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Developments in mycotoxin analysis: an update for 2013-2014

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REVIEW ARTICLE

Abstract

This review highlights developments in the determination of mycotoxins over a period between mid-2013 and mid-2014. It continues in the format of the previous articles of this series, emphasising on analytical methods to determine aflatoxins, *Alternaria* toxins, ergot alkaloids, fumonisins, ochratoxins, patulin, trichothecenes and zearalenone. The importance of proper sampling and sample preparation is briefly addressed in a dedicated section, while another chapter summarises new methods used to analyse botanicals and spices. As LC-MS/MS instruments are becoming more and more widespread in the determination of multiple classes of mycotoxins, another section is focusing on such newly developed multi-mycotoxin methods. While the wealth of published methods during the 12 month time span makes it impossible to cover every single one, this exhaustive review nevertheless aims to address and briefly discuss the most important developments and trends.

Keywords: aflatoxin, *Alternaria* toxins, ergot alkaloids, fumonisin, ochratoxin A, patulin, trichothecene, zearalenone, sampling, multi-mycotoxin, botanicals, method development

1. Introduction

This article is the latest instalment of a series of annual reviews highlighting analytical method developments for mycotoxin determination, seamlessly continuing from the previous paper covering the 2012/2013 period (Berthiller *et al.*, 2014). While the main focus of this work is clearly on new methods published between mid-2013 to mid-2014, some occurrence data are included as well, especially to demonstrate method applicability. The covered topics are sampling and sample preparation (Section 2), multi-mycotoxin liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods (Section 3), mycotoxins in botanicals and spices (Section 4), aflatoxins (Section 5), *Alternaria* toxins (Section 6), ergot alkaloids (Section 7), fumonisins (Section 8), ochratoxins (Section 9), patulin (PAT, Section 10), trichothecenes (Section 11) and zearalenone (ZEA, Section 12).

Several reviews have been published lately which discuss various analytical methodologies used for the determination of mycotoxins (and other biotoxins). The interested reader is referred to them for additional information. General analytical method reviews for the determination of mycotoxins in cereals or other agricultural commodities are provided by Huybrechts *et al.* (2013) and by Pereira *et al.* (2014). Amelin *et al.* (2013) reviewed chromatographic methods for the determination of mycotoxins in food

products. O'Mahony *et al.* (2013) specifically looked at methods employing ultra-high performance liquid chromatography (UHPLC) with tandem mass spectrometric (MS/MS) detection for the determination of agrochemical residues and mycotoxins in food. Anfossi *et al.* (2013b) reviewed lateral-flow immunoassays for mycotoxins and phycotoxins, while Dzantiev *et al.* (2014) summarised immunochromatographic methods in food analysis, focussing on the detection of toxic contaminants. The development of various microchips, including microfluidic chips and microarrays, for the detection of biotoxins has been reviewed by Zhang *et al.* (2014d). Vidal *et al.* (2013) critically reviewed electrochemical affinity biosensors for the detection of mycotoxins. Cell-based models are often used for toxicity evaluation, but also to screen for toxic compounds. Cheli *et al.* (2014b) reviewed those models regarding mycotoxins. Finally, applications of single kernel conventional and hyperspectral imaging near infrared spectroscopy in cereals were summarised by Fox and Manley (2014). While there is still much research and development to be done, these applications show promise for the sorting of grain infected by fungi and/or grain contaminated with mycotoxins.

2. Sampling and sample preparation

Improvements in sampling and sample preparation methods used to detect mycotoxins and other quality attributes in food and feed products continues to be a high priority among regulatory agencies, international organisations and commodity industries worldwide. Several articles were published in this area.

Cheli *et al.* (2014a) reviewed existing EU legislation associated with cereal safety with a focus on mycotoxins. The authors present a brief review of topics such as sampling and analysis and maximum levels (MLs) for contamination. Knowledge of EU sampling plans and MLs will help those who export cereal grains to the EU design sampling plans for use at origin to reduce lots rejected at destination in the EU. The authors also provide data related to the worldwide occurrence of mycotoxins in cereals. Related to this, Wesolek *et al.* (2014) described a mathematical approach for sampling plan performance assessment for aflatoxin B_1 (AFB_1) in pistachios and computed operating characteristic curves for different sampling scenarios with respect to the legislation on aflatoxins in the EU. Hallier *et al.* (2013) continued studies to reduce sampling variability associated with testing organic wheat grains for deoxynivalenol (DON). The authors studied sampling procedures that took into consideration different DON contamination levels among kernels of various sizes and kernel densities. Results indicated more DON contamination was associated with small kernels than large kernels and DON contamination was inversely proportional to kernel density (the least dense kernels had the highest DON contamination). Results of the study can be used to reduce the DON contamination of entire lots and can be used to scale down the size of the laboratory sample taken from the aggregate sample. Cheli *et al.* (2013) reviewed the effect of wheat milling procedures on mycotoxin distribution in products and by-products. Literature confirms that while milling can minimise mycotoxin concentrations in fraction used for human consumption, other fractions – typically used as animal feed – are enriched up to eight-fold compared to the original grain. Of course, other physical processes carried out before milling, e.g. sorting, cleaning or debranning, can be efficient to reduce mycotoxins in grain. Here, however, published data show a rather high variability and sometimes appears even conflicting. The reasons for this can be mainly associated to the level and extent of fungal contamination, different behaviours of different mycotoxins, and a failure to understand the complexity of the milling technology.

3. Multi-mycotoxin LC-MS(/MS) methods

A survey of the available literature relevant to the 2013/2014 period shows that with respect to quantitative multi-mycotoxin methods the main challenge remains the optimisation of suitable sample preparation protocols, and QuEChERS (quick, easy, cheap, effective, rugged and safe) based approaches have been this year the most widely investigated. For multi-mycotoxin screening methods, mostly based on high resolution mass spectrometry (HRMS), the use of powerful software tools is gaining importance rather than sample preparation.

A QuEChERS based protocol has been optimised for the UHPLC-MS/MS determination of 36 mycotoxins in wines (Pizzutti *et al.*, 2014). It is worth mentioning that after testing various sorbents and sorbent mixtures, the authors concluded that the dispersive solid phase extraction (dSPE) step did not give significant improvements either in terms of recoveries or matrix effects. This was probably due to the very small amount of matrix injected (3 µl wine) on column. Average recoveries were in the range 70-120%, with a relative standard deviation (RSD_r) generally lower than 20%. Only nivalenol (NIV) could not be quantified at any concentration level. Arroyo-Manzanares *et al.* (2014a) reported a QuEChERs-based sample treatment suitable for the extraction of fifteen mycotoxins (aflatoxins, ochratoxin A (OTA), trichothecenes, sterigmatocystin (STC), and ZEA) prior UHPLC-MS/MS analysis in pseudocereals (buckwheat, quinoa, and amaranth), spelt and rice. The recoveries in spiked samples ranged from 60 to 135% except for beauvericin (BEA), with both repeatabilites (RSD_r) and reproducibilities (RSD_R) ≤12%. Similarly, QuEChERS have been used for the simultaneous LC-MS/MS determination of 16 mycotoxins, including aflatoxins, OTA, trichothecenes, enniatins, and BEA in dried fruits (Azaiez *et al.*, 2014) and for determination of 33 mycotoxins in *Lentinula edodes* by LC-MS/MS (Han *et al.*, 2014).

The use of QuEChERS and solid phase extraction (SPE) clean-up was compared by Wang *et al.* (2014c) for the simultaneous determination of 17 mycotoxins, including aflatoxins, trichothecenes, ZEA, and Alternaria toxins, in traditional Chinese medicine (*Puerariae lobatae radix*). The SPE columns (TC-M160, r-Biopharm) gave better recoveries for all studied mycotoxins. However, it should be taken into account that the several available QuEChERS commercial kits or QuEChERS-like protocols, differing in extraction, partitioning or dSPE steps, may show different clean-up efficiencies. As an example, an extensive discussion about modifications/adaptations of the extraction and dSPE processes, to make them fit for the purpose of extracting mycotoxins of different polarity prior to UHPLC-MS/MS analysis, has been reported by Koesukwiwat *et al.* (2014). The authors applied the optimised QuEChERs protocol for the determination of 14 mycotoxins in rice, obtaining 70-98% overall recoveries with $RSD_r \leq 7\%$ for most analytes and satisfactory z-scores in a proficiency test. Another experimental design to optimise a QuEChERS based protocol for the simultaneous extraction of 58 mycotoxins from dairy products has been described by Jia *et al.* (2014). Response surface methodology was used to investigate the variations in recovery rates with respect to extraction solvent volume, amounts of sodium acetate, PSA (primary secondary amine) and C18 sorbents. The optimal composition of the four variables was determined by using a central composite design approach. The resulting optimised conditions, coupled to UHPLC-Q/Orbitrap detection, allowed reliable simultaneous analysis of the 58 target analytes with recoveries in the range of 87-114%. Dzuman *et al.* (2014b) optimised a QuEChERS method for the determination of 56 *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Claviceps* mycotoxins in animal feeds by UHPLC-MS/MS. In the optimisation of extraction/purification the most critical factor was found to be pH of extraction solvents, especially with regard to the pH-sensitive silages. Elimination of lipidic compounds was obtained using C18 silica sorbent for the dSPE step. According to other studies (Pizzutti *et al.*, 2014), after evaluation of matrix effects the authors concluded that while dSPE did not significantly influence matrix effects, the main benefit was the reduction of highly abundant non-polar matrix co-extractives.

Some authors proposed an additional clean-up step to improve performances of QuEChERS protocols, mainly for minimising matrix effects to achieve suitable detection limits. A sample treatment comprising QuEChERS extraction of fumonisins, trichothecenes, citrinin (CIT), STC, ZEA and OTA, followed by dispersive liquid-liquid microextraction (DLLME), was optimised in nuts and seeds by Arroyo-Manzanares *et al.* (2013). The DLLME step was necessary to reduce ion suppression in UHPLC-MS/MS analysis and achieve suitable aflatoxins detection limits. RSD_r values were <11% and recoveries ranged between 61 and 104%. A similar approach has been described by Desmarchelier *et al.* (2014), combining QuEChERS and immunoaffinity column (IAC) clean-up, for the simultaneous LC-MS/MS determination of EU-regulated mycotoxins in cereals, cocoa, oil, spices, infant formula, coffee and nuts. Also in this case the additional clean-up step was necessary to achieve satisfactory quantification limits (LOQ) for aflatoxins and OTA. Other performance parameters like recovery (71-118%) and precision (RSD_r<33%) were compliant with the analytical requirements stipulated in the CEN/TR/16059 document (CEN, 2011).

Even though less investigated if compared to QuEChERS, some applications of the dilute-and-shoot approach as generic sample pre-treatment can be found also this year. An LC-MS/MS method was developed for the determination of 12 mycotoxins (aflatoxins, DON, fumonisins, OTA, T-2 toxin (T-2), and ZEA) in milk-based infant formula and foods (Zhang *et al.*, 2013a). Samples were prepared by dilution and filtration, followed by LC-MS/MS analysis. The individual recoveries ranged from 72 to 136%, with RSD_r ranging from 2 to 25%. LOQs were from 0.01 µg/kg (aflatoxin M_1, AFM_1) to 2 µg/kg (fumonisin B_1, FB_1). It is worth noting that the most critical factor in achieving low LOQs was the high sensitivity of the MS detector, as stated by the authors themselves. This still represents the main limitation of methods omitting sample clean-up, and requiring sample dilution to manage matrix effects. As an example, Capriotti *et al.* (2014) reported poor detectability of target mycotoxins (trichothecenes, ZEA and its metabolites, fumonisins, OTA, enniatins and BEA) due to high matrix interference when injecting crude biscuits extracts. The problem was overcome by introducing a SPE step using graphitized carbon black prior LC-MS/MS determination. Recoveries of the optimised method ranged between 63 and 107% with RSD_r lower than 12%.

For LC-MS/MS methods focusing on major/regulated mycotoxins more selective clean-up strategies have been applied. The use of polymeric SPE columns continue to represent a good compromise between efficient interference removal and retention of structurally different mycotoxins. In particular, Oasis HLB® columns (Waters, Milford, MA, USA) have been re-proposed for the analysis of 9 regulated mycotoxins in maize (Wang *et al.*, 2013a) as well as for AFM_1, OTA, ZEA and α-zearalenol (α-ZOL) in milk (Huang *et al.*, 2014a). Merging multi-antibody IAC specificity with selectivity and sensitivity of LC-MS/MS detection is still considered a possible strategy mainly when low LOQs are desired or highly efficient removal of matrix interfering components is needed for complex food matrices or biological fluids. A multi-analyte column (Myco6in1™, Vicam, Milford, MA, USA) containing six different antibodies, for aflatoxins, OTA, fumonisins, DON, ZEA, T-2 and HT-2 toxin (HT-2), has been used for clean-up of extracts from cereals and nuts (Vaclavikova *et al.*,

2013). Recently this column, upgraded by introducing a new antibody able to retain DON and NIV, was used to develop an LC-MS/MS method for the quantitative determination of aflatoxins, OTA, fumonisins, ZEA, DON, NIV, T-2 and HT-2 in cereals and derived products (Lattanzio *et al.*, 2014). The intra-laboratory validation assay, carried out at 50, 100 and 150% of EU MLs for each mycotoxin, showed recoveries generally higher than 70% with RSD_r less than 37%. LOQs (calculated as the lowest amount of each analyte which could be determined with a precision of 10%) ranged from 1 μg/kg to 30 μg/kg. Two multi-mycotoxin methods, a dilute-and-shoot LC-MS/MS method and a method based on multi-toxin IAC before LC-MS/MS, were used for the determination of mycotoxins in good and mouldy maize samples harvested in the former Transkei (South Africa). A highly significant linear relationship ($P<0.001$) was shown to exist between the two methods for both FB_1 and FB_2 with r^2 values of 0.914 and 0.886, respectively, with the regression being forced through zero (Shephard *et al.*, 2013a).

New LC-MS(MS) methods for determination of multiple mycotoxins in biological fluids have been proposed also this year. A multi-mycotoxin method for the simultaneous determination of ZEA, DON and their metabolites in pig serum using Oasis HLB® columns and isotopically labelled internal standards has been developed by Brezina *et al.* (2014). Results for method precision ranged between 3 and 21% for inter-day and between 1 and 11% for intra-day. Recoveries were in the range of 82-131%. The method was tested for quantitative determination of ZEA, DON and their metabolites in pig serum from a feeding trial. A high-throughput LC-MS/MS method, involving pressurised liquid extraction (PLE), for the determination of 28 mycotoxins in various biological fluids and tissues of human and laboratory animals has been optimised (Cao *et al.*, 2013). The use of PLE pre-treatment without the need for any clean-up enabled to achieve limits of detection (defined as CCα) from 0.01 to 0.69 μg/kg (μg/l). Recoveries from spiked samples ranged from 71 to 100% (RSD_r<18%), except FB_1 and FB_2 recoveries, which were lower than 60%. A multi-biomarker approach to estimate the exposure of mothers and infants to mycotoxins, through the analysis of breast milk has been described by Rubert *et al.* (2014). The occurrence of mycotoxins in 35 human milk samples was evaluated by a UHPLC-HRMS after QuEChERS extraction enabling the detection of ZEA and metabolites, neosolaniol (NEO), NIV, enniatins, HT-2, DOM-1 and T-2 triol. Recoveries ranged between 64-93% and RSD_r lower than 20%. The value of multi-biomarker methods in measuring multiple mycotoxin exposure, especially in communities where it is difficult to collect food samples and food consumption data, is evidenced by a number of exposure studies that have been carried out this year. Multiple exposure biomarkers in the high oesophageal cancer region of the former Transkei (South Africa) have been studied by urinary single and multi-biomarker LC-MS/MS methods (Shephard *et al.*, 2013b) revealing a frequent co-presence of FB_1, DON, ZEA, α-ZOL, β-zearalenol (β-ZOL) and OTA. This was the first finding of urinary DON, ZEA, their conjugates, OTA and zearalenols in Transkei. An LC-MS/MS based multi-biomarker approach has been also used to evidence mycotoxin co-exposure in rural Nigeria population (Ezekiel *et al.*, 2014). A total of eight analytes were detected in 61/120 (51%) of studied urine samples, with OTA, AFM_1 and FB_1 being the most frequently occurring biomarkers. An improved UHPLC-MS/MS multi-biomarker method was developed and used to detect and measure incidence and levels of urinary biomarkers of the principal mycotoxins (DON, OTA, FB_1, ZEA and AFB_1) in Southern Italy. The high sensitivity of the method (LOQs between 0.006-1.5 ng/ml) enabled to measure biomarkers of two or more mycotoxins in all tested 52 samples. Urinary biomarker concentrations were used to estimate human exposure to multiple mycotoxins. For OTA and DON, 94 and 40% of volunteers, respectively exceeded the tolerable daily intake (TDI) for these mycotoxins, whereas the estimated human exposure to FB_1 and ZEA was largely below the TDI for these mycotoxins for all volunteers (Solfrizzo *et al.*, 2014).

Concerning the application of LC-HRMS techniques for screening of a large number of fungal metabolites, the main outcome derived from the studies reported this year is the importance of suitable software tools enabling reliable database searching and managing the huge amount of information generated by full scan acquisitions. A screening method using a database of over 600 metabolites to establish contamination profiles in food and feed has been set by Ates *et al.* (2014). Extracts were injected directly into an automated turbulent flow sample clean-up system, coupled to an LC-HRMS (Orbitrap) system. The ExactFinder software (Thermo Scientific, Waltham, MA, USA) was tested for database searching, and identification criteria were validated by spiking plant and fungal metabolites into cereals and feed. The optimised settings enabled effective compound screening with a false compliant rate of <1%. The screening of up to 3,000 metabolites in fungal extracts combining information generated by diode array detection (DAD) and quadrupole time of flight mass (QTOF) detector after UHPLC separation has been reported by Klitgaardet *et al.* (2014). The Target Analysis software from Bruker Daltonics (Billerica, MA, USA) was tested in this study (similar software is available from other suppliers, such as Waters, Thermo Scientific, Agilent (Santa Clara, CA, USA) or AB Sciex (Framingham, MA, USA)). Peaks corresponding to known compounds (reference standards, previously identified compounds, and major contaminants from solvents, media, filters, etc.) were labelled to differentiate these from compounds only identified by elemental composition. This enabled fast manual evaluation of both known peaks and potential novel-compound peaks, by manual verification of the adduct pattern, UV-Vis,

retention time compared with the distribution-coefficient log D, co-identified biosynthetic related compounds and elution order. System performance, including adduct patterns, in-source fragmentation, and ion-cooler bias, was investigated on reference standards, and the overall method was used on extracts of *Aspergillus carbonarius* and *Penicillium melanoconidium*, revealing new nitrogen-containing biomarkers for both species. The MarkerLynx™ software (Waters) has been evaluated to screen and identify mycotoxins in Chinese herbal materials by UHPLC-QTOF-MS (Fang *et al.*, 2013). The crude drug contaminated with fungi was analysed by comparison with uncontaminated ones. The software was employed to screen the excess components in analytes, compared with control samples, and those selected markers that were likely to be the fungal metabolites. The accurate masses of the markers were then searched in a mycotoxins/fungal metabolites database. Fragmentation data of the mycotoxin candidates were also compared with those of the most probable fragments generated by the MassFragment™ tool (Waters) from each of the possible structures of the mycotoxin candidates.

With respect to multi-class methods, i.e. methods intended for the simultaneous determination of different classes of small-molecular weight contaminants, the combined determination of mycotoxins and pesticides remains the most investigated topic. An UHPLC-MS/MS method for the simultaneous quantification of 10 mycotoxins and 29 pesticides in ginseng has been reported by Kuang *et al.* (2013). The method consisted of a one-step extraction procedure using acetonitrile (ACN):H_2O:HCOOH (99:33:1, v/v/v) without further clean-up. Analytical data were satisfactory with typical recoveries of 70-120% and RSDs below 20%. Untargeted full scan HRMS acquisition (UHPLC-QTOF) followed by database searching has been proposed by Masiá *et al.* (2014) for the identification of different contaminants in water, including mycotoxins, pesticides, pharmaceuticals and polyphenols. Again, this application shows the importance of software tools to manage data from HRMS acquisitions and in particular the use of proper settings for reliable database searching (retention time, accurate mass, isotopic pattern and MS/MS fragments) in combination with statistical data analysis using principal component analysis.

Finally, the latest advance in MS approaches for multi-mycotoxin and multi-contaminant detection reported this year is represented by the exploration of MS imaging techniques. Nielen and Van Beek (2014) investigated the use of laser-ablation electrospray ionisation (LAESI) mass spectrometry imaging (MSI) to obtain accurate mass ion-maps for macroscopic and microscopic distribution of pesticides, mycotoxins, and plant metabolites on rose leaves, orange and lemon fruit, ergot bodies, cherry tomatoes, and maize kernels, envisaging a future contribution of LAESI-MSI to research in food science, and plant metabolomics. Silica Plate Imprinting Laser Desorption/Ionisation Mass Spectrometry Imaging (SPILDI-MSI) has been shown to enable accurate identification of aflatoxins and resveratrol with unique fingerprint profiles in peanuts skin and kernels (De Oliveira *et al.*, 2014). Results, expressed as chemical images allowed the comparison between the spatial distribution of target compounds in kernel and skin. This novel application might become an innovative tool for rapid qualitative assessment of fungal contamination in nuts and other foodstuffs.

4. Mycotoxins in botanicals and spices

The advancement of method development pertaining to complex matrices including botanicals and spices has continued. Methods were developed utilising HPLC with fluorescence detection (FLD) and MS/MS detection. This further advancement is trending toward method development, optimisation and usage of multi-mycotoxin methodology (Section 3).

