al sample preparation is shown in Figure 5. Cunha et al achieved recoveries from 67-101% and from 52-103% for mycotoxins in breakfast cereal and flour respectively with good repeatability of 9- 21%. Some years later, Ferreira, in association with Cunha and Fernandes modified this methodology and used it for mycotoxin analysis in popped and unpopped popcorn. In the last years QuEChERS is being used more frequently as it is fast, inexpensive and simple. It is now being used for analysis of multiple mycotoxins in many food matrices such as grains, cereals, eggs, milk, wine and coffee. The challenge with this QuEChERS approach was developing an extraction procedure suitable for the extraction of multiple mycotoxins with adequate recovery for analysis.

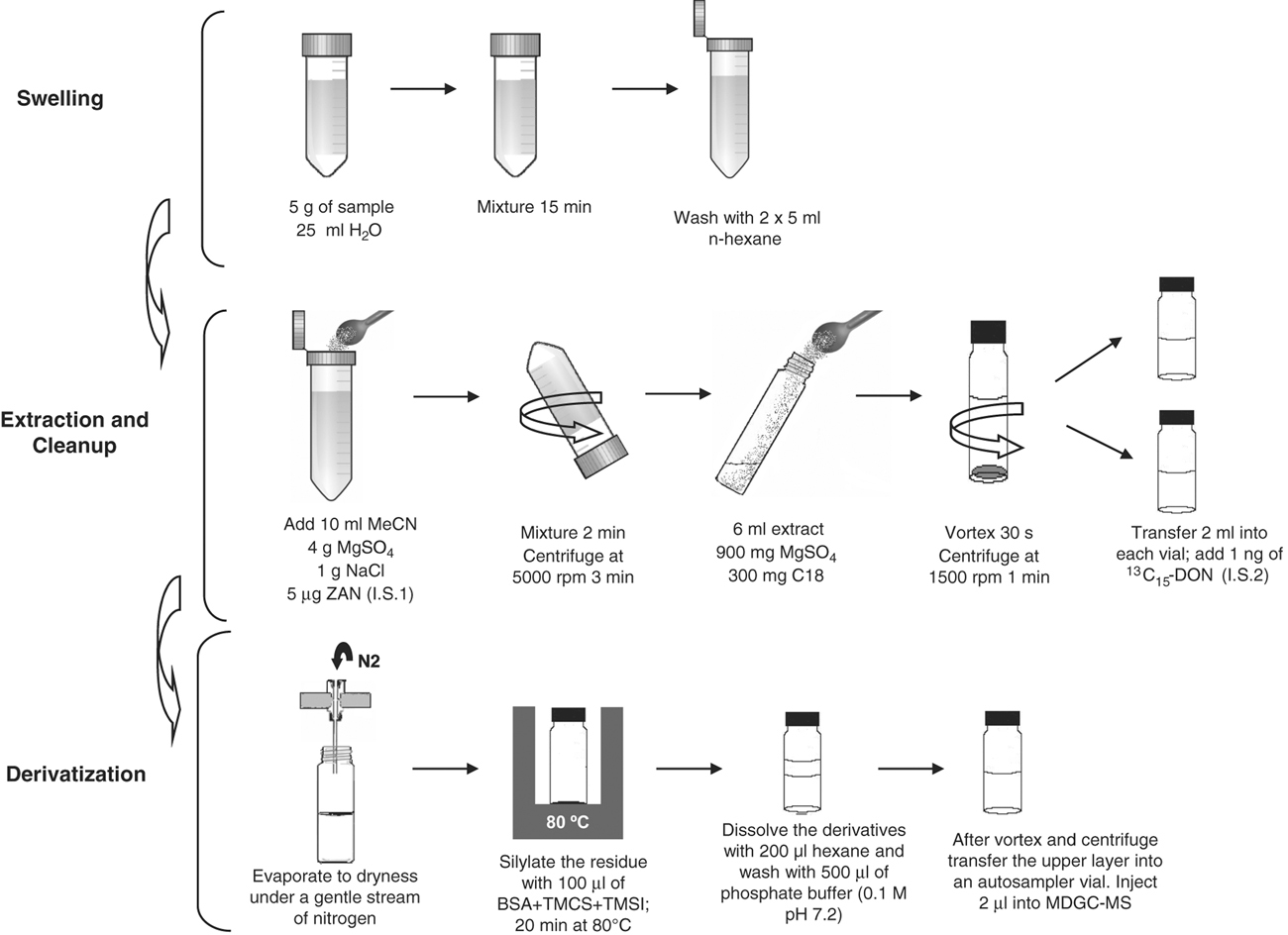


Figure 5 A visual representation of sample preparation completed by Cunha et al (13)

As previously mentioned, immunoaffinity columns are increasing as the clean-up method of choice. This method is rapid, efficient, reproducible and safe. In a paper from Hu X et al 2016, a rapid sample preparation method was developed using a single multiple immunoaffinity column (mIAC) using monoclonal antibodies produced in the laboratory. The mIAC allowed the specific capture of multiple mycotoxins which could be eluted for downstream processing using UPLC. This procedure performed better and reduced the amount of chemical organic solvents used. The study focused on the simultaneous determination method for eight mycotoxins in feeds by using UPLC– MS/MS via a mIAC.

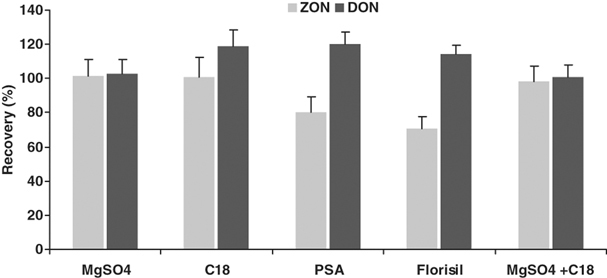


Figure 6 Recoveries of mycotoxins in breakfast cereal and flour (13)

Eighty feed samples were obtained from local markets in China and homogenised and extracted in a single step. 20 grams of sample were added to 100 mL acetonitrile (ACN)/water/acetic acid (80:18:2, v/v/v) and homogenised for 2 min. The resulting supernatant was then filtered and diluted with 1:3 with phosphate buffer saline. This solution (10mL) was then used for the mIAC clean-up step. The limits of detection and limits of quantification ranged from 0.02-025 ng/ml and 0.018-0.75ng/ml respectively. The recoveries ranged from 91-104.1% for all mycotoxins studied (AFB1, AFB2, AFG1, AFG2, OTA, ZEN, ST and T-2) and showed good repeatability.

Irrespective of the method of analysis the different structural and physicochemical properties of the toxins makes a universal samples preparation method a challenging prospect to ensure adequate recovery through validation for regulatory purposes. Historically sample preparation was optimised to the selected target to ensure the maximum recovery with the method of analysis. For multiple toxin analysis there has to be some compromise in optimnal sample preparation and hence recovery especially between polar and non-polar compounds for a detection method to remain fit for purpose.

***Analytical Methods***

Each of the methods described has been used or is currently in use for the detection of mycotoxins and mycotoxin mixtures in food and feed. Chromatography is by far the most commonly used method for mycotoxin analysis in food and feed with different detector systems applied whether these be ultra-violet (UV), photodiode array (PDA) or mass spectrometry (MS) (Shephard et al 2016). The chromatography component separates the toxins for easier identification using the detector applied.

*Thin Layer chromatography*

A chromatographic method applied to mycotoxins, thin layer chromatography is by far the most widely used in the detection, analysis and characterisation of fungal toxins. However, the traditional method of TLC has now been overtaken by other forms of analysis. The main toxin detected by TLC was aflatoxin but this technique can also be used for analysis of ochratoxin A especially in commodities such as nuts, wine and dairy products. The chromatography is used to separate mixtures of substances into their components. All forms of chromatography work on the same principle, the most basic of which is Thin Layer Chromatography. Thin layer chromatography was first used by H de Longh et al in 1964 in their paper ‘The occurrence and detection of aflatoxin in food’. According to Wacoo and Wendiro et al., (2014), TLC has been regarded by the Association of Official Analytical Chemist (AOAC), as the method of choice since the 1990s, and has been used widely since the discovery of Aflatoxins in the 1960s. It is still used today in conjunction with ELISA for the analysis of peanuts and walnuts to detect (Khalifa et al 2017) and analysis of groundnut samples (Watson et al 2015). The main toxin detected by TLC is Aflatoxin. It is used for detection in food samples such as baby food (Aidoo et al 2011) and in milk samples (Fallah A. A et al 2011). The study of Fallah et al aimed to determine the occurrence of aflatoxin M-1(AFM(1)). Sample collection involved a total of 682 dairy product samples composed of raw cow milk (n = 88), raw goat milk (n = 65), raw sheep milk (n = 72), Lighvan cheese (n = 75), industrial yoghurt (n = 61), traditional yoghurt (n = 60), industrial Kashk (n = 64), traditional Kashk (n = 61), industrial Doogh (n = 71) and traditional Doogh (n = 65) were obtained from dairy ranches, supermarkets and retail outlets in four large Iranian cities (Tehran, Esfahan, Tabriz and Shiraz) during year 2008. The samples were transported to the laboratory inside a digital portable refrigerator at 3 °C and stored at −20 °C until analysis by TLC for AFM1. The method followed for the analysis had been previously described in Fallah et al 2010 paper. The method was validated in lab and the recoveries were with 75-93% with both the coefficient of variation 4.8-11.3% and the lowest limit of detection of the method being 0.0125ppb.

TLC is simple, fast and inexpensive. It is qualitative and semi quantitative therefore mainly used for screening purposes of aflatoxin and ochratoxin A in commodities such as wine, nuts and dairy products. Simply described the method of TLC is done by spotting a sample on a plate along with silica gel (stationary phase) and placed into a tank with the developing solvent (mobile phase), and the solvent moves up the plate by capillary action and the compounds are separated. Depending on the compound, the analyte can be identified by fluorescence or UV light, although quantification may be carried out by densitometry. This traditional method seems to have been overtaken somewhat by other forms of chromatographic analysis.

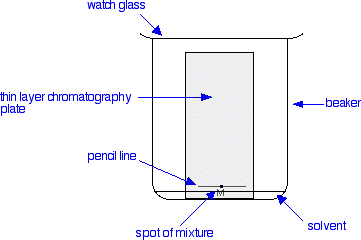
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Figure 7 Diagram to show layout procedure of TLC method

*HPLC – High Performance Liquid Chromatography / LC/MS/MS*

High performance liquid chromatography is a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures in the region of several hundred pounds per square inch (psi). This is a very versatile and widely used type of elution chromatography. This is another techniques used for separating and determining species of organic, inorganic and biological materials. In liquid chromatography, the mobile phase is a liquid solvent containing the sample as a mixture of solutes and a very finely divided stationary phase. Columns used for HPLC and packed with particle diameters as small as 3 to 10 µm. This smaller particle size for the column packing material gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it and allows much better separation of the components of the sample mixture. Highly automated and extremely sensitive detection methods are used.

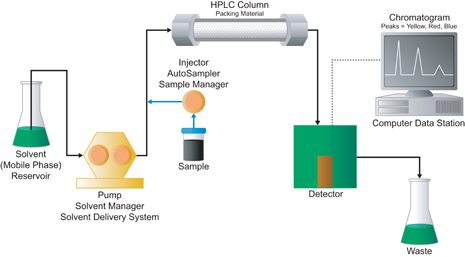


Figure 8 Simple block diagram showing the components of a HPLC system (14)

The most widely used detectors for liquid chromatography are based on absorption of UV or visible radiation. Spectrophotometric detectors are considerably more versatile than photometers. The combination of LC and mass spectrometry gives high sensitivity and the technique can provide fingerprinting of a particular eluate instead of relying on retention times. This combination also gives information regarding the molecular mass and structural information. For complex mixtures it is feasible to couple two mass analysers together in a technique called LC/MS/MS. The tandem systems are usually triple quadrupole systems and to attain even higher level of resolution, and the final mass analyser can be a time-of-flight mass spectrometer. Mycotoxin analysis has greatly advanced by coupling liquid chromatography techniques to mass spectrometry (Shephard et al 2016, Womack et al 2016, Wang et al 2016). From the diagram below is it clear that LC/MS/MS is the method of choice for the analysis of mycotoxins and in more recent years multiple mycotoxins.

Two types of partition chromatography are distinguishable based on the polarities of the mobile and the stationary phases. Normal phase chromatography is based on a highly polar stationary phase such as water or triethylene glycol and there is a relatively non polar mobile phase such as hexane. Reverse phase chromatography is when the stationary phases are non polar often a hydrocarbon and the mobile phase is relatively polar such as water or methanol. In normal phase the least polar analyte is eluted first and in reverse phase the least polar is eluted last.

UPLC is an emerging method of analysis for the detection of mycotoxins, and is in principle similar to HPLC, however there are some differences which make the time of analysis shorter than HPLC. The method has been modified to include higher pressure, the use of smaller sized particles (2µm over 5µm), which allows for a greater surface area, leading to better separation, and thus an improved time/cost/analysis. Detectors that may be used in conjunction with UPLC include UV light, fluorescence and mass spectrometry. According to the literature, reported limits of detection using UPLC with fluorescence detection, to detect ochratoxin A in ginger was 0.09 ng Ml-1 (Cao and Zhou et al., 2013). Amongst all non-MS chromatographic techniques, HPLC with fluorescence detection coupled with extensive clean- up methods is regularly used especially for the quantitative analysis of aflatoxins (Pereira et al 2014 and shepherd et al 2016). Using this method of analysis, it is possible to obtain sensitivity that is comparable to that of LC/MS/MS but HPLC-FLD is usually reserved for use when analysing single mycotoxins rather than multiple sets. (Kong et al 2013). In this work, a high‐performance liquid chromatography with fluorescence detection (HPLC‐FLD) method was successfully developed for analysing the contamination levels of zearalenone and its metabolite *α*-zearalenol in 100 widely consumed foods and medicinal plants in China. Samples were extracted with methanol–water (80:20, v/v), and cleaned up by using an immunoaffinity column. The limits of detection of this method for zearalenone and *α*‐zearalenol were 4 µg kg−1 and 2.5 µg kg−1, respectively. Recoveries for the samples ranged from 85.8% to 96.1% with relative standard deviation (RSD) of 2.6–7.1% for zearalenone, and from 89.9% to 98.7% with RSD of 1.9–9.2% for *α*‐zearalenol**.** Confirmatory analysis on positive samples was carried out using LC/MS/MS.

*Multiple mycotoxin Analysis*

Most recently, the use of liquid chromatography and tandem mass spectrometry analysis (LC/MS/MS) is continuing to be the most implemented method of analysis for multiple-mycotoxin determination. Most of the published methods are describing extensions of previous procedures rather than the development of new strategies. Key areas of the procedures remain to be sample preparation and extension validation of the method performance. A detailed description of LC/MS/MS methods used for analysis of multiple mycotoxins in food and feed is shown in Table 2 .

(The papers were searched for in Web of Science in all databases using the following search terms: TOPIC: (mycotoxins) AND YEAR PUBLISHED: (e.g. 2018) Refined by: TOPIC: (multi mycotoxins) AND TOPIC: (liquid chromatography) Timespan: All years. Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI. This gave 26 hits which were then screened and the papers were then included or excluded depending on whether they dealt specifically with LC/MS/MS and if the matrix was food or feed and not human or animal biological matrices. The title and abstract of each paper was scrutinised. A screen of the title of the article, and parts of its abstract, were read to try to determine whether or not to devote time to reading on. The findings described in the abstract were also key, either as the sole part of an article that will be read, or to determine whether reading the full text is required. All articles that met the aforementioned search terms were screened and the detailed description of the LC/MS method and conditions is shown in Table 2.

Table 2 Table Showing detailed description of the set-up of key LC-MS methods for multiple mycotoxin determination 2010-2018

