REVIEW

A comparative review on methods of detection and quantification of mycotoxins in solid food and feed: a focus on cereals and nuts

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Abstract

Many emerging factors and circumstances urge the need to develop and optimize the detection and quantification techniques of mycotoxins in solid food and feed. The diversity of mycotoxins, which have different properties and affinities, makes the standardization of the analytical procedures and the adoption of a single protocol that covers the attributes of all mycotoxins a tedious or even an impossible mission. Several modifications and improvements have been undergone in order to optimize the performance of these methods including the extraction solvents, the extraction methods, the clean-up procedures, and the analytical techniques. The techniques range from the rapid screening methods, which lack sensitivity and specificity such as TLC, to a spectrum of more advanced protocols, namely, ELISA, HPLC, and GC–MS and LC–MS/MS. This review aims at assessing the current studies related to these analytical techniques of mycotoxins in solid food and feed. It discusses and evaluates, through a critical approach, various sample treatment techniques, and provides an in-depth examination of different mycotoxin detection methods. Furthermore, it includes a comparison of their actual accuracy and a thorough analysis of the observed benefits and drawbacks.

Keywords Mycotoxins · Detection · Quantification · Solid food and feed · Cereals and nuts

Abbreviations

15-AcDON	15-Acetyl-deoxynivalenol	ACN	Acetonitrile
3-AcDON	3-Acetyl-deoxynivalenol	AFB1	Aflatoxin B1
AA	Acetic acid	AFB2	Aflatoxin B2
		AFG1	Aflatoxin G1

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AFG2	Aflatoxin G2
AFs	Aflatoxins
ATX	Altertoxin
BEA	Beauvercin
BSA	N,O-bis(trimethylsilyl) acetamide
DAS	Diacetoxyscirpenol
DON	Deoxynivalenol
dSPE	Dispersive solid phase extraction
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
ESI −	Negative electrospray ionization
ESI +	Positive electrospray ionization
FA	Formic acid
FBs	Fumonisins
FUS	Fusaproliferin
GC	Gas chromatography
GC-MS	Gas chromatography coupled to mass spectrometry
GC–MS/MS	Gas chromatography-tandem mass spectrometry
HPLC	High-performance liquid chromatography
HPLC-ELSD	High-performance liquid chromatography-evaporative light scattering detector
HPLC-FLD	High-performance liquid chromatography–fluorimetric detector
HPLC-UV	High-performance liquid chromatography–ultraviolet detector
HT-2	HT-2 toxin
IAC	Immuno-affinity columns
LC–MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
MPL	Maximum permissible limits
MRM	Multiple reaction monitoring
MTX	Mycotoxin
NA	Not available
NIV	Nivalenol
OTA	Ochratoxin A
OTB	Ochratoxin B
OTC	Ochratoxin C
PBS	Phosphate-buffered saline
PCD	Post-column derivatization
PDA	Photodiode array detector
QqQ	Triple quadrupole system
QuEChERS	Quick, easy, cheap, efficient, rugged, and safe method
RSDr	Relative standard deviation for repeatability
RT	Room temperature
SIDA	Stable isotope dilution analysis
SLE	Supported liquid extraction

sMRM	Scheduled multiple reaction monitoring
SPE	Solid phase extraction
SRM	Single reaction monitoring
T-2	T-2 toxin
TLC	Thin layer chromatography
TMCS	Trimethylchlorosilane
TMSI	N-trimethylsilylimidazole
UPLC-FLD	Ultra-high-performance liquid chromatography linked with fluorescence detection
UV	Ultraviolet
WAHSPE	Water acetonitrile heptane solid phase extraction
ZEN	Zearalenone

Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi as their natural chemical defense system against various aggressors such as grazing animals, insects, parasites, and microorganisms (Abrehame et al. 2023; Cinar and Onbaşı 2019). These metabolites are harmful to human and animal health due to their potential cytotoxicity, mutagenicity, neurotoxicity, carcinogenicity, and immunosuppressive effects (Alshannaq and Yu 2017; Mahdjoubi et al. 2020; Salvatore et al. 2023). They might be found on the crops during the pre- and post-harvest stages and during the processing steps as well (Alshannaq and Yu 2017; Chatterjee et al. 2023). The occurrence of mycotoxins in solid food and feed is a major health and food safety concern. In addition, and due to the global warming impact, this issue is becoming worse leading to the urgent need to develop new detection, quantification, and detoxification techniques and to optimize the currently available ones (Abou Dib et al. 2022; Gomez et al. 2022; Medina et al. 2017). The most common mycotoxins in cereals and nuts include aflatoxins (AFs: AFB_1, AFB_2, AFG_1, AFG_2) (Guo et al. 2021; Udomkun et al. 2017), ochratoxins (OTA), zearalenone (ZEN), deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), HT-2 toxin (HT-2), fumonisins (FBs), enniatins (EN), sterigmatocystins (STCs), moniliformin (MON), beauvercin (BEA), and fusaproliferin (FUS) (Awuchi et al. 2021; Fapohunda et al. 2018; Jajić et al. 2019).