Four methods have been published utilising HPLC-FLD detection. A method validation study was performed for the determination of OTA in paprika and chili (Kunsagi and Stroka, 2014). This method included methanol (MeOH) extraction, IAC clean-up and HPLC-FLD measurement. 21 laboratories participated in the validation and recoveries for this method ranged from 84-88% with RSD_r from 1.7-14% and RSD_R from 9.1 to 28%. This method demonstrated acceptable precision per matrix validated according to the EU legislation. A second method validation for the determination of OTA in Chinese spices was also published (Zhao *et al.*, 2014a). This method encompassed a large variety of Chinese spices including pepper, chili, prickly ash, cinnamon, aniseed, fennel, curry powder and cumin. It utilised IAC clean-up followed by HPLC-FLD detection. This method obtained a recovery ranging from 75-102% and has an RSD_r of less than 12%. With this method, 480 retail spice products in China were analysed for OTA. Of the 480 samples, 9.6% where found to be contaminated with OTA and 2 chili products were found to be above the EU levels permitted for safe consumption. The next published method was a multi-mycotoxin method developed and validated specifically for coix seed (Kong *et al.*, 2013). It included the determination of AFB_1, aflatoxin B_2 (AFB_2), G_1 (AFG_1), G_2 (AFG_2), ZEA, α-ZOL and β-ZOL. The sample extraction was performed using a rapid ultrasound-assisted methodology with MeOH/H_2O (80/20). This was followed by IAC clean-up, on-line photochemical derivatisation, and UHPLC-FLD detection. Recoveries ranged from 74-107% with RSDs of 7.7% or less. The limits of detection and quantification ranged from 0.01 to 50 μg/kg and from 0.04 to 126 μg/kg, respectively. The final method published last year pertaining to HPLC-FLD was also a multi-mycotoxin method (Wen *et al.*, 2014). This method, which included an IAC clean-up, was developed for ginger and related products

for the simultaneous determination of AFB_1, AFB_2, AFG_1, AFG_2 and OTA. The method validation produced limits of dectection (LODs) ranging from 0.005-0.2 μg/kg, LOQs from 0.0125-0.5 μg/kg, and RSDs from 0.6-7.9%. After the validation, 30 samples of ginger and related products were analysed utilising this methodology. A total of five samples were positive for aflatoxins and for OTA with all sample results being below the European MLs. All positive results were confirmed with LC-MS/MS measurements.

An additional three methods were published utilising LC-MS/MS detection. One method was developed and validated for the simultaneous mycotoxin screening in food-grade gum (Zhang *et al.*, 2014b). This method utilised stable isotope dilution with LC-MS/MS detection to determine eleven toxins including AFB_1, AFB_2, AFG_1, AFG_2, FB_1, FB2, fumonisin B_3 (FB_3), OTA, T-2 and ZEA. Samples were fortified with ^{13}C-labeled internal standards for use in quantitation, extracted with ACN:H_2O (4:1, v/v) and then analysed using LC-MS/MS. The average recoveries for the toxins of interest were 84-117% with RSDs of less than 20%. Method dependent LOQs were from 0.1 (AFB_1) to 25 μg/kg (FB_3). Using this methodology, 20 market samples were analysed with AFB_1 detected in Guar gum and a Tragacanth gum, and ZEA detected in xanthan gum above the method's LOQ. A second method was developed using the QuEChERS based extraction technique and LC-MS/MS detection (Desmarchelier *et al.*, 2014). This method included the EU regulated mycotoxins for the analysis of cereals, cocoa, oil, spices, infant formula, coffee, and nuts. Once again this is a duel clean-up process starting with QuEChERS based technology for all toxins followed by an IAC clean-up for aflatoxins and OTA. Quantification of the toxins was performed with the use of ^{13}C-labelled internal standards. Limits of quantification were at or below the MLs set in the EC/1886/2006. LOQs in cereals as low as 0.05 μg/kg for aflatoxins and 0.25 μg/kg for OTA were achieved due to the added IAC clean-up process. Additional performance specifications include recoveries between 71-118% and a precision (RSD_r) of <19%.

Four market surveys were performed including three multi-mycotoxin methodologies with LC-MS/MS detection and the other with HPLC-FLD detection. A total of 130 spices were collected from stores in Northern Italy and analysed for AFB_1, AFB_2, AFG_1, AFG_2 and OTA by LC-MS/MS and HPLC-FLD detection (Prelle *et al.*, 2014). From the samples analysed, 15.4% and 23.8% were positive for aflatoxins and OTA, respectively. All aflatoxins detected were below the ML admitted by the EU, however 2.3% of the samples found to be positive for OTA surpassed these limits. Of the 130 samples analysed, 6 samples were found to have co-occurrence of OTA and aflatoxins ranging from 1.6 to 16 μg/kg and 0.6 to 3.2 μg/kg, respectively. A survey was performed involving 121 dry chili samples from Sri Lanka and Belgium for contamination of aflatoxins, OTA, FB_2, STC, alternariol methyl ether (AME), and CIT using LC-MS/MS detection (Yogendrarajah *et al.*, 2014). AFB_1 was the most predominant toxin contaminating 77% of the samples from Sri Lanka, OTA was found in 41%, and STC was found in 38%. Of the samples from Sri Lanka, 67% exceeded the EU ML for AFB_1 and 44% exceeded the EU ML for total aflatoxins. Additionally of the samples tested, one third of the chilies from Sri Lanka were found to be contaminated with more than three toxins. A survey utilising LC-MS/MS detection involved the analysis of fumonisins and OTA in herbs and spices commercialised in Poland (Waskiewicz *et al.*, 2013). A total of 79 samples were purchased from markets in Poland in which 31% were found to be contaminated with fumonisins and 49% for OTA. The last survey utilised HPLC-FLD detection of mycotoxins in functional foods and spices in Chinese markets (Kong *et al.*, 2014). A total of 24 samples were analysed with 4 functional foods and 3 spice samples found positive at low levels of AFB_1 (up to 0.26 μg/kg) and OTA (5.0 μg/kg). AFB_1 was found at the highest frequency with 16.7% positive samples.

5. Aflatoxins

Publications related to methods of analysis for aflatoxins have a clear focus on the demonstration of a variety of interesting immunoassays and their principles. The segment is widespread and some authors give detailed insight into the development of a new principle, demonstrating the applicability of the described technology with samples analysed or only in solution. It appears that often the aim is not the determination of aflatoxins as such, but that aflatoxins serve as an important contaminant to prove the practical use. This shows that the current trend is characterised by various novel technologies and principles, either as whole test system or single aspect of a methodology. This must be taken into account to understand the development for the rapid screening of aflatoxins. Authors that test their methods for cross reactivity do so with mycotoxins currently regulated in a variety of countries or regions, rather making a selection from a chemical point of view. It has been however demonstrated that structurally related mycotoxins, such as STC, even though not regulated, are more critical in terms of cross-reactivity (Sasaki *et al.*, 2014). Another interesting development is on the other hand the effort to find new or alternative methodological approaches for extract clean-up. In some cases alternatives to proprietary products, such as IACs, are developed, in other cases approaches to minimise reagent use. The arguments used to promote findings have to be judged by readers case by case as laboratory scenarios might differ. Interesting progress has also made in the use of spectroscopy as a non-destructive tool. In the following methods are grouped for contributions to immunochemical based technologies, other novel technologies such as spectroscopy or sample clean-up and contributions to elucidate related principles.

Sheng *et al.* (2014) proposed a method for fluorescence labelling of aflatoxins for a fluorescence polarisation immunoassay. Park *et al.* (2014) developed a new fusion protein, having improved properties to bind to the gold layer and the antibody in surface plasmon resonance (SPR). Authors reported improved immobilisation rates of antibodies with their new approach demonstrated with AFB_1 as example. Also the synthesis of magnetic nanoparticles has been described for SPE purification of aflatoxin containing extracts from maize and rice (Hashemi *et al.*, 2014a). Various parameters influencing ad- and de-sorption were studied and analytical results of the finally optimised method were compared with those from an official AOAC International method. Authors reported LODs and LOQs far below existing MLs in the EU. Nanotechnology was also used by Masoomi *et al.* (2013) who developed a non-enzymatic nanomagnetic electro-immunosensor and Lv *et al.* (2014) who described a label-free electro-chemiluminescence immunosensor based on silver nanoparticles. A monoclonal antibody was developed by Zhang *et al.* (2013b) and used for a lateral flow device (LFD). The visual detection (LOD) of AFM_1 in milk was reported as 0.2 μg/kg. Authors further reported a cross reactivity lower than 5% to other aflatoxins, which is thought to be due to the duo-immunogen immunisation strategy employed. A LFD on the basis of polyclonal antibodies has been developed by Liu *et al.* (2013) and used to test samples over a contamination range of 1.3-234 μg/kg AFB_1. Also Anfossi *et al.* (2013a) describe a lateral flow immunoassay (LFIA) for AFM_1 determination in milk based on polyclonal antibodies. The authors make valuable remarks on current challenges in developing such assays to meet current fit-for-purpose criteria.

Next to classical antibodies various authors used fragmented antibodies (FABs), molecular imprinted polymers (MIPs) or aptamers for specific interaction to determine aflatoxins. For example an immunoassay for the determination of AFB_1 based on a FAB was proposed by Edupuganti *et al.* (2013b). The precision of the extraction procedure was established with maize test material at a level of 3.5 μg/kg, while the LOD was established by multiple measurements of a blank plus three times the standard deviation of these measurements. Interesting features of this immunoassay are that microtiter plates are re-usable and the test showed only little cross reactivity to other aflatoxins. Unfortunately, as done by many other authors as well, additional cross reactivity was tested only for other structurally different mycotoxins, but not for STC. An electrochemical sensor for determination of AFB_1 in solutions was described by Wang *et al.* (2014f). Authors used multi-walled carbon nanotubes in combination with MIPs and demonstrated the applicability with vegetable oils fortified with AFB_1. This type of sensor seems to be also an interesting candidate for screening of ZEA in vegetable oils in view of existing MLs in the EU. An extraction for AFM_1 with magnetic graphene and subsequent quantification with antibody labelled quantum dots carbon nanotubes was realised in another study (Gan *et al.*, 2013). Authors reported a limit of detection of 0.3 ng/l (s/n=3). Several other research groups used aptamers to determine aflatoxins. For example Nguyen *et al.* (2013) described an aptamer based sensor system for electrochemical detection of AFM_1. Authors describe this novel system in detail and proved the concept with neat AFM_1 solutions. A step further went Guo *et al.* (2014) to use an AFB_1 aptamer as a molecular recognition probe and its complementary DNA as a signal generator for amplification by real-time quantitative PCR. Authors tested the methods applicability with hay and food samples and reported satisfactory recoveries. In a different way Shim *et al.* (2014) used aptamers for the development of a chemiluminescence competitive assay for the detection of AFB_1 in maize. Authors report a wide dynamic range of their developed method over two orders of magnitude with little cross reactivity to other aflatoxins such as AFG_1 and AFM_1. While authors, like others for similar novel methodologies, describe the principle in detail with neat solutions, no experiments with complex food matrices were carried out.

A chemoluminescence based method for the determination of AFB_1 in rice was described by Yu *et al.* (2013). Their immunoassay was optimised and tested with rice samples to prove applicability. Method performance was tested for repeatability and showed quantitative behaviour for recovery. The title of the paper invites readers to assume a wider scope, however only data for rice are presented, which in turn showed fit-for-purpose performance. Also Li *et al.* (in press) described a chemoluminescence based immunosorbent assay on the basis of luminol. Authors optimised the method for a number of parameters and finally compared results with those of a commercial enzyme-linked immunosorbent assay (ELISA) kit for the determination of AFM_1 in milk. Both methods compared for precision and recovery based on fortified milk, indicating the suitability of the new developed assay. Another chemoluminescence method for the determination of AFM_1 was presented by Vdovenko *et al.* (2014). Authors focussed on optimising the method for detection capability to allow dilution of milk samples for measurement. As a result the dynamic working range was reported as 2-7.5 ng/l. Market samples from Taiwan were used to demonstrate the applicability, however no AFM_1 was found. Abhijith *et al.* (2013) presented an approach to determine AFB_1 in solution making use of gold nanoparticles to enhance chemiluminescence. The method was compared with a HPLC-UV method analysing fortified peanut butter and wheat flour in parallel. Unfortunately, the working range was only reported in ng/ml of the solution measured, while the sample extraction part is only referred to in a reference. Beloglazova *et al.* (2013a) developed two formats of immunosorbent assasys, a quantitative fluorescence labelled

immunosorbent assay (FLISA) and a gel SPE column, called on-site column. Both assays are based on quantum dot loaded liposomes. The quantitative method has shown to be able to determine AFM_1 precisely in milk and cream in a small inter-laboratory study using certified reference materials. The limit of detection is given with 0.014 µg/kg. Furthermore the on-site column showed potential for rapid qualitative screening of AFM_1 in milk at the ML in the EU. Another fluoro-immunoassay for the determination of AFB_1 in peanuts on the basis of quantum dots has been described by Zhang *et al.* (2014c) with acceptable recovery at levels far below those regulated in the EU.

Regarding non-immunochemical methods, Li *et al.* (2014) who did fundamental work on the use of chemoluminescence-based detection of AFB_1 in pure solution making use of magnesium chlorophyllin. The method allowed a limit of detection of 27 ng/l in solution. Several proposals to improve or tailor the extraction and purification of extracted aflatoxins for further determination were made by various research groups. Campone *et al.* (2013) described a DLLME clean-up coupled to HPLC-MS for the determination of AFM_1 in milk. Authors compared a variety of different DLLME parameters and tested various chlorinated and brominated solvents. The method was developed as alternative to the use of IACs. However, the arguments controverting the usefulness of IACs for the determination of AFM_1 in milk appear not fully conclusive as only a minor amount of organic solvent is actually required during the elution of the purified AFM_1. In addition, IACs have proven to be reusable for a number of replicates in former international studies. This is however not documented in peer-reviewed articles. Nevertheless, authors demonstrated the method as potential and valid alternative to an IAC clean-up, demonstrating a limit of quantification of 2.0 ng/kg and meeting the provisions of EU legislation on method performance (recovery) at various contamination levels ranging from 5-500 ng/kg AFM_1 in milk with various levels of fat. Also Lai *et al.* (2014) proposed a DLLME procedure for the determination of AFB_1, AFB_2 and OTA in rice. In a similar manner as Campone *et al.* (2013) authors compared various chlorinated solvents for extraction efficacy. Despite the impressive low measurement capability reported by the authors the re-introduction of chlorinated solvents into routine testing labs might be seen as an obstacle in some regions of the world. The derivatisation procedure for AFB_2 is also unclear. It is unfortunate that authors did no test other non-chlorinated solvents, such as *t*-butymethylether in combination with polarity modifiers (e.g. butanol), as this has been described as promising replacement for chloroform in many fields. An alternative clean-up was described by Wang *et al.* (2014a) using gel-permeation chromatography for the simultaneous determination of aflatoxins and benzo(a)pyrene from vegetable oils. The LODs for the four aflatoxins under investigation were estimated to be 1.0 µg/kg. The authors made use of two subsequent evaporation steps with different solvents prior to HPLC separation. Reported recoveries (82-98%) for all aflatoxins over various levels with precision values ranging from 7-10% are impressive.

Yet another approach was described by Sirhan *et al.* (2014) who compared a HPLC-FLD method with a QuEChERS clean-up against a fluorimetric method. Authors used for the QuEChERS clean-up an unusual extraction solvent (MeOH:ACN, 60:40, v/v), while for fluorimetry an existing protocol was followed. Poor precision was obtained with the fluorimeter method compared to QuEChERS. As both methods differ significantly, a comparison of the methodological concepts is rather difficult and judgements must be made carefully. Critical is that only small sample portions of 1 g were used for extraction. This is no problem when samples were fortified, but naturally contaminated samples are likely to give questionable results, without details on proven sample homogeneity at a 1 g level. As new experimental solvents were used, not only the recovery of fortified (spiked) samples are of interest, also relative extraction yields of naturally contaminated samples are crucial, but these have not been reported. Nonetheless, the concept of employing a QuEChERS clean-up for aflatoxin determination in food is an interesting approach. Manoochehri *et al.* (in press) proposed an ultrasound-assisted matrix solid-phase dispersion procedure for clean-up of rice samples for the subsequent HPLC determination of aflatoxins. Authors identified through a series of experiments the best conditions, which make use of C18 material in a ratio of 1/1 with the sample. One critical factor might be the cost of C18 material as consumable as the sample size for analysis usually recommends larger ones as the authors used in their study. A vast amount of clean-up alternatives, such as bentonites, celite, aluminium oxides and charcoal and mixtures thereof exist and have been applied successfully. Nonetheless chromatograms show clear interference-free peaks of all aflatoxins. A more conventional approach was followed by Khayoon *et al.* (2014) who described an optimised micro SPE method coupled with LC-MS for the determination of aflatoxins in coffee and malt beverage. The method was optimised for various parameters including a comparison of different sorbents, modifiers and for capacity exhaustion. Authors reported acceptable recoveries in the range of 86-109% with a working range of 0.4-50 µg/kg. The method was tested on commercial products (n=40) but no contamination was observed.

Two quite different methods have been described for the determination of aflatoxins in eggs. Pavšič-Vrtač *et al.* (2014) used a multi-stage clean-up method in combination with HPLC-FLD to analyse eggs for AFB_1. The analytical procedure is a combination of classical extraction, liquid-liquid extraction with lead acetate precipitation and IAC

clean-up. Authors used the method to determine AFB_1 but could not find any contamination in eggs. A different and straight forward approach with an IAC was followed by Khalil *et al.* (2013) for AFM_1. Despite an extensive evaluation and validation effort to describe the method authors did not report if they identified AFM_1 in unknown egg samples. Biancardi *et al.* (2013) developed another alternative for an IAC purification and enrichment of AFM_1 in milk. Authors used a single liquid-liquid extraction with ethyl acetate (EtAc) and obtained a concentration factor of 20 by evaporation/re-dissolution prior determination by LC-MS. The method was developed with the aim to avoid IACs. One main argument is that an IAC clean-up is a multistage process that inherits a larger risk of analytical errors, leading to irreproducibility. It is however unclear whether IAC is always more complex than alternatives such as liquid partitioning. The advantages to circumvent proprietary products are however a valid argument that can be important during unexpected analysis campaigns (e.g. mycotoxin outbreaks). Stroka and Seidler (2014) demonstrated the potential of an alternative approach to elute purified mycotoxins from IACs. Mycotoxins loaded on an IAC, were eluted with hot water, rather than organic solvents, a procedure that allows advantageous aliquotation prior to HPLC. Despite that the protocol was developed for Fusarium toxins, it proved to be also applicable for aflatoxins, which recently led to the commercialisation of a product (FREESTYLE ThermELUTE®; LCTech, Dorfen, Germany), taking advantage that the protocol reduces the size of IACs.

Another interesting IAC application was demonstrated by Ma *et al.* (2013) who developed a silica-gel IAC, by linking antibodies covalently to an amino-derivatised silica gel, a welcome approach for the re-use of clean-up columns. Authors identified that a nearly quantitative recovery (>94%) was obtained when aflatoxins were applied in 20% methanolic solution as a compromise between solubility of aflatoxins and antibody affinity. Authors tested the developed clean-up column for the analysis of various samples, such as peanuts, vegetable oils and tea. The aspect of possible microbiological decay and need of a stabilisation buffer with a suitable preservative such as sodium azide, was unfortunately not addressed. A potential problem that has no significance if the columns are re-used for a high number of sample replicates within a short time range, however an issue that might be critical if such products are reused for longer periods. An interesting analytical approach was followed by Sasaki *et al.* (2014) who used an aflatoxin IAC for the isolation and enrichment of the structurally related mycotoxin STC. Despite the fact that the method was developed for a mycotoxin that is not an aflatoxin member it is of interest, as it highlights the importance of cross-reactivity and its potential use and risks. A very interesting approach for the determination of AFM_1 in milk was followed by Hashemi *et al.* (2014b). Authors synthetised coatings for magnetic nanoparticles that sufficiently selective bind to AFM_1 in aqueous solutions, which they release quantitatively using a solvent mixture of acetone, ACN and dichloromethane. The purified AFM_1 was then quantified after derivatisation by fluorimetry without further need of separation from matrix constituents. Despite the need of dichloromethane the method is attention-grabbing. Authors tested the method by comparing results obtained with their method with those from a classical method using IAC followed by HPLC-FLD quantification. Since the method endeavours simplicity, it is a pity that authors demonstrated the potential of their method only with two contaminated test samples of similar contamination level. A more benefitting approach would have been to test over the initial calibration range to stress the potential of this novel method.

The non-destructive determination of aflatoxins in agricultural commodities has been explored by several authors, who used either infrared or fluorescence spectroscopy. Wang *et al.* (2014d) studied infrared imaging of healthy maize kernel surfaces for the identification of AFB_1 in combination with statistical/chemometric data processing. The system was tuned and tested with fortified maize kernels. The system proved to be able to discriminate various levels of AFB_1 down to 10 μg/kg. This approach is certainly an interesting first step and should be verified with kernels naturally infected with aflatoxin producing fungi as the presence of aflatoxins on maize kernels is accompanied with several other fungal metabolites and goes in line with the authors remarks on a step by step process of their research. Using fluorescence imaging spectroscopy, Hruska *et al.* (2013) described the performance of a non-destructive method to identify fungal-infected maize ears. Authors demonstrated that a spatial resolution is necessary to make predictions on the contamination status of test samples. This research was later accompanied by further studies in which Hruska *et al.* (2014) used the fluorescence excitation-emission features of AFB_1 and two additional related secondary metabolites to evaluate the potential of image-based (spacial) technology for detecting aflatoxins in grain. Authors identified an aflatoxin-specific signal signature as potential candidate to determine aflatoxins in the presence of kojic acid and other secondary metabolites that emit fluorescence close to the aflatoxin emission when excited at 365 nm.