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Extraction** | **Clean up** | **Toxin** | **Matrix** | **Instrument** | **Conditions** | **MS conditions** | **Reference** |
| 25 g of finely ground sample were extracted with 100 ml of methanol/water (4/1 v/v), after 30 min stirring the extract was passed through filter paper | Samples were passed on the DZT IAC (R‐Biopharm‐Rhône Ltd) at a flow rate of 1–2 drops/sec. | deoxynivalenol (DON), zearalenone (ZEA), T‐2 and HT‐2 toxin | Cereals | HPLC Alliance® 2695 (Waters) and a Tandem Mass Spectrometer Quattro Micro™ API (Waters) equipped with an ESI source. | A reversed‐phase column RP C18 (25 cm × 2.1 mm, 5 µm) (Discovery®, Supelco, USA) was used. The column was kept at room temperature. A mobile phase gradient programme was used for combining solvent A (Milli‐Q water + 10 mM ammonium acetate) and solvent B (methanol) | The mass spectrometer was operated in MRM mode with negative–positive–negative ion switching. | (15) |
| A simple pH-buffered sample extraction was used.Silage samples were frozen with liquid N2 and homogenised in a domestic blender. Extraction was performed by QuEChERS | No further clean up | FUT C, ENN B, AOH, AME, CIT, NIV, DON, GLI, MPA, CPA, OTA, PAT, ROQ C, T-2, TEA, ZEA, MEV, PEN A and STE, FB1 and FB2 | Maize silage | Agilent 1100 series HPLC system with a Quattro Ultima triple quadrupole MS without the high-collision hexapole | Gemini C6-Phenyl, (3 μm, 2.0 × 100 mm) column equipped with a Gemini Security guard cartridge. The mobile phases were (A) ammonium formiate 0.4 mM, 0.2% formic acid in water (pH 2.5) and (B) 100% ACN | The source and desolvation temperatures were 120 °C and 400 °C, respectively. Operated in the in the multiple-reaction monitoring (MRM) mode | (16) |
| QuEChERS-based method. 4 g of sample were weighted into the PTFE cuvette and 7.5 mL of 0.1% (v/v) formic acid and 10 mL of acetonitrile were added. The suspension was shaken vigorously for 3 min. After addition of 1 g of NaCl and 4 g of MgSO4, the mixture was shaken again | Separate aqueous and organic phase, the sample was centrifuged (5 min, 5000 rpm). The 0.5 mL aliquot of upper organic phase was diluted with deionized water in 1:1 (v/v) ratio. Sample solution was passed through the 0.2 μm filter. | nivalenol (NIV), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-Glc), 3-acetyldeoxynivalenol (3-ADON), fusarenon-X (FUS-X), HT-2 toxin (HT-2), T-2 toxin (T-2), zearalenone (ZEA), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 | Cereals and cereal based products | Acquity UPLC coupled to time-of-flight mass spectrometer LCT Premier XE with a dual electrospray source was used | separation of sample components. 100 mm × 2.1 mm i.d., 1.8 μm column, the column temperature was maintained at 40 °C. Mobile phases were 5 mM ammonium formate with pH 5.6 (eluent A1) and methanol (eluent B) | Parameters of the ion source were as follows: capillary voltage 3.5 kV, cone 40 V, desolvation temperature 350 °C, source temperature 120 °C, cone gas 10 L h−1, and desolvation gas 750 L h−1. The leucine–enkephalin lock mass calibrant (flow 10 mL min−1) was measured every 40 scans | (17) |
| Extraction carried out using a mixture of acetonitrile/water/acetic acid 79 + 20 + 1 (v + v + v), with ratios between 3 and 16 mL solvent/g | Samples were centrifuged, diluted 1 + 1 and injected as described in detail by Sulyok et al. (2007). | Brefeldin A, cytochalasins A, B, C, D, J and H, HC-toxin, kojic acid, and 3-nitropropionic acid, penicillic acid and roquefortine C, AAL TA toxin, Enniatin B3 and 2-amino-14,16-dimethyloctadecan-3-ol T2-tetraol and T2-triol. Alpha zearalenol-4-glucoside and beta zearalenol-4-glucoside | bread, fruits, vegetable cheeses, nuts and jam. | QTrap 4000 LC–MS/MS System equipped with a TurboIonSpray electrospray ionization (ESI) source and an 1100 Series HPLC System | C18-based column. | Quantification was performed in the Selected Reaction Monitoring (SRM) mode | (18) |
| Feed samples were blended using a blender. 5g of feed sample was extracted with 20 mL of acetonitrile/water/acetic acid (79/20/1, v/v/v), tumbled on a end-over-end tumbler for 1 h, and centrifuged for 15 min at 3300g. | The supernatant was passed through the C18-SPE column and immediately collected in a volumetric flask of 25 mL. | aflatoxin-B1, aflatoxin-B2, aflatoxin-G1, aflatoxin-G2, ochratoxin A, deoxynivalenol, zearalenone, fumonisin B1, fumonisin B2, fumonisin B3, T2-toxin, HT2-toxin, nivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, diacetoxyscirpenol, fusarenon-X, neosolaniol, altenuene, alternariol, alternariol methyl ether, roquefortine-C, and sterigmatocystin | Feed | Waters Acquity UPLC system coupled to a Micromass Quatro Micro triple-quadrupole mass spectrometer | The column used was a 150 mm × 2.1 mm i.d. 5 μm Symmetry C18, with a 10 mm × 2.1 mm i.d. guard column of the same material. mobile phase A (water/methanol/acetic acid, 94/5/1 (v/v/v), and 5 mM ammonium acetate) and mobile phase B (methanol/water/acetic acid, 97/2/1 (v/v/v) and 5 mM ammonium acetate) at a flow rate of 0.3 mL/min with a gradient elution program. | MS was operated in the positive electrospray ionization (ESI+) mode. The capillary voltage was 3.2 kV, and nitrogen was used as the spray gas. Source and desolvation temperatures were set at 150 and 350 °C, respectively. Mycotoxins were analyzed using selected reaction monitoring (SRM) channels. | (19) |
| Sample extracted using a QuEChERS-based extraction procedure (Quick, Easy, Cheap, Effective, Rugged and Safe) without applying any further clean-up step. | No further clean up | beauvericin, enniatin A, A1, B1, citrinin, aflatoxin B1, B2, G1, G2 and ochratoxin A | Eggs | ACQUITY UPLC™ system (Waters) with MS/MS detection was performed using an Acquity TQD tandem quadrupole mass spectrometer (Waters) | BEH C18 column (100 mm × 2.1 mm), with 1.7 μm particle size | operated using an electrospray (ESI) source in positive and negative ion mode | (20) |
| Extraction procedure based on two separate steps. In the first step, 15 mL of MeOH was added to 2 g of maize silage (wet weight) in a tube protected from light and put on a horizontal shaker for 30 min. After centrifugation at 4000g for 10 min, the supernatant was collected and kept apart in a light-protected tube. A second extraction step with 15 mL of MeCN/H2O (84:16, v/v) was introduced. | 50 mL was brought on an Oasis HLB column (Waters), which was equilibrated before starting cleanup by subsequently passing 4 mL of MeOH and 4 mL of HPLC H2O over the column., | aflatoxins, beauvericin, citrinin, cyclopiazonic acid, deoxynivalenol, fusaric acid, fumonisins, gliotoxin, HT-2 toxin, mycophenolic acid, nivalenol, ochratoxin A, patulin, salinomycin, sterigmatocystin, T-2 toxin, verrucarol, α-zearalenol, β-zearalenol, and zearalenone Roquefortine C Enniatin | Maize silage | Acquity Ultra Performance liquid chromatograph coupled to Xevo TQ mass spectrometer. | The column used was a 100 mm × 2.1 mm i.d., 1.7 μm, UPLC BEH C18. Mobile phase A H2O + 0.1% formic acid + 0.1% isopropanol + 1 mM ammonium acetate. Mobile phase B MeCN + 0.1% formic acid + 0.1% isopropanol. | Electrospray ionization (ESI) interface operated in both negative and positive mode. Source temperature and desolvation temperature were set at 150 and 600 °C, respectively | (21) |
| Samples were extracted with a mixture of acetonitrile/water (84:16, v/v) | clean up through a polymeric solid‐phase extraction column | aflatoxins (B1, B2, G1, G2), ochratoxin A, deoxynivalenol, zearalenone, T‐2 and HT‐2 toxins | Cereal based foods | QTrap MS/MS system equipped with an electrospray ionisation (ESI) interface and a 1100 series micro‐LC system comprising a binary pump and microautosampler from Agilent Technologies | Separation by Kinetex C18 (100 mm × 2.10 mm, 2.6 µm particle size) preceded by a KrudKatcher Ultra in‐line filter (0.5 µm porosity × 0.004" i.d | ESI interface was used in negative and positive ion mode, with the following settings: temperature (TEM) 350°C; curtain gas (CUR), nitrogen, 30 psi; nebulizer gas (GS1), air, 10 psi; heater gas (GS2), air, 30 psi; ion spray voltage +4500 V or −4500 V, respectively. | (22) |
| 10-mL sample of each beer-based drink was injected into a  50-mL polypropylene centrifuge tube after being degassed by  sonication for 15 min. 10 mL of acetonitrile was added | Sample was cleaned by passing it through an  InertSep C18 cartridge previously conditioned with 5 mL of  acetonitrile, followed by passing another 5 mL of acetonitrile  through the cartridge and by collecting it in a test tube. | PAT, NIV, DON, AFG2, AFM1, AFG1  AFB2, AFB1, HT-2,T-2, ZON, FMB1, FMB2  FMB3, OTA | Beer based drinks | UHPLC/MS/MS analysis was performed on an ACQUITY  UPLC system coupled with a Quattro PremierTM XE tandem  quadrupole mass spectrometer | ACQUITY UPLC BEH C18 (1.7 μm,  2.1 × 50 mm)  40°C  0.5 mL/min  Solvent A: water  Solvent B: 2 vol% acetic acid–0.1 mM  ammonium acetate–methanol | electrospray-ionization (ESI) source was operated in both  positive and negative modes. The operating parameters were:  capillary voltage, 3.0 kV (positive mode) and 2.8 kV (negative  mode); ion source temperature, 120°C; desolvation temperature,  450°C; cone gas flow, 50 L/h; desolvation gas flow, 800 L/h  (both gases were nitrogen) | (23) |
| Shaking with 25g sample in methanol/water (75/25). Centrifugation, dilution then filtration. | Claen up using a DZT Multi Myco Immunoaffinity column | deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2) and zearalenone (ZEN) | Wheat and biscuit | An Agilent 1200 series LC coupled to Agilent 6410 triple quadrupole mass spectrometer equipped with the ESI . | Separation carried out with 100 mm  ×  2.1 mm I.D. column packed with 1.8 μm ZORBAX Extend C18, solvent A is water containing 10 mM ammonium acetate and a solvent B is acetonitrile. | Ionisation mode ESI+, capillary 4 kV, Fragmentor 100(V), desolvation temperature 350°C, desolvation gas flow 10L/min, nebuliser gas pressure 345 (kPa) | (24) |
| matrix solid-phase dispersion (MSPD) method of extraction |  | AFB1, AFB2, AFG1, AFG2, OTA, ZEN, NIV, DON, DAS, FB1, FB2, BEA, T-2 and HT-2 toxin | Flour | Triple quadrupole mass spectrometry detector (QqQ) was equipped with an LC Alliance 2695 system that includes an autosampler and a quaternary pump coupled toQqQ mass spectrometer Quattro LC from Micromass | Separation was attained on a Phenomenex (Madrid, Spain) Gemini-NX C18 (150 mm × 4.6 mm I.D., 5 μm particle size) analytical column, preceded by a security guard cartridge C18 (4 mm × 2 mm I.D.), using a gradient that started at 100% of A (5 mM ammonium formate and 0.1% of formic acid in water) and 0% of B (5 mM ammonium formate in methanol) | Parameters were optimized in positive (ESI+) and negative (ESI−) ionization mode The ESI source values were capillary voltage, 3.50 kV positive ionization mode and 3 kV negative ion mode; extractor, 1 V; RF lens 0.5 V; source temperature, 120 °C; desolvation temperature, 400 °C; desolvation gas (nitrogen 99.99% purity) flow, 800 l h−1; cone gas 50 l h−1 | (25) |
| 10 g of sample were extracted with 50 mL Solvent A methanol/water/1 M ammonium formate/formic acid (95:4.9:0.1:0.002, v/v/v/v), centfrigued and filtered | crude extract was  cleaned through a MultiSep®226 column (flow rate of 1 mL/min, assisted by vacuum) | AFB1, B2, G1 and G2, FB1, FB2, OTA, DON,  ZEA, T-2, and HT-2 | Animal feed | Quaternary Surveyor HPLC system coupled to a TSQ Quantum Discovery triple  quadrupole mass spectrometer with an electrospray ionization (ESI) source | The HPLC column (Synergi Hydro-RP of 4 µm particle size and 150 × 2.0 mm  i.d) and the guard column (Security Guard cartridge 4.0 × 2.0 mm i.d) Water/methanol/1 M ammonium formate/formic acid (90:10:0.1:0.002,  v/v/v/v) (Eluent A); methanol/water/1 M ammonium formate/formic acid (95:4.9:0.1:0.002, v/v/v/v)  (Eluent B) | Quantification of each toxin performed in Selected Reaction Monitoring (SRM) mode. ESI run in both positive and negative modes | (26) |
| 5 g of ground samples weighed in a centrifuge tube (40 mL) and 10 mL of an acetonitrile/water solution (80/20, v/v) was added and the mixture was vortexed for 2 min. After that, the tubes were shaken on a rotary agitator for 10 min at 60 rpm. | 2 mL of the supernatant layer were taken and filtered through a Millex-GN nylon filter before injection | aflatoxins (AFB1, AFB2, AFG1 and AFG2), ochratoxin A (OTA), fumonisins (FB1 and FB2), deoxynivalenol (DON), T2 and HT2 toxins | Raw wheat, barley, sorghum and cereals derived products, pasta and semolina and breakfast cereals | ACQUITY UPLC™ system coupled to ACQUITY TQD tandem quadrupole mass spectrometer | BEH C18 column (100 × 2.1 mm), with 1.7 μm particle size, from Waters. Eluent A being methanol and Eluent B consisting of an aqueous solution of ammonium formate (5 mM). | Ionization source parameters were: capillary voltage 3.5 kV, extractor voltage 3 V, source temperature 120 °C, desolvation temperature 350 °C, cone gas flow 50 L/h and desolvation gas flow 650 L/h (both gases were nitrogen). | (27) |
| acetonitrile:water or methanol:water mixtures for extraction | Some samples run with clean up using IAC followed by SPE columns | (aflatoxins B1, B2, G1  and G2, fumonisins B1 and B2, ochratoxin A, deoxynivalenol, T-2 and HT-2 toxins and zearalenone | Maize | LC/MS/MS equipment not specified | HPLC columns containing  a C18 stationary phase. The mobile phases used were composed of MeOH:water  or ACN:water  with or without addition of salts, acids, or bases. | electrospray ionization (ESI) was used for all analysis | (28) |
| Samples (10 g) were first extracted with 40 mL of an organic extraction solvent (acetonitrile: water: acetic acid, 79:20:1) by shaking for 60 min | “No-clean up” sample preparation method as a fast, reliable and sensitive method. | aflatoxins (AFB1, AFB2, AFG1 and AFG2), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), fumonisins (FB1 and FB2), T-2 and HT-2 toxins | rice, wheat, oat, barley and maize-meal | Finnegan TSQ quantum ultra mass system | C18 column, 50 × 2.1 mm i.d., 1.9 μm particle size. The column temperature was kept at 30 °C. | The capillary voltage was 3 kV, and nitrogen was used as the spray gas. The source and desolvation temperatures were set at 120 and 400 °C, respectively. Ionization methods including electro spray-ionization (ESI) and atmospheric-pressure chemical ionization (APCI) in positive- and negative-ion modes | (29) |
| After homogenization of ground maize samples and the addition of internal standards, mycotoxins were extracted with acetonitrile/water/acetic acid (79:20:1, v/v/v). | Supernatents analysed directly | MON, moniliformin; DON, deoxynivalenol; NIV, nivalenol; 3‐ADON, 3‐acetyl‐deoxynivalenol; 15‐ADON, 15‐acetyl‐deoxynivalenol; ZEN, zearalenone; FB1 and FB2, fumonisins; BEA, beauvericin. | Maize | TSQ Quantum Ultra AM | reliable HPLC‐ESI‐MS/MS method Herebian et al., 2009 | reliable HPLC‐ESI‐MS/MS method Herebian et al., 2009 | (30) |
| Solid-liquid extraction of 2 g plant material with 20 ml MeCN/Milli-Q water (80:20, v/v) was carried out for 2 h on a SM 30 orbital shaker at 200 rpm | clean-up step using a Varian Bond Elut Mycotoxin® After extraction, the suspended material was filtered over a paper filter. For clean-up, all of the filtrate was passed through a Varian Bond Elut Mycotoxin® cartridge | 3-Acetyl-deoxynivalenol (3-AcDON), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1), citrinin (CIT), deoxynivalenol (DON), HT-2 toxin (HT-2), patulin (PAT), and T-2 toxin (T-2). Alternariol (AOH), alternariol monomethylether (AME), altenuene (ALT), beauvericin (BEA), diacetoxyscirpenol (DAS), ergocornine (ECO), fusarenone-X (FUS-X), neosolaniol (NEO), nivalenol (NIV;), sterigmatocystin (STC;), sulochrin (SUL;), tentoxin (TET;), verrucarin A (VERA;), zearalenone (ZEA;), α-zearalenol (α-ZOL; β-zearalenol (β-ZOL | Whole wheat | Varian 1200 L LC-MS instrument | Separated using a 125 mm × 2.0 mm i.d., 3 μm, Pyramid C18 column, with a 2.1 mm × 20 mm i.d. guard column of the same material. eluent A consisted of Milli-Q water/MeOH/acetic acid (89:10:1, v/v/v) and eluent B of Milli-Q water/MeOH/acetic acid (2:97:1, v/v/v). | ESI- mode were: needle voltage -4,000 V, nebulising gas (compressed air) 3.01 bar, drying gas (N2, 99.5%) 275°C and 1.24 bar, shield voltage -600 V. ESI+ mode: needle voltage +4,500 V, nebulising gas (compressed air) 3.01 bar, drying gas (N2, 99.5%) 275°C and 1.24 bar, shield voltage +600 V | (31) |
| The first extraction was performed with the fourfold amount (20 mL) of extraction solvent 1 (acetonitrile/water/formic acid, 80:19.9:0.1, v/v/v) for 60 min. Extraction done a second time with 20 mL extraction solvent 2 (acetonitrile/water/formic acid, 20:79.9:0.1, v/v/v) on the rotary shaker for 30 min |  | aflatoxin B1, B2, G1 and G2 (AFB1, AFB2, AFG1, AFG2) AFB1 aflatoxin M1); fumonisin B1 and B2 (FB1, FB2); ochratoxin A (OTA); patulin; deoxynivalenol (DON); and zearalenone (ZEN). The MLs for HT-2 and T-2 toxin (HT-2 and T-2) | Maize samples | 1290 series UHPLC system coupled to a 6490 Triple Quadrupole (QqQ) mass spectrometer | ZORBAX RRHD Eclipse Plus C18 (100 × 2.1 mm, 1.8 μm) separation was performed at 30 °C with a flow rate of 350 μL min−1. Eluent A was composed of water/formic acid (99.9:0.1, v/v) and eluent B of methanol/formic acid (99.9:0.1, v/v); both contained 5 mM ammonium formate. | Source settings in the positive and negative ionisation modes were as follows: gas temperature, 140 °C; gas flow, 16 L min−1; nebulizer, 25 psi; sheath gas temperature, 350 °C; sheath gas flow, 11 L min−1; capillary voltage, 4,000 V (pos.) and 3,000 V (neg.); and nozzle voltage, 0 V. | (32) |
| Water:methanol (25:75 v/v) | No further clean up | AFB1, AFB2, AFG2, OTA, ZEN, DON, FB1, FB2, T2, HT-2 | Wheat grain | Nexera X2 UHPLC (Shimadzu, Tokyo, JapanAPI 6500 hybrid triple quadrupole/linear ion trap mass spectrometer (Sciex, Concord, ON, Canada), equipped with a turbo-ion electrospray (ESI) ion source.) | equipped with 100 × 2.1 mm, 2.6 µm Kinetex C18 columnThe column was maintained at 40 °C and the injection volume was 2 µLThe mobile phase consisted of 2.5 mM ammonium acetate acidified with 0.1% acetic acid (A), and methanol (B). The methanol (B) concentration was raised gradually from 5% to 95% within 8 min, brought back to the initial conditions at 9 min, and allowed to stabilize for 3 min. The mobile phase was delivered at a flow rate of 0.4 mL/min. | Source temperature was set at 350 °C, ion-spray voltages at −4500 V (negative mode) and 5000 V (positive mode), curtain gas at 35 arbitrary units (au), nebulizer gas at 60 au, and turbo gas at 40 au. | (33) |
| acetonitrile/water/acetic acid (80:19:1, v/v/v) | cleanup by multiple immunoaffinity column. | AFB1, AFB2, AFG1, AFG2, OTA, ZEA, T-2 | Peanut, corn and wheat | Accela HPLC system coupled to a TSQ Quantum Ultra EMR (Thermo Fisher Scientific, USA) in a selected reaction monitoring (SRM) mod | C18 column (Hypersil Gold, 100 mm × 2.1 mm, 3.0 μm) at 35 °C. Elution A consisted of water with 0.05% formic acid while elution B was acetonitrile with 0.05% formic acid. The linear gradient elution program was as follows: 0–10 min, 15–100% B; 11–13 min, 100% B; 14–15 min, 100–15% B; 16–18 min, 15% B. The elution flow rate was set at 250 μL min−1 and 10 μL sample solution was injected into HPLC. | MS was obtained by a triple quadrupole coupled with electrospray interface (ESI) The conditions were set as the follows: spray voltage at 4.0 kV for ESI+ and −3.0 kV for ESI−, respectively; capillary temperature at 350 °C; sheath gas pressure (N2) at 30 units; auxiliary gas pressure (N2) at 5 units; collision gas (Ar) at 1.5 mTorr; scan time at 0.1 s. | (34) |
| Mycotoxins in milk were extracted and purified with a multi-mycotoxins AOZ immunoaffinity column in a single run, which made the matrix effect be negligible. The deliberate addition of small amount of acetonitrile was found to be beneficial for the extraction of mycotoxins from the complex milk matrix, especially for zearalenone and its derivatives. | the diluted sample was cleaned up through an AOZ immunoaffinity column at a flow-rate of about 1–2 drops per second. The column was washed with 20 mL pure water at a flow-rate of 1–2 drops per second. Mycotoxins were eluted with 3 mL methanol at a flow-rate of 2–3 s per drop | AFB1, AFB2, AFG1, AFG2, AFM1, AFM2, OTA, OTB, ZEN, α-zeralanol, β-zeralanol, α-zeralenol, and β-zeralenol. | Milk | The UHPLC/Q-Orbitrap system consisted of an U3000 UHPLC system coupled with a Q-Exactive Focus mass spectrometer | mobile phases A and B consisted of 0.1 mM ammonium formate, 0.01% formic acid, and 5% methanol in water (v/v) and methanol, respectively. The UHPLC column utilized was a Kinetex C18 core-shell column (100 mm × 2.1 mm, 1.7 μm) from Phenomenex. The flow rate was 0.3 mL min−1. The injection volume was 10 μL, and the total run-time was 12 min. | HESI MS parameters were as the following: spray voltage 4.0 kV, capillary temperature 250 °C, heater temperature 350 °C, sheath gas flow rate 45 arbitrary units (a.u.), auxiliary gas flow rate 10 a.u, and S-lens level 50.0. | (35) |
| Simple solid–liquid extraction method successfully applied by Beltrán et al., (Beltrán et al., 2013) for solid matrices was adapted for these liquid matrices. To specify, the QuEChERS method involved mixing 250 μL of plant-based beverage with 1 mL of ACN 0.1% HCOOH, which was then shaken for 20 min, and centrifuged at 4000 rpm for 10 min, |  | AFB1, AFB2, AFG1,AFG2 OTA,DON, ZEA, T-2, HT-2, FB1 FB2 | Soy, oat and rice plant beverages | Agilent 1290 Infinity LC Series coupled to a 6495 iFunnel Triple Quadrupole MS/MS with an electrospray ionisation (ESI) interface, positive ion mode. | Chromatographic separation was performed using a Cortecs UHPLC C18 column (100 mm × 2.1 mm, 1.6 μm) from Waters (Wexford, Ireland). | parameters were a capillary voltage of 4000 V for aflatoxins and 3500 V for the rest of compounds, desolvation gas flow and temperature of 18 L min−1 and 160 °C, nebulizer pressure of 35 psi, nozzle voltage of 500 V, fragmentor voltage of 380 V, cell acceleration voltage of 5 V, and sheath gas flow of 11 L min−1 and 350 °C. | (36) |
| Samples were milled with a blender and 2 g was weighted and placed into 50 mL polypropylene centrifuge tubes. 10 mL of an extraction solvent composed by a mixture of acetonitrile/water (84:16, v/v) were added. The tubes were shaken using a horizontal shaking device (IKA KS 260 basic) (250 shakes/min) for 1 h. | - | DON, 15AcDON, NEO, NIV, HT2, FB1, OTA, ENA, ENA1, ENB and ENB1 | Barley | LC Agilent 1200 using a binary pump and an automatic injector, and coupled to a 3200 QTRAP®ABSCIEX equipped with a Turbo-VTM source (ESI) interface. | conducted at 25 °C with a reverse phase analytical column Gemini®C18 (3 μM, 150 × 2 mm ID) and a guard column C18 (4 × 2 mm, ID; 3 μM). Mobile phase was a time programmed gradient using methanol (0.1% formic acid and 5 mM ammonium formate) as phase A, and water (0.1% formic acid and 5 mM ammonium formate) as phase B. | The mass spectrometer was operating in electron impact ionization (EI, 70 eV). The source and transfer line temperatures were 230 °C and 280 °C, respectively. The collision gas for MS/MS experiments was nitrogen, and the helium was used as carrier gas at fixed pressure of 20.3 psi, both at 99.999% purity | (37) |