Regulatory agencies around the world have established strict limits to control the levels of mycotoxins in food and feed, which vary based on the mycotoxin classification, the product type, and the considered country (Pisuttu et al. 2023). The Codex Alimentarius Commission of FAO-WHO has established the codex standard CXS 193–1995, which sets maximum levels of AFs and OTA in food. While many countries still lack regulations in this regard, the most extensive standards concerning levels of mycotoxins are decreed in the European Union (Abou Dib et al. 2022; Altomare

et al. 2021; Luo et al. 2021; Mahato et al. 2019). The European Commission (EC) has established the maximum permissible limits (MPLs) for several of mycotoxins in different food products. The MPLs of AFs and AFB$_1$ on groundnuts and nuts and processed products intended for direct human consumption or used as an ingredient in foodstuffs are set to be 4–15 and 2–12 µg/kg, respectively. Meanwhile, the MPLs of AFs, AFB$_1$, OTA, FBs, ZEN, DON, and T-2/HT-2 on all cereals and all products derived from cereals, including processed cereal products, are set to be 4, 2, 3, 200–2000, 75, 750, and 50–200 µg/kg, respectively. Similar MPLs have been established by the US Food and Drug Administration with higher tolerable limits for some mycotoxins such as 20 µg/kg for AFs on foods and nuts, 2000–4000 µg/kg for FBs in different corn and corn products, and 1000 µg/kg for DON on grains and grains finished wheat products potentially consumed by humans (Chen and Inbaraj 2022). The 2020 annual report of the Rapid Alert System for Food and Feed (RASFF) reported that aflatoxins were among the top 10 hazards detected in crops from countries like the USA, Turkey, Spain, and Argentina, and there has been a rapid increase in non-compliance cases in recent years (Chatterjee et al. 2023).

Many strategies have been adopted to reduce mycotoxins in food matrices. Preventive measures implementing the Good Agricultural Practices (GAP) and the control of the storage conditions are necessary, but not sufficient alone (Cheli et al. 2017; Stroka and Gonçalves 2019). After the failure of these measures in achieving the required safety level, further detoxification techniques could be used individually, subsequently, or simultaneously, in order to reduce or eliminate these toxins (Abou Dib et al. 2022). Physical methods may include invasive treatments like milling (Scarpino et al. 2021) and extrusion (Janić Hajnal et al. 2021; Massarolo et al. 2021) and non-invasive treatments such as irradiation (Pérez-Santaescolástica et al. 2019). Chemical agents could be used also to neutralize or destruct the mycotoxins in food like lime water (Maureen et al. 2020), acids (Jubeen et al. 2020), ozone (Li et al. 2015), and ammonia (Nyandieka et al. 2009). Other novel detoxification methods involve the use of microbial adsorbents (Assaf et al. 2018a, 2019b), or bacterial biofilms for contaminated liquid food matrices (Assaf et al. 2019a; Nahle et al. 2022a, b), the adsorption of mycotoxins on treated shrimp shells or chitin in milk (Assaf et al. 2018b), their degradation in maize by detoxifying through bacterial fermentation (Chen et al. 2019; Zadeike et al. 2021), their biocontrol by yeast addition to baked products (Podgórska-Kryszczuk et al. 2022), and their enzymatic degradation by fuminosin esterase in maize (Alberts et al. 2021).