A completely different spectroscopic approach was followed by Lee *et al.* (2014b), as they produced silver nano-crystals that were used as amplifier in subsequent Raman spectroscopy measurements of extracts from maize for aflatoxin estimation. Authors presented impressive data on the ability to cluster samples with various contamination levels and making predictions on the aflatoxins level when compared with results from a conventional HPLC method. The method was tested at contamination levels

relevant for a rapid identification of unfit samples at an early stage of crop processing. Also Ivanova and Spiteller (2014) describe Raman spectroscopy as well as mass spectrometric approaches for the determination of aflatoxins. Authors claim that for a reliable assessment of human health a high level of analytical method performance parameters are required. These are beyond the capability of optical spectroscopic and ELISA methods and only mass spectrometry meets those requirements for the purpose of food quality control. The authors however do not provide any supporting data, such as a comparison of studies with performance characteristics. With respect to LOD and LOQ authors unfortunately remain unclear whether pure solutions of analytes or fortified sample extracts were used to establish performance parameters.

Yet another unusual approach has been described by Busman *et al.* (2014) using direct analysis real time (DART) mass spectrometry to quantify AFB_1 in maize extracts. Authors tested and optimised various parameters to demonstrate the quantitative nature of their method. Test samples were extracted in classical fashion with an aqueous organic solvent, the extract transferred on a medium for drying (chromatographic paper) and analysed. The use of stable isotope standards allowed a linear working range over three orders of magnitude, with precision and recovery figures comparable to what can be achieved with LC-MS methods. Only a few methods have been published describing conventional analytical methods to determine aflatoxins in less common matrices of which two are listed below. Luan *et al.* (2014) identified that grape pomace is commonly contaminated with aflatoxins, STC and mycophenolic acid and therefore developed a method for the determination thereof. The method is based on an SPE clean-up with tailored columns followed by LC-MS identification and quantification. Authors compared various extraction solvents and SPE absorbents for optimising the simultaneous determination of mycotoxins. Xue *et al.* (2014) present an LC-MS/MS method with IAC clean-up for the determination of aflatoxins and OTA in bee pollen. The method is based on already published principles and was used to screen bee pollen from northern China. Authors could not identify any mycotoxins in the products tested.

6. *Alternaria* toxins

The methods published in the last year were very few and most of the published articles were on the occurrence of *Alternaria* toxins in food commodities. A new method was developed by Tölgyesi and Stroka (2014) for the determination of alternariol (AOH), AME, altenuene (ALT), tentoxin and tenuazonic acid (TeA) in wheat, tomato juice and sunflower seeds. The method involves a solid-liquid extraction with MeOH and a subsequent derivatisation with 2,4-dinitrophenylhydrazine for TeA. Then sample extracts were purified with solid-phase extraction on polymeric-based columns and toxins were separated and quantified by LC-MS/MS using matrix-matched standard calibration. The limits of quantification ranged between 1 and 10 μg/kg, recoveries between 71 and 113%, RSD_r between 1 and 18%. The method was applicable also to CIT. A stable isotope dilution assay that was recently developed for the determination of TeA in food commodities was extensively re-validated for human urine matrix (Asam *et al.*, 2013). Urine samples were spiked with labelled standard, adjusted to pH 2-3, mixed with the derivatisation reagent prepared in ethyl acetate, ultrasonicated, shaken, mixed with the quenching reagent, shaken and centrifuged. The organic phase was collected and combined with another portion of EtAc that was used to further extract the urine sample. The organic phase was dried, reconstituted in ACN and H_2O and purified on a C18-SPE column before LC-MS/MS determination. The limits of detection and quantification were 0.2 and 0.6 μg/l, respectively. The mean recovery was 102±3% in the range between 1.0 and 100 μg/l. The method was used to monitor urinary concentration of TeA in 6 volunteers that were all tested positive (1.3-17 μg/l). The urinary excretion of TeA was monitored in two volunteers that ingested 30 μg TeA. Most of the ingested TeA (87-93%) was excreted in 24 h urine. Experiments with β-glucuronidase/sulfatase treatments showed that TeA is not metabolised to a glucuronide or sulfate derivative because its urinary concentration remains constant after enzymatic deconjugation. A method based on solid-liquid extraction with ACN/KCl solution, followed by liquid-liquid extractions of sample extract with dichloromethane and aqueous sodium bicarbonate and HPLC with UV-Vis and FLD was used in a large survey on the occurrence of TeA, AOH, AME and ALT in 1064 freshly-harvested winter wheat samples from commercial farmers in Germany in the years from 2001 to 2010 (Müller and Korn 2013). Maximum levels and incidence of positive samples were 4.2 mg/kg and 30% for TeA, 830 μg/kg and 8% for AOH, 900 μg/kg and 3% for AME, 200 μg/kg and 2.6% for ALT. The main factors that affected levels and incidence of positive samples were the crop year, tillage practices and the preceding crop. The occurrence of TeA in different infant foods and beverages collected in Germany was investigated by Asam and Rychlik (2013). It was concluded that the threshold of toxicological concern for TeA (1.5 μg/kg body weight per day), established by the European Food Safety Authority, may be exceeded by infants consuming predominantly sorghum-based food.

7. Ergot alkaloids

LC-MS/MS has continued to be the most useful and versatile analytical method for application to both surveys of food and feed and for research studies. A strategy for the identification of novel ergot alkaloids by the detection of specific fragmentations from collision induced dissociation in LC-MS/MS was devised by Arroyo-Manzanares *et al.*

(2014b). The fragmentation patterns were elucidated by studying the fragmentation of 12 known ergot alkaloids (ergometrine, ergosine, ergotamine, ergocornine, α-ergokryptine, ergocristine, methylergometrine, methysergide, dihydroergotamine, ergocornam, ergocryptam and ergocristam). Product ions of *m/z* 223 and *m/z* 251 were found to be common for all ergopeptines, ergoamides and ergopeptams, therefore parent ion scan experiments could be used to screen samples for the presence of known and unknown ergot alkaloids. The six common ergot alkaloids and their corresponding epimers proposed as priority targets by the European Food Safety Authority (EFSA 2012), and eleven other ergot alkaloid derivatives were identified in samples of feed. Alkaloids were extracted from the feed according to previous work (Di Mavungu *et al.*, 2012) comprising extraction with a mixture of EtAc:MeOH:0.2 M ammonium bicarbonate at pH 8.5, addition of buffer (ammonium bicarbonate at pH 10) and saturated ammonium sulphate, followed by centrifugation. An aliquot of the EtAc layer was evaporated and the residue reconstituted in MeOH/ACN/H_2O. After washing with n-hexane the mixture was analysed by LC-MS/MS using a triple quadrupole instrument in positive electrospray ionisation (ESI) mode. New and prior but recent data from the LC-MS/MS analysis of cereals and cereal products in Europe including those submitted to EFSA were collated and presented by Malysheva *et al.* (2014). 1,065 samples intended for human consumption and animal feeding were analysed. Ergot alkaloid contamination affected 59% of the samples. Those showing the highest degree and levels of contamination were rye-based foods (84%) followed by wheat-based foods (67%) and multigrain foods (48%). Of the feed samples, 52% of rye, 27% of wheat, and 44% of triticale feeds contained total ergot alkaloid levels from ≤1 μg/kg (LOQ) to 12.3 mg/kg. The median value for the -ine forms of the six major main ergot alkaloids was 1 μg/kg and for the -inine forms was 2 μg/kg. Contamination frequencies were higher in foods but overall levels were higher in feeds, especially Swiss rye feed. Both the frequency and the levels of contamination were significantly lower in organically produced samples. Ergosine, ergokryptine and ergocristine were the most frequently occurring ergot alkaloids.

Beaulieu *et al.* (2013) measured ergot alkaloids in the tissues of four species of morning glory by LC-FLD to evaluate the diversity and distribution of alkaloids in seeds and seedlings as well as the variation in alkaloid distribution among species. The dried plants were pulverised in a homogeniser and the powder steeped in cold MeOH for three days with daily vortex mixing. The compounds determined were ergobalansine, chanoclavine, lysergol, ergonovine. In addition to these cycloclavine, festuclavine, ergine and lysergic acid α-hydroxyethylamide were detected. A comparison was made between fungicide-treated and non-treated plants to differentiate seed-borne alkaloids from alkaloids produced *de novo* post-germination. There was differential allocation of individual alkaloids to various plant parts, which did not differ with fungicide treatment. Each species contained four to six unique ergot alkaloids. An HPLC-FLD method with SPE and clean-up was developed for the 12 priority ergot alkaloids and applied to rye flour and wheat germ oil (Köppen *et al.*, 2013). The use of acidic and alkaline partitioning conditions in the sample preparation stage was enabled by neutralising the strong cation exchange (SCX) medium with sodium hydroxide solution which reduced epimerisation. The epimerisation rates of ergot alkaloids were reported for a number of solvent systems. The ergot alkaloids were eluted from the SCX-column by ion pairing with sodium hexanesulphonate. The presence of sodium hexanesulphonate in solutions of ergot alkaloids in mixtures of ACN and H_2O eliminated epimerisation for 96 h. The optimum solution mixture for the elimination or inhibition of epimerisation in the absence of sodium hexanesulphonate was ACN:H_2O (84:16, v/v). Alkaline modifiers did not affect the extraction efficiency. The SPE conditions were optimised, and as hexanesulphonate does not fluoresce the use of fluorescence detection of the ergot moiety was not affected. In-house validation of the method showed it to have recoveries of 80-120% when applied to rye flour and 71-96% for the analysis of wheat germ oil, with an LOQ for both matrices of 2 μg/kg per alkaloid. Nine rye flours contained a maximum of 178 μg/kg total ergot alkaloids. The alkaloids were also quantified for the first time in 7 wheat germ oils which had a maximum of 57 μg/kg, with the less polar alkaloids being predominant in the oil.

Himmelsbach *et al.* (2014) used capillary zone electrophoresis to determine lysergic acid, iso-lysergic acid and paspalic acid in reaction mixtures derived from synthesis. An important part of the method development focused on optimisation of the running background electrolytes (BGEs) to give the best separation selectivity, analysis time and compatibility with a quadrupole time-of-flight mass spectrometer detector. The electrolytes compared were asparagine, ammonium hydrogen carbonate, and ammonium acetate. Asparagine precipitated even from low concentration in 40% MeOH. The best BGE was 15 mM disodium tetraborate in MeOH:H_2O, 40:60. The addition of 0.5% ammonia to the mobile phase was required to improve solubility and separation of the alkaloids. When the system was used with UV detection, the LODs for paspalic acid and lysergic acid were 0.45 and 0.40 mg/l respectively with day-to-day precision of 2.4 and 4.0%, respectively. However, for certain complex matrices (such as fermentation broth) there were matrix interferences. Therefore a study of mass spectrometric compatible BGEs was made which led to the selection of an optimised phase of 25 mM ammonium acetate in MeOH:H_2O, 40:60. The ESI conditions (nebulizer pressure and drying gas flow rate and temperature) were also optimised. Using this system, the detection was more sensitive, lowering LODs to 0.09 mg/l

for paspalic acid and 0.07 mg/l for lysergic acid. Method performance data for repeatability and recovery were good and matrix effects limited.

Metabolites produced by *Penicillium* fungi isolated from cheese-making and meat-processing plants included various mycotoxins, some of which were identified as clavine ergot alkaloids (Kozlovsky *et al.*, 2014). One strain of *Penicillium* produced cyclopiazonic acid, rugulovasin A and rugulovasin B, and another strain produced the clavine ergot alkaloids festuclavine and its isomers pyroclavin, costaclavin and epicostaclavin. The exo-metabolites were extracted from acidified culture broth by stirring with chloroform. The solvent was evaporated and the culture broth made alkaline (pH 8) and extracted with chloroform. Both acidic and alkaline extracts were analysed by thin layer chromatography (TLC) and indole alkaloids detected with Ehrlich's reagent. Isolation and purification of exo-metabolites was carried out by preparative TLC on silica gel plates. Zones containing fungal metabolites were detected by UV light, isolated and the metabolites eluted with MeOH. The exo-metabolites were identified by co-chromatography with reference standards and structures elucidated by LC-MS/MS.

8. Fumonisins

Due to their toxicity and widespread occurrence, substantial interest continues to exist in the fumonisins, which is reflected in the very large number of publications on this group. Many of the aspects of the fumonisins were recently reviewed (Bryła *et al.*, 2013). An assessment of the challenges associated with risk assessment and risk management of fumonisins was also conducted (Shephard *et al.*, 2013c). Widely used methods generally fit into one of three categories: those based upon physical properties (i.e. mass/charge ratio), those based upon detecting a labelled derivative (usually fluorescent, as with LC-FLD), or those based upon indirect detection (i.e. competitive assays, as with many immunoassays). For many years the methods based upon derivatisation, primarily LC-FLD, predominated. In recent years there has been much effort dedicated to detection with mass spectrometric techniques. However, this past year there were also a substantial number of articles describing indirect methods, primarily immunoassays and biosensors, as well as the binding materials upon which such methods are based (such as antibodies and aptamers).

Increasingly the trend has been to include fumonisins in multi-mycotoxin assays. Many forms of MS are readily amenable to multi-analyte detection, and the use of LC-MS-based methods in maize was recently reviewed (De Girolamo *et al.*, 2013). Multi-mycotoxin detection is also covered in another section of the current manuscript, and so this section will focus on the performance with the fumonisins. Historically, the fumonisins have tended to fit somewhat poorly with multi-toxin methods, perhaps because the extraction procedures that are optimal for many of the other mycotoxins were sub-optimal for the fumonisins. Therefore, this section will focus on extraction techniques. In all cases the cited methods have limits of detection or quantification well below the regulatory guidelines, so interested readers are directed to the primary literature for that information. Also, except where noted otherwise, the MS-based methods that are described herein use an LC separation with either MS/MS or HRMS detection. De Girolamo *et al.* (2013) reported on a proficiency test of multi-toxin methods that included fumonisins. 41 laboratories reported results and these laboratories used a total of 21 different extraction solvent mixtures. The majority used mixtures of ACN and H_2O for extraction. Other extraction solutions included MeOH and H_2O mixtures, or multiple extractions. To a lesser extent ACN:H_2O:MeOH, H_2O:EtAc, or buffer alone were used. Most of the laboratories used shaking (66%) or blending (17%) for the extraction, while the others used sonication with or without shaking/blending. Interestingly, unacceptably high recoveries (average of 159% for FB_1, and 163% for FB_2) and high variabilities were obtained by the majority of the participating laboratories. Unfortunately, this meant that the effects of extraction solvent and clean-up method were not evaluated for the fumonisins as they were for the other mycotoxins. The authors concluded that, with regards to FB_1 and FB_2, the current performance of many of the LC-MS multi-mycotoxin methodologies may be insufficient to meet the precision and accuracy requirements established in Europe, suggesting the need for further investigation.

For several years the combination of extraction and clean-up known as QuEChERS has been employed for multi-toxin analysis. The process involves extraction under conditions that salt-out certain matrix components (usually with $MgSO_4$ and NaCl) while simultaneously yielding a biphasic extract. Sometimes a dSPE step (such as C18 or amine-based SPE) is also incorporated. Recently Arroyo-Manzanares *et al.* (2014a) applied a QuEChERS technique to several pseudocereals (buckwheat, quinoa, amaranth), spelt and several varieties of rice. Extraction was accomplished with H_2O and acidified ACN, and phase separation was induced with a commercially available QuEChERS mixture ($MgSO_4$, NaCl, citrate). Analyses were conducted on the ACN phase. Average recoveries for FB_1 and FB_2 ranged from 63% (red rice) to 100% (buckwheat). Of the 42 combinations tested (2 toxins, 7 matrices, 3 levels per matrix), recoveries greater than or equal to 75% were obtained 35 times, indicating that in general the recoveries of FB_1 and FB_2 from these matrices were good. Hu *et al.* (2014) developed a method applicable to foods for infants and young children. The clean-up was by QuEChERS. Acidification of the solution used for extraction resulted in improved recoveries at pH 3 relative to pH 6 or 9. Recoveries were not improved with the inclusion of an SPE clean-up.

Biscuits, because of their fat content, represent a challenge and Capriotti *et al.* (2014) examined three types of extraction procedures. These included a solid-liquid extraction (SLE) 'dilute and inject' method, a QuEChERs-based extraction, and SLE combined with a graphitized carbon black SPE clean-up. The SLE procedure involved extraction with acidified ACN:H_2O (80:20). The QuEChERS procedure used an extraction with acidified H_2O and ACN, with addition of $MgSO_4$ and NaCl. The SLE-SPE procedure used acidified ACN:H_2O (80:20), with extracts cleaned-up over a Carbograph-4 cartridge. The SLE procedure gave good recoveries of FB_1 and FB_2 (above 80%), while the QuEChERS method gave poorer recoveries (approximately 70% for FB_1 and below 60% for FB_2). The SLE-SPE procedure yielded average recoveries from 80-89% (FB_1) and 84-93% (FB_2). While the SLE 'dilute and inject' procedure was adequate for recovery of FB_1 and FB_2, the authors chose the SLE-SPE procedure to facilitate detection of other mycotoxins and to reduce the extent of the matrix effects observed. Acidified ACN/H_2O extraction coupled to QuEChERS has also been applied to other matrices, such as cereals, oils, infant formula, and nuts (Desmarchelier *et al.*, 2014). The QuEChERS salts used were $MgSO_4$ and NaCl. The ACN phase was defatted with hexane and a portion was taken to dryness, reconstituted, centrifuged, a further sub-portion was diluted with H_2O, centrifuged again and then analysed. With two laboratories, the recoveries ranged from 71% (FB_2 in infant formula, laboratory 1) to 118% (FB_1 in infant formula, laboratory 2).

Acidified ACN:H_2O was also the extraction solvent used in combination with a multi-toxin IAC clean-up. The method was applied to peanuts, barley, and maize-breakfast cereals (Vaclavikova *et al.*, 2013). An aliquot of extract was taken to dryness, re-dissolved in aqueous buffer and then cleaned up over the IAC. Unlike many protocols, this procedure also included FB_3. Average recoveries from spiked barley ranged from 72 to 90%. Recoveries from spiked maize breakfast cereals, 76 to 85%; and recoveries from peanuts, 72 to 78%. Wang *et al.* (2013a) also extracted with acidified ACN/H_2O, however they used an SPE clean-up based upon OASIS HLB® cartridges. The method was applied to multiple toxins, including FB_1 in maize. Interestingly, the extract was filtered and taken to dryness then reconstituted into an appropriate solvent before application to the SPE cartridge. Recoveries of FB_1 averaged 88%. A multi-toxin assay for analysis of 24 different food matrices also used acidified ACN:H_2O for extraction for most of the matrices (Beltrán *et al.*, 2013). The extracts were centrifuged or filtered, and diluted with H_2O before injection. Certain matrices, such as beverages (fruit juice, wine, and beer) were simply diluted, centrifuged, and filtered. For oil samples, hexane was added, followed by partitioning into ACN. The ACN phase was diluted with H_2O before injection. Matrix-matched calibration was used to compensate for matrix effects. Recoveries ranged from 43% (FB_2 in eggs) to 117% (FB_1 in yoghurt), with 65 of the 79 combinations of toxin (FB_1, FB_2) and matrix having recoveries greater than or equal to 75%. Food oils gave problems with FB_1 and FB_2 extraction. Given the number of aforementioned papers that use acidified ACN:H_2O it is clear that such mixtures are very widely used and can yield acceptable results for extraction of fumonisins.

While most of the methods described above are multi-toxin methods that included FB_1 and FB_2 among the analytes, several articles used LC-MS with a focus upon fumonisin (as opposed to multi-toxin) detection. An LC-HRMS method was developed for the determination of fumonisins, their partial hydrolysis products (i.e. PHFBs) and their 'full' hydrolysis products (i.e. HFBs) in maize and maize-based products (De Girolamo *et al.*, 2014). Structural isomers of the partial hydrolysis of FB_1 (e.g. $PHFB_1a$, $PHFB_1b$) and FB_2 (e.g. $PHFB_2a$, $PHFB_2b$) were identified and produced by hydrolysis from FB_1 or FB_2, respectively. For isolation of the fumonisins and their hydrolysis products samples were extracted with an acidic mixture of MeOH and ACN with citrate/phosphate buffer. For maize and maize-products extraction was conducted at 55 °C, while for nixtamalised products the extraction was carried out at ambient temperature. Filtered extracts were diluted with acidified MeOH/H_2O and filtered once more before injection. For maize, masa flour and tortilla chips the average recoveries for all 6 analytes ranged from 81 to 99%.

Before the widespread use of acidified aqueous ACN for multi-toxin detection, analytical methods for fumonisins typically used MeOH:H_2O, ACN:H_2O or other aqueous solvent mixtures for extraction, with the extract cleaned up using strong anion exchange (SAX) or C18 SPE cartridges. An inter-laboratory study of an LC-MS/MS method for fumonisins in maize was reported this year (Yoshinari *et al.*, 2013b). The method used extraction with MeOH/H_2O (3/1) and clean-up of the extract using SAX SPE. Data from 9 laboratories were included in the study, which involved measurements of three spiked samples, two naturally contaminated samples, and a fumonisin-negative sample. SPE columns from two manufacturers were studied. Mean recoveries from FB_1-spiked maize ranged from 80 to 87% while recoveries of FB_2 ranged from 79 to 103%, and recoveries of FB_3 ranged from 80 to 93%. The method met EU criteria for analytical precision (RSD_r and RSD_R), and the HorRat values reported were low (0.2-0.6 for FB_1, 0.3-0.9 for FB_2 and 0.4-0.7 for FB_3). While one type of SAX cartridge tended to have slightly higher recoveries than the other, there was no significant difference in trueness between the two (P>0.05). Lastly, with regard to MS-based methods, a new stable isotope labelled fumonisin was produced using ^{18}O (Bergmann *et al.*, 2013). This material will give researchers another tool to use as an internal standard for LC-MS methods for FB_1.