| Sample (12.5 g) was weighed and placed in 100 mL flasks after being ground  into powders by a food grinder. 50ml of ACN containing acetic  acid (ACN: water: acetic acid = 79.5:20.0:0.5; v:v:v) as a selected solvent was added to it, and the  13 toxins were extracted by shaking at 320 rpm for 1 h | 5ml of each filtrate  was diluted with 75 mL of PBS (pH 7.4) and then filtered through a GF/A filter paper. 65mls of the filtrate was loaded onto an IAC (Myco6in1+ column, and passed through at a flow rate of 1-2 drops/sec. The column  was washed with 10 ml of PBS and distilled water until 2–3 mL of air passed through it, and toxins  were eluted from the column with 5 mL of 80% MeOH containing 0.5% acetic acid | (deoxynivalenol, nivalenol, 3-acetylnivalenol, aflatoxin  B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, fumonisin B1, fumonisin B2, T-2, HT-2, zearalenone, and  ochratoxin A) | Cereal grains | HPLC (1260 series, Agilent, Santa Clara, CA, USA) equipped with a AB SCIEX QTRAP mass  spectrometer | a Scherzo SM-C18 column (3 mm × 150 mm, 3 µm particle  size; Imtakt, Kyoto, Japan). The two elution solutions used were (A) 0.1% formic acid in water  containing 2 mM ammonium acetate and (B) 0.1% formic acid in MeOH containing 2 mM ammonium  acetate. The solutions were pumped at a flow rate of 0.5 mL/min and a gradient elution program was  applied as shown in Table 2. The injection volume of the samples was 10 µL. | s operated in the positive ESI (electrospray ionization) mode  with MRM (multiple reaction monitoring) at unit resolution. The main MS parameters were optimized  and finally set as follows: curtain gas, 20 psi; collision gas (CAD), medium; capillary temperature  (TEM), 500 ◦C; ion spray voltage, ± 4500 V; ion source gas 1 (GS1), 50 psi; ion source gas 2 (GS2), 50 psi;  interface heater (ihe), on. Nitrogen was used | (38) |
| Infant cereals (5.00 ± 0.01 g) were weighed into 50 mL polypropylene tubes. 20 mL of acidified acetonitrile (acetonitrile/water/  formic acid, 80:19.9:0.1, v/v/v) was added, and the tubes were then shaken on a horizontal shaker for 60 min at room temperature | After extraction, the tubes were centrifuged for 5 min (3500 rpm). An aliquot (80 µL) of the centrifuged extract was transferred into an  HPLC vial with a microinsert and 20 µL of the [13C]-labeled working  solution was added. Each cereal sample was analyzed in triplicate. A 10 µL injection of the  sample extract was made into the UHPLC-MS/MS system | aflatoxins B1, B2, G1, G2, ochratoxin A, fumonisins B1 and B2, zearalenone,  22 deoxynivalenol, T-2 toxin, and HT-2 | Infant cereals: Rice, Barley, oats and mixed grains | An Agilent 1290 Infinity UHPLC system with an Agilent 6460 Triple  200 Quadrupole LC-MS/MS with Jet Stream Technology. | C18 (100 mm × 2.1 mm, 1.8 µm) column was used for chromatographic separation of the mycotoxins. Mobile phase A was composed of 0.1% formic acid in water and mobile phase B  was composed of 0.1% formic acid in methanol, and both eluents contained 5 mM ammonium  formate. The column temperature was kept at 35°C and the flow rate was 0.3 mL/min. | In positive electrospray ionization mode (ESI+), the gas temperature was 300°C and the flow rate  was 10 L/min. The nebulizer was set at 45 psi, and sheath gas temperature and flow rate were  10 350°C and 11 L/min, respectively. The capillary and nozzle voltages were 3500 V and 0 V. | (39) |
| 10g of sample were weighed into a blender jar and extracted with 40 mL of water by blending at high speed for 2 min. Then 60 mL of methanol was added to the sample (without removing the first extract) and extracted again by high speed blending for 2 min. The extract was filtered through paper filter (Whatman N.4). 5mls (0.5 g sample) of filtered extract were evaporated under air stream at about 40–50 °C to reduce the volume to approximately 2 mL. Then 5 mL PBS was added | The samples were passed through the Myco6in1+™ column. After drying the column by a gentle air flow, toxins were eluted with 3 mL water and 2 mL methanol Afterwards 2 mL water were passed through the column and collected in the same tube. The eluate was dried under air stream at 40 °C and reconstituted with 200 μL of methanol/water 20:80. 20 μL were injected. | aflatoxins (B1, B2, G1, G2), ochratoxin A, fumonisins (B1, B2), zearalenone, deoxynivalenol, nivalenol, T-2 and HT-2 | Cereal and cereal food samples (wheat and maize, corn flakes and maize snacks) | LC–MS/MS analyses were performed on a QTrap MS/MS system, from Applied Biosystems (Foster City, CA, USA), equipped with an ESI interface and a 1100 series micro-LC system comprising a binary pump and a microautosampler from Agilent | The analytical column was a Gemini® C18 column (150 mm × 2 mm, 5 μm particles) (Phenomenex, Torrance, CA, USA), preceded by a Gemini C18 guard column (4 mm × 2 mm, 5 μm particles). | The ESI interface was used in negative and positive ion mode | (40) |
| mixture of solvents for extraction (79:20:1, v/v/v, acetonitrile/water/acetic acid) | Then, defatting of the obtained crude extracts with hexane was introduced into the sample preparation procedure in order to remove the lipids that might interfere with the mycotoxin analysis by UHPLC–HESI-MS/MS. Such prepared crude extracts of the samples were used for further analysis without any purification step. | aflatoxins B1, G1, B2 and G2, ochratoxin A (OTA), zearalenone (ZEA), HT-2 toxin, T-2 toxin and fumonisins B1 and B2 | Nuts;  Walnut (*Juglans*)  Hazelnut (*Corylus avellana*)  Peanut (*Arachis hypogaea*)  Almond (*Prunus dulcis*) | ultra-high performance liquid chromatography (UHPLC) performed by Accela with a triple quadrupole mass spectrometer (MS/MS) TSQ Vantage equipped with heated-electrospray ionization probe HESI-II (Thermo Fisher Scientific). | Hypersil GOLD™, 50 mm × 2.1 mm i.d., 1.9 μm column (Thermo Fisher Scientific) was used with a flow rate of 0.5 mL/min, and the column temperature was maintained at 25 °C. The injection volume was 10 μL. The mobile phase consisted of eluent A containing water/acetic acid (99:1, v/v), and eluent B consisting of methanol/acetic acid (99:1, v/v). Both eluents contained 5 mM ammonium acetate | Parameters of the ion source were as follows: spray voltage: 3.4 kV, vaporizer temperature: 350 °C, sheath gas pressure: 40 arbitrary units, auxiliary gas pressure: 10 arbitrary units, and capillary temperature: 270 °C. | (41) |
| 2.0 g were macerated with 10 mL of acetonitrile/water (84:16, v/v) for 5 min, and then ultrasonicated for 40 min at 35 °C. After centrifugation at 4000 × g for 5 min, an aliquot of 5 mL of the supernatant was collected and dried by nitrogen gas at 50 °C. The residues were firstly re-dissolved in 0.5 mL acetonitrile/water (20:80, v/v) and then diluted with 4.5 mL water. | sample solution was passed through the MWCNTs-packed SPE cartridges, and then the cartridge was washed first with 5 mL of n-hexane, subsequently with 5 mL of methanol/water (5:95, v/v) | T-2 toxin (T-2), HT-2 toxin (HT-2), diacetoxyscirpenol (DAS) and neosolaniol (NEO) | Maize, Rice and Wheat | Waters Acquity UHPLC system. MS/MS detection was carried out on a Waters XEVO TQ-S mass spectrometer | Separation was performed on an Agilent Poroshell EC-C18 column (100 mm × 3.0 mm, 2.7 μm; Agilent, USA) at 40 °C with a mobile phase flow rate of 0.4 mL/min. The mobile phase consisted of water containing 5 mmol/L ammonium acetate (A) and methanol (B) | electrospray ionization source operated in positive ionization mode (ESI+). The source parameters were set as follows: source temperature of 150 °C; desolvation temperature of 500 °C | (42) |
| Finely ground and homogenized 2.0 ± 0.05 g of MEA was weighed in a 50 mL extraction tube (prior to weighing, agar was smashed in to fine pieces using a spatula). For method validation, MEA was spiked with a mixture of mycotoxin standards at different concentrations. A fixed concentration (50 μg/kg) of ZAN internal standard (IS) was added. The samples were left for an hour in the dark for equilibration. Thereafter, 10 mL of the extraction solvent (MeCN/1% formic acid (v/v)) | the supernatant was filtered using a folded filter paper (Whatman® Schleicher & Schuell® qualitative filter paper, grade 595 ½: 4–7 μm) into a new extraction tube. The filtrate was evaporated under N2 at 40 °C. The residue was reconstituted in 200 μL of the injection solvent (mobile phase A/B, 60/40 (v/v) mL as | deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), neosolaniol (NEO), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), fumonisin B1 (FB1), fumonisin B2 (FB2), HT-2 toxin (HT-2), alternariol methyl ether (AME), zearalenone (ZEN), sterigmatocystin (STERIG) and zearalanone (ZAN) were purchased | Black pepper | Liquid chromatography was performed using a waters ACQUITY ultra-performance liquid chromatography (UPLC) system. Mass spectrometry (MS/MS) was performed with a Quattro Premier XE tandem quadrupole mass spectrometer (Waters) | Mobile phase A was MeOH/water (20/80 v/v) and mobile phase B was MeOH/water (90/10 v/v), both contained 5 mM ammonium formate and 0.1% formic acid. | operated at electrospray ionization in positive mode (ESI+). | (43) |
| QuEChERS extraction procedure without any clean-up step  The fresh eggs were first thoroughly mixed manually, and then 1.0 ± 0.05 mL of the homogenized egg sample was transferred into a 50 mL polypropylene centrifuge tube. After adding 4 mL water into the tube, the tube was vortexed for 1 min. Then 5 mL of acidified ACN (1% FA) was added and the system was subjected to extraction using a horizontal electric shaker for 30 min at 120 rpm. MgSO4 (2.0 g) and NaCl (0.5 g) were subsequently added into the solution |  | aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, aflatoxin M2, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, de-epoxy-DON, zearalenone, α-zearalenol, β-zearalenol, α-zearalanol, and β-zearalanol | Eggs | Shimadzu LC-MS-8030 Tandem Quadrupole LC–MS/MS equipped with a Shimadzu LC-30 AD Nexera Liquid Chromatography, a Shimadzu SIL-30 AC Nexera autosampler, and a Shimadzu CTO-20 AC Prominence Column Oven | The analytical column was a C18 column (Agilent Poroshell 120, 100 mm × 3.0 mm, 2.7 μm). The mobile phase consisting of the eluent A (water with 5 mM AA) and eluent B (MeOH) was used at a flow rate of 0.2 mL/min. A 15 min linear gradient program was applied | Mass spectrometry was set at both positive and negative electrospray ionization mode (ESI), with selected reaction monitoring | (44) |
| A 5-g sample was extracted with 20 mL of an acidified acetonitrile/water mixture | Described in (Sulyok et al. 2006 Sulyok M, Berthiller F, Krska R, Schuhmacher R. 2006. Rapid Commun Mass Sp. 20:2639–2649. | aflatoxins, trichothecenes, fumonisins, ZEN, OTA and two ergot alkaloids | Corn, Rice, Cassava | 1290 Series HPLC System by Agilent, coupled with QTrap 5500 LC-MS/MS System equipped with Turbo Ion Spray electrospray ionisation (ESI) source | Chromatographic separation was performed at 25°C on a Gemini® C18-column, 150 × 4.6 mm i.d., 5 μm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge | scheduled multiple reaction monitoring (MRM) mode both in positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte. | (45) |
| Samples were then extracted with 2 mL of acetonitrile/  water/acetic acid 79:20:1, v/v/v for 90 min on a rotary shaker (GFL) and subsequently centrifuged for 2 min  at 3000 rpm | The extracts were transferred into glass  vials using Pasteur pipettes, and 350 pL aliquots were  diluted with the same volume of acetonitrile/water/acetic  acid 20:79:1, v/v/v. Finally, 5 pL of the diluted extract  was injected into a LC-MS-MS system without further  pre-treatment. | AFB1, AFB2, AFG1, AFG2, OTA, DON, Nivalenol, T-2, HT-2, ZEN | Peanut, Marzipan, Raisin, Bran, Hot pepper, Coffee, Liquorice, Pistachio | QTrap 5500 MS/MS system  (Sciex) equipped with a TurboV electrospray ionization  (ESI) source and a 1290 series UHPLC system (Agilent  Technologies) | Chromatographic separation was  performed at 25 °C on a 150 x 4.6 mm, 5-pm Gemini  C18-column equipped with a C18 security guard  cartridge, 4 x 3 mm i.d. | ESI-MSMS  was performed in the scheduled multiple reaction  monitoring (sMRM) mode both in positive and negative  polarities | (46) |
| One gram of ground maize silage was placed into a 50 ml polypropylene conical centrifuge tube and treated with 10 ml of MeCN/water (84:16 v/v) containing 1% acetic acid. The mixture was shaken for 90 min | clean-up step with Mycospin 400 Multi-toxin columns | HT-2 Toxin (HT-2), zearalenone (ZEA), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) Penicillic acid, verruculogen (VERR), T-2 toxin (T-2) and roquefortine C (ROQ-C) aflatoxin B1 (AFB1) and aflatoxin G1 (AFG1) each ochratoxin A (OTA) Fumonisin B1 (FB1) and Fumonisin B2 (FB2) enniatin B, enniatin B1, sterigmatocystin, α-zearalenol and β-zearalenol. | Maize silage | Thermo Fisher Scientific instrument consisting of a Dionex Ultimate 3000 pump and an Accela autosamplerand a Quantum Access triple quadrupole mass spectrometer equipped with a heated electrospray ionisation (HESI) source. | Twenty millilitres of standard solution or silage extract were injected into the Kinetex C18 column (100 × 2.1 mm, 2.6 µm) (Phenomenex, Torrance, CA, USA). The column and the sample temperature were maintained at 25°C and 10°C, respectively. water (mobile phase A) and methanol (mobile phase B), both with 3 mmolL−1 ammonium formate, 0.1% formic acid | The HESI-MS/MS interface was working with the following parameters: sheath gas (38 au (arbitrary unit)), auxiliary gas (5 au), skimmer offset (4 V), capillary temperature (350°C) and ion sweep cone gas (3 au); all in both positive and negative modes. | (47) |
| 3g of homogenized sample was weighed into a 50 mL centrifuge tube. Ten mL of MeCN was added. The sample and solvent mixtures were allowed to mix in an automated shaker at 180 rpm for 10 min. Then, the sample was centrifuged at 3700 rpm for 2 min. | Three mL of supernatant was transferred to a glass tubes. The extract was evaporated under nitrogen stream at 40 °C water bath until dry. The residue was reconstituted with 1 mL MeOH (50%) before filtered through nylon filter 0.2 μm into vials. The extract was diluted to 1:2 before injected to LC-MS/MS. | aflatoxins (AFs), ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZEA) | Vegetable oil | Chromatography analyses were performed using the Thermo Finnigan TSQ quantum ultra-mass system connected to Finnigan Surveyor MS pump, Surveyor auto-sampler and TSQ Quantum Discovery TM mass spectrometer. | 30 °C on Phenomenex Gemini C18 110A separation column, 150 × 3.0 mm i.d., 5 μm particle size, equipped with a Phenomenex Gemini C18 4 × 3.0 mm i.d. security guard cartridge | The capillary voltage was 4 kV, source and desolvation temperatures were 120 °C and 400 °C respectively. Nitrogen and argon gases were used as the spray gas and collision gas respectively. Mass optimization for each analyte was performed in ESI by switching the polarity of the ion source to positive and negative mode. | (48) |
| 5g of sample was extracted with acetonitrile/water/acetic acid (79/20/1,  v/v/v). The supernatant was passed through a preconditioned C18-SPE column, and the extract was defatted | , 12.5 mL of the defatted extract was added to 27.5 mL of acetonitrile/acetic acid (99/1, v/v),  and passed through a MultiSep®226 AflaZon+ multifunctional column. In the second pathway, 10 mL  of defatted extract was filtered using a glass microfilter. 2ml of the filtered extract were  combined with the Multisep226 eluate and evaporated to dryness. | fumonisin  B1, B2, and B3; DON; 3-acetyl-DON (3ADON); 15-acetyl-DON (15ADON); DON-3G; ZEN;  α-zearalenol (α-ZEL); β-zearalenol (β-ZEL); ZEN-14G; NIV; FUS-X; T-2 toxin (T-2); HT-2 toxin (HT-2);  diacetoxyscirpenol (DAS); and neosolaniol (NEO) | maize, sorghum, millet | Waters Acquity UPLC system coupled to a Micromass Quattro Micro triple-quadrupole mass  spectrometer | The column  was a Symmetry C18 (150 mm × 2.1 mm i.d. 5 µm) column & guard column (10 mm × 2.1 mm  i.d.) of the same material and was kept at room temperature. The injection  volume was 20 µL. Mobile phase consisting of water/methanol/acetic acid (94/5/1, v/v/v) and 5 mM  ammonium acetate (mobile phase A), and methanol/water/acetic acid (97/2/1, v/v/v) and 5 mM  ammonium acetate (mobile phase B) were used at a flow rate of 0.3 mL/min | The mass spectrometer  was operated using selected reaction monitoring (SRM) channels in positive electrospray ionization  (ESI+) mode. | (49) |
| Sample extraction method was based on QuEChERS method (Lacina et al., 2012). The quantification was achieved by interpolation from a standard curve prepared by spiking the blank matrix samples at 7 different levels with a mixture of mycotoxins before extraction. |  | aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), fumonisin B1 (FB1), fumonisin B2 (FB2), deoxynivalenol (DON), ochratoxin A (OTA) and zearaleneone (ZEN) | Maize based Porridge | Waters Acquity UPLC coupled to a Xevo TQ-S triple quadrupole mass spectrometer. | As used in study (Oplatowska-Stachowiak et al., 2015). |  | (50) |
| Finely ground and homogenized sample (1.0 g) were weighted into a 10-mL centrifuge tube, and 4 mL of methanol-water-formic acid (79:20:1, v/v/v) was added, then shaken by vortex for 2 min. Afterward, the mixture was extracted by ultrasonication in an ultrasonic bath at 500 W for 20 min. The extraction was centrifuged at 10,000 rpm for 10 min. | 1 mL of the supernatant was transferred to a 2-mL eppendorf tube and evaporated to dryness under a stream of nitrogen gas at 40 °C. The residue was re-dissolved with 0.5 mL methanol-water (50:50, v/v). After being mixed by vortex for 15 s, the sample extraction was passed through a 0.22 μm syringe filter. 2 μL of the filtrate was injected into the UFLC–MS/MS system | AFB1, AFB2, AFG1, AFG2, OTA, FB1, FB2 and ZEA | Chinese Yam | ultra-fast liquid chromatography (UFLC) system (Shimadzu, Kyoto, Japan) coupled to an Applied Biosystem 5500 QTRAP® mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with electrospray ionization (ESI). | Chromatographic separation of analytes was performed on a SHISEIO Capcell core C18 column (50 × 2.1 mm, 2.7 μm; Shiseio, Japan). 0.1% formic acid in acetontrile (solvent A) and 0.1% formic acid in aqueous (solvent B) were selected as the mobile phase. | Detection was operated at ESI in both positive and negative ionization modes. | (51) |
| Blended to a powder-like consistency. 1.0g was weighed into a 50-mL centrifuge  tube and 5 mL of water was added and mixed. To this suspension  was added 5 mL of 10% (v/v) HOAc in ACN and vortexed for  1 min. Four salts (2.0 g anh. MgSO4, 0.50 g NaCl,  0.50 g sodium citrate tribasic dihydrate and 0.25 g sodium citrate dibasic  sesquihydrate) were added into the mixture and then vigorously  shaken by hand for 1 min | 2 mL of supernatant was transferred into a 15-mL  centrifuge tube containing 300 mg anh. MgSO4, 50 mg C18, 25 mg  PSA and 25 mg silica, shaken and centrifuged. 1 mL of the supernatant evaporated to dryness under nitrogen gas. An aliquot was reconstituted in 1 mL of  water with a 1:1 (v/v) ratio of 0.1% (v/v) FA:MeOH and 0.5 µg/L  of an SMX IS. Then filtered through  0.22-µm PTFE syringe filters | AFB1, AFB2, AFG1, AFG2, FB1,  OTA, ZON and DON | Brown Rice | A Model 1290 UHPLC system (Agilent Technologies, CA, USA), consisting  of a vacuum degasser, binary pump, Agilent jet weaver, autosampler  and a column oven. A Model 6490 (Agilent Technologies) triple quadrupole mass analyzer  with electrospray ionization (ESI) and MassHunter software processing  was used | The UHPLC  column used was an ACQUITY UPLC® HSS T3 C18, 100 × 2.1 mm ID, 1.8 µm (Waters, MA, USA), with a mobile-phase flow rate of  0.3 mL/min. The column temperature was controlled at 40 °C. Standard  solutions or extracted solutions were injected at 10 µL. Mobile  phase A was 0.5% (v/v) FA in water containing 5 mM ammonium formate,  and mobile phase B was MeOH | ESI was operated in both the positive and negative  modes with multiple-reaction monitoring (MRM) 3,000 V capillary  voltage, 1,000 V nozzle voltage, 16 L/min gas flow, 150 °C gas temperature,  20 psi nebulizer pressure, 11 L/min sheath gas flow, 400 °C  sheath gas temperature, 380 V fragmentor and a 50 ms dwell time. | (52) |
| 3 procedures: Acetate QuEChERS, dilute and shoot, dilute and shoot clean up |  | aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, deoxynivalenol, fumonisin B1, fumonisin B2, HT-2 toxin, ochratoxin A, T-2 toxin and zearalenone | Green tea and royal jelly | Transcend 600 LC (Thermo Scientific Transcend™, Thermo Fisher Scientific, San Jose, CA, USA) coupled to a single stage Orbitrap mass spectrometer (Exactive™, Thermo Fisher Scientific, Bremen, Germany) operating with a heated electrospray interface | A mixture of an aqueous solution of ammonium formate 4 mM and formic acid 0.01% (v/v) (eluent A) and methanol with ammonium formate 4 mM and formic acid 0.01% (v/v) (eluent B) was used as mobile phase at a constant flow rate of 0.3 mL/min. The column used for the separation of the compounds was a Hypersil GOLD aQ column (100 × 2.1 mm, 1.9 μm particle size). Column temperature was set at 30 °C and the injection volume was 10 μL. | heated electrospray interface (HESI-II), in positive (ESI+) and negative ionization mode (ESI−) | (53) |
| Wheat samples (250g) were previously finely milled with a blender. Representative sub-samples of 2 g each were weighed and placed into 50 mL polypropylene centrifuge tubes and 10 mL of an extraction solvent composed by a mixture of acetonitrile/water/formic acid (84:15:1 v/v/v) were added. | Residue was reconstituted to a final volume of 1 mL with methanol/water (70/30) (v/v) and filtered through a 13-mm/0.22 μm nylon filter purchased from Análisis Vínicos S.L. (Tomelloso, Spain) before LC–MS/MS analysis. | AFB1, AFB2, AFG1, AFG2, OTA, STG, ZEN, NIV, DON, 3-AcDON, 15-AcDON, DAS, NEO, T-2 and HT-2 toxin, FB1, FB2, FB3, BEA, ENNs (A, A1, B, B1), AOH, AME, and TEN | Durum wheat samples | LC Agilent 1200 using a binary pump and an automatic injector, and coupled to a 3200 QTRAP® AB SCIEX (Applied Biosystems, Foster City, CA) equipped with a Turbo-V™ source (ESI) interface. | separation of the analytes was conducted at 25 °C with a reverse phase analytical column Gemini® C18 (3 μM, 150 × 2 mm ID) and a guard-column C18 (4 × 2 mm, ID; 3 μM) Mobile phase was a time programmed gradient using water (0.1% formic acid and 5 mM ammonium formiate) as phase A, and methanol (0.1% formic acid and 5 mM ammonium formiate) as phase B. | 3200 QTRAP® System AB SCIEX was used as triple quadrupole mass spectrometry detector (MS/MS). Used in positive mode to analyse the 26 mycotoxins with Source/Gas Parameters: Vacuum Gauge (10e−5 Torr) 2.7, curtain gas (CUR) 20, ionspray voltage (IS) 5500, source temperature (TEM) 450 °C, ion source gas 1 (GS1) and ion source gas 2 (GS2) 50. | (54) |