The nature of food matrices and low concentrations of mycotoxins makes the extraction process an essential yet a critical step for a better detection (Chen and Inbaraj 2022). The extraction solvent must be selected according to its polarity, selectivity, and reactivity. Hydrophobic solvents, e.g., acetonitrile (ACN) and methanol (MeOH), are suitable for extracting non-polar mycotoxins. The extraction protocol may also result in the recovery of other components that can interfere with the analysis (Magoke et al. 2022). Sample pretreatment must be conducted before any analytical procedure, especially when it comes to complex matrices (Yang et al. 2020). The clean-up could be performed using methods like solid-phase extraction (SPE) or immuno-affinity columns (IAC) (Singh and Mehta 2020; Smaoui et al. 2020). In IAC, mycotoxin-specific antibodies are bound to a solid phase support. After selective binding of the mycotoxin, a deposit is formed either by passing a miscible solvent through the column or by denaturation of the antibodies (Magoke et al. 2022). This method has its drawbacks like reduced recovery rates, high cost, long time, and the need for technical expertise (Jedziniak et al. 2019; Kim et al. 2022). The acronym QuEChERS refers to a QUick, Easy, CHeap, Efficient, Rugged, and Safe method. Anastassiades et al. firstly developed QuEChERS method to provide a quick and low-cost method for the analysis of pesticides residues in fruits and vegetables (Anastassiades et al. 2003). It is a widely used phase partition method for the analysis of mycotoxins in solid food and feed. Hence, this method is performed after acetonitrile-based extraction, and it is designed to extract both moderately polar and non-polar molecules (Tölgyesi et al. 2023). MgSO$_4$, NaCl, tri-sodium citrate dehydrate, and sodium hydrogen citrate sesquihydrate are used as the partitioning salts (Silva et al. 2021). This method has been found to be efficient for a wide range of mycotoxins providing an acceptable degree of selectivity (Kim et al. 2022; Seo et al. 2021). The dilute-and-shoot is another commonly used method for the analysis of mycotoxins. It involves a direct injection of a diluted sample which ensures its cleanliness and eliminates the need for any further expensive and time-consuming clean-up. This method is fast, valid for a large number of mycotoxins, and relatively cost-effective as it prevents the loss of the analyte that may occur during pretreatments and excessive handling of the sample compared to other methods (Magoke et al. 2022; Turner et al. 2015). Ultimately, the detection or the quantification of mycotoxins could be conducted using many techniques. Among others, thin layer chromatography (TLC) is considered as an old and basic technique, and the following four methods are the most commonly used nowadays: enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC–MS/MS), or gas chromatography-tandem mass spectrometry (GC–MS/MS) (Salvatore et al. 2023; Smaoui et al. 2020).

This review outlines the abovementioned analytical techniques of mycotoxins in solid food and feed, with a particular emphasis on cereals and nuts. It investigates and compares different extraction and quantification methods of mycotoxins.

Most papers published over the last 10 years were covered. The selection was chiefly based on the food matrices in which the detection and quantification were performed, specifically cereals and nuts. Data were critically discussed based on a comparison of the LOD and LOQ, the accuracy (recovery rates), the precision under repeatability conditions known as relative standard deviation for repeatability (RSDr), and the advantages and disadvantages of the techniques.

Thin layer chromatography (TLC)

TLC is one of the earliest and most common chromatographic techniques used for the separation and detection of non-volatile compounds, such as mycotoxins in foods or else (Bueno et al. 2013; Zhang and Banerjee 2020). TLC is considered as a qualitative or semi-quantitative techniques (Elkenany and Awad 2021; Ji et al. 2019). It is a liquid–solid adsorption technique consisting in separating a mobile phase and a sample on a stationary phase by difference of affinity (Cai 2014). TLC is performed on a glass, a plastic, or an aluminum foil coated with a thin layer of adsorbent material composed of cellulose, aluminum, or silica gel which is considered the stationary phase. On the other hand, the mobile phase consisting of a solvent or a mixture of solvents (methanol, acetonitrile, and water) acts as a carrier of the sample on the solid stationary phase (Janik et al. 2021). The separation of the analytes results from the difference in their migration rates caused by a difference in their adsorption to the stationary phase and in their solubility in the used solvent (Bueno et al. 2013). It is an economical technique characterized, however, by low accuracy and sensitivity, and requiring specific sample preparation and clean-up steps that depend on the physical and chemical properties of the mycotoxins being detected (Agriopoulou et al. 2020). TLC is widely used to determine the aflatoxins in food with LOD ranging from 1 to 20 µg/kg (Abbas 2021). The retention factor (R_f) is not an absolute physical constant. It is calculated as follows (Ekwomadu et al. 2021):

$$Rf = \frac{X}{Z} \times 100$$

where

X is the distance the compound traveled;
Z is the distance the solvent traveled.