While much of the research effort has been directed towards detection of fumonisins by MS methods, there was a substantial body of research reported in the past year on indirect methods mediated by a binding event, such as immunoassays. A commercial fumonisin ELISA was compared to an LC-MS/MS method for quantification of multiple toxins in maize distiller's dried grains with soluble (DDGS) (Tansakul *et al.*, 2013). Using a Bland-Altman plot for the comparison of 30 samples, it was noted that the ELISA and LC methods were not different at fumonisin (FB_1+FB_2) levels below 20 mg/kg, but there were differences for the two samples above 20 mg/kg. The authors determined that the ELISA method had a bias towards underestimation of the fumonisin content, relative to the LC-MS/MS method. The first antibodies for FB_1 were developed many years ago, soon after the fumonisins were discovered. However, improvements to immunoassay formats have continued, as have the development of alternative recognition elements. Therefore, while antibodies and ELISAs for fumonisins continue to be developed (Ling *et al.*, 2014, Wang *et al.*, 2014e), the focus has shifted to novel fumonisin binding elements, or multiplexed assays. More novel binding elements include single chain variable fragments (scFv), which contain the toxin-binding region of the antibody without many of the framework (structural) regions of the protein. Zou *et al.* (2014) developed a monoclonal antibody (mAb) and, using recombinant technologies, a plasmid containing the sequence for a fumonisin-specific scFv. The plasmid was expressed in *Escherichia coli* and scFv was used to construct a competitive ELISA. The sensitivity of the scFv ELISA was very similar to that of the parent mAb, with IC_{50} for FB_1 of 12.7 and 10.2 μg/l, respectively. The scFv was very specific for FB_1, with low cross-reactivity to FB_2 (5.23%). Results from the scFv-based ELISA correlated favourably with an LC method for detection of FB_1 in naturally contaminated maize.

Nucleotide-based recognition elements, such as aptamers, represent an alternative to protein-based recognition. Food safety applications of the technology used to produce aptamers (selection of ligands by exponential enrichment, SELEX) were recently reviewed (Wu *et al.*, 2014). An improvement to the SELEX procedure for making aptamers to small molecules was described by Chen *et al.* (2014) for the selection of an aptamer to FB_1. The aptamer had a dissociation constant of 62 nM, indicating a high affinity for FB_1. In another report aptamers were used to develop a sensor based on the principle of Förster resonance energy transfer. The sensor used a molecular beacon and magnetic nanoparticles (Wu *et al.*, 2013). The molecular beacon consisted of part of the sequence of a fumonisin aptamer, with gold nanoparticles (AuNP) attached at the 5' end and upconversion fluorescent nanoparticles (UCNPs) attached to the 3' end. With the molecular beacon in its closed position, the AuNP (5' end) and UCNP (3' end) were in close proximity, resulting in quenching of the fluorescence of the UCNP by the AuNP. Magnetic nanoparticles (MNPs) were constructed that contained both the FB_1 aptamer and a shorter complementary oligonucleotide. In the presence of FB_1 the aptamer bound the toxin, displacing the complementary oligonucleotide. The complementary oligonucleotide then bound to the molecular beacon, changing the configuration to one that was open and separating the quencher from the fluorophore. Thus addition of FB_1 led to an increase in fluorescence signal from the UCNPs. In aqueous solution the limit of detection was a remarkable 0.01 ng FB_1/ml. The assay agreed well with a commercial ELISA when used to analyse 15 samples of naturally contaminated maize. ELISAs are traditionally conducted on microtiter plates, however a recent article changed the format so that immunomagnetic beads were used instead, allowing most of the assay steps to be conducted in microfuge tubes (Wang *et al.*, 2014e). Many of the electrochemical biosensors are similar to ELISAs but use electrochemical, rather than optical, signal detection. The group, based upon amperometric, impedance, potentiometric or conductimetric detection, was recently reviewed by Vidal *et al.* (2013).

As with the MS techniques, research in the area of indirect assays continues to move towards multiplexed detection. One technology amenable to multiplexing uses colour coded microspheres and instruments (such as the Luminex analysers) that can rapidly distinguish the identity of the microspheres. In one assay format the microspheres were coated with anti-toxin antibodies then the microspheres were exposed to a mixture of sample and toxin-protein-biotin conjugates. The toxin competed with the toxin-protein-biotin conjugate for binding to the microsphere. The microsphere-conjugate complex was then labelled with a fluorescent phycoerythrin-streptavidin. The instrument distinguished the type of microsphere, which established the type of toxin, and the fluorescence of the phycoerythrin label, which was inversely related to toxin concentration. An assay of this type was used to determine FB_1 with an IC_{50} of 41.5 ng/ml. Recoveries from spiked maize were greater than 100% (Wang *et al.*, 2013c). Another form of suspended microsphere assay used silicon photonic crystal microspheres (SPCMs). The identity of the SPCMS (and therefore toxin identity) was encoded by the reflectance spectra of the microspheres (as opposed to their fluorescence), and the detection was based upon the generation of a chemiluminescent enzyme product (as opposed to the fluorescence of phycoerythrin) (Xu *et al.*, 2014). In this case the competition was between FB_1 and FB_1-BSA-SPCM for anti-fumonisin antibody. The antibody-FB_1-BSA-SPCM complex was determined through the attachment of an enzymatically-labelled secondary antibody, with the enzyme converting a substrate into a chemiluminescent product. The IC_{50} for FB_1 with this assay was 0.036 ng/ml, and the LOD was a remarkable 0.60 pg/ml (corresponding to 30 pg/g). Recovery rates of FB_1 from spiked rice, maize, and wheat ranged from 64 to 116%.

A mainstay of screening immunoassays are immunochromatographic test strips, also known as LFDs. A new LFD for FB_1 in cereals was also recently described (Venkataramana *et al.*, 2014). The signalling mechanism in LFD is often derived from a coloured label (such as colloidal gold), however fluorescent labels have also been used. Wang *et al.* (2014g) developed an LFD for fumonisins that used fluorescent microspheres. The microspheres were solid materials that contained a fluorescent dye distributed throughout the bead, rather than on the surface. The method that was developed detected FB_1 over a relevant concentration range, and a quantitative comparison with an LC-MS/MS method showed good agreement. An interesting aspect of this work is that the spheres are available in several colours, potentially allowing for multiplexed detection. In this regard, another LFD was developed to test ZEA in combination with fumonisins (Wang *et al.*, 2013b). Labelling was with colloidal gold nanoparticles, and a strip reader was used to aid in quantification. Recoveries from spiked maize ranged from 93 to 97%, compared to 86 to 110% by LC-MS/MS.

For any analytical method, particularly one where widespread application is desired, validation is essential. Lattanzio *et al.* (2013) described the design of validation protocols and application of those protocols to a commercial LFD. The validation design is of significant interest, because it was directed at determining how well a numerical response from a method could be used when it was compared to a cut-off or threshold value. That is, for validation of a quantitative LFD method used qualitatively. The target level (threshold value) for fumonisins (FB_1+FB_2) was 4,000 μg/kg. The validation used blank samples, as well as those spiked at 25%, 50%, and 100% of the target level. It is beyond the scope of this article to describe the experimental design for the, necessarily complicated, validation protocol. The interested reader is referred to Von Holst and Stroka (2014), who discuss performance criteria for rapid screening methods to detect mycotoxins. Not surprisingly, the probability of misclassification increased as the fumonisin content came closer to the threshold value. The rates of false positives were: 5.8% (for blank maize), 12% (for maize fortified at 25% of threshold), and 64% (for maize fortified at 50% of threshold). These values were used to estimate the rate of false positives in a larger data set obtained on the occurrence of fumonisins in 1,693 European food samples, which was calculated to be 8.6%. The authors further conducted a cost analysis and determined that the method was fit for the purpose of verifying compliance of cereals relative to the EU regulatory limits.

The number of commodities and foods in which fumonisins are detected has continued to increase. Unfortunately, space does not permit us to discuss all of these methods, most of which use LC-MS or LC-MS/MS. However, interested readers should know that such methods were developed for fumonisins in medicinal plants (Sharma *et al.*, 2014), ginseng (Kuang *et al.*, 2013), milk-based infant formula and foods (Zhang *et al.*, 2013a) and gums (Zhang *et al.*, 2014b). Kim *et al.* (2014) also described the production of fumonisins by black Aspergillus. Likewise, the number of fumonisins and their derivatives that are known to science continues to expand. Of particular interest are fumonisins esterified to fatty acids in maize (Falavigna *et al.*, 2013), N-fatty acyl fumonisins in alkali-processed maize foods (Park *et al.*, 2013), ceramide analogs of FB_1 in *F. verticillioides* culture material (Bartók *et al.*, 2013a), new isomers of fumonisin B_5 (Bartók *et al.*, 2013b), and new analogs of fumonisin P (Bartók *et al.*, 2014). Assessment of the exposure of individuals to fumonisins is aided by methods that can measure biomarkers. Several reports examined biomarkers of fumonisin exposure in human urine (Van der Westhuizen *et al.*, 2013; Shephard *et al.*, 2013b; Solfrizzo *et al.*, 2013, 2014). Lastly, while the focus of this review has been on methods that estimate toxin content, there was also a method that combined near infrared spectroscopy with multivariate statistical regression to predict fumonisin content in maize (Della Riccia and Del Zotto, 2013).

9. Ochratoxins

Compared to last year, no relevant changes in the diagnostic tools used for OTA determination in foods and feeds were noted. No interlaboratory trials for OTA method validation in food and feed were organised in the last year. The most common principle in EU member states remains HPLC-FLD, which is the basis for all CEN standards for OTA. As a general trend, in the last year a strong increase in multi-mycotoxin analysis (see Section 3) including OTA was noted, together with the enlargement of the spectrum of foods investigated for OTA content, such as sorghum, wine including rice type, pork meat, pet food and spices.

The Food Analysis Performance Assessment Scheme (FAPAS) includes proficiency tests (PTs) for OTA analysis across a wide variety of matrices. From a consultation of FAPAS® PTs for OTA, 11 PTs were performed in the period June 2013 to May 2014 (FAPAS, 2013-2014). The performance data are summarised in Table 1. For each PT, the assigned value was calculated from the robust mean of participants' results. The standard deviation for proficiency assessment (data not shown) was derived from the Thompson-modified Horwitz equation, i.e. 22% relative to the assigned value. The test materials were all prepared from matrices that were initially screened for the presence of incurred OTA. No incurred OTA was detected in any of the test materials, so these were all prepared by spiking with OTA solution. A majority (71%) of participants stated that their labs were accredited. In the extraction step, participants tended to use either ACN or MeOH, usually in combination with H_2O. With a few exceptions, MeOH was the preferred extraction solvent (63%). It is common

Table 1. Performance data summary of FAPAS® Series 17 ochratoxin A proficiency tests 2013-2014.

PT	Date	Matrix	Assigned value, μg/kg	Total number labs	% |z| ≤2
17122	June 2013	Wheat flour	7.26	67	94
17123	July 2013	Dried vine fruit	8.47	54	94
17124	August 2013	Barley flour	2.96	53	85
17125	September 2013	Green coffee	3.82	39	87
17126	October 2013	Paprika	31.4	52	87
17127	December 2013	Instant coffee	12.04	46	80
17128	January 2014	Wine	3.35	46	91
17129	February 2014	Maize flour	2.92	44	80
17130	March 2014	Roasted coffee	2.69	48	85
17131	April 2014	Animal feed	33.66	52	88
17132	May 2014	Cocoa powder	2.20	44	86

to use IAC as a selective clean-up step. HPLC continues to be the determination method of choice with only a few participants (7%) using ELISA. Of the participants using HPLC, conventional fluorescence detection is still used by the majority (78%) although mass spectrometry is increasingly being used.

Bazin *et al.* (2013) showed that the extraction of OTA in alkaline medium or addition of polyethylene glycol (PEG) to wine induced an underestimation of OTA content in wheat or wine, respectively. The analyses of OTA contents in wheat, following purification onto IAC after alkaline or neutral extraction, led to an overestimation of OTA. This bias could be also due to the interferences between OTA and CIT, a mycotoxin generally co-occurring with OTA, but also to the similarity of ochratoxin quinone and dechlorinated OTA (ochratoxin B, OTB) formed by auto-oxidation of OTA induced by CIT. The pH is crucial for reliable detection of OTA and should be below 7 for both accurate liquid/liquid extraction and IAC clean-up. Regarding extraction methodology an increase in the use of QuEChERS as promising tool for enhancing the extraction power from the matrix was registered, not for OTA as single mycotoxin but exclusively in multi-mycotoxin studies.

Considering methods of analysis for OTA determination in foodstuffs as single mycotoxin, Akdeniz *et al.* (2013) analysed grape samples (dried grapes, grape juices and pekmez by IAC for clean-up and HPLC-FLD. A mixture of MeOH:H_2O (70:30, v/v) was used for extraction with a Waring Blender at high speed for 2 min. Clean-up with IAC was performed prior to HPLC-FLD quantification using a reversed phase column and a mobile phase of ACN:H_2O:CH_3COOH (99:99:2, v/v/v). Confirmation of OTA in positive samples was done by the formation of the methyl ester derivative. The retention time was about 12 min for OTA and 35 min for OTA methyl ester using the same chromatographic conditions. A paper describing the preparation of reusable IACs and the development of an UHPLC-MS/MS method combined with IAC clean-up for the determination of OTA in cereals and feeds was published by Meng *et al.* (2014). The mAb was produced from a stable hybridoma cell line, which belongs to the immunoglobulin G1 (κ-light chain) isotype. A competitive indirect ELISA was used to characterise the mAb. The concentrations causing 50% inhibition of binding of mAb to OTA-ovalbumin by free OTA, OTB, and ochratoxin C were 1.29, 4.78, and 0.94 ng/ml, respectively. The IAC-UHPLC-MS/MS method offered a limit of quantification ranging from 0.5 to 1.0 μg/kg and a limit of detection ranging from 0.2 to 0.3 μg/kg in cereal and feed samples. The IAC-UHPLC-MS/MS method offered low LOQs for OTA in cereal and feed samples. This methodology has been validated in four different matrices (millet, maize, soybean, and swine finisher diet) with highly satisfactory results and applied to the analysis of samples collected from the markets. Pork meat based foodstuffs such as sausages and other dry-cured meat product samples were investigated for OTA presence (Pleadin *et al.*, 2013) by using 1% aqueous sodium hydrogen carbonate solution mixing for 2 min. After addition of MeOH, the sample was mixed and extracted on an overhead shaker. Samples were degreased and purified using IAC. The LOD and LOQ estimated both for the muscle tissue and all analysed meat products were 0.15 and 0.20 μg/kg, respectively.

Brera *et al.* (2014) published a comparison between two methods in cured ham, namely, one based on HPLC-FLD and one based on UHPLC-MS/MS. The obtained data showed the advantages but also the limits of the two methods in terms of accuracy and sensitivity. The goal of this study was to identify the most suitable strategy for OTA determination in cured ham. From the obtained results, it was possible to conclude that within official control activities, the use of HPLC-FLD should be preferred since it is less affected by matrix interferences and leads to lower detection limits. *Vice versa*, for research or monitoring studies the use of a mass spectrometer

should be used as run times can be quicker, allowing a higher number of samples to be analysed. Due to the tendency to use IAC as clean-up for different foods without checking and validating the best procedure for each matrix, the aim of a study performed by Prelle *et al.* (2013), was to compare IAC clean-up performance of four different commercially available OTA selective cartridges (AffiniMIP – Affinisep, Val-de-Reuil, France; Mycosep™ – Romer Labs, Tulln, Austria; Mycospin™ – Romer Labs; and HLB® SPE). The study was carried out on four different matrices susceptible to OTA contamination: wine, beer, coffee, and chili. Recovery, repeatability and reproducibility were calculated for each method and matrix. OTA was determined by HPLC-FLD. The two categories of clean-up strategies tested ('pass through' and 'capture the analyte'), demonstrated different ability to remove interferences depending on the matrix. IAC columns revealed high efficacy for OTA clean-up in each matrix, with recovery values never below 70%. MIP columns were more efficiently recovering OTA only in coffee (consistently >80%), whilst for other matrices recovery values ranged from 54% (wine) to 84% (chilli). Several studies will be necessary to understand which matrix compounds and method conditions interfere with specific bond-capacity of MIP. Despite the different strategy of clean-up used by Mycosep™ cartridge which does not permit sample enrichment, validation results demonstrated high efficacy on wine and chilli matrix with recovery values above 80%.

Easy, sensitive, rapid and low cost OTA biosensors are strongly demanded in food analysis. Todescato *et al.* (2014) developed a novel plasmonic-based optical biosensor prototype for OTA. It exploits the metal-enhanced fluorescence phenomenon due to the silver film over nanosphere plasmonic substrate. Since OTA could be present in different food commodities, sensor performances have been tested on three different matrices (dried milk, juices and wheat). After defining a common extraction solvent, as well as a labelling and detection protocol, the efficiency of the silver film over nanosphere (Ag-FON) surfaces in signal amplification for the detection of low OTA concentrations was elucidated. Using samples spiked with OTA levels below of 0.5 μg/kg in wheat, milk and apple juice could be detected. The test performances were comparable to those of ELISA kits but with some perspective advantages, such as low cost, fast assay time, versatility of the protocol for the investigation of different matrices, employment also in non-qualified laboratories, small dimensions that allow its integration in a compact device for OTA on-site detection. The limit of quantification for OTA detection on Ag-FON substrates was as low as 3.6 ng/kg that is 20 times lower than what observed for commercial array slides (71 ng/kg). Plasmonic surfaces allow detecting OTA at concentrations comparable with that of commercial ELISA kits as reported in the specific data sheets by the producers. A comparison between ELISA and HPLC was performed by analysis of 115 blood serum samples of women in the child rearing age from the Czech Republic (Dohnal *et al.*, 2013). OTA was determined in a broad range of concentrations from 0.05 to 1.13 μg/l. The outcome of ELISA and HPLC measurements was well correlated (r=0.907). However, it was observed that ELISA tended to result in underestimating the OTA level at the low serum concentrations. Both methods had the same limits of quantification of 0.05 μg/l under standard operation conditions.

To overcome the various limitations of antibodies, an alternative approach may be adopted by the use of synthetic bioreceptors, such as aptamers and MIPs. Aptamers are artificial nucleic acids produced by chemical synthesis. MIPs result from the polymerisation of monomeric units in the presence of a template molecule. These promising recognition elements are characterised by high affinity and specificity to their targets. Indeed, in comparison to antibodies, aptamers, and MIPs are very stable and their production is easier and cost-effective. Giovannoli *et al.* (2014) published a method for OTA determination in 17 Italian red wines where the extraction method was based on a MIP prepared through a mimic template approach. Under optimised conditions, recoveries of OTA from spiked samples ranged from 88 to 102% with sample volumes up to 20 ml. HPLC-FLD allowed limits of detection and quantification, respectively, of 0.075 and 0.225 ng/ml. Sample extractions by an immunoaffinity protocol showed the method to be comparable, demonstrating the potential of the imprinting approach to substitute current immunoaffinity methods.

An exhaustive review of the use of biochemical methods for OTA analysis is reported by Rhouati *et al.* (2013b). These methods are mainly based on the interaction between a recognition element and its target inducing a mechanism of molecular recognition. They are mainly based on the integration of antibodies or aptamers as biorecognition elements in sensing platforms. However, aptamers have gained more attention in affinity-based assays because of their high affinity, specificity, stability, and their easy chemical synthesis. In the review an overview of aptamer-based assays and their applications in OTA purification and detection methods, that have appeared in the literature during the last five years is presented. Yang *et al.* (2013) proposed a new label-free colorimetric assay based on self-assembly of DNAzyme-aptamer conjugates. The structure of the new DNA includes the OTA-specific aptamer and a G-rich sequence of nucleotides mimicking peroxidase activity. The binding of OTA to aptamer results in the dehybridisation between the oligonucleotides. Thus, the activity of the non-hybridised DNAzyme is linearly correlated with the concentration of OTA, with a limit of detection of 1.6 ng/ml.

Based on the combination of target-induced strand release and cleavage of nicking endonuclease technology, Hun *et al.* (2013) developed an electrochemiluminescent bioassay based on target-induced strand release coupling cleavage of nicking endonuclease for OTA detection. The method was successfully applied in naturally contaminated wheat samples with a detection limit of 0.3 pg/ml and the RSD was 3.4% at 0.1 ng/ml. The LODs in solid matrix were not given, nor could be estimated from the publication. Rhouati *et al.* (2013a) designed an automatic system by injecting the functionalised magnetic beads onto the surface of screen-printed carbon electrodes (SPCEs) integrated into a central flow cell. The device was connected with a flow injection system and on-line detection of OTA in beer samples was performed amperometrically with a detection limit of 0.05 ng/ml. Hayat *et al.* (2013a,b,c) investigated an original strategy of immobilisation in the production of a highly sensitive label-free aptasensor for OTA detection in beer samples. The immobilisation of the aptamer was based on the combination of 'click chemistry' and binary film grafting. This new strategy provided a uniform, controlled and efficient immobilisation improving the sensitivity and reducing the non-specific signal. The impedimetric aptasensor showed a detection limit of 0.25 pg/ml and the authors demonstrated that the aptasensor is reusable at least up to 10 times without significant loss of performance. The same authors have subsequently described an impedimetric and an amperometric label-free aptasensor, based on the aptamer's conformational change upon target analyte binding. OTA's aptamer was covalently immobilised on SPCE via a bifunctional spacer, forming diblock macromolecules. The spacer forms a long tunnel while the aptamer acts as a gate of the tunnel. After OTA binding, the gates were closed decreasing the electrochemical signal. Both aptasensors were successfully applied for beer samples where the detection limit was 0.12 pg/ml by using a polyethylene glycol spacer and 0.1 ng/ml by using hexamethyldiamine.