| Samples were extracted with acetonitrile-water (84:16, v:v, containing 1% acetic acid) using ultrasonic extraction. | The extracts were purified with a dispersive SPE method using C18 as a cleaning agent. The final clear extracts were dried by nitrogen blowing and subsequently redissolved in methanol-water (5:5, v:v). | aflatoxins (B1, B2, G1, G2), fumonisins (B1, B2, B3), zearalenone, Deoxynivalenol | Corn | A Waters Acquity UPLC instrument was used with Waters XEVO-G2  Q-TOF-MS fitted with an electrospray ionization (ESI) probe  operated in the positive ion mode. | Hypersil GOLD C18 column (1.9 µm, 2.1 mm×100 mm).  Sample room temperature and the column temperature  were set at 15 and 35°C, respectively. 0.1% formic acid in ammonium acetate-methanol as mobile phase | The mass spectra was acquired  over the mass range of 100 to 1 000 m/z: the detection was carried out in [M+H]+ mode, the source temperature  was fixed at 120°C, the capillary voltage was set  at 3.0 kv, the cone energy was 40 kv, the collision energy  was 4 ev, and the desolvation temperature was 400ºC. | (55) |
| sample (2.0 g) was accurately weighed into a 50 mL centrifuge tube. After maceration with 10 mL of acetonitrile/water (84/16, v/v) for 5 min, the sample was ultrasonicated for 40 min and then centrifuged at 4000 g for 5 min. | An aliquot (5 mL) of supernatant was collected for M-SPE purification. The supernatant was first dried by nitrogen gas at 50 °C, and re-dissolved with 5 mL of acetonitrile/water (5/95, v/v). Then, 20 mg of MWCNT-MNPs were added. The mixture was vortexed for 3 min to enable the targeted mycotoxins to interact with and be adsorbed on MWCNT-MNPs. | ZEA, α-ZOL, β-ZOL, ZAN, α-ZAL and β-ZAL | Maize | UHPLC was performed via a Waters Acquity UHPLC system and Waters XEVO TQ-S mass spectrometer with an electrospray ionization | Separation was achieved at 40 °C on a Poroshell EC-C18 column (100 mm × 3.0 mm, 2.7 μm) (Agilent, USA). The mobile phase consisted of (A) methanol and (B) water containing 5 mol/L ammonium acetate | source operated in negative mode (ESI−). The MS/MS conditions were set as follows: source temperature, 150 °C; desolvation temperature, 500 °C; cone gas flow, 30 L/h; desolvation gas flow, 1000 L/h. A multiple reaction monitoring (MRM) acquisition method was developed | (56) |
| one gram (1.0 g ± 0.1 g) of freeze-dried sample was accurately weighed into a 15 mL centrifuge tube. Samples were extracted by shaking with 8 mL of AcCN:H2O (80:20) 1% HCOOH on a mechanical wrist shaker for 30 min. | 4mls of supernatant were dried under a stream of nitrogen, redissolved with 400 μL of injection solution (mobile phase A:B, 50:50), and then centrifuged at 10,000 rpm for 10 min. | aflatoxins; ochratoxin A; deoxynivalenol; fumonisins; T-2 and HT-2 toxins; zearalenone; | Bread,pastapizza, rice wheat, cereals, and flours,breakfast cereals, biscuits, | A Waters UPLC system (Waters, Milford, MA, USA) was used to perform a reverse phase coupled with a Waters Quattro Premier XE TQ mass spectrometer | Kinetex Biphenyl column (50 mm × 3 mm i.d., 2.6 μm particle size) preceded by a SecurityGuardTM ULTRA Holder pre-column | ESI source operating in positive ionization mode (ESI+).Capillary voltage, source temperature, desolvation gas flow rate, and its temperature were set at 3 kV, 120 °C, 600 L h−1, and 350 °C, respectively. Collision-induced dissociation was performed using argon as collision gas at a pressure of 3.5 × 10−3 mbar in the collision cell. | (57) |
| 20ml of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) was added to five grams of maize sample weighed in a 50-ml polypropylene tube. Samples were extracted for 90 min on a GFL 3017 rotary shaker |  | fumonisin B1 (FB1) and fumonisin B2 (FB2). Aflatoxin B1 (AFB1) and aflatoxin G1 (AFG1) | Maize | QTrap 5500 LC-MS/MS System equipped with a Turbo Ion Spray electrospray ionisation (ESI) source and a 1290 Series HPLC System | Separation was undertaken at 25 °C on a Gemini C18-column, 150 × 4.6 mm i.d., 5 μm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge | ESI-MS/MS was performed in the time-scheduled multiple reaction monitoring (MRM) mode, both in positive and negative polarities, in two separate chromatographic runs per sample | (58) |
| (2.00 g) and placed into 50 mL PTFE centrifugal tubes, followed by the addition of 10 mL mixture acetonitrile/water (84:16, v/v). The tubes were stirred for 1 h at 300 shakes min−1 using a horizontal shaking device | 5 mL of the supernatant were placed in 15 mL PTFE centrifugal tubes and were evaporated to dryness at 35 °C with a gentle stream of nitrogen using a multi-sample Turbovap LV Evaporator. The residue was reconstituted to a final volume of 1 mL with methanol/water (50/50, v/v) and filtered through a syringe nylon filter. | ZEA, NIV, DON, 3AcDON, 15AcDON, DAS, NEO, T-2, HT-2, BEA ENs (A, A1, B, B1) | Wheat | LC Agilent 1200 using a binary pump and an automatic injector, and coupled to a 3200 QTRAP ® AB SCIEX equipped with a Turbo-V™ source (ESI) interface. | Separation of the compounds was conducted at 24°C on a reverse phase analytical column Gemini® C18 (3 μM, 150 × 2 mm ID) and a guard-column C18 (4 × 2 mm, ID; 3 μM) Mobile phase was methanol (0.1% formic acid and 5 mM ammonium formate) as phase A, and water (0.1% formic acid and 5 mM ammonium formate) as phase B. | source was used in positive mode to analyze the 14 mycotoxins with the following settings for Source/Gas Parameters: Vacuum Gauge (10e-5 Torr) 3.1, curtain gas (CUR) 20, ionspray voltage (IS) 5500, source temperature (TEM) 450 °C, ion source gas 1 (GS1) and ion source gas 2 (GS2) 50 | (59) |
| One mL of whole cow milk was added to four milliliters of ACN (2% HCOOH) and this mixture was mixed using a rotary agitator during 15 min. Next, the tube was centrifuged for 10 min at 5000 rpm, allowing separation of a supernatant. | 4ml of supernatant were separated and added to a tube with approximately 60 mg of sodium acetate. The tube was shaken for 10 min in a rotary agitator and then centrifuged for 10 min (5000 rpm). Next, 3.2 mL of the acetonitrile phase (upper phase) were separated and placed into another tube and evaporated at 65 °C until dryness. Finally, 200 μL of 40%B-mobile phase were added, and the tube was vortexed until the residue was dissolved (2 min). Once filtered (PVDF, 0.45 μm, 20 μL were injected for chromatographic analysis. | aflatoxins M1, B1, B2, G1 and G2, OTA and OTB, fumonisins B1, B2 and B3, DOM-1, T-2 and HT-2, ZEA and STC | Cow milk | liquid chromatograph 1200 series with degasser, binary pump, thermostated autosampler and column compartment coupled to a mass spectrometer (triple quadrupole), 6410 Agilent Technologies with an electrospray ionization source. | Ascentis Express C18, 150 × 2.1 mm, 2.7 μm particle size, fused core technology (1.7 μm solid core and a 0.5 μm porous shell) from Supelco | 4000 V in the capillary voltage, nitrogen was used as nebulizing and drying gas at 350 °C, at 9 L/min and 40 psi. Nitrogen was used in the collision cell. | (60) |
| 5 mL degassed beer, 10 mL acetonitrile and the internal standards were added The mixture was vortexed for 20 s and centrifuged at 4000 rpm for 5 min. The residue was extracted two more times with 3 mL acetonitrile/water (70/30, v/v), respectively. | The combined supernatants were evaporated to dryness. The residue was solved in 4 mL acetonitrile/water (84/16, v/v), vortexed for 20 s and completely applied on a Bond Elut Mycotoxin cartridge (500 mg, 3 mL, Agilent Technologies) | deoxynivalenol-3-glucoside, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyl-deoxynivalenol, HT2-toxin, T2-toxin, enniatin B, B1, A1, A, beauvericin and zearalenone |  | Shimadzu LC-20A Prominence system interfaced with a hybrid triple quadrupole/linear ion trap mass spectrometer (API 4000 QTrap;) | Hydrosphere RP-C18 column (150 × 3.0 mm2, S-3 μm, 12 nm, with a C18-guard column The binary gradient system consisted of (A) 0.1% formic acid and (B) methanol with 0.1% formic acid at a flow rate of 0.2 mL/min. | operated in the negative ESI mode for the analyte ZEA and in the positive ESI mode for the analytes D3G, DON, 3-ADON, 15-ADON, FUSX, HT2, T2, ENN B, B1, A1, A, and BEA. The ion source parameters for the negative mode were set as follows: curtain gas 20 psi, CAD gas pressure medium, ion spray voltage −4500 eV, spray gas 50 psi, dry gas 65 psi, and temperature 525 °C | (61) |
| QuEChERS extraction. 4 g of homogenous representative sample were weighted into the PTFE cuvette and 7.5 mL of 0.1% (v/v) formic acid and 10mL of MeCN were added. | After addition of 1 g of NaCl and 4 g of MgSO4, the mixture was shaken. The sample was centrifuged (5 min, 5000 rpm (1960 g)). The 0.5 mL aliquot of upper organic phase was diluted with deionized water in 1:1 (v/v) ratio. The sample solution was filtered through the 0.2 µm filter prior to instrumental analysis. | ochratoxin-A (OTA), aflatoxin B1 (AFB1), zearalenone (ZON), deoxynivalenol (DON), 3-and 15-acetyl-deoxynivalenol (3-AcDON and 15-AcDON), nivalenol (NIV), neosolaniol (NEO), fusarenon-X, (FUS-X), T-2 toxin (T-2), HT-2 toxin (HT-2), fumonisin B1 and B2 (FB1 and FB2), (ENA, ENA1, and ENB), beauvericin (BEA) | Durum wheat pasta | UHPLC, equipped with a degassing system, a Dionex Ultimate 3000 a Quaternary UHPLC pump working at 1250 bar, an auto sampler device, Q Exactive Orbitrap LC-MS/MS has been used. | Accucore aQ C18 column (100 × 2.1 mm 2.6 µm particle size), Injection volume was 5 µL. Eluent phase was formed as follows: phase A (H2O in 0.1% formic acid and 5mM ammonium formate), phase B (methanol in 0.1% formic acid and 5mM ammonium formate). | Ion source parameters in both ESI− and ESI+ mode were: spray voltage 3.50 kV, sheath gas (N2> 95%) 30, auxiliary gas (N2> 95%) 10, capillary temperature 320°C, S-lens RF level 50, auxiliary gas heater temperature 300°C. | (62) |
| 40 g slurry (1:1 w/v) or 50 g slurry (1:1.5 w/v) were mixed with 60 mL acetonitrile, and the mixture was shaken for 2 h in a horizontal shaker. | After filtration, 1 mL of the extract was taken and was diluted in 3 mL of water (in the case of 1:1 w/v slurry) or 2.55 mL (in the case of 1:1.5 w/v slurry). The solution was filtered using a 0.45-μm membrane syringe filter (Agilent, USA) and was directly injected into the UPLC-MS/MS system | Aflatoxins, Ochratoxin A, Zearalenone, Deoxynivalenol, Fumonisins, T-2 and HT-2 toxins, | nuts (peanuts, almonds and pistachio) and cereals (wheat and maize) | UPLC system (Acquity I-Class, Waters, Milford, MA, USA) coupled with a triple quadruple mass spectrometer (XEVO TQ-S, Waters Micromass, Manchester, UK) using an orthogonal Z-spray-electrospray interface (ESI) | Acquity UPLC BEH C18 analytical column (2.1 mm × 100 mm, 1.7 μm particle size, Waters), at a flow rate of 0.4 mL –1, with an injection volume of 10 μL. Mobile phase consisting of A (H2O, 0.1% HCOOH) and B (ACN, 0.1% HCOOH) was used. | ESI-MS/MS was performed in a multiple reaction monitoring mode (MRM) at positive polarity, at a capillary voltage of 3 kV, a desolvation temperature of 400°C, a source temperature of 150°C, a desolvation gas flow rate of 800 L-1 | (63) |
| Reagent-1, was added to 5g of sample and vortexed for 30 sec. Reagent-2 was  added and mixed for two min; then centrifuged  for five min at 4000 rpm. 5 ml was taken from upper phase  and transferred into a tube and evaporated under nitrogen. Reagent-3 was added into the tube, solved and filtrated through a 0.45 µm filter and put into LC MS/MS |  | aflatoxin B1, ochratoxin A, zearalenone, T-2 toxin,  HT-2 toxin, fumonisin and deoxynivalenol | Poultry feed | LC/MS/MS Zivak Technologies Turkey | ZV-reagent1-Mobile Phase A, ZV-reagent2-Mobil Phase B, 0,20 mL/min, ZV-1034-02C1 150x2  mm, HPLC Column, 50 psi API Nebulising gas pressure,  350 ºC drying gas temperature, 35 psi drying gas pressure,  0,5 min scanning time | 1800 V detector, | (64) |
| beer samples was done using a QuEChERS method5 g of beer samples were degassed by sonication for 10 min 2 g of MgSO4, and 20 mL of acetonitrile/acetic acid (99/1, v/v) were added. Beans and spices were extracted using 20 mL acetonitrile/water/acetic acid (79/20/1, v/v/v). | passed through a preconditioned C18 solid phase extraction (SPE) column and the eluent was collected into a 25 mL volumetric flask. The extraction was repeated by adding 5 mL of acetonitrile/water/acetic acid (79/20/1, v/v/v), | fumonisin B1 (FB1), fumonisin B2 (FB2), deoxynivalenol (DON), 3-acetyl-DON (3-ADON), 15-acetyl-DON (15-ADON), deepoxydeoxynivalenol (DOM), fusarenon-X (FUS-X), nivalenol (NIV), HT-2 toxin (HT-2) neosolaniol (NEO) zearalenone (ZEN) zearalanone (ZAN) α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL) Fumonisin B3 (FB3), Diacetoxyscirpenol (DAS) DON-3-glucoside (DON-3G) and T-2 toxin | Beans, spices and beers | Waters Acquity UPLC system coupled to a Micromass Quattro Micro triple-quadrupole mass spectrometer | C18 (150 mm × 2.1 mm i.d. 5 μm) column and a guard column (10 mm × 2.1 mm) of the same material. The mobile phases: water/methanol/acetic acid (94/5/1, v/v/v) and 5 mM ammonium acetate (mobile phase A), and methanol/water/acetic acid (97/2/1, v/v/v) and 5 mM ammonium acetate (mobile phase B) and were used at a flow rate of 0.3 mL/min | Mass spectrometer was operated with selected reaction monitoring (SRM) channels in positive electrospray ionization (ESI+) mode. The capillary voltage was 3.2 kV and nitrogen was used as desolvation gas. Source and desolvation temperatures were set at 150 °C and 350 °C, respectively. | (65) |
| QuEChERS approach)  With a spatula an amount of 10 g of slurry was transferred into a 50 mL PTFE centrifuge tube and weighed on an analytical balance | Without clean up | aflatoxins B1, B2, G1 and G2, and ochratoxin A; group 2 (G2): altenariol, altenariol-methyl, citrinin, cyclopiazonic acid (CPA), diacetoxyscirpenol (DAS), deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, fumonisin B1, B2 and B3, fusarenon-X, HT2-toxin, mycophenolic acid, penicilic acid, roquefortine C, sterigmatocystine, T2-toxin, zearalanone (ZAN), zearalenone (ZEN), α-zearalanol (α-ZAL), β-ZAL, α-zearalenol (α-ZEL); and the group 3 (G3): ergocornin, ergocristin, ergokryptin, ergonovin, ergosin, ergotamin, and mevinolin. | Coffee | Waters Acquity (UPLC) integrated to a Waters Quattro Premier XE mass spectrometer. | Waters BEH C18 (1.7 μm) analytical column (100 mm × 2.1 mm I.D.) with a 0.2 μm prefilter. mobile phase consisted of ultrapure water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B). Injection volume was 5 μL | (ESI) source operating in the positive ion mode. Data acquisition performed in the multiple reaction monitoring mode (MRM). temperatures of the source and desolvation gas, 100 °C and 450 °C. Nitrogen (N2) was used | (66) |
| QuEChERS technique reduced to a simple “salting-out liquid-liquid extraction” (SO-LLE) to obtain the most efficient extraction of themycotoxin classes in a very short time | QuEChERS-based extraction procedure was also optimized, in order to avoid an additional clean-up step. | AFB1, aflatoxins B2 (AFB2), G1 (AFG1), and G2 (AFG2), sterigmatocystin (STE), citrinin (CIT), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), HT-2 toxin (HT-2), T-2 toxin (T-2), ochratoxin A (OTA), nivalenol (NIV), DON, neosolaniol (NEO), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), roquefortine C (ROQ-C), deoxynivalenol-3-glucoside (DON-3-Glu), fusarenon-X (F-X), diacetoxyscirpenol (DAS), alpha zearalenol (α-ZOL), and ZEN, as well as the 6 major EAs (Em, Et, Ecr, Ekr, Eco, Es) and their epimers (ergotaminine (Etn), egometrinine (Emn), egocristinine (Ecrn), ergokryptinine (Ekrn), ergocroninine (Econ), and ergosinine (Esn). | Wheat and Maize samples | UPLC Waters Acquity system (binary solvent manager, auto sampler, and column heater units) The mass spectrometer measurements were performed on a triple quadrupole mass spectrometer with electrospray ionization (ESI), a Xevo TQ-S, also from Waters | ACQUITY HSS UPLC T3 (150 mm × 2.1 mm, 1.8 μm) column from Waters Corp. was used. mobile phase consisting of 0.3% formic acid aqueous solution with 5 mM ammonium formate (solvent A), and MeOH with 0.3% formic acid and 5 mM ammonium formate (solvent B) at a flow rate of 0.4 mL/min. | Mass spectrometer was operated in positive ESI mode under the multiple reaction monitoring (MRM) The ionization source parameters were source temperature 150 °C; nebulizer gas (nitrogen) 7 bar; source offset voltage +50 V; cone gas flow of 150 L/h and desolvation gas set to 400 °C, with flow at 1000 L/h. | (67) |
| Liquid-liquid extraction (LLE) commonly used for multi-residue analyzes in food products, and adapted from Mol et al. (2008). Second sample treatment tested, named dilute and shoot (D&S), comes from metabolomic approaches (Tengstrand, Rosen, Hellenas, & Aberg, 2013). | No clean up | DON, FB1, FB2, OTA | Tea | Waters® Acquity H-Class UPLC® system, composed of a quaternary solvent manager pump (QSM), a refrigerated sample manager Flow-Through-Needle (SM-FTN) and a column oven, coupled to a Waters® high resolution mass spectrometer with a Time of Flight analyzer Xevo® G2-S ToF (UHPLC/MS-ToF) | Separation was done on a column made of silica based particles bonded with C18-pentafluorophenyl functions (C18-PFP) (dimensions were 150 × 2.1 mm; 2 µm particles diameter | An electrospray ionization source was used in both positive (ESI+) and negative (ESI−) modes.  ESI+ the mobile phase was composed of water (A), ACN (B), both acidified with 0.1% FA, and MeOH (C), flowing at 0.4 mL/min. ESI−, the mobile phase was composed of water buffered with 10 mM of ammonium formate (A) and MeOH (B) flowing at 0.3 mL/min | (68) |
| 30 g of raw milk was centrifugated for 10 min at 4500 rpm. Then after filtration with filter paper, 20 g of the supernatant was mixed with 20 mL water containing 5% (v/v) acetonitrile. | the diluted sample was cleaned up through an AOZ immunoaffinity column at a flow-rate of about 1–2 drops per second. The column was washed with 20 mL pure water at a flow-rate of 1–2 drops per second. | aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1, aflatoxin M2, ochratoxin A, ochratoxin B, zearalenone, zearalanone, α-zeralanol, β-zeralanol, α-zeralenol, and β-zeralenol. | Milk | UHPLC/Q-Orbitrap system consisted of an U3000 UHPLC system coupled with a Q-Exactive Focus mass spectrometer | mobile phases A and B consisted of 0.1 mM ammonium formate, 0.01% formic acid, and 5% methanol in water (v/v) and methanol, respectively. Column was C18 core-shell column (100 mm × 2.1 mm, 1.7 μm) | The ion source was equipped with a heated electrospray ionization (HESI) probe, and the Q-Orbitrap was tuned and calibrated using positive and negative calibration solutions | (69) |
| extraction of analytes from matrices was achieved by a QuEChERS-based approach. 2.50 g ± 0.01 g of homogenized eggs (5.00 g milk) were weighted into a 50-mL centrifuge tube. Subsequently, 5.0 mL of water (2.5 mL of water for milk) and 10.0 mL of ACN containing 3.35% of FA (v/v) were added in tube. The mixture was vortexed vigorously for 2 min and then subjected to ultrasonic extraction for 25 min | addition of 4.0 g of anhydrous Na2SO4, 1.2 g of NaCl and 0.5 g of anhydrous NaAc, which was shaken by hand for 3 min and then centrifuged at 8500 rpm for 3 min (7606 rcf, 4 °C). Subsequently, 3.0 mL of the upper organic layer was transferred to a 15-mL dispersive tube containing 300 mg of C18, 140 mg of PSA and 1.5 g of anhydrous Na2SO4. mixture was vortexed for 3 min and then centrifuged. 2.0 mL of the resulting supernatant was transferred into a new glass tube and evaporated under a gentle nitrogen flow at 45 °C. Finally, the residue was reconstituted with 1.0 mL of ACN/water (20/80, v/v) | aflatoxin B1/B2/G1/G2 and ochratoxin A/B; sterigmatocystin; T-2 toxin, diacetoxyscirpenol, neosolaniol, deoxynivalenol-3-glucuronide and tentoxin; HT-2 toxin, de-epoxydeoxynivalenol, alternariol methyl ether, chaetoglobosin and ochratoxin α; deoxynivalenol, 3/15-acetyl-deoxynivalenol, fusarenone X and fumonisin B1; fumonisin B2 and fumonisin B3; zearalenone and its derivatives; aflatoxin M1/M2; verruculogen and gliotoxin; cyclopiazonic acid penicillic acid fumonisin B1/B2 | Eggs and Milk | UPLC–MS/MS system was composed of Waters AcquityTM UPLC and mass spectrometer, namely Xevo TQ-S (Manchester, UK), which equipped with Z-spray electrospray ionization (ESI) interface. | separation was performed with an Acquity UPLC CORTECS column (3.0 mm × 150 mm, 1.6 μm) at 40 °C column temperature. A flow rate of 0.4 mL/min was used, and the injection volume was set at 10 μL | Acquisition was performed in multiple-reaction-monitoring (MRM) mode, | (70) |
| Buckwheat seeds were pulverized into powder sufficiently fine to pass through a 50-mesh  sieve. Finely ground sample was weighed (1 g) and placed in a 50 mL centrifuge tube. After addition  of 10 mL methanol-water (8:2, v/v) the mixture was extracted by ultrasonic treatment | 5.0 mL supernatant was  immediately transferred to a 5.0 mL EP tube and evaporated under a stream of nitrogen gas at room  temperature. Finally the residue was re-dissolved in 1.0 mL methanol-water (5:5, v/v) and the solution  was filtered through a 0.22 µm filter | aflatoxin B1, B2, G1, G2, HT-2, T-2 toxin, ochratoxin  A, fumonisin B1, B2, zearalanone, zearalenone, and deoxynivalenol | Buck-wheat | (UFLC) system  equipped with two Shimadzu LC-20 AD pumps, a CBM-20A system controller, a SIL-20 AC  auto-sampler and a CTO-20A column oven. Coupled to a QTRAP® 5500 mass spectrometer  via a Turbo Ion Spray ionisation interface was used for the UFLC-QTrap-MS/MS analysis. | Separation was  performed on a SHISEIDO Capcell core C18 column (2.1 mm × 50 mm, 2.7 µm) with a gradient  elution with the flow rate of 0.35 µL·min−1 by a mobile phase consisting of 0.1% formic acid aqueous  solution (A) and acetonitrile with 0.1% formic acid (B) | (ESI) in positive and negative modes under the  multiple reaction monitoring (MRM) condition. Curtain gas and source gas (GS 1 and GS 2) were set to  35, 55 and 55 psi, respectively. The spray voltage was 4500 V for positive ESI and −4500 V for negative  ESI and the source temperature was set at 550 ◦C. | (71) |