Optimisation of hairpin DNA was introduced by Lee *et al.* (2014a) to detect OTA by chemically conjugating the cofactor hemin toward the 5´-end. The OTA aptasensor showed good stability and sensitivity with a detection limit close to 1 nM (0.4 ng/ml; no values in matrix were given). In addition, an optimal spacer for hemin conjugation was investigated for stable responses towards diluted OTA solutions. Silver nucleation on gold has been exploited by Anfossi *et al.* (2013c) for signal amplification and has found application in several qualitative and quantitative biosensing techniques, thanks to the simplicity of the method and the high sensitivity achieved. This technique has also been tentatively applied to improve the performance of gold-based immunoassays. The exploitation of the signal amplification due to silver deposition on gold nanoparticles has been first applied to a competitive LFIA. The signal enhancement due to silver allowed to reduce the amount of the competitor and of specific antibodies employed to build an LFD for measuring OTA, with a sensitivity gain of more than 10-fold compared to the gold-based LFIA that used the same immunoreagents and to all previously reported LFIA for measuring OTA. In addition, a less sensitive, quantitative LFIA could be established, by suitably tuning competitor and antibody amounts, which was characterised by reproducible and accurate OTA determinations (82-117% recovery, 6-12% RSD). The quantitative system allowed OTA quantification in wines and grape musts at μg/l levels, as demonstrated by results obtained through the quantitative silver-enhanced LFIA and a reference HPLC-FLD method for 30 samples.

10. Patulin

Zhang *et al.* (2014a) developed a dopant-assisted atmospheric pressure photoionisation with LC-MS/MS method to determine PAT in apple juice and apple-based food. Parameters such as the selection of dopant, dopant flow rate, and LC flow rate that can affect the sensitivity of PAT were evaluated and optimised. Using toluene as the dopant, the method achieved a linear calibration from 12.5 to 2,000 μg/l (r^2>0.99). Matrix-dependent limits of quantitation were from 8 μg/l (solvent) to 12 μg/l (apple juice). [^{13}C]-PAT-fortified apple juice samples were directly analysed by the method. Other apple-based food was fortified with [^{13}C]-PAT, diluted using H_2O (1% HCOOH), centrifuged, and filtered, followed by atmospheric pressure photoionisation with LC-MS/MS analysis. In clear apple juice, unfiltered apple cider, applesauce, and apple-based baby food, average recoveries were 101±6% (50 μg/kg), 103±5% (250 μg/kg), and 102±5% (1000 μg/kg). Beltrán *et al.* (2014) developed a method for analysis of PAT in regulated foodstuffs by using UHPLC-MS/MS. Ionisation in the atmospheric pressure chemical ionisation source was found to reduce matrix effect allowing the determination of PAT in juices by direct injection. The method was validated in four different apple matrices. Solid samples were extracted with EtAc, while liquid samples were directly injected into the chromatographic system after dilution and filtration without any clean-up step. Chromatographic separation was achieved in less than 4 min. The method was validated in juice, fruit, puree and compote at two concentrations at the low μg/kg level. Average recoveries ranged from 71 to 108%, with RSDs<14%.

Abu-Bakar *et al.* (2014) developed a technique using salting out-vortex-assisted liquid-liquid microextraction for the extraction of furfurals and PAT in apple, mango and grape juice samples. The extraction solvent was 1-hexanol with salt addition. Samples were vortexed for 45 s. The simultaneous determination of the furfurals and PAT was achieved using a reversed phase column under gradient elution and diode array detection. The detection wavelengths used were 280 nm and 210 nm. The method was linear (r^2>0.99) within the

range 1-5,000 μg/l for all compounds except for 3-furfural (10-5,000 μg/l) and PAT (0.5-100 μg/l). LODs of 0.28-3.2 μg/l were estimated at a signal-to-noise ratio of 3/1. A method based on enzyme-assisted extraction and ionic liquid-based DLLME was developed and optimised for isolation and determination of PAT in apple juice samples (Mohammadi *et al.*, 2013). Apple juice samples were treated with pectinase and amylase. The volume of extraction and disperser solvents, pH and salt effect were optimised using response surface methodology based on central composite design. 1-hexyl-3-methylimidazolium hexafluorophosphate was selected as a suitable extraction solvent and MeOH as disperser solvent. After final partitioning PAT was quantified by LC-UV. The calibration curves showed high levels of linearity (r^2>0.99) for PAT in the range of 1-200 μg/kg. The RSD_r was 7.5%, while LOD and LOQ were 0.15 μg/kg and 0.5 μg/kg, respectively.

11. Trichothecenes

The EFSA Panel on Contaminants in the Food Chain (CONTAM panel) published a scientific opinion on the risk to human and animal health related to the presence of NIV in food and feed. Based on the data available, the CONTAM Panel concluded that the overall weight of evidence was that NIV was unlikely to be genotoxic. The CONTAM panel, established a TDI of 1.2 μg/kg b.w. All chronic human dietary exposures to NIV estimated, based on the available occurrence data in food, were below the TDI, and therefore not a health concern (EFSA, 2013a). EFSA also published a statement on the risks to public health with respect to a possible increase in the ML of DON. It was concluded an increase of the ML could be expected to be associated with an increase in contamination levels of products reaching the market and therefore also of exposure (EFSA, 2013b).

Three reviews of methods have been published. An updated review of methods for T-2 and HT-2 was published (Krska *et al.*, 2014), based on the scientific opinion published by the EFSA CONTAM panel. The review discussed chromatographic and immuno-analytical methods for the determination of T-2 and HT-2. Approaches including biosensor-based methods in SPR and electrochemical formats, as well as DNA microchip assays and rapid screening immunochemical methods were reviewed. The article stated that no chromatographic or immunochemical methods have been formally validated in interlaboratory validation studies. However, a method for baby food with IAC clean-up and GC-MS was validated and reported in 2008 (Breidbach *et al.*, 2008). Another review based on the scientific opinion published by the EFSA CONTAM panel, provides an update on the determination of NIV (Malachova *et al.*, 2014). Chromatographic methods as well as other approaches are discussed. The authors state that accurate quantification of NIV is mostly carried out by LC-MS/MS, often within a multi-analyte approach. None of the currently available analytical methods have been formally validated in interlaboratory validation studies. While a certified calibrant for NIV is available, no matrix reference materials have been developed yet. Due to the scarcity of appropriate antibodies also no rapid immunochemical methods specific for NIV have become available. Finally, currently available analytical methods for DON and its derivatives, both qualitative and quantitative, were presented in a review. Basic principles as well as advantages and limitations of each method and highlights of new emerging technologies and their potential applications were discussed (Ran *et al.*, 2013).

Reports describing methods using HPLC were limited, however there were some interesting publications. Geng *et al.* (2014) reported a new hydrophilic interaction liquid chromatography (HILIC) method for the determination of deoxynivalenol-3-glucoside (D3G) in cereals. D3G was extracted with H_2O and cleaned up with an IAC, followed by chromatographic separation on a Syncronis HILIC column (Thermo Scientific) with ACN:H_2O (90:10, v/v) as the mobile phase, and detected at 220 nm by UV detection. Limits of detection and quantification were 8 and 25 μg/kg, respectively. A fast and simple method for the extraction of DON from wheat flour using DLLME followed by high-performance liquid chromatography-UV detection has been developed (Karami-Osboo *et al.*, 2013). It was compared with IAC clean-up. Average recoveries of DON for DLLME and IAC from spiked wheat samples at levels of 500 μg/kg were 73±1.6 and 86±3.1%, respectively. A good correlation between DLLME and IAC methods was found for spiked samples. In another study, UHPLC was used for the simultaneous determination of DON and NIV in wheat (Pascale *et al.*, 2014). Ground sample was extracted with H_2O and cleaned up by IAC containing a monoclonal antibody specific for DON and NIV. Toxins were separated and quantified by UHPLC with photodiode-array detector (220 nm) in less than 3 min, showing a significant reduction in analysis time compared to standard HPLC. Mean recoveries from blank wheat samples spiked with DON and NIV at levels of 100-2,000 μg/kg (each toxin) ranged from 85 to 95% for DON and from 81 to 88% for NIV, with RSDs <7%. Aqueous extraction was shown to give increased toxin concentrations (P<0.001) compared to MeOH:H_2O (80:20, v/v). The LOD of the method was 30 μg/kg for DON and 20 μg/kg for NIV (signal-to-noise ratio 3/1).

The use of GC was rarely reported and there were few developments. Shar *et al.* (2014) compared GC-FID, GC-ECD and GC-MS methods for analysis of poultry feed. All three methods gave comparable results. The GC-FID method was compared in two laboratories and performed well with recovery of 93% and limit of quantification of 6 μg/kg. A GC-MS method for DON, 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), NIV, T-2, HT-2, diacetoxyscirpemol (DAS) and fusarenone

X (FUS-X) in cereal grain was validated and used for monitoring shipments of Canadian cereal grain (Tittlemier *et al.*, 2013).

ELISAs are frequently used for rapid screening of DON in cereals and derived products. However some anti-DON antibodies also bind to structurally similar DON metabolites, such as DON-3G and 3-ADON, leading to a significant overestimation of DON concentrations. Dzuman *et al.* (2014a) carried out a validation study of six commercial DON-dedicated ELISA kits, namely Ridascreen DON, Ridascreen FAST DON (both R-Biopharm, Darmstadt, Germany), DON EIA (Elisa Technologies, Gainsville, FL, USA), AgraQuant DON Assay (Romer Labs), Veratox 5/5, and Veratox HS (both Neogen, Lansing, MI, USA) on wheat, barley and malt matrices. Performance characteristics of all the tested ELISAs were determined using aqueous solutions, extracts of artificially spiked blank cereals and matrix-matched standards. The accuracy of data was assessed by comparison of DON concentrations determined by ELISAs and a reference UHPLC-MS/MS method. High cross-reactivities were proved for both DON-3G and 3-ADON in the majority of examined assays, and the contribution of some matrix components to overestimation of DON results was confirmed. Similarly, Ogiso *et al.* (2013) examined whether immunochemical-based test kits designed for analysis of DON screening in grains were applicable to maize processing by-products. Two types of immunochromatographic kits and three ELISA kits were used to test three types of maize processing by-products and mixed feed. The results obtained with some kits were significantly different from LC-MS analysis. Extraction times were standardised and neutralisation and centrifugation processes were introduced to prevent denaturation of antibody. After these modifications, the recovery for all kits in assays of maize gluten meal was within the range of 80-120%, and all kits showed acceptable accuracy. Hiraoka *et al.* (2013) took the modification approach one step further by introducing a clean-up method using a MultiSep® 226 column (Romer Labs) for analysis of rice and maize silage before using ELISA. Overestimation of DON by the influence of specific cross-reaction with ADON was confirmed by LC-MS analysis. Therefore, the overestimation of DON by the influence of nonspecific cross-reaction with sample matrix was reduced by using a MultiSep® 226 column, and analysis of DON in silage was improved.

The most commonly used approach reported was LC-MS/MS. Several new methods were reported. Bryła *et al.* (2014) evaluated the usefulness of HPLC ion trap mass spectrometry for simultaneous determination of selected trichothecenes (NIV, DON, FUS-X, NEO, 3-ADON, DAS, HT-2 and T-2) in grain products. These compounds were extracted from the grain products and then cleaned up with a mixture of neutral alumina, charcoal and diatomaceous earth. Method recovery was 88-125% depending on the combination of the analysed mycotoxins, sample matrix and the fortification level. The method precision expressed by RSD ranged from 2.6 to 27%. Another method developed for simultaneous analysis of nine trichothecenes (NIV, DON, FUS-X, NEO, 3-ADON, 15-ADON, DAS, HT-2 and T-2) used extraction with ACN:H_2O (1:1) and purification using a QuEChERS kit and a multi-functional cartridge (Tamura *et al.*, 2014). Toxins were quantified by LC-MS/MS using internal standards and a pentafluorophenyl LC column allowed complete separation and highly sensitive quantification of the nine trichothecenes. Method validation with powdered maize soup samples demonstrated high linearity and accuracy ranging from 95% to 111% with RSD_r of 0.9 to 6.6% and RSD_R of 0.6 to 11.6%. A simple and reliable method for simultaneous determination of D3G and major type B trichothecenes (DON, NIV, FUS-X, 3-ADON, 15-ADON and DOM-1) in animal feed and raw materials was developed and validated in another study. The method was based on an improved dSPE followed by analysis using HPLC-MS/MS. Matrix-matched calibration curves (r^2>0.99) were employed to minimise matrix effects and ensure accurate quantification. The recoveries during sample preparation process (including extraction and clean-up) ranged from 79 to 118%. The limit of quantification ranged from 5.0 μg/kg for DON to 13.6 μg/kg for FUS-X (Zhao *et al.*, 2014b).

Yoshinari *et al.* (2013a) validated an LC-MS/MS method for simultaneous determination of DON and its acetylated derivatives, 3-ADON and 15-ADON, in wheat using a multifunctional column by an inter-laboratory study with 9 laboratories. The recoveries ranged from 99 to 103% for DON, 89 to 99% for 3-ADON, and from 85 to 90% for 15-ADON. RSD_r and $rRSD_R$ values of DON were in the ranges of 7.2-11.3% and 9.5-23%, respectively. The HorRat values for the three analytes ranged from 0.4 to 1.2. These results validate this method for the simultaneous determination of DON and its acetylated derivatives, 3-ADON and 15-ADON.

The main novel aspect of methods developed were the matrices the methods were applied to. Two methods were published for analysis of trichothecenes in chicken tissue samples. Both methods used LC-MS/MS. Lingchen *et al.* (2013) developed a method that allows for the determination of T-2, HT-2 and DAS in heart, liver, spleen, lung, kidney, glandular stomach, muscular stomach, small intestine, muscle, bone and brain samples from broilers. The samples were initially extracted with EtAc before being filtered through a 0.22 μm nylon syringe filter and subjected to chromatographic separation on a reversed-phase column. The limit of detection was in the range of 0.02-0.05 μg/kg, whereas the limit of quantification was in the range of 0.08-0.15 μg/kg. Lixiao *et al.* (2014) developed and validated a similar method; however they made use of an Oasis

HLB® SPE cartridge to purify the sample extracts. Again separation was performed on a C18 column by detection with MS in selected reaction monitoring (SRM) mode. The decision limits and the detection capabilities of the analytes in the chicken tissues ranged from 0.16 to 0.92 μg/kg and 0.68 to 2.07 μg/kg, respectively. Another novel application was a method that was developed for rapid simultaneous determination of two type A (T-2 and DAS) and two type B (3-ADON and FUS-X) trichothecenes in potato tubers by UHPLC-MS/MS (Huali *et al.*, 2013). The recovery determined for three levels of spikes for the four toxins ranged from 78 to 113%, the lowest recovery was for T-2 spiked at 10 μg/kg. Limits of detection ranged from 2-5 μg/kg and limits of quantification from 5-15 μg/kg. The method proved to be suitable for simultaneous determination of T-2, DAS, 3-ADON, and FUS-X in potato tubers inoculated with *Fusarium sulphureum* and was used to measure these toxins in lesions and normal potato tuber tissue. An interesting application for the analysis of DON in wheat dust by LC-MS/MS was reported by Sanders *et al.* (2013). The method that was developed and validated used extraction with ACN:H_2O:CH_3COOH (79:20:1, v/v/v) followed by a hexane defatting step, analysis was performed by LC-MS/MS. The method was used for a small survey on raw wheat materials and their corresponding dust samples. Although only a limited number of samples were analysed (n=12) a linear correlation (r^2=0.941) was found for the DON concentration in dust versus the DON concentration in wheat. Further work would be required, however it does seem that it would be possible to estimate the cereal DON contamination through analysis of dust resulting in the potential to save large amounts of effort in sample collection and homogenisation.

Masked mycotoxins are an important area of research with the number of publications increasing each year. A glucoside of NIV was isolated from NIV-contaminated wheat and was identified as nivalenol-3-*O*-β-D-glucopyranoside using LC-TOF-MS, this was confirmed by ^{1}H and ^{13}C NMR (Yoshinari *et al.*, 2014). Analytical methods using a multifunctional column or a commercially available IAC were developed for the simultaneous determination of NIV, NIV-3-*O*-β-D-glucopyranoside, DON, and DON-3G in wheat. The methods were single laboratory-validated, and recovery from wheat samples spiked at four levels ranged between 86 and 104% for the IAC clean-up. Many of publications on masked mycotoxins this year report studies of their fate during different processes (Huijie and Bujun, 2014; De Angelis *et al.*, 2013), and the fate and bioavailability during digestion (De Angelis *et al.*, 2014a,b). Many of these studies reported use of high-resolution mass spectrometry. This technology is extremely useful for analysis and discovery of new masked mycotoxins (mycotoxin glucosides) where analytical standards are not available. Nakagawa *et al.* (2013) described the new glucosides neosolaniol-glucoside and diacetoxyscirpenol-glucoside on the basis of accurate mass measurements of characteristic ions and fragmentation patterns using LC-Orbitrap MS analysis. The absolute structures were not clarified, however 3-OH glucosylation appeared to be the most probable when the fragmentation profiles and structures of neosolaniol and diacetoxyscirpenol were considered.

Overall the main trends were increased interest in trichothecenes other than DON, NIV is gaining more attention especially, and masked mycotoxins. This latter area will continue to grow, but routine analysis of masked mycotoxins will not be possible until analytical standards are more widely available. Isolation from naturally contaminated materials is not necessarily the most efficient method to achieve this as Yoshinari *et al.* (2014) reported a yield of 9.2 mg of NIV-3-*O*-β-D-glucopyranoside from 12 kg wheat, so alternative approaches should be found to meet this important need.

12. Zearalenone

Most methods for the determination of ZEA in food and feed are based on LC-MS(/MS) multi-toxin methods, which do also cover a range of other mycotoxins beyond ZEA and its metabolites. These are discussed in the respective chapter of this review. Moreover, dedicated chromatographic methods based on LC-UV/FLD and LC-MS(/MS) as well as immunoanalytical methods (e.g. ELISAs, LFDS) have been developed that exclusively focus on the determination of ZEA and its metabolites.

Ok *et al.* (2014) developed and compared HPLC-FLD and UHPLC-FLD methods for the determination of ZEA in noodles, cereal snacks and infant formula. Both methods used IAC for purification and yielded good recoveries (HPLC-FLD: 94-98%, UHPLC-FLD: 97-104%) on average for all matrices as well as good repeatabilities (HPLC-FLD: 4-6%, UHPLC-FLD: 3-5%). LOQs between 8-10 μg/kg were obtained for all matrices with both methods. The authors concluded that the main advantage of the UHPLC method is – unsurprisingly – the shorter run time and therefore reduced solvent consumption. Kong *et al.* (2013) presented their LC-FLD method for the determination of ZEA, α-ZOL, β-ZOL and aflatoxins in Coix seeds. After extraction in an ultrasonic bath the seeds were purified using a multiple-antibody IAC. FLD settings were changed within the run after the aflatoxins eluted from the column. Recoveries ranged from 71-95% for ZEA, from 82-102% for α-ZOL, and from 94-99% for β-ZOL. RSDs were lower than 8% for all analytes.

Regarding LC-MS/MS, at least four methods describe the determination of ZEA (and its metabolites) in various matrices. ZEA, α-ZOL, β-ZOL, zearalanone (ZAN), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL) were determined together with chloramphenicol in pig

meat (Wang *et al.*, 2014b). After enzymatic digestion by β-glucuronidase/sulfatase, the sample was extracted multiple times with diethyl ether and cleaned-up using IAC. The used IAC columns were regenerated with buffer and could be used multiple times, albeit the recoveries were decreasing after each round of sample purification (by about 20% after five times). ESI in negative mode was followed by MS/MS determination in the SRM mode. Typical RSD_r were below 10%, with recoveries of 75-105% and LOQs from 0.1-0.4 μg/kg for all analytes. Wozniak *et al.* (2013) describe the determination of ZEA, α-ZOL, β-ZOL, ZAN, α-ZAL, β-ZAL and stilbenes in bovine, porcine and poultry muscle tissue after QuEChERS clean-up. After spiking the ground sample with deuterated internal standards, the analytes were partitioned in H_2O:EtAc. Magnesium sulphate and sodium acetate were added and the organic layer was treated with dSPE. Purified extracts were measured by LC-MS/MS using negative ESI. 84-104% of the ZEA metabolites were recovered in all three matrices and the repeatabilities were around or less than 20% at levels of 1 μg/kg. Detection capabilities of about 0.3 μg/kg were achieved. Another method describes the LC-MS/MS determination of ZEA, α-ZOL, β-ZOL, ZAN, α-ZAL, β-ZAL in feed using multi-walled carbon nanotubes as a dSPE sorbent (Ying *et al.*, 2013). After extraction with ACN:H_2O and dilution with H_2O, dSPE was carried out with the nanotubes of an outer diameter <8 nm and a length of 10-30 μm. The analytes were desorbed by EtAc before analysis with UHPLC-ESI-MS/MS in negative mode. Deuterated ZEA was used as internal standard. Recoveries of 96-104% were achieved for all analytes in mixed feed at a level of 1 μg/kg (which was slightly higher than the LOQs), with RSDs <10%. Finally, Drzymala *et al.* (2014) prepared and used ^{13}C-labelled cis-ZEA as internal standard for the LC-MS/MS quantification of cis-ZEA in contaminated food and feed products. While ZEA occurs naturally in the trans-configuration, isomerisation can take place after the sample is exposed to light. In an isomerisation study the authors showed that about 25% of ZEA were converted to cis-ZEA in edible oil stored on a window sill with daylight exposure for three weeks. cis-ZEA is eluting after ZEA on a C-18 reversed phase column. A small survey of maize germ oils resulted in only a single sample with detectable amounts of cis-ZEA (>0.3 μg/kg), while two samples were contaminated with ZEA at 17 and 31 μg/kg.