*Mass Spectrometry*

Apart from the advantages of LC methods mentioned above, mass spectrometry offers several advantages over most LC methods for mycotoxin analysis. Mass spectrometry MS is among the most powerful tools in bioanalytical chemistry. It can determine the molecular weights of both small and large molecules with high accuracy and give highly valuable information regarding the structural information of a molecule. The mass spectrometer is an instrument that produces ions, separates them according to their mass to charge ratio, m/z, detects them and subsequently plots the mass spectrum. The sample molecules are first ionised in what is called the ion source. The gaseous ions are then introduced into the mass analyser and separate according to their m/z values. To avoid collisions between ions and air molecules, the system in under a high vacuum. The sample can be ionised by one of several techniques;

1. Electron Impact Ionisation (EI) – electrons are fired at the sample molecules
2. Chemical Ionisation (CI) – the sample molecules are collided with a reactive gas
3. Fast Atom Bombardment (FAB) – the sample molecules are bombarded with Argon atoms
4. Electrospray Ionisation (ESI) – the dissolved sample can be sprayed into an electric field
5. Matrix Assisted Laser Desorption Ionization (MALDI) – the sample is co-crystallised with a matrix and then ions can be generated by exposure to photons
6. Matrix Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry - MALDI-TOF/MS – with this technique molecular weights of 500,000Da with sensitivities as low as fmol (femtomole) and mass accuracies as high as 0.1-0.01%

All the above techniques will yield positively and/or negatively charge ions in the gaseous phase. Hard ionisation techniques like FAB or EI lead to the breakdown of the sample molecules into smaller fragments and these fragments act as a fingerprint of the sample. Soft ionisation techniques such as ESI or MALDI lead to molecular ions [M]+ and quasi molecular ions [M+H]+ which are in turn used for molecular weight determination. As previously mentioned, MS can be coupled to liquid chromatography (LC) or capillary electrophoresis (CE) to analyse more complex molecules and therefore is useful in the detection of mycotoxins and mixtures of mycotoxins in food and feed. MS offers higher sensitivity and selectivity and well as chemical structure identification. MS detection reduces the time by removing the need for sample derivatisation and clean up steps needed when using fluorescence detection. There are many different types of mass analysers and interfaces used in the determination of mycotoxins, some include quadruple, time of flight (TOF), ion trap and electrospray ionisation (ESI). Currently, there are a wide range of LC-compatible MS instruments available on the market. the MS detectors used in mycotoxin analysis include triple quadrupole (QqQ), ion trap, time-of-flight (TOF), and orbital ion trap mass analyzers, as well as hybrid systems that combine two types of analyzers. The latter group includes quadrupole–linear ion trap (QLIT), double quadrupole–TOF (QqTOF), quadrupole–orbital ion trap quadrupole–Orbitrap; Q–Orbitrap, and linear ion trap–orbital ion trap systems. The methods applied using these instruments have been summarised in previous reviews though some examples are provided in Table 2.

An LC/MS/MS multi-mycotoxin method was developed for the major fusarium toxins which included modified mycotoxins (deoxynivalenol-3-glucoside, 3- and 15-acetyldeoxynivalenol, HT-2 and T2 toxins, zearalenone and enniatins (ENNs) in beer (Habler et al 2017). The aim of the study was to develop a generally applicable solid phase extraction for trichothecenes including modified mycotoxins, enniatins, and zearalenone to analyze mycotoxins in beer samples. The resulting multi-mycotoxin stable isotope dilution LC–MS/MS method could be applied to beers from different continents to provide a current survey of *Fusarium* toxins in beer worldwide. As sample preparation and purification of beer a combined solid phase extraction for trichothecenes, enniatins, and zearalenone was developed. Internal standards were prepared from stock solution of each of the mycotoxins to a concentration of 10–100 μg/mL in acetonitrile. Sample preparation involved several sequential extraction steps with acetonitrile (70:30 v/v ACN:water) followed by a pass through a SPE cartridge. Method validation was carried out in lab and resulted in LODs in the range of 0.05 and 6.9 μg/L and the LOQs in the range of 0.15 and 20 μg/L. The intra-day precision varied between 1 and 5% and the inter-day precision between 2 and 8%. The recoveries ranged between 72 and 117% with the relative standard deviations (RSD) below 7%.

Another example of an LC/MS/MS method being used for multiple mycotoxin determination was developed by Dagnac et al 2016 for the simultaneous determination of 24 mycotoxins in maize silage. A simple liquid/solid extraction was performed either with clean-up on Mycospin 400 columns or without any clean-up. The method was validated by using ISO 11843. The recoveries ranged from 60% to 122% with relative standard deviations (RSDs) below 11%. The limits of detection (LODs) and limits of quantification (LOQs) were between 0.02–17.1 µg kg−1 and 0.06–57 µg kg−1. The calculated repeatability and within-lab reproducibility ranged from 5.2 to 23.2% and from 7.2 to 23.9%, respectively. The validated method was applied to 148 samples collected over two years in 19 dairy farms from Galicia (NW Spain).

According to recent publications, validated official methods for the analysis of multi-mycotoxin analysis based on LC/MS/MS will be issued in the near future by the European Committee for Standardisation (CEN) within the framework of the current mandate M520 (EC, 2013b). Nowadays, MS is used to analyse over 100 mycotoxins in a single run making it the current method of analysis of choice for use in multi-mycotoxin analysis especially when used in conjunction with liquid chromatography. Krska et al (2017) have conducted a review on mutli-mycotoxin analysis to metabolomics whereby mass spectrometry was a key focus. Mass-spectrometry-based analytical methods have been key for the determination of a variety of mycotoxins and their metabolites in plants and foods but also for the investigation of the metabolism of these toxic compounds in body fluids such as serum and urine. One example is a multi-analyte LC-MS/MS method which has recently been developed by Sulyok et al., and which is capable of determining more than 380 fungal, bacterial and plant metabolites, respectively, in cultures, cereals, food and feed products. LC-MS based screening is also playing a vital role in the discovery of novel conjugated (masked) mycotoxins, in the past, which is also believed to continue in the future. This unique multiple mycotoxin LC-MS method has been employed in BIOMIN´s Spectrum 380® screening programme. Spectrum 380® employs latest state-of-the-art liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) in a single analytical step. This program revealed that a typical agricultural commodity contains on average 30 different mycotoxin metabolites. The results of a worldwide survey utilizing this fully in-house validated LC-MS/MS method have very recently been published. Malachová et al (2018) also conducted a significant review on advanced LC–MS-based methods and validation parameters to study the co-occurrence and metabolization of multiple mycotoxins in cereals and cereal-based food. An interesting trend is the use of LC high resolution (HR) MS methods, with time-of-flight or Orbitrap mass analysers. While those types of mass spectrometers are not as sensitive, they offer a virtually unlimited number of compounds to be analysed. For instance, a method for the analysis of mycotoxins (but also other analytes such as pesticides) in bakery products by LC-Orbitrap-MS was developed. While good quantitative findings were obtained for the analysed mycotoxins, the limits of quantification were not suitable to verify the levels of OTA and aflatoxin B1 with respect to maximum permitted levels in the European Union. An additional advantage of LC-HR-MS method is the acquisition of full scans, which allows the detection of substances which were not considered at the time of method development and even retrospective data analysis.

Though in both these reviews the importance of sample preparation is highlighted as are thorough method up set up and validation criteria for multiple mycotoxin analysis.

*Gas Chromatography/GCMS*

Gas Chromatography is a widely used technique for qualitative and quantitative analysis. Various detection systems may be used with GC analysis such as mass spectrometry, MS. In GC the components of a vaporised sample are separated by being distributed between a gaseous mobile phase and liquid or solid stationary phase held within a column. The sample is injected into the head of the chromatographic column and elution is brought about by the flow of an inert gaseous mobile phase. GC is based on the partitioning of the analyte between a gaseous mobile phase and a liquids phase immobilised onto the surface of an inert solid packing or on the walls of capillary tubing. The concept of gas chromatography was first proposed in 1941 by Martin and Synge. In 1955 the first commercial apparatus for GC was launched onto the market.

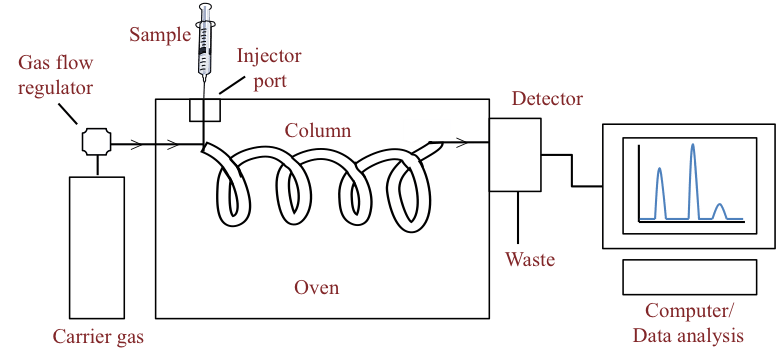


Figure 9 Diagram of typical GC apparatus (72)

A typical schematic of a Gas Chromatograph is shown in Figure 9. The mobile phase in a GC is called a carrier gas and must be chemically inert. The most common used gas is Helium although Argon, Nitrogen and Hydrogen can also be used. Regulators are used to control the flow rates typically this is set to between 25-150 ml / min under a packed column.

In a GC analysis, a known volume of gaseous or liquid sample is injected into the head of the column. A carrier gas then moves the sample through the column, this motion is inhibited by the adsorption of the sample molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the sample mixture are separated as they progress along the column and reach the end of the column at different times, also known as the retention time. A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Typically, substances are identified by the order in which they elute from the column and by their retention time.

Chromatographic columns vary in length from 2m to 60m and are usually constructed of stainless steel, fused silica or Teflon. For them to be able to fit into the GC’s oven they are coiled up. The column temperature is very important variable that must be controlled for precise work. The optimum column temperature is one which is equal to or a few degrees above the boiling point of the sample. Temperature programming maybe used for sample with a broad boiling range.

Many Different types of detector may be used in association with GC. The most common are Flame Ionization Detector (FID) and Thermal Conductivity Detector (TCD). Both detector are sensitive over a wide range of components and both work over a wide range of concentrations. Fourier transform IR (FTIR) can also be used and is best for analyzing organic compounds with typical detection limits of 0.2 – 40 ng. One of the most powerful detectors for GC is the Mass Spectrometer (MS). The combination of both is known as GC/MS.

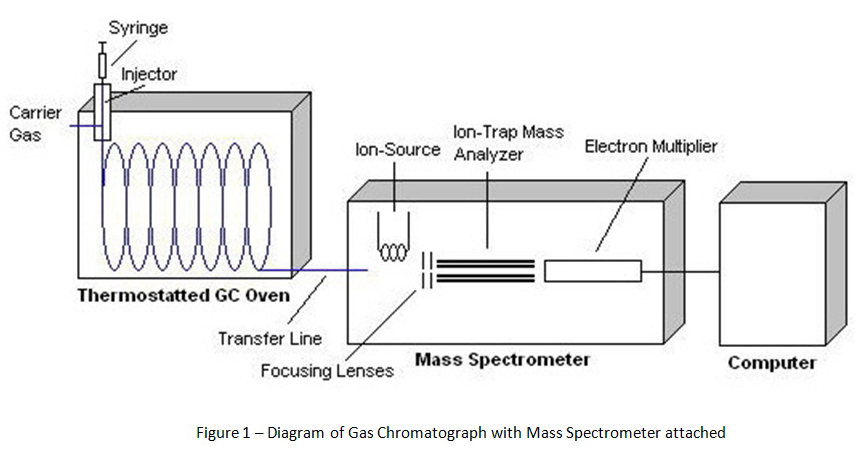


Figure 10 Diagram of Gas chromatograph with mass spectrometer attached (73)

A mass spectrometer measures the mass-to-charge ratio *(m/z)* of the ions produced from a sample. GC is regularly used for identification and quantification of the presence of mycotoxins in food samples and many protocols have been developed for these materials. Usually the system is linked up to a MS in order to evaluate the volatile products. Due to the low volatility and high polarity of most mycotoxins, a derivatisation step needs to be included before analysis on the GC. This is one of the reasons why GC is not used regularly as the method of choice, and the use of GC detection for mycotoxin analysis in commercial protocols is not viable as a much cheaper and faster method of analysis is available - HPLC.