Several LC-MS based biomarker methods to quantify ZEA and its metabolites in biological fluids – mostly urine – for exposure assessment have been developed and employed last year. Belhassen *et al.* (2014) describe a UHPLC-ESI-MS/MS method for the quantification of ZEA, α-ZOL, β-ZOL, α-ZAL, β-ZAL and ZAN in human urine. After enzymatic hydrolysis, the samples were partitioned with acidified EtAc and defatted with hexane. LC-MS/MS analysis was performed in ESI negative mode with deuterated ZEA as internal standard. 96-104% of the analytes were recovered with RSDs from 4-8%. LOQs ranged from 0.1 μg/l for ZEA to about 1 μg/l for its metabolites. Three studies used multi-biomarker approaches to determine the exposure of mycotoxins, including ZEA in humans. Abia *et al.* (2013) verified the mycotoxin exposure in Cameroon using 175 urine samples, from which 7 were positive for ZEA, ZEA-14-glucuronide or α-ZOL. Shephard *et al.* (2013b) analysed the maize-based evening meals and morning urine of 53 female rural subsistence farmers in the former Transkei region of South Africa. After enzymatic treatment and IAC clean-up, 100% of all urine samples were tested positive for ZEA with mean values of 0.53 ng ZEA/mg creatinine, 0.61 ng α-ZOL/mg creatinine and 0.70 ng β-ZOL/mg creatinine. Solfrizzo *et al.* (2014) used their method to evaluate the mycotoxin exposure of 52 volunteers from the Apulia region in Southern Italy. While again all urine samples were contaminated with ZEA (on average with 0.06 ng/ml), α-ZOL or β-ZOL, the estimated human exposure to ZEA was far below the TDI established by EFSA (0.25 μg/kg bodyweight). Finally, Songsermsakul *et al.* (2013) verified the level of ZEA and its metabolites in plasma, urine and faeces of horses. The degree of glucuronidation was approximately 100% in urine and plasma. Their results indicate that ZEA is mainly metabolised to β-ZOL in horses.

Immuno-analytical methods for the determination of ZEA remain very popular, as can be seen from the number of recent publications. Huang *et al.* (2014b) developed a biotin-avidin amplified ELISA to detect ZEA in maize. The monoclonal antibody was biotinylated, while avidin-horseradish peroxidase was the used enzyme. The recovery in maize ranged from 87-94% with RSDs<8%. The authors gained a six-fold higher sensitivity of the biotin-avidin amplified ELISA compared to the traditional ELISA method. Tang *et al.* (2014) developed a monoclonal antibody versus ZEA and used it for IAC clean-up and/or in an indirect competitive ELISA. Cross-reactivities of the antibody were 88% for β-ZOL and 4% for α-ZOL. The additional use of the IAC before ELISA raised recoveries from 46-54% to 83-93%. Using a reference HPLC-FLD method with the newly developed IAC 94-108% was recovered. The IC_{50} value of the ELISA against ZEA was 0.02 ng/ml. Edupuganti *et al.* (2013a) generated single chain antibody fragments versus ZEA and used it in both a competitive inhibition ELISA and a SPR-based assay. The developed antibody fragment showed minimal cross-reactivities (≤0.1%) to α-ZOL and β-ZOL. The SPR assay was more sensitive than the ELISA, yielding a LOQ of 13 μg/kg in sorghum. Recoveries of 88-112% and RSDs<7% were obtained. He *et al.* (2014) used a peptide library of random 12-mers displayed on phage to screen for binding to an anti-ZEA antibody. Selected phage-borne 'peptidomimetics' were than used as coating antigen and applied in a rapid yes/no dot-immunoassay. In cereal samples a cut-off level of 50 μg/kg ZEA was achieved. Beloglazova *et al.* (2013b) developed FLISAs for the determination of ZEA using quantum dot

loaded liposomes as labels. Both water-soluble and water-insoluble quantum dots were tested. Between 84-121% were recovered in spiked cereal samples with LODs ranging from 0.02-0.08 μg/kg. Furthermore, qualitative on-site tests using the different labels were developed which showed cut-off levels of 20, 50 and 100 μg/kg. Also several immunoassays for the determination of multiple mycotoxins, including ZEA, were recently developed. Song *et al.* (2014) describe a lateral flow immunoassay for the qualitative or semi-quantitative determination of ZEA, AFB_1 and DON in cereal samples using three different class specific antibodies. For ZEA a LOD of about 1 μg/kg, recoveries of 87-120% in wheat and maize as well as RSDs ≤20% were obtained. Hu *et al.* (2013) presented their fluorescent competitive immunoassay microarray to detect ZEA, AFB_1 and OTA. Wide linear ranges of almost two to three orders of magnitude were achieved for all toxins, with a LOD of 3 ng/l for ZEA (corresponding to 90 ng/kg in solid matrices). The non-fouling antigen microarray showed recovery rates of 86-109% for all three toxins in peanuts, which were used as sample matrix. Wang *et al.* (2013c) developed a suspension array immunoassay for ZEA, FB_1, DON and AFB_1 in cereals. Monoclonal antibodies were conjugated to the surface of different encoding microspheres, mycotoxin-protein conjugates were coupled with biotin and streptavidin-phycoerythrin was used as a signal reporter protein. The direct competitive multiple immunoassay yielded detection limits of 0.5 μg/l (corresponding to 20 μg/kg in cereals), recovery rates of 93-106% and RSDs of about 7% for ZEA.

Other methods include the determination of oestrogenic compounds, including ZEA, α-ZOL, β-ZOL, α-ZAL and β-ZAL, in water samples by DLLME and micellar electro-kinetic chromatography coupled to mass spectrometry (D'Orazio *et al.*, 2014). DLLME, using chloroform and ACN as extraction and dispersion solvents, was employed to extract target analytes from water samples containing 30% NaCl at pH 3. An electrolyte containing aqueous perfluoro octanoic acid adjusted to pH 9 with ammonia solution, and MeOH as organic modifier, was used. The limits of detection were around 0.2 μg/l in mineral water (about 80% recovery) and around 0.8 μg/l (about 60% recovery) in waste water. Stroka and Seidler (2014) published an IAC clean-up procedure using hot water at 85 °C as an alternative for organic elution solvents. The eluate was transferred into a 3 ml stainless steel injection loop connected to a reversed phase analytical column with a 6-port valve. The main advantage of this method is the complete injection of the eluate. For ZEA 82% recovery and 5% RSD were obtained in maize.

Regarding conjugated forms of ZEA the following publications are of interest. A new masked mycotoxin – zearalenone-16-O-β-glucoside (ZEA-16-Glc) was recently discovered (Kovalsky Paris *et al.*, 2014). The authors identified a UDP-glucosyltransferase from barley, able to convert ZEA into an almost equimolar mixture of zearalenone-14-O-β-glucoside (ZEA-14-Glc) and ZEA-16-Glc. Incubation of the compound with human faeces resulted in the rapid release of ZEA, suggesting the likely fate of the ZEA-16-Glc during digestion. ZEA-16-Glc and ZEA-16-sulfate were also synthesised to gain sufficient amounts for further analysis and potential toxicological studies (Mikula *et al.*, 2014). In addition, also ZEA-14-β,D-gentiobioside is now available due to chemical synthesis (Weber *et al.*, 2013).

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The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the authors of this article or any of their institution. The institutions of all authors are equal opportunity providers and employers. C. Brera thanks Dr. Mark Sykes for his appreciated cooperation in providing useful and valuable information on OTA proficiency testing.

References

Abhijith, K.S., Ragavanab, K.V. and Thakur, M.S., 2013. Gold nanoparticles enhanced chemiluminescence – a novel approach for sensitive determination of aflatoxin B_1. Analytical Methods 5: 4838-4845.

Abia, W.A., Warth, B., Sulyok, M., Krska, R., Tchana, A., Njobeh, P.B., Turner, P.C., Kouanfack, C., Eyongetah, M., Dutton, M. and Moundipa, P.F., 2013. Bio-monitoring of mycotoxin exposure in Cameroon using a urinary multi-biomarker approach. Food and Chemical Toxicology 62: 927-934.

Abu-Bakar, N.B., Makahleh, A. and Saad, B., 2014. Vortex-assisted liquid-liquid microextraction coupled with high performance liquid chromatography for the determination of furfurals and patulin in fruit juices. Talanta 120: 47-54.

Akdeniz, A.S., Ozden, S. and Alpertunga, B., 2013. Ochratoxin A in dried grapes and grape-derived products in Turkey. Food Additives and Contaminants Part B 6: 265-269.

Amelin, V.G., Karaseva, N.M. and Tret'yakov, A.V., 2013. Chromatographic methods for the determination of mycotoxins in food products. Journal of Analytical Chemistry 68: 195-205.

Anfossi, L., Baggiani, C., Giovannoli, C., Biagioli, F., D'Arco, G. and Giraudi G., 2013a. Optimization of a lateral flow immunoassay for the ultrasensitive detection of aflatoxin M_1 in milk. Analytica Chimica Acta 772: 75-80.

Anfossi, L., Baggiani, C., Giovannoli, C., D'Arco G. and Giraudi, G., 2013b. Lateral-flow immunoassays for mycotoxins and phycotoxins: a review. Analytical and Bioanalytical Chemistry 405: 467-480.

Anfossi, L., Di Nardo, F., Giovannoli, C., Passini, C. and Baggiani, C., 2013c. Increased sensitivity of lateral flow immunoassay for ochratoxin A through silver enhancement. Analytical and Bioanalytical Chemistry 405: 9859-9867.

Arroyo-Manzanares, N., Huertas-Pérez, J.F., Gámiz-Gracia, L. and García-Campaña, A.M., 2013. A new approach in sample treatment combined with UHPLC-MS/MS for the determination of multiclass mycotoxins in edible nuts and seeds. Talanta 115: 61-67.

Arroyo-Manzanares, N., Huertas-Pérez, J.F., García-Campaña, A.M. and Gámiz-Gracia, L., 2014a. Simple methodology for the determination of mycotoxins in pseudocereals, spelt and rice. Food Control 36: 94-101.

Arroyo-Manzanares, N., Malysheva, S.V., Vanden Bussche, J., Vanhaecke, L., Di Mavungu, J.D. and De Saeger, S., 2014b. Holistic approach based on high resolution and multiple stage mass spectrometry to investigate ergot alkaloids in cereals. Talanta 118: 359-367.

Asam, S. and Rychlik M., 2013. Potential health hazards due to the occurrence of the mycotoxin tenuazonic acid in infant food. European Food Research and Technology 236: 491-497.

Asam, S., Habler, K. and Rychlik M., 2013. Determination of tenuazonic acid in human urine by means of a stable isotope dilution assay. Analytical and Bioanalytical Chemistry 405: 4149-4158.

Ates, E., Godula, M., Stroka, J. and Senyuva, H., 2014. Screening of plant and fungal metabolites in wheat, maize and animal feed using automated on-line clean-up coupled to high resolution mass spectrometry. Food Chemistry 142: 276-284.

Azaiez, I., Giusti, F., Sagratini, G., Mañes, J. and Fernández-Franzón, M., 2014. Multi-mycotoxins analysis in dried fruit by LC/MS/MS and a modified QuEChERS procedure. Food Analytical Methods 7: 935-945.

Bartók, T., Szécsi, A., Juhász, K., Bartók, M. and Mesterházy, A., 2013a. ESI-MS and MS/MS identification of the first ceramide analogues of fumonisin B_1 mycotoxin from a *Fusarium verticillioides* culture following RP-HPLC separation. Food Additives and Contaminants Part A 30: 1651-1659.

Bartók, T., Tölgyesi, L., Szécsi, A., Mesterházy, A., Bartók, M., Gyimes, E. and Véha, A., 2014. Detection of previously unknown fumonisin P analogue mycotoxins in a *Fusarium verticillioides* culture by high-performance liquid chromatography-electrospray ionization time-of-flight and ion trap mass spectrometry. Journal of Chromatographic Science 52: 508-513.

Bartók, T., Tölgyesi, L., Szécsi, A., Varga, J., Bartók, M., Mesterházy, A., Gyimes, E. and Véha, A., 2013b. Identification of unknown isomers of fumonisin B_5 mycotoxin in a *Fusarium verticillioides* culture by high-performance liquid chromatography/electrospray ionization time-of-flight and ion trap mass spectrometry. Journal of Liquid Chromatography and Related Technologies 36: 1549-1561.

Bazin, I., Faucet-Marquis, V., Monje, M.C., El Khoury, M., Marty, J.L. and Pfohl-Leszkowicz, A., 2013. Impact of pH on the stability and the cross-reactivity of ochratoxin A and citrinin. Toxins 5: 2324-2340.

Beaulieu, W.T., Panaccione, D.G., Hazekamp, C.S., McKee, M.C., Ryan, K.L. and Clay, K, 2013. Differential allocation of seed-borne ergot alkaloids during early ontogeny of morning glories (*Convolvulaceae*). Journal of Chemical Ecology 39: 919-930.

Belhassen, H., Jimenez-Diaz, I., Ghali, R., Ghorbel, H., Molina-Molina, J.M., Olea N. and Hedili, A., 2014. Validation of a UHPLC-MS/MS Method for quantification of zearalenone, alpha-ZEL, beta-ZEL, alpha-ZAL, beta-ZAL and ZAN in human urine. Journal of Chromatography B 962: 68-74.

Beloglazova N., Shmelin, P., Goryacheva, I. and De Saeger, S., 2013a. Liposomes loaded with quantum dots for ultrasensitive on-site determination of aflatoxin M_1 in milk products. Analytical and Bioanalytical Chemistry 405: 7795-7802.

Beloglazova, N.V., Shmelin, P.S., Speranskaya, E.S., Lucas, B., Helmbrecht, C., Knopp, D., Niessner, R., De Saeger, S. and Goryacheva, I.Y., 2013b. Quantum dot loaded liposomes as fluorescent labels for immunoassay. Analytical Chemistry 85: 7197-7204.

Beltrán, E., Ibáñez, M., Portolés, T., Ripollés, C., Sancho, J.V., Yusa, V., Marín, S. and Hernández, F., 2013. Development of sensitive and rapid analytical methodology for food analysis of 18 mycotoxins included in a total diet study. Analytica Chimica Acta 783: 39-48.

Beltrán, E., Ibáñez, M., Sancho, J.V. and Hernández, F., 2014. Determination of patulin in apple and derived products by UHPLC-MS/MS. Study of matrix effects with atmospheric pressure ionisation sources. Food Chemistry 142: 400-407.

Bergmann, D., Hübner, F. and Humpf, H.U., 2013. Stable isotope dilution analysis of small molecules with carboxylic acid functions using 18O labelling for HPLC-ESI-MS/MS: analysis of fumonisin B_1. Journal of Agricultural and Food Chemistry 61: 7904-7908.

Berthiller, F., Burdaspal, P.A., Crews, C., Iha, M.H., Krska, R., Lattanzio, V.M.T., MacDonald, S., Malone, R.J., Maragos, C., Solfrizzo, M., Stroka, J. and Whitaker, T.B., 2014. Developments in mycotoxin analysis: an update for 2012-2013. World Mycotoxin Journal 7: 3-33.

Biancardi, A., Piro, R., Dall'Asta, C. and Galaverna, G., 2013. Simple and reliable liquid chromatography-tandem mass spectrometry method for the determination of aflatoxin M_1 in milk. Food Additives and Contaminants Part A 30: 381-388.

Breidbach, A., Povilaitye, V., Mischke, C., Doncheva, I., Van Egmond, H. and Stroka, J., 2008. Validation of an analytical method to determine the content of T-2 and HT-2 toxins in cereals and baby food by immunoaffinity column clean-up and GC-MS. JRC Scientific and Technical Reports: EUR 23559 EN-2008.

Brera, C., Pannunzi, E., Guarino, C., Debegnach, F., Gregori, E. and De Santis, B., 2014. Ochratoxin A determination in cured ham by high performance liquid chromatography fluorescence detection and ultra performance liquid chromatography tandem mass spectrometry: a comparative study. Journal of Liquid Chromatography and Related Technologies 37: 2036-2045.

Brezina, U., Valenta, H., Rempe, I., Kersten, S., Humpf, H.U. and Dänicke, S., 2014. Development of a liquid chromatography tandem mass spectrometry method for the simultaneous determination of zearalenone, deoxynivalenol and their metabolites in pig serum. Mycotoxin Research 30: 171-86.

Bryła, M., Jedrzejczak, R., Szymczyk, K., Roszko, M. and Obiedzinski, M. W., 2014. An LC-IT-MS/MS-based method to determine trichothecenes in grain products. Food Analytical Methods 7: 1056-1065.

Bryła, M., Roszko, M., Szymczyk, K., Jedrzejczak, R., Obiedziński, M.W. and Sekul, J., 2013. Fumonisins in plant-origin food and fodder – a review. Food Additives and Contaminants Part A 30: 1626-1640.

Busman, M., Liu, J., Zhong, H., Bobell, J.R. and Maragos, C.M., 2014. Determination of the aflatoxin AFB_1 from corn by direct analysis in real time-mass spectrometry (DART-MS). Food Additives and Contaminants Part A 31: 932-939.

Campone, L., Piccinelli, A.L., Celano, R., Russo M. and Rastrelli, L., 2013. Rapid analysis of aflatoxin M_1 in milk using dispersive liquid-liquid microextraction coupled with ultrahigh pressure liquid chromatography tandem mass spectrometry. Analytical and Bioanalytical Chemistry 405: 8645-8652.

Cao, X., Wu, S., Yue, Y., Wang S., Wang, Y., Tao, L., Tian, H., Xie, J. and Ding, H., 2013. A high-throughput method for the simultaneous determination of multiple mycotoxins in human and laboratory animal biological fluids and tissues by PLE and HPLC-MS/MS. Journal of Chromatography B 942-943: 113-125.

Capriotti, A.L., Cavaliere, C., Foglia, P., Samperi, R., Stampachiacchiere, S., Ventura, S. and Laganà, A., 2014. Multiclass analysis of mycotoxins in biscuits by high performance liquid chromatography-tandem mass spectrometry. Comparison of different extraction procedures. Journal of Chromatography A 1343: 69-78.

Cheli, F., Battaglia, D., Gallo, R. and Dell'Orto, V., 2014a. EU legislation on cereal safety: an update with a focus on mycotoxins. Food Control 37: 315-325.

Cheli, F., Fusi, E. and Baldi, A. 2014b. Cell-based models for mycotoxin screening and toxicity evaluation: an update. World Mycotoxin Journal 7: 153-166.

Cheli, F., Pinotti, L., Rossi, L. and Dell'Orto, V., 2013. Effect of milling procedures on mycotoxin distribution in wheat fractions: a review. LWT – Food Science and Technology 54: 307-314.

Chen, X., Huang, Y., Duan, N., Wu, S., Xia, Y., Ma, X., Zhu, C., Jiang, Y., Ding, Z. and Wang, Z., 2014. Selection and characterization of single stranded DNA aptamers recognizing fumonisin B_1. Microchimica Acta 181: 1317-1324.

De Angelis, E., Monaci, L. and Visconti, A., 2014a. Investigation on the stability of deoxynivalenol and DON-3 glucoside during gastro-duodenal *in vitro* digestion of a naturally contaminated bread model food. Food Control 43: 270-275.

De Angelis, E., Monaci, L., Mackie, A., Salt, L. and Visconti, A., 2014b. Bioaccessibility of T-2 and HT-2 toxins in mycotoxin contaminated bread models submitted to *in vitro* human digestion. Innovative Food Science and Emerging Technologies 22: 248-256.

De Angelis, E., Monaci, L., Pascale, M. and Visconti, A., 2013. Fate of deoxynivalenol, T-2 and HT-2 toxins and their glucoside conjugates from flour to bread: an investigation by high-performance liquid chromatography high-resolution mass spectrometry. Food Additives and Contaminants Part A 30: 345-355.

De Girolamo, A., Lattanzio, V.M.T., Schena, R., Visconti, A. and Pascale, M., 2014. Use of liquid chromatography-high-resolution mass spectrometry for isolation and characterization of hydrolyzed fumonisins and relevant analysis in maize-based products Journal of Mass Spectrometry 49: 297-305.

De Girolamo, A., Solfrizzo, M., Lattanzio, V.M.T., Stroka, J., Alldrick, A., Van Egmond, H.P. and Visconti, A., 2013. Critical evaluation of LC-MS-based methods for simultaneous determination of deoxynivalenol, ochratoxin A, zearalenone, aflatoxins, fumonisins and T-2/HT-2 toxins in maize. World Mycotoxin Journal 6: 317-334.

De Oliveira, D.N., Ferreira, M.S. and Catharino, R.R., 2014. Rapid and simultaneous *in situ* assessment of aflatoxins and stilbenes using silica plate imprinting mass spectrometry imaging. PLoS ONE 9: e90901.

Della Riccia, G. and Del Zotto, S., 2013. A multivariate regression model for detection of fumonisins content in maize from near infrared spectra. Food Chemistry 141: 4289-4294.

Desmarchelier, A., Tessiot, S., Bessaire, T., Racault, L., Fiorese, E., Urbani, A., Chan, W.C., Cheng, P. and Mottier, P., 2014. Combining the quick, easy, cheap, effective, rugged and safe approach and clean-up by immunoaffinity column for the analysis of 15 mycotoxins by isotope dilution liquid chromatography tandem mass spectrometry. Journal of Chromatography A 1337: 75-84.

Di Mavungu, J.D., Malysheva, S.V., Sanders, M., Larionova, D., Robbens, J., Dubruel, P., Van Peteghem, C. and De Saeger, S., 2012. Development and validation of a new LC-MS/MS method for the simultaneous determination of six major ergot alkaloids and their corresponding epimers. Application to some food and feed commodities. Food Chemistry 135: 292-303.

Dohnal, V., Dvořák, V., Malíř, F., Ostrý, V. and Roubal T., 2013. A comparison of ELISA and HPLC methods for determination of ochratoxin A in human blood serum in the Czech Republic. Food Chemical Toxicology 62: 427-431.

D'Orazio, G., Asensio-Ramos, M., Hernandez-Borges, J., Fanali, S. and Rodriguez-Delgado, M.A., 2014. Estrogenic compounds determination in water samples by dispersive liquid-liquid microextraction and micellar electrokinetic chromatography coupled to mass spectrometry. Journal of Chromatography A 1344: 109-121.