A paper by Rodriguez et al 2012, proposed a method to analyse multi-mycotoxins in wheat semolina using an acetonitrile-based extraction procedure and gas chromatography–tandem mass spectrometry. In this paper, a multi-mycotoxin method based on the QuEChERS extraction methodology previously discussed and using GC–MS/MS analysis with triple quadrupole for detection, quantification and reliable identification of the analytes present in the samples, was developed and validated in order to attain the legal limits established by EU regulation for these mycotoxins. The mycotoxins in question were patulin, diacetoxyscirpenol, neosolaniol, HT2, T2, deoxynivalenol, 3-acetyl-deoxynivalenol, fusarenon X, nivalenol and zearalenone. Wheat was the commodity for investigation. A total of fifteen wheat semolina samples were randomly purchased in supermarkets located in Valencia Metropolitan Area (Spain). All samples were homogenised using a laboratory mill and stored in a dark and a dry place and analysed within 2 days of sampling.

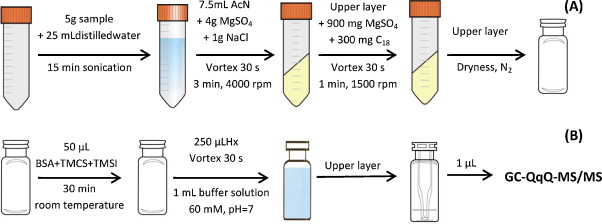


Figure 11 A schematic flow diagram summarising the QuEChERS procedure employed by Rodriguez et al 2012 (74)

Derivatization was then employed as these samples are to be analysed via GCMS. The dry extract was added with 50 μL of BSA (N,O-bis(trimethylsilyl)acetamide) + TMCS (trimethylchlorosilane) + TMSI  (N-trimethylsilyimidazole) (3:2:3) and the sample left for 30 min at room temperature. The derivatized sample was diluted to 250 μL with hexane and mixed thoroughly on a vortex for 30 s. Then the hexane was washed with 1 mL of phosphate buffer (60 mM, pH 7) and mixed until the upper layer was clear. Finally, the hexane layer was transferred to an autosampler vial for the chromatographic analysis. The method was validated and the limits of quantification ranged from 1.25-10 µg/kg and high recoveries for the assayed mycotoxins were obtained with distilled water. Data ranged from 83 to 111% with RSDs lower than 3% in all analytes except for zearalenone which showed a recovery of 63% and a RSD of 21%. Nevertheless, the results obtained were in accordance with the Commission Regulation (EC) No. 401/2006. The recovery results were very similar to those reported by Dermarchelier et al.  (55–112%), Sospedra et al. (86–108%), Cunha-Fernandes et al. (52–103%), Ferreira et al. (61–118%), and Romero-González et al. (70–120%). The repeatability data expressed as intra-day precision was <13%. No apparent difference in repeatability results among the tested concentration levels was found. The inter-day precision was <17% for all studied mycotoxins except for zearalenone which was slightly upper than 20% and lies well within the legislative regulations. Table 3 provides a detailed description of the set-up of GC-MS methods for multiple mycotoxin determination.

Current trends in mycotoxin analysis, especially multi-mycotoxin analysis in food and feed are focusing on the application of robust, fast, efficient and inexpensive technologies to detect and quantify many different mycotoxins with high sensitivity and selectivity in a single run. As observed many chromatographic methods are used high pressure liquid chromatography (HPLC) coupled with Fluorescence detector (FLD), UV detector or mass spectrometer (MS). Gas chromatography (GC) is also used and this is usually coupled to a flame ionisation detector (FID) or more frequently a mass spectrometer (MS) though derivatization as an additional sample preparation step makes this method more difficult to apply.

Table 3 Table showing detailed description of the set-up of GC/MS methods for multiple mycotoxin determination

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Extraction** | **Clean up** | **Toxin** | **Matrix** | **Instrument** | **GC Conditions** | **MS conditions** | **Reference** |
| Extraction with acetonitrile/water (84/16) | cleanup with MycosepÒ 227/derivatization with PFPA | NIV, DON, FUS-X, 3-AcDON, 15-AcDON, NEO, DAS, T-2, HT-2 | Barley | Agilent Technologies 6890N gas chromatography system with a G2613A autosampler injector and coupled to a 5973 quadrupole mass spectrometer controled by ChemStation software (Agilent Technologies). | Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm i.d.) coated with a 0.25 μm film of stationary phase (5% phenyl-methylsiloxane) from Agilent. Helium was used as carrier gas at a constant flow of 1.1 mL min−1. Injection volume was 2.0 μL The injector was held at 250  °C and the transfer line to detector at 300  °C. The GC oven temperature was programmed as follows: 120 °C for 2 min, 30  °C min−1 to 175 °C, then 10 °C min−1 to 220 °C and finally 25 °C min−1 to 275 °C holding 6 min. The source temperature was 230 °C | mass spectrometer was operated in electron impact mode (EI, 70 eV). A solvent delay of 4 min was used in order to avoid overloading the mass spectrometer with the solvent. | (75) |
| Modified QuEChERS (extraction with acetonitrile, | d-SPE cleanup with C18)/derivatization with Tri-Sil TBT | NIV, FUS-X, DAS, 3-AcDON, NEO, DON, T-2, HT-2 | Wheat semolina | GC system Agilent 7890A coupled with an Agilent 7000A triple quadrupole mass spectrometer with inert electron-impact ion source and an Agilent 7693 autosampler | HP-5MS 30 m × 0.25 mm × 0.25 μm capillary column. One microliter of the final clean extract of mycotoxins was injected in splitless mode at 250 °C in programmable temperature vaporization (PTV) inlet employing helium as carrier gas at fixed pressure of 20.3 psi. The oven temperature program was initially 80 °C, and the temperature was increased to 245 °C at 60 °C min−1. After a 3 min hold time, the temperature was increased to 260 °C at 3 °C min−1 and finally to 270 °C at 10 °C min−1 and then held for 10 min. | The mass spectrometer was operated in electron impact ionization (EI, 70 eV). The transfer line and source temperatures were 280 and 230 °C, respectively. The collision gas for MS/MS experiments was nitrogen, and the helium was used as quenching gas, both at 99.999% purity | (74) |
| Fast sample preparation procedure is based on a pre-extraction of fat compounds with n-hexane, extraction of target analytes with acetonitrile and removing of matrix co-extracts | The upper layer (6 ml) was submitted to a dispersive SPE cleanup with 900 mg of MgSO4 and 300 mg of C18. | deoxynivalenol, nivalenol, 15-acetyl-deoxynivalenol, fusarenon X and zearalenone | Popcorn | GC–MS analyses were performed on an Agilent (Little Falls, DE, USA) gas chromatograph 6890 equipped with an electronically controlled split/splitless injection port and an inert 5975N mass selective detector with electron impact (EI) ionization chamber. | GC separation was conducted with a DB-5 MS (30 m × 0.25 μm × 0.25 mm film thickness; J&W Scientific, Folsom, CA, EUA) analytical column. The injection was made in splitless mode (60 s) at 280 °C. The oven temperature program was as follows: 140 °C held for 0.5 min, ramped to 300 °C at 20 °C/min, and held for 7.5 min. Total run time was 16 min. The MS transfer line temperature was held at 280 °C. | Mass spectrometric parameters were set as follows: electron impact ionization with 70 eV energy; ion source temperature, 230 °C; MS quadrupole temperature, 150 °C and solvent delay 4.5 min. | (76) |
| A 25 g sample of finely ground cereal was homogenised with 100 mL of acetonitrile:water (84:16, v/v) for 3 min, using an UltraTurrax T 25 at 1832.6 rad/s. | The extracted sample was then filtered through a filter paper (Whatman grade 2V) from Whatman (Maidstone, UK). The filtrate was defatted with n-hexane (2× 20 mL). A total of 8 mL defatted extract was purified by MycoSep 227 column | Deoxynivalenol (DON), nivalenol (NIV), 3-acetyl-DON (3-AcDON), 15-acetyl-DON (15-AcDON) and fusarenone X (Fus-X) | Breakfast cereals | The GC was an Agilent model 6890N equipped with an Agilent 7683 B autosampler injector and coupled to an Agilent 5975 quadrupole mass selective detector. | Separation was achieved on the HP-5 capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) from Agilent Technologies (Waldbrom, Germany).  The temperature of injection port was 270 °C and the mode of injection was splitless. The carrier gas was helium at 1.8 mL/min flow rat and injection volume was of 2 μL in splitless mode. The initial GC oven temperature was 80 °C, and held for 1 min. It was increased from 80 to 240 °C at a rate of 30 °C/min | The mass spectrometer worked at electron impact mode at 70 eV. Interface, ion source and quadrupole temperatures were 300, 230 and 150 °C, respectively. Mass scan range covered from 35 to 500 *m*/*z*. All spectra were monitored with a total ion current (TIC) and selected ion monitoring (SIM) modes. | (77) |
| Extraction with acetonitrile/water (84/16); | cleanup with MycosepÒ 227/derivatization with TMSI/TMCS (1/0.2, v/v) | DON, 3-AcDON, 15-AcDON, NIV, T-2, HT-2 | Maize, durum wheat | The GC–MS system consisted of Trace GC Ultra equipped with an ISQ single-quadrupole mass spectrometer; the system was controlled by Excalibur 2.1 software | The analysis was carried out using a capillary column Rtx-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Restek Corporation, Bellefonte, PA, USA). Helium was the carrier gas with a column head pressure of 55 kPa. The sample was injected (2 μL, split ratio 1:30) into the GC–MS by a programmed temperature vaporisation (PTV) injector | MS transfer-line and ion source temperature were 300 °C and 200 °C, respectively. Electron ionisation at 70 eV and selected ion monitoring (SIM) were used for detection. | (78) |
| Modified QuEChERS method (extraction with acetonitrile | d-SPE cleanup with C18)/derivatization with Tri-Sil TBT | DON, 3-AcDON, PAT, FUS-X, DAS, NIV, NEO, HT-2, T-2, ZEN | Wheat, rice, maize | Agilent 7890A GC system coupled with an Agilent 7000A triple quadrupole mass spectrometer with inert electron-impact ion source and an Agilent 7693 autosampler (Agilent Technologies, Palo Alto, CA). | HP-5MS 30 m × 0.25 mm × 0.25 μm capillary column. The oven temperature program was initially 80 °C for 2 min, and the temperature was increased to 245 °C at 80 °C min−1. After a 5 min hold time, the temperature was increased to 250 °C at 5 °C min−1 and finally to 270 °C at 10 °C min−1 and then held for 3 min | The mass spectrometer was operated in electron impact ionization (EI, 70 eV). The transfer line and source temperatures were 280 °C and 230 °C, respectively. The collision gas for MS/MS experiments was nitrogen, and the helium was used as carrier gas at fixed pressure of 20.3 psi, both at 99.999% purity | (79) |
| Aliquots (2 g) of vegetable oil samples were diluted to 10 mL with an ethyl acetate-cyclohexane solution (1:1, *v*/*v*), shaken vigorously and vortex-mixed at 2000 rpm for 1 min, |  | *α*-ZOL, *β*-ZOL, *α*-ZAL, *β*-ZAL, ZON and ZAN |  | Trace GC Ultra equipped with a split/splitless injector. A TSQ Quantum GC mass spectrometer (Thermo Fisher Scientific) operated in the electron impact (EI) mode was used. | The analytical column used was a TR-5 MS (30 m × 0.25 mm, ID 0.25 μm film thickness) coated with a 5% phenylmethylpolysiloxane stationary phase (Thermo Fisher Scientific). High-purity helium (⩾99.999%) at a constant flow rate of 1 mL min−1 was used as the carrier gas. Argon | operated in the electron impact (EI) mode was used. The ion source and transfer line temperatures were set at 250 °C and 280 °C, respectively. The emission current of the ionisation filament was set at 100 μA, generating electrons with 70 eV. The analyses were carried out with a filament-multiplier delay of 5 min | (80) |
| Feed (5 g) accurately weighed (precision 0.1 mg) were transferred to centrifuge tubes (50 ml) and homogenised in a vortex with 10 ml of acetonitrile/water (80:20) 0.1% HCOOH | Samples were shaken for 1 h, the tubes were placed in an ultrasonic bath for 15 min followed by centrifugation at 4500 rpm for 10 min. A total of 2 ml of supernatant extract were transferred to an Eppendorff vial and stored in a freezer (for a minimum of 2 h). After this time, the extract was centrifuged again at 14 000 rpm for 10 min, filtered with a 0.22-μm filter | DON, 3-ADON, 15-ADON, NIV, NEO, DAS and FUS-X | laboratory rat feed | GC system Agilent 7890A coupled with an Agilent 7000A triple quadrupole mass spectrometer and an Agilent 7693 autosampler | Analytes were separated on a HP-5MS 30 m × 0.25 mm × 0.25 μm capillary column. A total of 1 μl of extract of mycotoxins was injected in splitless mode in a programmable temperature vaporisation (PTV) inlet (150°C for 0.1 min then 600°C min–1 to 250°C for 5 min) employing helium as the carrier gas at a fixed pressure of 20.3 psi. The oven temperature programme was initially 80°C, and the temperature was increased to 245°C at 60°C min–1 | Data was acquired at selection reaction monitoring (SRM) mode and the MS was operated in electron ionisation (EI, 70 eV). The transfer line and source temperatures were 280 and 230°C, respectively. The collision gas for MS/MS experiments was nitrogen (1.5 ml min–1), and helium was used as the quenching gas (2.25 ml min–1), both at 99.999% purity | (81) |
| First, the grain samples were milled with a blender and 2 g was weighted and placed into 50 mL polypropylene centrifuge tubes. 10 mL of an extraction solvent composed by a mixture of acetonitrile/water (84:16, v/v) were added. | Sample were centrifuged for 5 min at 4500 rpm in 5 °C with Eppendorf Centrifuge 5810R; the supernatant was filtered on Whatman filter paper No. 4 and 5 mL of the supernatant were evaporated to dryness at 38 °C under a gentle stream of nitrogen using a multi-sample Turbo-vap LV Evaporator | DON, 15AcDON, NEO, NIV, HT2, FB1, OTA, ENA, ENA1, ENB and ENB1 | Barley and soup of barley | Agilent 7890A GC system coupled with an Agilent 7000A triple quadrupole mass spectrometer with inert electron-impact ion source and an Agilent 7693 autosampler | Analytes have been separated on a HP-5MS 30 m × 0.25 mm x 0.25 μm capillary column. The oven temperature program was initially 80 °C, and the temperature increased to 245 °C progressively at 60 °C/min. After a 3 min hold time, the temperature was increased to 260 °C progressively at 3 °C/min and finally to 270 °C at 10 °C/min and then held for 10 min. | The mass spectrometer was operating in electron impact ionization (EI, 70 eV). The source and transfer line temperatures were 230 °C and 280 °C, respectively. The collision gas for MS/MS experiments was nitrogen, and the helium was used as carrier gas at fixed pressure of 20.3 psi, both at 99.999% purity | (37) |

*Capillary Electrophoresis*

Capillary electrophoresis (CE) is an instrumental evolution of traditional electrophoretic techniques, where separation occurs in fused-silica capillaries and involves application of high voltages across buffer filled capillaries in order to achieve separation. Due to its speed of analysis, high efficiency, automated analytical equipment, low reagents and sample consumption and rapid method development, it is an effective analytical technique. Capillary electrophoresis (CE) employs narrow-bore (20-200 µm i.d.) capillaries, made of fused silica, to separates ions based on their electrophoretic mobility with the use of an applied voltage. The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius. The rate at which the particle moves is directly proportional to the applied electric field--the greater the field strength, the faster the mobility. Neutral species are not affected, only ions move with the electric field. If two ions are the same size, the one with greater charge will move the fastest. For ions of the same charge, the smaller particle has less friction and overall faster migration rate. It is a useful technique because there is a large range of detection methods available. CE is actually a range of separation techniques based on different separation principles: one of which is MECE - micellar electrokinetic capillary chromatography which enables separation of neutral compounds by using surfactant micelles. Aflatoxin production was first confirmed by micellar electrokinetic capillary electrophoresis.

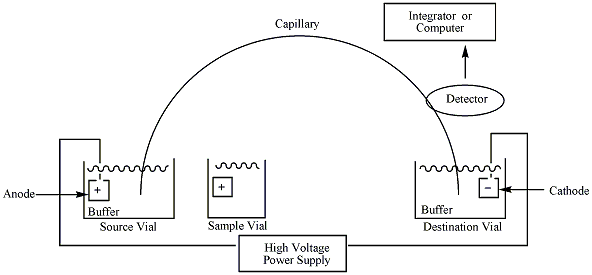


Figure 12 Schematic of the apparatus used for Capillary Electrophoresis (82)

Capillary electrophoresis can be used to detect mycotoxins. It is mainly used in conjunction with another analytical method when determining mycotoxins. A number of mycotoxins such as aflatoxins, deoxynivalenol, ochratoxin and zearalenone have been separated by CE. CE or more specifically MECE - micellar electrokinetic capillary chromatography has been used in conjunction with HPLC-MS to detect ochratoxin A production in molds (Martin et al 2004) in dry cure meats and (Rodriguez et al 2011) and Aflatoxin detection This method of analysis can also be used to detect Aflatoxin production using it conjunction with HPLC. The method has been compared to HPLC when detecting ochratoxin A in wine (Gonzalez-Penas, et al 2006). A good correlation of the results had been obtained when 27 fortified wine samples were analyzed by capillary electrophoresis with diode array detector (CE-DAD) and HPLC-FL. The work demonstrated that the CE technique is a valuable alternative to HPLC-FL analysis of ochratoxin A in wine if fluorescence detection is available. A methodology for simultaneous separation of ergot alkaloids by capillary electrophoresis after cloud point extraction from cereal samples has been determined by Felici et al 2014. The high sensitivity achieved for Ergot Alkaloids determinations in real samples suggests Cloud point extraction procedure as an interesting approach when used in association with a CE-UV visible detection system. The limits of detection were 2.6 and 2.2 µg/kg for ergotamine and ergonovine, respectively. The validation procedure revealed suitable linearity, accuracy and precision. The average extraction and clean-up recoveries were compared with the theoretical values and were better than 92%. The reproducibility was <1.5 and 2.0% for intra and interday respectively. Overall the whole process could be considered as a contribution to green chemistry because nonorganic solvents were involved, demonstrating its great potential over conventional techniques. Capillary electrophoresis analysis advantages over other forms of chromatography are that a low sample and reagent consumption involved with a much reduced analysis time. Capillary electrophoresis has also been used for determination of zearalenone in poultry feed and cereals (Guray et al 2013). CE has also been used in conjunction with UV detection in the determination of zearalenone in poultry feed and cereals (Guray et al 2013.) Different brands of commercial animal feeds, maize, flour, crisp from popcorn and rice, biscuits, and grain samples were purchased from local markets in Eskisehir, Turkey. In the separation, a run buffer consisting of 20 mM sodium tetraborate at pH 9.0 with 15% acetonitrile applying 15 kV, injecting 10 s at 50 mbar was utilized. Phenobarbital was a suitable internal standard and the signals were recorded at 254 nm. The repeatability results are in the range of 0.01–1.58 for inter-day. The LOD and LOQ were calculated to be 8.25 µg/L and 25 µg/L respectively.

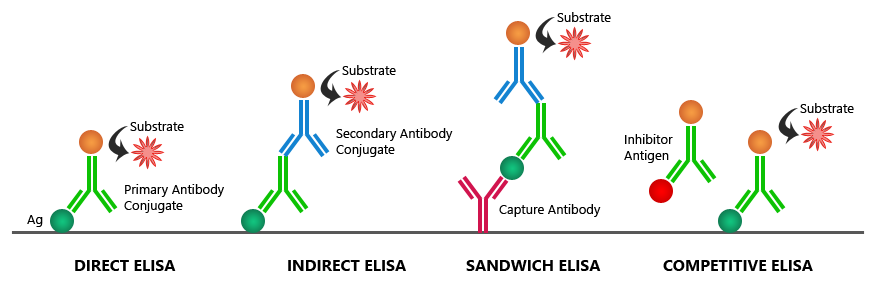
In the study by Guray et al 2013, a simple, precise, and sensitive capillary zone electrophoresis-uv detection method that requires less chemicals and time for the determination of zearalenone had been studied. Previously, zearalenone had been determined using a variety of analytical methods which included thin layer chromatography with UV detection (Binder et al 2007), HPLC using different detection principles like UV diode array (Curticapean et al 2011) and mass spectrometry (Soleimany et al 2012) and fluoresence (Rahmani et al 2010) as well as LC/MS-MS (ref) and GC/MS (ref). These methods however are costly and require expensive equipment and complicated clean up procedures and require skilled operators. The usual determination of ZEN is by immunochemical assay (Panini et al 2011). This method of analysis is faster and can turnover many samples per day but again the drawbacks are cost and availability is limited to only some mycotoxins and the possible phenomena of false positive result from cross reactivity. Capillary electrophoresis can overcome some of these disadvantages for example it uses less organic solvents and only small volumes are required and can be more efficient and have better resolution than other conventional methods (Holland et al 1993). A recent interrogation of the literature showed how capillary electrophoresis is trending when it comes to being used for single mycotoxin analysis.