Drzymala, S., Riedel, J., Köppen, R., Garbe, L.A. and Koch, M., 2014. Preparation of C-13-labelled cis-zearalenone and its application as internal standard in stable isotope dilution analysis. World Mycotoxin Journal 7: 45-52.

Dzantiev, B.B., Byzova, N.A., Urusov, A.E. and Zherdev, A.V., 2014. Immunochromatographic methods in food analysis. Trends in Analytical Chemistry 55: 81-93.

Dzuman, Z., Vaclavikova, M., Polisenska, I., Veprikova, Z., Fenclova, M., Zachariasova, M. and Hajslova, J., 2014a. Enzyme-linked immunosorbent assay in analysis of deoxynivalenol: investigation of the impact of sample matrix on results accuracy. Analytical and Bioanalytical Chemistry 406: 505-514.

Dzuman, Z., Zachariasova, M., Lacina, O., Veprikova, Z., Slavikova, P. and Hajslova, J., 2014b. A rugged high-throughput analytical approach for the determination and quantification of multiple mycotoxins in complex feed matrices. Talanta 121: 263-272.

Edupuganti, S.R., Edupuganti, O.P. and O'Kennedy, R., 2013a. Generation of anti-zearalenone scFv and its incorporation into surface plasmon resonance-based assay for the detection of zearalenone in sorghum. Food Control 34: 668-674.

Edupuganti, S.R., Edupuganti, O.P., Hearty, S. and O'Kennedy, R., 2013b. A highly stable, sensitive, regenerable and rapid immunoassay for detecting aflatoxin B_1 in corn in corporating covalent AFB_1 immobilization and a recombinant fab antibody. Talanta 115: 329-335.

European Committee for Standardization (CEN), 2011. Food analysis – performance criteria for single laboratory validated methods of analysis for the determination of mycotoxins. Technical Report: CEN/TR 16059.

European Food Safety Authority (EFSA) 2012. Panel on Contaminants in the Food Chain, Scientific Opinion on Ergot alkaloids in food and feed. EFSA Journal 10: 2798.

European Food Safety Authority (EFSA) 2013a. Panel on Contaminants in the Food Chain, Scientific opinion on risks for animal and public health related to the presence of nivalenol in food and feed. EFSA Journal 11: 3262.

European Food Safety Authority (EFSA) 2013b. Panel on Contaminants in the Food Chain, Statement on the risks for public health related to a possible increase of the maximum level of deoxynivalenol for certain semi-processed cereal products. EFSA Journal 11: 3490.

Ezekiel, C.N., Warth, B., Ogara, I.M., Abia, W.A., Ezekiel, V.C., Atehnkeng, J., Sulyok, M., Turner, P.C., Tayo, G.O.,Krska, R. and Bandyopadhyay, R., 2014. Mycotoxin exposure in rural residents in Northern Nigeria: a pilot study using multi-urinary biomarkers. Environment International 66: 138-345.

Falavigna, C., Lazzaro, I., Galaverna, G., Battilani, P. and Dall'Asta, C., 2013. Fatty acid esters of fumonisins: first evidence of their presence in maize. Food Additives and Contaminants Part A 30: 1606-1613.

Fang, L.X., Xiong, A.Z., Wang, R., Ji, S., Yang, L. and Wang, Z.T., 2013. A strategy for screening and identifying mycotoxins in herbal medicine using ultra-performance liquid chromatography with tandem quadrupole time-of-flight mass spectrometry. Journal of Separation Science 36: 3115-3122.

Fox, G. and Manley, M., 2014. Applications of single kernel conventional and hyperspectral imaging near infrared spectroscopy in cereals. Journal of the Science of Food and Agriculture 94: 174-179.

Gan, N., Zhou, J., Xiong, P., Hu, F., Cao, Y., Li, T. and Jiang, Q., 2013. An ultrasensitive electrochemiluminescent immunoassay for aflatoxin M_1 in milk, based on extraction by magnetic graphene and detection by antibody-labelled CdTe quantum dots-carbon nanotubes nanocomposite. Toxins 5: 865-883.

Geng, Z., Yang, D., Zhou, M., Zhang, P., Wang, D., Liu, F., Zhu, Y. and Zhang, M., 2014. Determination of deoxynivalenol-3-glucoside in cereals by hydrophilic interaction chromatography with ultraviolet detection. Food Analytical Methods 7: 1139-1146.

Giovannoli, C., Passini, C., Di Nardo, F., Anfossi, L. and Baggiani, C., 2014. Determination of ochratoxin A in Italian red wines by molecularly imprinted solid-phase extraction and HPLC analysis. Journal of Agricultural and Food Chemistry 62, 5220-5225.

Guo, X., Wen, F., Zheng, N., Luo, Q., Wang, H., Wang, H., Li, S. and Wang, J., 2014. Development of an ultrasensitive aptasensor for the detection of aflatoxin B_1. Biosensors and Bioelectronics 56: 340-344.

Hallier, A., Celette, F., Coutarel, J. and David, C. 2013. A contribution to reduce sampling variability in the evaluation of deoxynivalenol contamination of organic wheat grain. Food Additives and Contaminants Part A 30: 2159-2164.

Han, Z., Feng, Z., Shi, W., Zhao, Z., Wu, Y. and Wu, A., 2014. A quick, easy, cheap, effective, rugged, and safe sample pretreatment and liquid chromatography with tandem mass spectrometry method for the simultaneous quantification of 33 mycotoxins in *Lentinulaedodes*. Journal of Separation Science 37: 1957-1966.

Hashemi, M., Taherimaslaka, Z. and Rashidiba, S., 2014a. Application of magnetic solid phase extraction for separation and determination of aflatoxins B_1 and B_2 in cereal products by high performance liquid chromatography-fluorescence detection. Journal of Chromatography B 960: 200-208.

Hashemi, M., Taherimaslaka, Z. and Rashidiba, S., 2014b. Enhanced spectrofluorimetric determination of aflatoxin M_1 in liquid milk after magnetic solid phase extraction. Spectrochimica Acta Part A 128: 583-590.

Hayat A., Andreescu, S. and Marty J.L., 2013b. Design of PEG-aptamer two piece macromolecules as convenient and integrated sensing platform: application to the label free detection of small size molecules. Biosensors and Bioelectronics 45: 168-173.

Hayat A., Haider W., Rolland M. and Marty J.L., 2013c. Electrochemical grafting of long spacer arms of hexamethyldiamine on a screen printed carbon electrode surface: application in target induced ochratoxin A electrochemical aptasensor. Analyst 138: 2951-2957.

Hayat, A., Sassolas, A., Marty, J.L. and Radi, A.E., 2013a. Highly sensitive ochratoxin A impedimetric aptasensor based on the immobilization of azido-aptamer onto electrografted binary film via click chemistry. Talanta 103: 14-19.

He, Q.H., Xu, Y., Zhang, C.Z., Li, Y.P. and Huang, Z.B., 2014. Phage-borne peptidomimetics as immunochemical reagent in dot-immunoassay for mycotoxin zearalenone. Food Control 39: 56-61.

Himmelsbach, M., Ferdig, M. and Rohrer, T., 2014. Analysis of paspalic acid, lysergic acid, and iso-lysergic acid by capillary zone electrophoresis with UV-and quadrupole time-of-flight mass spectrometric detection. Electrophoresis 35: 1329-1333.

Hiraoka, H., Yamamoto, K., Mori, Y., Asao, N., Fukunaka, R., Deguchi, K., Iida, K., Miyazaki, S. and Goto, T., 2013. Modified use of a commercial ELISA kit for deoxynivalenol determination in rice and corn silage. Mycotoxin Research 29: 79-88.

Hruska, Z., Yao, H., Kincaid, R., Brown, R., Cleveland, T. and Bhatnagar, D., 2014. Fluorescence excitation-emission features of aflatoxin and related secondary metabolites and their application for rapid detection of mycotoxins. Food and Bioprocess Technology 7: 1195-1201.

Hruska, Z., Yao, H., Kincaid, R., Darlington, D., Brown, R.L., Bhatnagar, D. and Cleveland, T.E., 2013. Fluorescence imaging spectroscopy (FIS) for comparing spectra from corn ears naturally and artificially infected with aflatoxin producing fungus. Journal of Food Science 78: 1313-1320.

Hu, W., Xu, L., Yang, J. and Ling, R., 2014. QuEChERS-based extraction procedure and rapid resolution liquid chromatography coupled to triple quadrupole mass spectrometry for the determination of nine mycotoxins in cereal-based complementary foods for infants and young children. Chinese Journal of Chromatography 32: 133-138.

Hu, W.H., Li, X., He, G.L., Zhang, Z.W., Zheng, X.T., Li, P.W. and Li, C.M., 2013. Sensitive competitive immunoassay of multiple mycotoxins with non-fouling antigen microarray. Biosensors and Bioelectronics 50: 338-344.

Huali, X., Yang, B., Jinmei, W., Yamei, T., Ying, Z. and Yi, W., 2013. New method for the simultaneous analysis of types A and B trichothecenes by ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry in potato tubers inoculated with *Fusarium sulphureum*. Journal of Agricultural and Food Chemistry 61: 9333-9338.

Huang, L.C., Zheng, N., Zheng, B.Q., Wen, F., Cheng, J.B., Han, R.W., Xu, X.M., Li, S.L. and Wang, J.Q., 2014a. Simultaneous determination of aflatoxin M_1, ochratoxin A, zearalenone and α-zearalenol in milk by UHPLC-MS/MS. Food Chemistry 146: 242-249.

Huang, Y.H., Xu, Y., He, Q.H., Chu, J.S., Du, B.B. and Liu, J., 2014b. Determination of zearalenone in corn based on a biotin-avidin amplified enzyme-linked immunosorbent assay. Food and Agricultural Immunology 25: 186-199.

Huijie, Z. and Bujun, W., 2014. Fate of deoxynivalenol and deoxynivalenol-3-glucoside during wheat milling and Chinese steamed bread processing. Food Control 44: 86-91.

Hun, X., Liu, F., Mei, Z., Ma, L., Wang, Z. and Luo, X., 2013. Signal amplified strategy based on target-induced strand release coupling cleavage of nicking endonuclease for the ultrasensitive detection of ochratoxin A. Biosensors and Bioelectronics 39: 145-151.

Huybrechts, B., Tangni, E.K., Debongnie, P., Geys, J. and Callebaut, A., 2013. A review of analytical methods for determining mycotoxins in agricultural products. Cahiers Agricultures 22: 202-215.

Ivanova, B. and Spiteller, M., 2014. Raman spectroscopic and mass spectrometric determination of aflatoxins. Food Analytical Methods 7: 242-256.

Jia, W., Chu, X., Ling, Y., Huang, J. and Chang, J., 2014. Multi-mycotoxin analysis in dairy products by liquid chromatography coupled to quadrupole orbitrap mass spectrometry. Journal of Chromatography A 1345: 107-114.

Karami-Osboo, R., Maham, M., Miri, R., AliAbadi, M. H. S., Mirabolfathy, M. and Javidnia, K., 2013. Evaluation of dispersive liquid-liquid microextraction-HPLC-UV for determination of deoxynivalenol (DON) in wheat flour. Food Analytical Methods 6: 176-180.

Khalil, M.M.H., Gomaa, A.M. and Sebaei, A.S., 2013. Reliable HPLC determination of aflatoxin M_1 in eggs. Journal of Analytical Methods in Chemistry Article 2013: 817091.

Khayoon, W.S., Saad, B., Salleh, B., Manaf, N.H.A. and Latiff, A.A., 2014. Micro-solid phase extraction with liquid chromatography-tandem mass spectrometry for the determination of aflatoxins in coffee and malt beverage. Food Chemistry 147: 287-294.

Kim, N.Y., Lee, I. and Ji, G.E., 2014. Reliable and simple detection of ochratoxin and fumonisin production in black *Aspergillus*. Journal of Food Protection 77: 653-658.

Klitgaard, A., Iversen, A., Andersen, M.R., Larsen, T.O., Frisvad, J.C. and Nielsen, K.F., 2014. Aggressive dereplication using UHPLC-DAD-QTOF: screening extracts for up to 3000 fungal secondary metabolites. Analytical and Bioanalytical Chemistry 406: 1933-1943.

Koesukwiwat, U., Sanguankaew, K. and Leepipatpiboon, N., 2014. Evaluation of a modified QuEChERS method for analysis of mycotoxins in rice. Food Chemistry 153: 44-51.

Kong, W., Li, J., Qiu, F., Wei, J., Xiao, X., Zheng, Y. and Yang, M., 2013. Development of a sensitive and reliable high performance liquid chromatography method with fluorescence detection for high-throughput analysis of multi-class mycotoxins in coix seed. Analytica Chimica Acta 799: 68-76.

Kong, W., Wei, R., Logrieco, A., Wei, J., Wen, J., Xiao, X. and Yang, M., 2014. Occurrence of toxigenic fungi and determination of mycotoxins by HPLC-FLD in functional foods and spices in China markets. Food Chemistry 146: 320-326.

Köppen, R., Rasenko, T., Merkel, S., Mönch, B. and Koch, M., 2013. Novel solid-phase extraction for epimer-specific quantitation of ergot alkaloids in rye flour and wheat germ oil. Journal of Agricultural and Food Chemistry 61: 10699-10707.

Kovalsky Paris, M.P., Schweiger, W., Hametner, C., Stückler, R., Muehlbauer, G.J., Varga, E., Krska, R., Berthiller, F. and Adam, G., 2014. Zearalenone-16-O-glucoside: a new masked mycotoxin. Journal of Agricultural and Food Chemistry 62: 1181-1189.

Kozlovsky, A.G., Zhelifonova, V.P., Antipova, T.V., Baskunov, B.P., Ivanushkina, N.E. and Ozerskaya, S.M., 2014. Exo-metabolites of mycelial fungi isolated in production premises of cheese-making and meat-processing plants, Food Additives and Contaminants Part A 31: 300-306.

Krska, R., Malachova, A., Berthiller, F. and Van Egmond, H.P., 2014. Determination of T-2 and HT-2 toxins in food and feed: an update. World Mycotoxin Journal 7: 131-142.

Kuang, Y., Qiu, F., Kong, W., Luo, J., Cheng, H. and Yang, M., 2013. Simultaneous quantification of mycotoxins and pesticide residues in ginseng with one-step extraction using ultra-high performance liquid chromatography-electrospray ionization tandem mass spectrometry. Journal of Chromatography B 939: 98-107.

Kunsagi, Z. and Stroka, J., 2014. Determination of ochratoxin A in *Capsicum* spp. (paprika and chili) by immunoaffinity column cleanup and liquid chromatography: collaborative study. Journal of AOAC International 97: 876-883.

Lai, X.W., Sun, D.L., Ruan, C.Q., Zhang, H. and Liu, C.L., 2014. Rapid analysis of aflatoxins B_1, B_2, and ochratoxin A in rice samples using dispersive liquid-liquid microextraction combined with HPLC. Journal of Separation Science 37: 92-98.

Lattanzio, V.M., Von Holst, C. and Visconti, A., 2013. Experimental design for in-house validation of a screening immunoassay kit. The case of a multiplex dipstick for Fusarium mycotoxins in cereals. Analytical and Bioanalytical Chemistry 405: 7773-7782.

Lattanzio, V.M.T., Ciasca, B., Powers, S. and Visconti, A., 2014. Improved method for the simultaneous determination of aflatoxins, ochratoxin A and *Fusarium* toxins in cereals and derived products by liquid chromatography-tandem mass spectrometry after multi-toxin immunoaffinity clean up. Journal of Chromatography A 1354: 139-143.

Lee, J., Jeon, C.H., Ahn, S.J. and Ha, T.H., 2014a. Highly stable colorimetric aptamer sensors for detection of ochratoxin A through optimizing the sequence with the covalent conjugation of hemin. Analyst 139: 1622-1627.

Lee, K.M., Herrman, T.J., Bisrat, Y. and Murray, S.C., 2014b. Feasibility of surface-enhanced raman spectroscopy for rapid detection of aflatoxins in maize. Journal of Agricultural and Food Chemistry 62: 4466-4474.

Li, P., Zhang, Y., Lei, H.T., Wang, H., Xu, Z.L., Shen, Y., Sun, Y.M., Pang J. and Yang, J.Y., in press. Development of chemiluminescent enzyme immunoassay for the determination of aflatoxin M_1 in milk products. Food and Agricultural Immunology. DOI: http://dx.doi.org/10.1080/09540105.2013.878898.

Li, X., Yuan, H., Li, J. and Xiao, D., 2014b. Electrogenerated chemiluminescence of magnesium chlorophyllin a aqueous solution and its sensitive response to the carcinogen aflatoxin B_1. Biosensors and Bioelectronics 55: 350-354.

Ling, S., Pang, J., Yu, J., Wang, R., Liu, L., Ma, Y., Zhang, Y., Jin, N. and Wang, S., 2014. Preparation and identification of monoclonal antibody against fumonisin B_1 and development of detection by Ic-ELISA. Toxicon 80: 64-72.

Lingchen, Y., Zhiyong, Z., Aibo, W., Yifeng, D., Zhenlei, Z., Jianpeng, Z. and Jiafa, H., 2013. Determination of trichothecenes A (T-2 toxin, HT-2 toxin, and diacetoxyscirpenol) in the tissues of broilers using liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography B 942-943: 88-97.

Liu, B.H., Hsu, Y.T., Lu C.C. and Yu F.Y., 2013. Detecting aflatoxin B_1 in foods and feeds by using sensitive rapid enzyme-linked immunosorbent assay and gold nanoparticle immunochromatographic strip. Food Control 30: 184-189.

Lixiao, X., Guijun, Z., Chunna, G., Yaping, Z., Yi, Z., Jianlong, Z., Haicui, Y., Dexue, Y., Limin, H., Zhenling, Z. and Binghu, F., 2014. Simultaneous determination of major type-B trichothecenes and the de-epoxy metabolite of deoxynivalenol in chicken tissues by HPLC-MS/MS. Journal of Separation Science 37: 642-649.

Luan, L., Chen, N., Han, Z., Liu, X., Zheng, Y. and Wu, Y., 2014. Simultaneous determination of aflatoxin B_1, aflatoxin B_2, mycophenolic acid and stergmatocystin in grape pomace by UHPLC-MS/MS. World Mycotoxin Journal 7: 121-129.

Lv, X., Li, Y., Cao, W., Yan, T., Li, Y., Du, B. and Wei, Q., 2014. A label-free electrochemiluminescence immunosensor based on silvernanoparticle hybridized mesoporous carbon for the detection of aflatoxin B_1. Sensors and Actuators B 202: 53-59.

Ma, F., Chen, R., Li, P., Zhang, Q., Zhang, W. and Hu, X., 2013. Preparation of an immunoaffinity column with amino-silica gel microparticles and its application in sample cleanup for aflatoxin detection in agri-products. Molecules 18: 2222-2234.

Malachova, A., Van Egmond, H.P., Berthiller, F. and Krska, R., 2014. Determination of nivalenol in food and feed: an update. World Mycotoxin Journal 7: 247-255.

Malysheva, S.V., Larionova, D.A., Di Mavungu, J.D. and De Saeger, S., 2014. Pattern and distribution of ergot alkaloids in cereals and cereal products from European countries. World Mycotoxin Journal 7: 217-230.

Manoochehri, M., Asgharinezhad, A.A. and Safaei, M., in press. Determination of aflatoxins in rice samples by ultrasound-assisted matrix solid-phase dispersion. Journal of Chromatographic Science. DOI: http://dx.doi.org/10.1093/chromsci/bmu018.

Masiá, A., Campo, J., Blasco, C. and Picó, Y., 2014. Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry to identify contaminants in water: an insight on environmental forensics. Journal of Chromatography A 1345: 86-97.

Masoomi, L., Sadeghib, O., Banitabaa, M.H., Shahrjerdia, A. and Davarania, S.S.H., 2013. A non-enzymatic nanomagnetic electro-immunosensor for determination of aflatoxin B_1 as a model antigen. Sensors and Actuators B 177: 1122-1127.

Meng, U., Wang, Z., De Saeger, S., Wang, Y., Wen, K., Zhang, S. and Shen, J., 2014. Determination of ochratoxin A in cereals and feeds by ultra-performance liquid chromatography coupled to tandem mass spectrometry with immunoaffinity column clean-up. Food Analytical Methods 7: 854-864.

Mikula, H., Weber, J., Svatunek, D., Skrinjar, P., Adam, G., Krska, R., Hametner, C. and Fröhlich, J., 2014. Synthesis of zearalenone-16-beta,D-glucoside and zearalenone-16-sulfate: a tale of protecting resorcylic acid lactones for regiocontrolled conjugation. Beilstein Journal of Organic Chemistry 10: 1129-1134.

Mohammadi, A., Tavakoli, R., Kamankesh, M., Rashedi, H., Attaran, A. and Delavar, M., 2013. Enzyme-assisted extraction and ionic liquid-based dispersive liquid-liquid microextraction followed by high-performance liquid chromatography for determination of patulin in apple juice and method optimization using central composite design. Analytica Chimica Acta 804: 104-110.

Müller, M.E.H. and Korn, U., 2013. Alternaria mycotoxins in wheat. A 10 years survey in the Northeast of Germany. Food Control 34: 191-197.

Nakagawa, H., Sakamoto, S., Sago, Y., Kushiro, M. and Nagashima, H., 2013. Detection of masked mycotoxins derived from type A trichothecenes in corn by high-resolution LC-Orbitrap mass spectrometer. Food Additives and Contaminants Part A 30: 1407-1414.

Nguyen B.H., Tran, L.D., Do, Q.P., Nguyen, H.L., Tran, N.H. and Nguyen P.X., 2013. Label-free detection of aflatoxin M_1 with electrochemical Fe_3O_4/polyaniline-based aptasensor. Materials Science and Engineering C 33: 2229-2234.

Nielen, M.W. and Van Beek, T.A., 2014. Macroscopic and microscopic spatially-resolved analysis of food contaminants and constituents using laser-ablation electrospray ionization mass spectrometry imaging. Analytical and Bioanalytical Chemistry 406: 6805-6815.