Figure 13 Number of publications per year relating to capillary electrophoresis as method of analysis for each named mycotoxin.

TLC and Capillary Electrophoresis are mainly used for detection of single mycotoxins and when searched in the literature for use exclusively as a detection method for multi mycotoxin analysis between 2010-2018, no articles are returned.

*Enzyme Immunoassays (ELISA)*

On examination of the published methods, it is evident that enzyme linked immunosorbent assay (ELISA) is a common screening technique used for mycotoxin detection. It can be used in conjunction with other methods of analysis such as LC-MS/MS to be used as a combination screening and confirmatory style method of analysis. Enzymes are the most commonly used labels in immunoassay. ELISA is an immunological technique frequently for the rapid screening and detection of various mycotoxins, using antibodies that will interact accordingly to a biological recognition element that is specific towards the analyte of interest (the toxin). ELISA assays generally don’t require a clean-up step, therefore detection may occur after extraction. ELISA techniques can be divided into 4 different processes e.g. Direct, Indirect, Sandwich assay and Competitive (Inhibition) assays.



According to Li and Liu, (2012), as mycotoxins are generally low molecular weight compounds competitive (inhibition) assays are used in mycotoxin detection. Direct ELISAs involve attachment of the antigen to the solid phase, followed by an enzyme-labelled antibody. Indirect ELISAs also involve attachment of the antigen to a solid phase, but in this case, the primary antibody is not labelled. An enzyme-conjugated secondary antibody, directed at the first antibody, is then added. The third type of ELISA is the Competition Assay, which involves the simultaneous addition of 'competing' antibodies. The decrease in signal of samples where the second antibody is added gives a highly specific result. The last type of assay is the sandwich ELISA. Sandwich ELISAs involve attachment of a capture antibody to a solid phase support. Samples containing known or unknown antigen are then added in a matrix or buffer that will minimize attachment to the solid phase. An enzyme-labelled antibody is then added for detection. These basics of ELISA principles can then be applied to any lateral flow, microarray or biosensor application whereby the standard ELISA 96 well microplate format can be adapted into more portable or user friendly technologies.

ELISAs are adaptable to high-throughput screening because results are rapid, consistent and relatively easy to analyse. They are very popular for single mycotoxin detections as they are low cost and have relatively easy application. ELISA has also become popular as a screening test used in the lab and then followed up by confirmatory analysis done by HPLC especially when analysing for the aflatoxin group of toxins. Pleadin et al 2014 used ELISA coupled with HPLC to determine the level of aflatoxin B1 (AFB1) in maize sampled from farms and feed factories situated in Northern, Central and Eastern Croatia, following the occurrence of cow milk AFM1 contamination. 633 maize samples were analysed using Enzyme-Linked Immunosorbent Assay (ELISA) as a screening method and High Performance Liquid Chromatography Tandem Mass Spectrometry (LC–MS/MS) as a confirmatory method. The ELISA method was performed using an auto-analyzer ChemWell 2910 and an Agilent 1260 series HPLC system coupled with a 6410 triple quad-mass spectrometer. For the ELISA method the maize grain were prepared using 5g of homogenised sample added to 25ml of 70% methanol and shaken vigourously for 3 minutes and then filtered. The aflatoxin concentration was determined by a Ridascreen® test kit provided by R-biopharm. The sample preparation for the LC/MS involved taking 25 g of the sample, and adding 100 mL of the extraction solution (ACN/H2O = 80/20). The mixture was shaken for 2 hours and then filtered. 1 mL of the obtained filtrate was diluted with 3 mL of ultrapure water, mixed and filtrated through 0.45 μm-RC filter. 40 μL of the sample was injected into the LC system. Validation of the ELISA method gave limits of detection of 1.1 μg/kg with the mean recovery and the intermediate precision determined to be 91.0 and 92.8%, respectively, with acceptable mean relative standard deviations of 5.8 and 7.4%, respectively. For the LC/MS method validation the recoveries ranged from 99.-101.1% with relative standard deviations of between 2.6-17.2%. Given these results it was concluded that the applied ELISA and LC/MS methods were capable and reliable to efficiently determine and confirm the presence of aflatoxin B1 in these samples.

Shown below is a graphical representation of the number of publications per year that ELISA has been used as either a screening method or a proof of principle method or in conjunction with another method of analysis to determine presence of mycotoxins in food or feed. The ELISA technique does have certain disadvantages in that there can be cross-reactivity and dependence on a specific matrix. The kits only detect a single mycotoxin so for analysis requiring multiple mycotoxin detection that can be quite costly and any positive result will need to be confirmed by a suitable chromatographic method. However, McNamee et al (2017) demonstrated the capability for multi-spot ELISA for multiple mycotoxin detection.

Figure 14 Number of publications per year relating to ELISA as a method of analysis for each named mycotoxin.

Lateral flow Devices

Basically, a lateral flow device is classed as a simple to use diagnostic device used to confirm the presence or absence of a target analyte, such as mycotoxins or biomarkers in humans or animals, or contaminants in water supplies, foodstuffs, or animal feeds. LFDs typically contain a control line to confirm the test is working properly, along with one or more target or test lines. They are designed to incorporate intuitive user protocols and require minimal training to operate. They can be qualitative and read visually. They are an immunological method, based on antibody and antigen interactions, some have gold or silver nanoparticles to increase the sensitivity of the reaction. Raeisossadati and Danesh et al., (2016), highlights that lateral flow technology is a simple, cost effective method, and competitive or sandwich assays are generally the two standard formats of a lateral flow test strip. The competitive assay is used to analyse mycotoxins as they are low molecular weight compounds. Lateral flow devices are composed of four parts, this includes a sample pad, conjugate pad, nitrocellulose membrane, and adsorbent pad. Within the membrane are two important features- the test line and control line.

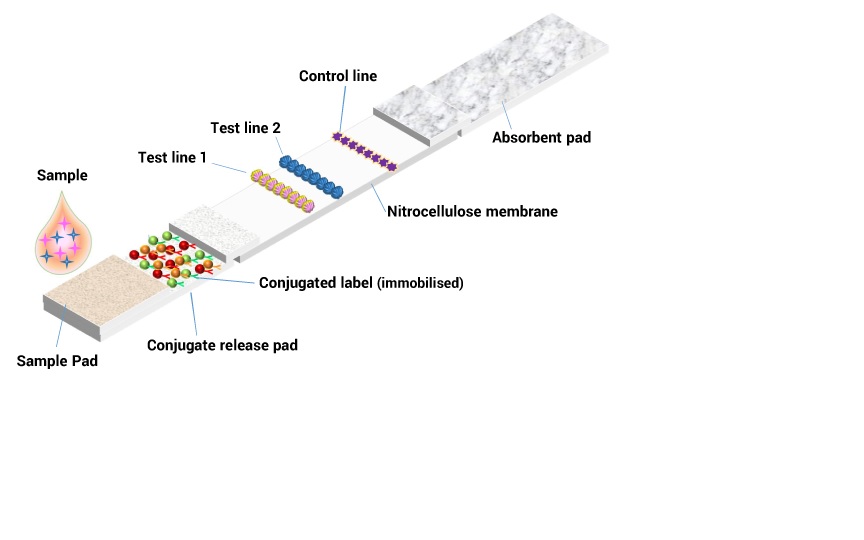
**

Figure 15 Illustration to show application of a basic lateral flow device

The sample pad acts as the first stage of the absorption process, and in some cases contains a filter, to ensure the accurate and controlled flow of the sample.The conjugate pad, which stores the conjugated labels and antibodies, receives the sample. If the target is present, the immobilised conjugated antibodies and labels will bind with the target and continue to migrate along the test.As the sample moves along the device the binding reagents situated on the nitrocellulose membrane will bind with the target at the test line. A coloured line will form and the density of the line will vary depending on the quantity of the target present. The sample will pass through the nitrocellulose membrane into the absorbent pad. The absorbent pad will absorb the excess sample. The specification of the absorbent pad will have an impact on the volume of sample the test can incorporate.A search of the literature for lateral flow devices as the method of analysis in multi mycotoxin detection was completed in Web of Science in all databases (Web of Science core collection, KCI- Korean Journal Database, MEDLINE, Russian Science Citation index, SciELO Citation index) using the following search terms:Searched for: TOPIC: (mycotoxins) AND TOPIC: (lateral flow\*)Refined by: TOPIC: (multiplex)Timespan: 2010-2018. A flowchart schematic of the search is provided and the key results are presented in Table 4 providing a description of lateral flow methods for multiple mycotoxin determination.

Flowchart for Lateral flow methods literature search 2010-2018

Full-text articles excluded, with reasons  
(n = 4 )

Full-text articles assessed for eligibility  
(n = 11 )

Records excluded  
(n = 1 )

Records after duplicates removed  
(n = 107)

Additional records identified through All database search in WOS   
(n =28 )

## Identification

## Eligibility

## Included

## Screening

Records identified through WOS database searching ‘mycotoxins’ AND ‘Lateral Flow\*’  
(n = 79 )

After refinement by ‘multiplex’ Records screened  
(n = 12 )

Studies included in reference table  
(n = 7 )

Table 4 Table showing detailed description of Lateral Flow methods for multiple mycotoxin determination 2010-2018

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Toxin** | **Matrix** | **Lateral Flow methods** | **LOD** | **Advantages** | **Reference** |
| deoxynivalenol,  T-2 toxin,  zearalenone | Maize | Multiplex Lateral Flow Immunoassays Based on Amorphous Carbon Nanoparticles | 20 μg/kg for deoxynivalenol, 13 μg/kg for T-2 toxin, and 1 μg/kg for zearalenone | Simultaneously analyzing three mycotoxins in maize samples. The ACNP-LFAs in this study provide a simple, sensitive, rapid, and reliable tool for monitoring multiple mycotoxins in cereals. | (83) |
| deoxynivalenol, zearalenone, and T2/HT2-toxin | Barley | Multiplex lateral flow immunoassay (LFIA) with luminescent quantum dots (QDs) as label | 1000 μg/kg deoxynivalenol,  80 μg/kg zearalenone,  80 μg/kgT2/HT2-toxin, | Give a fast result (15 min) with a low false-negative rate (<5%), and the results are easy to interpret without any sophisticated equipment. | (84) |
| zearalenone and deoxynivalenol | Maize and Wheat | Multiplex immunochemical technique based on indium phosphide (InP) Quantum Dots | 50 μg kg−1 zearralenone  500 μg kg−1 deoxynivalenol | Simultaneous detection, simple, cost-effective and easy to perform and do not require the use of any expensive equipment | (85) |
| deoxynivalenol (DON), zearalenone (ZEN) and T2/HT2-toxin (T2/HT2) | Cereals | Colloidal gold- and quantum dot-based multiplex lateral flow immunoassay. | 1000 μg kg−1 DON  80 μg kg−1 ZEN and  80 μg kg−1 T2/HT2 | Consume less immunoreagents and was more sensitive and economically beneficial. Easier to interpret, resulting in a lower false negative rate (<5%) | (86) |
| aflatoxin B1, zearalenone and ochratoxin A | Corn, rice and peanut. | Gold nanoparticle Multiplex lateral flow immunoassay | 10 μg/kg aflatoxin B1, 50 μg/kg zearalenone 15 μg/kg ochratoxin A | simultaneous detection of the three mycotoxins within 15 min, rapid on-site screening, feasible. | (87) |
| aflatoxin B1 (AFB1), zearalenone (ZEA), deoxynivalenol (DON) | Cereals  (Wheat and maize) | Multiplex Lateral Flow Immunoassay label AuNP | 0.03 μg/kg AFB1  1.6 μg/kg ZEA  10 μg/kg DON | 15 min assay time, simultaneous detection, simple, rapid, sensitive, cost-effective, and time-efficient. | (88) |
| zearalenone, T-2 and HT-2 toxins, deoxynivalenol | Wheat, Maize , Oats | A multiplex dipstick immunoassay label gold nanoparticles | 1400 μg kg−1 DON, 280 μg kg−1 ZEA,  400 μg kg−1  (200 μg kg−1 each) T-2 and HT-2 toxins | 30 min assay time, rapid, cost effective, easy-to-use and fit for purpose of on-site mycotoxin screening of cereals | (89) |

*Biosensors*

Biosensors have received much attention from the research community in recent years as rapid, reliable and low cost tools for quantitative determination of mycotoxins in food (Toothill et al 2011). Compared to classical mycotoxin determination methods biosensors have emerged as an attractive alternative tool for offering high selectivity and sensitivity, ease of use and low cost and portability in a self-contained simple design. The basic concept of biosensors is to convert biological responses resulting from interactions between an analyte and a biological element (bioreceptor) into electrical signals. A biosensor consists of several main parts; a bioreceptor, an immobilising matrix and a transducer. The bioreceptor may be an enzyme or an antibody used for the recognition of the analyte of interest. The immobilising matrix consists of various materials including conducting polymers, nanomaterials or sol-gel films (Malhotra *et al.,* 2014). A given biosensor is only as effective as the bioreceptor employed. Consequently, a range of mycotoxin specific recognition molecules have been developed. To date the most common biosensors used for single mycotoxin detection (in particular aflatoxins) have been optical or electrochemical, however piezoelectric sensors (*i.e* quartz mass micobalance, QCM) based on the change in mass on the sensor surface have been developed. Electrochemical biosensors have the greatest potential for mycotoxin determination in the form of simple high sensitive and cost effective portable devices. Most biosensors require minimal sample preparation. Solid samples may require extraction and clean-up procedures and tend to be adapted from those that would be used for HPLC or MS techniques. Though optical biosensors for mycotoxin detection based on Surface Plasmon Resonance (SPR) were reported by Li and Liu (2012). SPR is a physical optic phenomenon due to changes of the refractive index on the metal surface. A plane of polarised light shines directly through a prism to the metal interface a wide variety of incident angles, the evanescent wave will be generated under total internal reflection. At a selected incident light wavelength or angle the evanescent wave can resonate with surface plasmon produced by free electrons on the metal film of a sensor surface. The energy of the incident light will be absorbed by surface plasmon. The SPR angle is sensitive to the refractive index of the sample contacting the metal surface so it highly influenced by the sample contacting the sensor surface a reported limit of detection for optical biosensors for the detection of Aflatoxin B1 is 0.16ng Ml-1, with a response range of 0.5- 20 ng Ml-1, (Xu et al., 2013). A major advantage for biosensors is their potential for recycled use which distinguishes them from single use ELISA test kits and other rapid screening methods like lateral flow devices. Multiple mycotoxin analysis has also been conducted using SPR for example Meneely et al 2012 evaluated the simultaneous screening for T-2/HT-2 and deoxynivalenol in cereals using a surface plasmon resonance immunoassay. Though for biosensors these do not tend to have the same capacity to date in the numbers of toxins that can be analysed simultaneously for multiplexing in the same manner as the physiocohemical methods of analysis.

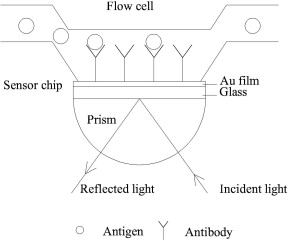
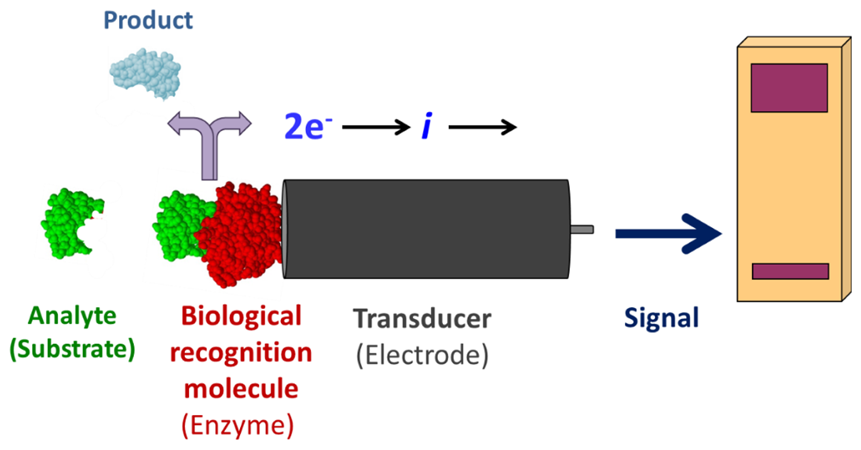


Figure 16 principle of an SPR biosensor Li and Liu et al., (2012)

*Electrochemical biosensors-*

The principle of electrochemical sensors in mycotoxin analysis lies in two directions depending on whether the total toxin load of a sample or a specific toxin is sought – which can be based on enzyme inhibition or affinity sensors- most sensors are affinity based on antibody – antigen reactions that produce or consume electrons. The resulting signal can be measured as current or potential from the corresponding concentration of the analyte can be exploited. According to the literature, the recorded limit of detection using an electrochemical biosensor for the detection of Aflatoxin B1, reported as 0.07ng/kg, with a response range of 0.6-2.4ng/mL, and a relative standard deviation of 2-5%, (Owino et al., 2008). Previously, biosensors have been mainly used for single mycotoxin detection (90-92).



*Figure 17 basic illustration of an electrochemical biosensor*

In great contrast to the large availability of many commercial products for single mycotoxin detection testing for example ELISAs and lateral flow strips, the number of commercially available multi mycotoxin biosensors is relatively small highlighting challenges facing the preparation and using multiplexed biosensors. The future for biosensors for mycotoxin detection is moving towards a simple, fast, less complex sample preparations and the analysis of multiple mycotoxins in one run. A proof of concept paper published in 2010 by Mak *et al* alludes to the next step of detection is multiplexed detection of mycotoxins from different families in a single run. In their paper they suggest that sample is added to the antibody-immobilized sensor array prior to the addition of the biotinylated detection antibody. The sensor response is then recorded in real time upon the addition of streptavidin-linked magnetic NanoTags on the chip. They demonstrate the simultaneous detection of multiple mycotoxins (aflatoxins B1, zearalenone and HT-2) and show that a detection limit of 50 pg/mL can be achieved. A search of the literature for biosensors as the method of analysis for multi mycotoxin detection was completed in Web of Science in all databases (Web of Science core collection, KCI- Korean Journal Database, MEDLINE, Russian Science Citation index, SciELO Citation index) using the following search terms: Searched for: TOPIC: (biosensors) AND TOPIC: (mycotoxins) Refined by: TOPIC: (multiplex) Timespan: 2010-2018. A flowchart schematic of the search is provided and the key results are presented in Table 5 providing a description of biosensor methods for multiple mycotoxin determination.

Flowchart for Biosensors search

Full-text articles excluded, with reasons  
(n = 0 )

Full-text articles assessed for eligibility  
(n = 10 )

Records excluded  
(n =3 )

Records after duplicates removed  
(n = 203 )

Additional records identified through All database search in WOS   
(n = 131 )

## Identification

## Eligibility

## Included

## Screening

Records identified through WOS database searching ‘mycotoxins’ AND ‘Biosensor’  
(n = 72 )

Studies included in reference table  
(n =10 )

After refinement by ‘multiplex’ Records screened  
(n = 12 )

Table 5 Table showing detailed description of biosensor methods for multiple mycotoxin determination

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Toxin** | **Matrix** | **Biosensor** | **LOD** | **Advantages** | **Reference** |
| aflatoxins B1, zearalenone and HT-2 |  | Magnetoresistive-based immunoassay | 50 pg/mL | Ultra-high sensitivity, multiplexing capability, simplicity of detection, point-of-use testing not only for mycotoxin detection but also many proteomic applications. | (93) |
| nivalenol (NIV) and deoxynivalenol (DON) | Cereals and Barley | A surface plasmon resonance (SPR) immunoassay using a monoclonal antibody | 0.1 mg kg(-1) NIV  0.05 mg kg(-1) DON | practical method to rapidly screen the NIV and DON. designed simply, without time-consuming cleanup procedures, and good accuracy and reproducibility | (94) |
| ochratoxin A (OTA), aflatoxins B1 (AFB1), fumonisin B1 (FB1) | Rice, Corn, and Wheat | Aptamer microarray method on TiO2-Psi surface | 15.4pg/ml OTA  1.48pg/ml AFB1  0.21 pg/ml FB1, | cost efficiency, versatile fabrication, easy modification, large surface area, and good biological compatibility | (95) |
| deoxynivalenol (DON), zearalenone (ZEA) and T-2 toxin. | Wheat | Gold nanoparticle-enhanced iSPR (imaging surface plasmon resonance) assay | 15 µg/kg DON,  24 µg/kg ZEA  12 µg/kg T-2 toxin. | low detection limits, short analysis time, and low reagent consumption, reduction in costs. | (96) |
| ochratoxin A (OTA), zearalenone (ZEA) and aflatoxins B1 (AFB1) | White wine | Label free Interferometric Biosensor | 0.25ng/ml OTA, 0.48ng/ml ZEA  1 ng/mL AFB1 | Label free biosensor with precise, rapid, sensitive and multiplex detection. Employs affordable single-used consumables (sensor chips) | (97) |
| deoxynivalenol (DON), zearalenone (ZEA),  T-2 toxin (T-2), ochratoxin A (OTA), fumonisin B1 (FB1) aflatoxin B1 (AFB1) | Barley | Multiplex surface plasmon resonance biosensor  (A 6-plex competitive inhibition immunoassay) | 26 μg kg−1 DON,  6 μg kg−1 ZEA,  0.6 μg kg−1 T-2,  3 μg kg−1 OTA,  2 μg kg−1 FB1  0.6 μg kg−1 AFB1. | Simultaneous detection of six toxins using one chip. Rapid detection both targeted and other less frequently occurring mycotoxins in real samples. Save costs in terms of the chip, reagents as well as assay time. | (98) |
| FMB1, AFB1, aflatoxin B2, aflatoxin G1, aflatoxin G2, ochratoxin A, deoxynivalenol, and zearalenone | Maize | Multiplex chemiluminescent biosensor | 6 μg kg−1 FMB1  1.5 μg kg−1 AFB1 | Simple, rapid and ultrasensitive on-site quantification of aflatoxin B1 and type B-fumonisins. Inexpensive, easy-to-use, and fit for the purpose of rapid screening of mycotoxins | (99) |
| Aflatoxin B1 (AFB1), fumonisin B1 (FB1) and ochratoxin A (OTA | Cereals | Multiplex chemiluminescent mycotoxin immunoassay suspension array system (CLIA) | 1.19 pg/ml AFB1,  0.60 pg/ml FB1, and 0.73 pg/ml OTA | Simple, rapid, low cost and high throughput for multiplex mycotoxin assay. | (100) |
| aflatoxin B1, AFB1; zearalenone, ZEA; ochratoxin A, OTA | Corn, Rice and Wheat | Microarray surface enhanced Raman scattering based immunosensor | 0.061–0.066 μg/kg AFB1,  0.53–0.57 μg/kg ZEA, 0.26–0.29 μg/kg OTA | simultaneously and rapidly monitoring multiple-mycotoxin levels in foodstuff. High assay sensitivity and wide detection range. | (101) |
| aflatoxin B1, ochratoxin A and deoxynivalenol | Corn | Multiplexed microfluidic fluorescence immunoassay with photodiode array signal acquisition | Below 1 ng mL−1 for all mycotoxins | Portable, rapid, cost effective and simple analytical tool | (102) |

An immunoassay is a biochemical test that measures the presence or concentration of a macromolecule or a small molecule in a solution through the use of an antibody (usually) or an antigen (sometimes). The molecule detected by the immunoassay is often referred to as an "analyte" and is in many cases a protein, although it may be other kinds of molecules, of different size and types, as long as the proper antibodies that have the adequate properties for the assay are developed (14). An additional search was carried out using the search term ‘immunoassay’. The search was completed in Web of Science in all databases (Web of Science core collection, KCI- Korean Journal Database, MEDLINE, Russian Science Citation index, SciELO Citation index) using the following search terms: Searched for: TOPIC: (mycotoxins) AND TOPIC: (immunoassay) Refined by: TOPIC: (multiplex) Timespan: 2010-2018. Any duplicates were removed and any articles that appeared in the previous search for lateral flow devices were also removed. A flowchart schematic of the search is provided and the key results are presented in Table 6 providing a description of immunological methods for multiple mycotoxin determination. Nolan et al (2019) also conducted a review of the rapid test for mycotoxin analysis and emerging techniques including immunological procedures.

Flowchart for additional immunoassay methods (not already listed above) search 2010-2018

Full-text articles assessed for eligibility  
(n = 38 )

Records after duplicates removed  
(n = 589)

## Identification

## Eligibility

## Included

## Screening

Records identified through WOS database All databases searching ‘mycotoxins’ AND AND ‘immunoassay’  
(n = 589)

After refinement by ‘multiplex’ Records screened  
(n = 59 )

Records excluded because already shown in Biosensors and Lateral Flow Reference tables  
(n = 21 )

Studies included in reference table  
(n = 26 )

Table 6 Table showing detailed description of additional immunoassay methods for multiple mycotoxin determination 2010-2018

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Toxin** | **Matrix** | **Immunoassay methods** | **LOD** | **Advantages** | **Reference** |
| T-2 toxin, aflatoxin B1, ochratoxin A, and zearalenone | Corn | Automated immunoassay instrument based on microfluidics and microarray. | between 0.03 and 1.24 ng/ml for all toxins | small-sized and user-friendly. | (103) |
| AFB1, OTA and FB1 | Cereals | Aptamer photonic crystal microsphere (PHCM) suspension array | 15.96 fg/mL AFB1,  3.96 fg/mL OTA  11.04 pg/mL FB1 | High throughput, ultrasensitive, high selectivity, and small volume reagent | (104) |
| zearalenone, deoxynivalenol, aflatoxin B1, and ochratoxin A) | wheat, barley, soybean, wheat bran, rice, rice bran, maize, rapeseed meal, and sunflower meal, and various types of complete feed | Membrane-based Flow-through Immunoassay | 50 μg/kg ZEA,  200 μg/kg DON,  1 μg/kg AFB1,  10 μg/kg OTA  (cut off levels) | Simple, cost-effective, and easy to perform on-site in a non-laboratory environment | (105) |
| deoxynivalenol, fumonisin B1, and ochratoxin A, | Wheat and corn flours | Magnetic particle-based enzyme immunoassay | 5.0 ± 1.4 ng mL−1 FB1, 4.3 ± 1.8 ng mL−1 DON, 0.1 ± 0.05 ng mL−1 OTA | decrease the incubation times, improve greatly the efficiency of the separation. high-throughput and accurate for rapid determination of 3 mycotoxins | (106) |
| zearalenone, deoxynivalenol, T‐2 toxin, HT‐2 toxin, aflatoxins, ochratoxin A, and fumonisin B1 | Barley | Immune‐affinity monolithic array with chemiluminescent detection | 0.029 µg L−1 ZEN  0.036 µg L−1 DON  0.025 µg L−1T-2  0.035 µg L−1HT-2  0.019 µg L−1 AFs  0.013 µg L−1 OTA  0.031 µg L−1 FB1, | Highly sensitive, stable and economical tool, able to minimise manipulation steps | (107) |
| zearalenone (ZEA), T2-toxin (T2) and fumonisin B1 (FUM) | Cereal grain, animal feed and forages | A nanoarray for the semi-quantitative and simultaneous analysis of mycotoxins. | Not stated in article | A sensitive and novel multiplex nanoarray, high throughput detection method | (108) |
| ochratoxin A (OTA), aflatoxin B1 (AFB1) and deoxynivalenol (DON) | feed | Microfluidic device with integrated photodetector array for immunoassay multiplexing | 0.1ng/mL AFB1 ,  0.3 ng/mL DON  1 ng/mL OTA, | Point of use, simple to operate, utilizing negative pressure and magnetic valves. | (109) |
| Zearalenone and T2 Toxin | Cereals | Competitive fluorescent microsphere immunoassay (CFIA) | - | portable cytometry gives rapid multiplexed Point Of Need testing to assure the safety of small food processing installations. | (110) |
| deoxynivalenol, zearalenone and aflatoxin B1 | Maize and Wheat | QD@SiO2-based immunoassay | 6.1 and 5.3 µg kg−1 DON, 5.4 and 4.1 µg kg−1 ZEA, 2.6 and 1.9 µg kg−1 AFB1 in maize and wheat respectively | immobilization of three antibodies specific towards different analytes into the same well of microtiter plate for multiplex detection. | (111) |
| Aflatoxin B1 (AFB1), fumonisin B1 (FB1) and ochratoxin A (OTA) | Rice, Corn and Wheat | Chemiluminescence immunoassay by silica-hydrogel photonic crystal microsphere suspension arrays | 1.7 pg/mL AFB1,  2.1 pg/mL FB1  0.4 pg/mL OTA | high throughput and sensitive screening for multiplex mycotoxins in cereal samples. | (112) |
| aflatoxins,  fumonisins, ochratoxins, type A trichothecenes  (T-2 toxin and HT-2 toxin), type B trichothecenes  (deoxynivalenol), and zearalenones. | Feed | Biochip Array Immunoassays | (IC50 values : 0.08 ng/mL (fumonisin B1),  0.03 ng/mL (ochratoxinA,  0.015 ng/mL (aflatoxin G1),  3.2 ng/mL (DON),  0.1 ng/mL (T-2  toxin),  0.0075  ng/mL  (aflatoxin  B1),  0.044 ng/mL  (zearalenone). | A multiplex, semiquantitative screening method for  Mycotoxins, sensitive, reliable, and rapid  detection of over 20 mycotoxins from all the main groups, cost effective tool. | (113) |
| deoxynivalenol (DON), T-2 toxin and fumonisin B1 (FB1) | Maize | Universal multi-wavelength fluorescence polarization immunoassay | 242.0 μg kg−1 DON,  17.8 μg kg−1 T-2 toxin  331.5 μg kg−1 FB1, | Detection time less than 30 mins | (114) |
| deoxynivalenol (DON)  ochratoxin A (OTA)  Aflatoxin B1 (AFB1) | - | Chemiluminescence-based microfluidic immunoassay | Stated ‘LODs comparable with state of the art methodologies’  No values given | simultaneously detects 3 different mycotoxins, in three independent samples using a direct competitive immunoassay Less than 15 mins run time. Simple Point of use tool | (115) |
| zearalenone and aflatoxin B1 | Wheat and Maize | multiplex fluorescent immunoassay  (Silica-coated liposomes loaded with quantum dots as labels) | (IC50 values)  16.2 and 18 µg kg−1 for zearalenone in wheat and maize  2.2 and 2.6 µg kg−1 for aflatoxin B1 in wheat and maize | Novel, sensitive, high-throughput and easy-to-operate multiplex immunoassay | (116) |
| deoxynivalenol, zearalenone, aflatoxin B1, T2-toxin and fumonisin B1 | Cereals | Novel multiplex fluorescent immunoassays (based on quantum dots) | 3.2 µg kg−1, DON  0.6 µg kg−1 ZEA,  0.2 µg kg−1 AFB1,  10 µg kg−1 T-2 and  0.4 µg kg−1 FB1 | Easy-to-operate. Provides cheap, simple and reliable analytical technique. | (117) |
| aflatoxin B1, ochratoxin A, zearalenone, deoxynivalenol, T2-toxin, HT-2 toxin and fumonisin B1 | Barley | 6-Plex microsphere immunoassay with imaging planar array detection | (Cut off values)  2 μg kg−1 aflatoxin B1,  2.5 μg kg−1 ochratoxin A,  625 μg kg−1 deoxynivalenol, 50 μg kg−1 zearalenone,  1000 μg kg−1 fumonisin B1  25 μg kg−1 T-2 toxin. | on-site testing, portable, cost effective screening method. The multiplex capacity of the color-coded microspheres allows the addition of more mycotoxin assays up to a 50-plex. | (118) |
| fumonisin B1 FB1, ochratoxin A OTA,  zearalenone ZEN | Maize and wheat | Colour-encoded paramagnetic microbead-based direct inhibition triplex flow cytometric immunoassay | For OTA, the sensitivities were 0.7 and 3.4 μg/kg, respectively (ML = 5 μg/kg); for ZEN, 5.8 and 32 μg/kg, respectively (ML = 100 μg/kg); and for FB1 + FB2, 170 and 1270 μg/kg, respectively (ML = 2000 μg/kg). | Faster and requires less procedural steps than the previously developed indirect assay format | (119) |
| aflatoxins,  ochratoxin A (OTA),  fumonisin B1 (FB1), deoxynivalenol, DON | Cereals | Automated flow-through multi-mycotoxin immunoassay using the stand-alone Munich Chip Reader 3 platform and reusable biochips | 0.06 μg L−1 AFB1  0.07 μg L−1 OTA  9.9 μg L−1 FB1  2.5 μg L−1 DON | microarray chip is reusable for at least 50 times. Total assay time 19 mins. | (120) |
| Aflatoxin B1 (AFB1), fumonisin B1 (FB1), and citrinin (CIT) | Cereals | Immunoassay with Photonic Crystal Microsphere Suspension Array | 0.5 pg/mL AFB1  1 pg/mL,FB1  0.8 pg/mL CIT | method saves a large amount of reagents and has a reduced detection time | (121) |
| Ochratoxin A (OTA), Aflatoxin B1 (AFB1), Fumonisin B1 (FB1),  T-2 toxin (T-2), Deoxynivalenol (DON) and Zearalenone (ZEA) | Corn | A flow cytometry based competitive fluorescent microsphere immunoassay (CFIA) | - | Help to avoid false negative results. more compact, robust and cost effective. | (122) |
| Ochratoxin A (OTA) Fumonisin B1 (FB1) | Maize | Aptasensor based on multiplexed fluorescence resonance energy transfer (FRET) | OTA 0.02 ng·mL–1  FB1 0.1 ng·mL–1 | novel sensor for the simultaneous determination of OTA and FB1 using a multiplexed FRET. High level of accuracy. Feasible | (123) |
| T-2/HT-2 toxins (T-2/HT-2)  deoxynivalenol (DON) | Cereals and cereal based products | Rapid surface plasmon resonance (SPR) immunoassay | 12, 1 and 29 µg/kg for  DON and 31, 47 and 36 µg/kg for HT-2 in wheat, breakfast cereal and maize-based baby food, |  | (124) |
| ochratoxin A, fumonisin B1, deoxynivalenol, and zearalenone | Peanut cake, maize, and cassava flour | Multiplex flow-through immunoassay | (cut off values)  3 μg kg−1 OTA  5 μg kg−1,FB1  700 μg kg−1, DON,  175 μg kg−1 ZEA | Efficient, cost-effective analytical method and can be used for the detection of mycotoxins in low-income communities. | (125) |
| deoxynivalenol (DON) and zearalenone (ZEN) | Maize and Wheat | Imaging surface plasmon resonance for multiplex microassay | 84 μg/kg DON (wheat)  68 μg/kg (wheat)  and 64 μg/kg ZEN (maize) and 40 μg/kg for ZEN in (wheat) | Rapid screening of maize and wheat extract without a complex and time-consuming sample preparation procedure | (126) |
| aflatoxins, ochratoxin A, deoxynivalenol, fumonisins, zearalenone and T-2-toxin | Feed | Multi-mycotoxin immunoassay—using the MultiAnalyte Profiling (xMAP) technology | AFB1 <0.005 mg/kg,  DON <0.50 mg/kg,  FB1 <0.10 mg/kg,  FB2 <0.10 mg/kg,  FB3 <0.10 mg/kg,  OTA <0.025 mg/kg,  T2 <0.5 mg/kg, and  ZEA <0.05 mg/kg) | an emerging technology that uses small carboxylated polystyrene microspheres. A promising qualitative multiplex immunoassay and can be easily extended with other mycotoxins of interest. | (127) |
| fumonisin B1 and ochratoxin A | Oats and corn | An indirect competitive fluid array fluoroimmunoassay | - | detection of two foodborne mycotoxins at sub-ppm levels | (128) |

**Biomarkers of exposures**

The investigation of adverse health effects associated with fungal mycotoxins requires the measurement of human exposure. Most frequently, this exposure is estimated from contamination levels of raw foodstuffs, which are the primary source of toxin exposure. However, variations in food preparation methods, food intake, contamination level, intestinal absorption, toxin distribution and excretion lead to individual variations in toxin exposure that are more readily measured with a biomarker. Contamination of humans by dietary intake may result in various pathophysiological effects, such as nephrotoxicity, gastro-enteric distress, hyperestrogenic medical conditions (which are characterised by an excessive amount of estrogenic activity in the body) and cancer. Therefore, exposure of these compounds to the human population needs to be highly controlled and assessed. Risk analysis of a toxin is evaluated with the help of biomarkers. These provide information on the biological response following the contamination or allow the quantification of the toxin or its biotransformation products in bodily fluids such as urine and blood (biomarkers of exposure). The biomarkers are needed to establish the oral dose (intake from food), the internal dose (biologically active) and the dose-response relationship. A series of biomarkers of exposure and effect for aflatoxins, fumonisins, ochratoxin A, zearalenone and deoxynivalenol include blood, urine and milk (aflatoxins and ochratoxins), serum, urine, faeces, hair and nails (fumonisins) and urine (deoxynivalenol). There are no reliable biomarkers for zearalenone.

Animal exposure to mycotoxins is also important to observe, due to the possibility of human contamination from animal product consumption most notable through milk. The assessment of exposure to any dietary contaminant is presently evaluated based on its intake from food (or feed), also known as ‘external exposure’ or ‘oral dose’. The link between the observed disease occurrence and a certain dietary contaminant is made by biomarkers. These provide information on factors that are original or informative towards the respective condition. Direct biomarkers are biomarkers of exposure. A biomarker of exposure indicates the quantification of the specific compound, its metabolites or interaction products in the body or biological fluid, which indicates the presence and magnitude of exposure to the toxin. Ideally, such a marker should reflect the toxicokinetics, transformation and fate of the assessed contaminant in the body. Currently, the main method of analysis of mycotoxins (aflatoxin, ochratoxin A and fumonisins in urine is by HPLC-MS/MS. For the simultaneous detection of multiple urinary biomarkers of DON, AFB1, FB1, ZEA, and OTA can be achieved using a LC-ESI-MS/MS method with a Myco6in1 immunoaffinity column enabling good purification and concentration of the mycotoxins. On performing screening and confirmatory methods of analysis for multiple mycotoxins, information will be gathered about the presence and the toxicokinetic behaviour of these mycotoxins in humans and animals. Biomarkers can therefore be selected to assess exposure not only in humans but in pigs, and poultry hence minimising agricultural economic losses. Finally, two kinds of detoxifying agents exist: mycotoxin binders and mycotoxin modifiers and the effect of these detoxifying agents can be evaluated and the most promising agents used to counteract the effects of mycotoxins in animals and humans.

**Conclusion**

There are a multitude of analytical and bioanalytical techniques presented in the literature for mycotoxin analysis though many of these are very much single toxin or toxin family specific depending on the cross-reactivity of antibodies raised in the case of immunological methods. There are still significant limitations in technologies that can analyse multiple mycotoxins in a timely cost effective manner without requiring specific skills or advanced analytical training. LC-MS and in particular HRS-LC-MS not only appears to be leading the way in multi-toxin analysis but also in screening for new unknown toxins or analogues that may be present. One of the challenges still posed for high quality quantification are routinely affordable internal radiolabelled standards. For ELISA and biosensor methodologies these are lacking behind in multitoxin capabilities primarily due to the availability of high quality or broad specificity antibodies for all toxins and analogues. There are also limitations to the number of toxins that could be analysed together with currently available technology or microarray equipment and readers. However, the bottleneck for all approaches remains the sample preparation for multiple toxins and the requirements in validation without having analytical standards being routinely available at affordable prices. The market today for mycotoxin test kits is already  
a competitive one with multiple companies selling variations of similar products. However, judging from the literature it is likely that within the next decade, the market will consist of test kits that are hand-held digital biosensors capable of performing multiplex analysis. One contrasting assay development, for mainly biosensors, is that which regards either the use of label-free detection or the addition of labels such as nanoparticles, to increase sensitivity. Here, a trade-off between sensitivity and cost can be made in assay development. However, immunoassays are still a popular detection method for screening mycotoxins due to the cost and availability, though further work is required to consider multiple toxin analysis. Until that time expensive LC-MS methods are leading the way in multiple toxin analysis though additional considerations need to be applied for the validation of multiple toxin assays and in the matrix to which the assay is applied.

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