Ogiso, M., Morita, T., Harada, C., Isagawa, S., Miyazaki, H., Shikada, N., Kimura, A., Kibune, N. and Watai, M., 2013. Investigation of the suitability of immunochemical-based test kits for quantitative analysis of deoxynivalenol in corn-derived feed and feed ingredients. Food Hygiene and Safety Science 54: 351-357.

Ok, H.E., Choi, S.W., Kim, M. and Chun, H.S., 2014. HPLC and UPLC methods for the determination of zearalenone in noodles, cereal snacks and infant formula. Food Chemistry 163: 252-257.

O'Mahony, J., Clarke, L., Whelan, M., O'Kennedy, R., Lehotay, S.J. and Danaher, M., 2013. The use of ultra-high pressure liquid chromatography with tandem mass spectrometric detection in the analysis of agrochemical residues and mycotoxins in food – challenges and applications. Journal of Chromatography A 1292: 83-95.

Park J.H., Kim Y.P., Kim, I.H. and Ko, S., 2014. Rapid detection of aflatoxin B_1 by a bifunctional protein crosslinkerbased surface plasmon resonance biosensor. Food Control 36: 183-190.

Park, J., Scott, P.M. and Lau, B.P., 2013. Analysis of N-fatty acyl fumonisins in alkali-processed corn foods. Food Science and Biotechnology 22: 147-152.

Pascale, M., Panzarini, G., Powers, S. and Visconti, A., 2014. Determination of deoxynivalenol and nivalenol in wheat by ultra-performance liquid chromatography/photodiode-array detector and immunoaffinity column cleanup. Food Analytical Methods 7: 555-562.

Pavšič-Vrtač, K., Ojanperä, S., Apajalahti, J., Šrimpf, K. and Tavčar-Kalcher, G., 2014. Analytical procedures for the determination of aflatoxin B_1 in eggs of laying hens using immunoaffinity columns and liquid chromatography with post-column derivatisation and fluorescence detection. Food Analytical Methods 7: 1917-1924.

Pereira, V.L., Fernandes, J.O. and Cunha, S.C., 2014. Mycotoxins in cereals and related foodstuffs: a review on occurrence and recent methods of analysis. Trends in Food Science and Technology 36: 96-136.

Pizzutti, I.R., De Kok, A., Scholten, J., Righi, L.W., Cardoso, C.D., NecchiRohers, G. and Da Silva, R.C., 2014. Development, optimization and validation of a multimethod for the determination of 36 mycotoxins in wines by liquid chromatography-tandem mass spectrometry. Talanta 129: 352-363.

Pleadin, J., Perši, N., Kovačević, D., Vahčić, N., Scortichini, G. and Milone, S., 2013. Ochratoxin A in traditional dry-cured meat products produced from sub-chronic-exposed pigs. Food Additives and Contaminants Part A 30: 1827-1836.

Prelle, A., Spadaro, D., Denca, A., Garibaldi, A. and Gullino, M.L., 2013. Comparison of clean-up methods for ochratoxin A on wine, beer, roasted coffee and chili commercialized in Italy. Toxins 5: 1827-1844.

Prelle, A., Spadaro, D., Garibaldi, A. and Gullino, M. 2014. Co-occurrence of aflatoxin and ochratoxin A in spices commercialized in Italy. Food Control 39: 192-197.

Ran, R., Wang, C., Han, Z., Wu, A., Zhang, D. and Shi, J., 2013. Determination of deoxynivalenol (DON) and its derivatives: current status of analytical methods. Food Control 34: 138-148.

Rhouati, A., Hayat, A., Hernandez, D.B., Meraihi, Z., Munoz, R. and Marty, J.L., 2013a. Development of an automated flow-based electrochemical aptasensor for on-line detection of ochratoxin A. Sensors and Actuators B 176: 1160-1166.

Rhouati, A., Yang, C., Hayat, A. and Marty, J.L., 2013b. Aptamers: a promising tool for ochratoxin A detection in food analysis. Toxins 5: 1988-2008.

Rubert, J., León, N., Sáez, C., Martins, C.P., Godula, M., Yusà, V., Mañes, J., Soriano, J.M. and Soler, C., 2014. Evaluation of mycotoxins and their metabolites in human breast milk using liquid chromatography coupled to high resolution mass spectrometry. Analytica Chimica Acta 820: 39-46.

Sanders, M., De Boevre, M., Dumoulin, F., Detavernier, C., Martens, F., Van Poucke, C., Eeckhout, M. and De Saeger, S., 2013. Sampling of wheat dust and subsequent analysis of deoxynivalenol by LC-MS/MS. Journal of Agricultural and Food Chemistry 61: 6259-6264.

Sasaki, R., Hossain, M.Z., Abe, N., Uchigashima, M. and Goto, T., 2014. Development of an analytical method for the determination of sterimatocystin in grains using LCMS after immunoaffinity column purification. Mycotoxin Research 30: 123-129.

Shar, Z.H., Sumbal, G.A., Sherazi, S.T.H., Bhanger, M.I. and Nizamani, S.M., 2014. Determination of deoxynivalenol in poultry feed by three gas chromatographic detection techniques. Chromatographia 77: 337-346.

Sharma, H.K., Bajpai, K., Srivastava, R. and Singh, S.K., 2014. Identification and effect of aflatoxins and fumonisins on medicinal plants from Indian sub-continent viz., *Withania somnifera* and *Ocimum sanctum*. International Journal of Pharma and Bio Sciences 5: 1-10.

Sheng Y.J., Eremin S., Mi T.J., Zhang S.X., Shen J.Z. and Wang Z.H., 2014. The development of a fluorescence polarization immunoassay for aflatoxin detection. Biomedical and Environmental Sciences 27: 126-129.

Shephard, G.S., Burger, H.M., Gambacorta, L., Gong, Y.Y., Krska, R., Rheeder, J.P., Solfrizzo, M., Srey, C., Sulyok, M., Visconti, A., Warth, B. and Van der Westhuizen, L., 2013b. Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the former Transkei, South Africa. Food and Chemical Toxicology 62: 217-225.

Shephard, G.S., Burger, H.M., Gambacorta, L., Krska, R., Powers, S.P., Rheeder, J.P., Solfrizzo, M., Sulyok, M., Visconti, A., Warth, B. and Van der Westhuizen, L., 2013a. Mycological analysis and multimycotoxin in maize from rural subsistence farmers in the former Transkey, South Africa by LC-MS/MS. Journal of Agricultural and Food Chemistry 61: 8232-8240.

Shephard, G.S., Kimanya, M.E., Kpodo, K.A., Gnonlonfin, G.J.B. and Gelderblom, W.C.A., 2013c. The risk management dilemma for fumonisin mycotoxins. Food Control 34: 596-600.

Shim, W.B., Mun, H., Joung, H.A., Appiah Ofori, J., Chung, D.H. and Kim, M.G., 2014. Chemiluminescence competitive aptamer assay for the detection of aflatoxin B_1 in corn samples. Food Control 36: 30-35.

Sirhan, A.Y., Tan, G.H., Al-Shunnaq, A., Abdulra'uf, L. and Wong, R.C.S., 2014. QuEChERS-HPLC method for aflatoxin detection of domestic and imported food in Jordan. Journal of Liquid Chromatography & Related Technologies 37: 321-342.

Solfrizzo, M., Gambacorta, L. and Visconti, A., 2014. Assessment of multi-mycotoxin exposure in Southern Italy by urinary multi-biomarker determination. Toxins 6: 523-538.

Solfrizzo, M., Gambacorta, L., Warth, B., White, K., Srey, C., Sulyok, M., Krska, R. and Gong, Y.Y., 2013. Comparison of single and multi-analyte methods based on LC-MS/MS for mycotoxin biomarker determination in human urine. World Mycotoxin Journal 6: 355-366.

Song, S.Q., Liu, N., Zhao, Z.Y., Ediage, E.N., Wu, S.L., Sun, C.P., De Saeger, S. and Wu, A.B., 2014. Multiplex lateral flow immunoassay for mycotoxin determination. Analytical Chemistry 86: 4995-5001.

Songsermsakul, P., Böhm, J., Aurich, C., Zentek, J. and Razzazi-Fazeli, E., 2013. The levels of zearalenone and its metabolites in plasma, urine and faeces of horses fed with naturally, *Fusarium* toxin-contaminated oats. Journal of Animal Physiology and Animal Nutrition 97: 155-161.

Stroka, J. and Seidler, J., 2014. Immunoaffinity clean-up of mycotoxins with organic solvent-free elution. World Mycotoxin Journal 7: 115-120.

Tamura, M., Nakagawa, H., Uyama, A. and Mochizuki, N., 2014. Simultaneous trichothecenes analysis by LC-MS/MS with a pentafluorophenyl column. Food Hygiene and Safety Science 55: 19-24.

Tang, X.Q., Li, X., Li, P.W., Zhang, Q., Li, R., Zhang, W., Ding, X.X., Lei, J.W. and Zhang, Z.W., 2014. Development and application of an immunoaffinity column enzyme immunoassay for mycotoxin zearalenone in complicated samples. PLoS ONE 9: e85606.

Tansakul, N., Jala, P., Laopiem, S., Tangmunkhong, P. and Limsuwan, S., 2013. Co-occurrence of five *Fusarium* toxins in corn-dried distiller's grains with solubles in Thailand and comparison of ELISA and LC-MS/MS for fumonisin analysis. Mycotoxin Research 29: 255-260.

Tittlemier, S. A., Gaba, D. and Chan, J. M., 2013. Monitoring of *Fusarium* trichothecenes in Canadian cereal grain shipments from 2010 to 2012. Journal of Agricultural and Food Chemistry 61: 7412-7418.

Todescato, F., Antognoli, A., Meneghello, A., Cretaio, E., Signorini, R. and Bozio, R., 2014. Sensitive detection of Ochratoxin A in food and drinks using metal-enhanced fluorescence. Biosensors and Bioelectronics 57: 125-132.

Tölgyesi, A. and Stroka, J., 2014. Report on the development of a method for the determination of *Alternaria* toxins and citrinin in wheat, tomatoes juice and sunflower seeds by liquid chromatography-tandem mass spectrometry. JRC Scientific and Policy Reports EUR 26496 EN.

Vaclavikova, M., MacMahon, S., Zhang, K. and Begley, T.H., 2013. Application of single immunoaffinity clean-up for simultaneous determination of regulated mycotoxins in cereals and nuts. Talanta 117: 345-351.

Van der Westhuizen, L.V., Shephard, G.S., Gelderblom, W.C.A., Torres, O. and Riley, R.T., 2013. Fumonisin biomarkers in maize eaters and implications for human disease. World Mycotoxin Journal 6: 223-232.

Vdovenko, M.M., Lu, C.C., Yu, F.Y. and Sakharov, I.Y., 2014. Development of ultrasensitive direct chemiluminescent enzyme immunoassay for determination of aflatoxin M_1 in milk. Food Chemistry 158: 310-314.

Venkataramana, M., Navya, K., Chandranayaka, S., Priyanka, S.R., Murali, H.S. and Batra, H.V., 2014. Development and validation of an immunochromatographic assay for rapid detection of fumonisin B_1 from cereal samples. Journal of Food Science and Technology 51: 1920-1928.

Vidal, J.C., Bonel, L., Ezquerra, A., Hernandez, S., Bertolin, J.R., Cubel, C. and Castillo, J.R., 2013. Electrochemical affinity biosensors for detection of mycotoxins: a review. Biosensors and Bioelectronics 49: 146-158.

Von Holst, C. and Stroka, J., 2014. Performance criteria for rapid screening methods to detect mycotoxins. World Mycotoxin Journal 7: 439-447.

Wang, H., Zhao, L., Yang, H.M., Guo, Q.L., Shi, H. L., Pan, H.Y., Zhao, L.P. and Qian, C., 2014a. Determination of benzo(a)pyrene and aflatoxins (B_1, B_2, G_1, G_2) in vegetable oil by GPC-HPLC-FLD. Analytical Methods 6: 1545-1549.

Wang, Q., Zhao, H., Xi, C.X., Wang, G.M., Chen, D.D. and Ding, S.J., 2014b. Determination of chloramphenicol and zeranols in pig muscle by immunoaffinity column clean-up and LC-MS/MS analysis. Food Additives and Contaminants Part A 31: 1177-1186.

Wang, S., Cheng, L., Ji, S. and Wang, K., 2014c. Simultaneous determination of seventeen mycotoxins residues in *Puerariae lobatae radix* by liquid chromatography-tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 98C: 201-209.

Wang, W., Heitschmidt, G.W., Ni, X., Windham, W.R., Hawkins, S. and Chu, X., 2014d. Identification of aflatoxin B_1 on maize kernel surfaces using hyperspectral imaging. Food Control 42: 78-86.

Wang, Y., Xiao, C., Guo, J., Yuan, Y., Wang, J., Liu, L. and Yue, T., 2013a. Development and application of a method for the analysis of 9 mycotoxins in maize by HPLC-MS/MS. Journal of Food Science 78: M1752-1756.

Wang, Y.K., Wang, Y.C., Wang, H.A., Ji, W.H., Sun, J.H. and Yan, Y.X., 2014e. An immunomagnetic-bead-based enzyme-linked immunosorbent assay for sensitive quantification of fumonisin B_1. Food Control 40: 41-45.

Wang, Y.K., Yan, Y.X., Ji, W.H., Wang, H.A., Li, S.Q., Zou, Q. and Sun, J.H., 2013b. Rapid simultaneous quantification of zearalenone and fumonisin B_1 in corn and wheat by lateral flow dual immunoassay. Journal of Agricultural and Food Chemistry 61: 5031-5036.

Wang, Y.K., Yan, Y.X., Li, S.Q., Wang, H.A., Ji, W.H. and Sun, J.H., 2013c. Simultaneous quantitative determination of multiple mycotoxins in cereal and feedstuff samples by a suspension array immunoassay. Journal of Agricultural and Food Chemistry 61: 10948-10953.

Wang, Z., Li, H, Li, C., Yu, Q., Shen, J. and De Saeger, S., 2014g. Development and application of a quantitative fluorescence-based immunochromatographic assay for fumonisin B_1 in maize. Journal of Agricultural and Food Chemistry 62: 6294-6298.

Wang, Z., Li, J., Xu, L., Feng, Y. and Lu, X., 2014f. Electrochemical sensor for determination of aflatoxin B_1 based on multiwalled carbon nanotubes-supported Au/Pt bimetallic nanoparticles. Journal of Solid State Electrochemistry 18: 2497-2496.

Waskiewicz, A., Beszterda, M., Bocianowski, J. and Golinski, P., 2013. Natural occurrence of fumonisins and ochratoxin A in some herbs and spices commercialized in Poland analyzed by UHPLC-MS/MS method. Food Microbiology 36: 426-431.

Weber, J., Mikula, H., Fruhmann, P., Hametner, C., Varga, E., Berthiller, F., Krska, R. and Fröhlich, J., 2013. Gentiobiosylation of beta-resorcylic acid esters and lactones: first synthesis and characterization of zearalenone-14-beta, D-Gentiobioside. Synlett 24: 1830-1834.

Wen, J., Kong, W., Hu, J., Wang, J. and Yang, M., 2014. Multi-mycotoxins analysis in ginger and related products by UHPLC-FLR detection and LC-MS/MS confirmation. Food Control 43: 82-87.

Wesolek, N., Ramirez-Martinez, A. and Roudot, A.C., 2014. Mathematical approach for sampling plan performance assessment for aflatoxin B_1 in pistachios. Food Research International 62: 448-455.

Wozniak, B., Zuchowska, I.M. and Zmudzki, J., 2013. Determination of stilbenes and resorcylic acid lactones in bovine, porcine and poultry muscle tissue by liquid chromatography-negative ion electrospray mass spectrometry and QuEChERS for sample preparation. Journal of Chromatography B 940: 15-23.

Wu, J., Zhu, Y., Xue, F., Mei, Z., Yao, L., Wang, X., Zheng, L., Liu, J., Liu, G., Peng, C. and Chen, W., 2014. Recent trends in SELEX technique and its application to food safety monitoring. Microchimica Acta 181: 479-491.

Wu, S., Duan, N., Li, X., Tan, G., Ma, X., Xia, Y., Wang, Z. and Wang, H., 2013. Homogenous detection of fumonisin B_1 with a molecular beacon based on fluorescence resonance energy transfer between NaYF4: Yb, Ho upconversion nanoparticles and gold nanoparticles. Talanta 116: 611-618.

Xu, K., Sun, Y., Li, W., Xu, J., Cao, B., Jiang, Y., Zheng, T., Li, J. and Pan, D., 2014. Multiplex chemiluminescent immunoassay for screening of mycotoxins using photonic crystal microsphere suspension array. Analyst 139: 771-777.

Xue, X.F., Selvaraj, J.N., Zhao, L., Dong, H., Liu, F., Liu, Y. and Li, Y., 2014. Simultaneous determination of aflatoxins and ochratoxin A in bee pollen by low-temperature fat precipitation and immunoaffinity column cleanup coupled with LC-MS/MS. Food Analytical Methods 7: 690-696.

Yang, C., Lates, V., Prieto-Simón, B., Marty, J.L. and Yang, X., 2013. Rapid high-throughput analysis of ochratoxin A by the self-assembly of dnazyme-aptamer conjugates in wine. Talanta 116: 520-526.

Ying, Y.F., Wu, Y.L., Wen, Y., Yang, T., Xu, X.Q. and Wang, Y.Z., 2013. Simultaneous determination of six resorcylic acid lactones in feed using liquid chromatography-tandem mass spectrometry and multi-walled carbon nanotubes as a dispersive solid phase extraction sorbent. Journal of Chromatography A 1307: 41-48.

Yogendrarajah, P., Jacxsens, L., Saeger, S. and Meulenaer, B. 2014. Co-occurrence of multiple mycotoxins in dry chilli (*Capsicum annum* L.) samples from the markets of Sri Lanka and Belgium. Food Control 46: 26-34.

Yoshinari, T., Sakuda, S., Furihata, K., Furusawa, H., Ohnishi, T., Sugita-Konishi, Y., Ishizaki, N. and Terajima, J., 2014. Structural determination of a nivalenol glucoside and development of an analytical method for the simultaneous determination of nivalenol and deoxynivalenol, and their glucosides, in wheat. Journal of Agricultural and Food Chemistry 62: 1174-1180.

Yoshinari, T., Tanaka, T., Ishikuro, E., Horie, M., Nagayama, T., Nakajima, M., Naito, S., Ohnishi, T., and Sugita-Konishi, Y., 2013a. Inter-laboratory study of an LC-MS/MS method for simultaneous determination of deoxynivalenol and its acetylated derivatives, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol in wheat. Journal of the Food Hygienic Society of Japan 54: 75-82.

Yoshinari, T., Tanaka, T., Ishikuro, E., Horie, M., Nagayama, T., Nakajima, M., Naito, S., Ohnishi, T. and Sugita-Konishi, Y., 2013b. Inter-laboratory study of an LC-MS/MS method for simultaneous determination of fumonisin B_1, B_2 and B_3 in corn. Journal of the Food Hygienic Society of Japan 54: 266-276.

Yu, F.Y., Gribas, A.V., Vdovenko, M.M. and Sakharov, I.Y., 2013. Development of ultrasensitive direct chemiluminescent enzyme immunoassay for determination of aflatoxin B_1 in food products. Talanta 107: 25-29.

Zhang, K., Wong, J.W., Hayward, D.G., Vaclavikova, M., Liao, C.D. and Trucksess, M.W., 2013a. Determination of mycotoxins in milk-based products and infant formula using stable isotope dilution assay and liquid chromatography tandem mass spectrometry. Journal of Agricultural and Food Chemistry 61: 6265-6273.

Zhang, K., Wong, J.W., Jia, Z., Vaclavikova, M., Trucksess, M.W. and Begley, T.H., 2014b. Screening multimycotoxins in food-grade gums by stable isotope dilution and liquid chromatograph/tandem mass spectrometry. Journal of AOAC International 97: 889-895.

Zhang, K., Wong, J.W., Mai, H. and Trucksess, M.W., 2014a. Dopant-assisted atmospheric pressure photoionization of patulin in apple juice and apple-based food with liquid chromatography-tandem mass spectrometry. Journal of Agricultural and Food Chemistry 62: 4112-4118.

Zhang, X., Liu, L., Chen, X., Kuang, H., Song, S. and Xu C., 2013b. Immunochromatographic strip development for ultrasensitive analysis of aflatoxin M_1. Analytical Methods 5: 6567-6571.

Zhang, Z., Li, Y., Li, P., Zhang, Q., Zhang, W., Hua, X. and Ding, X., 2014c. Monoclonal antibody-quantum dots CdTe conjugate-based fluoroimmunoassay for the determination of aflatoxin B_1 in peanuts. Food Chemistry 146: 314-319.

Zhang, Z.W., Yu, L., Xu, L., Hu, X.F., Li, P.W., Zhang, Q., Ding, X.X. and Feng, X.J. 2014d. Biotoxin sensing in food and environment via microchip. Electrophoresis 35: 1547-1559.

Zhao, X., Yuan, Y., Zhang, X. and Yue, T. 2014a. Identification of ochratoxin A in Chinese spices using HPLC fluorescent detectors with immunoaffinity column cleanup. Food Control 46: 332-337.

Zhao, Z., Rao, Q., Song, S., Liu, N., Han, Z., Hou, J. and Wu, A., 2014b. Simultaneous determination of major type B trichothecenes and deoxynivalenol-3-glucoside in animal feed and raw materials using improved DSPE combined with LC-MS/MS. Journal of Chromatography B 963: 75-82.

Zou, L., Xu, Y., Li, Y., He, Q., Chen, B. and Wang, D., 2014. Development of a single-chain variable fragment antibody-based enzyme-linked immunosorbent assay for determination of fumonisin B_1 in corn samples. Journal of the Science of Food and Agriculture 94: 1865-1871.