The determination of LOD is usually achieved visually. A serial dilution of the mycotoxin standard to be tested is performed, followed by a TLC of the diluted solutions on the same plate. The last spot detected corresponds to the lowest concentration representing the LOD (Aasa et al. 2022).

TLC results may show great variability depending on the type of TLC plate; the operating conditions such as the temperature, humidity, and limited length of the plate; and the

measurement method (Bueno et al. 2013; Singh and Mehta 2020). Hypothetically, it is irrelevant to compare the results conducted on different plates or in different studies. The comparison can only be done on the same plate under the same conditions to eliminate the difference that may occur in temperature, adsorbent type and thickness, and used solvents. TLC is typically used as a rapid screening method to indicate the presence of mycotoxins in a sample (Acuña-Gutiérrez et al. 2022; Agriopoulou et al. 2020; Almeida-Ferreira et al. 2013; Felšöciová et al. 2021). If greater accuracy and sensitivity are required, TLC must be complemented by another quantification method (Aasa et al. 2022).

Enzyme-linked immunosorbent assay (ELISA)

ELISA is a conventional immunological detection method based on the binding of an antigen to its specific antibody (Shi et al. 2018). It is commonly used for the detection of mycotoxins (Janik et al. 2021; Omar et al. 2020). ELISA involves three key components: the sorbent substrate, the immune recognition, and the enzyme labels (Liang et al. 2021). It is a cost-effective, simple, and rapid method often used as a screening technique for multiple mycotoxins. The enhanced sensitivity and the decreased cross-reactivity of ELISA are being continuously improved (Shi et al. 2018). ELISA test kits offer several advantages over TLC and HPLC, such as requiring lower volume and easier sample preparation, without the need of extensive clean-up procedures. Table 1 provides an overview of numerous studies that have utilized ELISA for the detection of mycotoxins in solid food and feed.

Prior to performing ELISA, mixtures of methanol/water with varying ratios have been utilized for the extraction of mycotoxins from solid matrices. The extraction process typically involves shaking, followed by centrifugation and filtration (Ekwomadu et al. 2021; Horváth et al. 2022; Liu et al. 2022; Maggira et al. 2022; Ochieng et al. 2022; Wu et al. 2020). However, only water is used for the extraction of DON (Foerster et al. 2022; Sanders et al. 2016). Additionally, some studies utilized supplied buffer extraction (D'Agnello et al. 2021). Notably, not all studies implemented dilution during the extraction process. For aflatoxins, low LOD values between 0.5 and 1.75 µg/kg have been reached by ELISA methods (Ekwomadu et al. 2021; Foerster et al. 2022). Wu et al. achieved an even lower LOD value (0.03 µg/kg) of AFB_1 with satisfactory recovery rates and repeatability (Wu et al. 2020). The study conducted by Horváth et al. highlighted the importance of drying and pH adjustment of the fermented forages before AFB_1 extraction. The low pH obtained was due to the action of lactic acid producing bacteria during fermentation. Increasing the sample/extraction solvent ratio from 1:5 to 1:8 also led to higher

Table 1 Comparative table of extraction protocols and ELISA analyses of mycotoxins in cereals and nuts

Materials	Extraction protocol	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Wheat	- Solvent H_2O - Maceration 30 min - Centrifugation 3000×g 5 min	DON	233	NA	96–102	(Sanders et al. 2016)
Cereals, nuts, and legumes	- Solvent MeOH:H_2O (4:1) - Shaking 10×g 5 min - Centrifugation 3000×g 5 min - Optimization: • Diluent 1 (D1) Tris–HCL (0.1 mol/mL) • Diluent 2 (D2) PBS (0.1 mol/mL) pH 7.4 • Diluent 3 (D3) PB (0.1 mol/mL) pH 7.4	AFB_1 DON ZEN	0.03 1 5	NA	(D1) 63–100 (D2) 81–119 (D3) 81–119 (D1) 65–99 (D2) 81–119 (D3) 81–119 (D1) 65–99 (D2) 81–119 (D3) 81–119	(Wu et al. 2020)
Cereals	- Solvent buffer - Addition of NaCl - Shaking 3 min - Centrifugation 4000×g 10 min 4 °C	T-2 HT-2	3	NA	102	(D'Agnello et al. 2021)
Maize	- Ridascreen$^{(R)}$	AFs ZEN OTA	1.75 0.75 0.5	NA	80–98	(Ekwomadu et al. 2021)
Breakfast cereals	- Solvents • MeOH:H_2O (70:30) for AFs, ZEN, and FBs • MeOH:H_2O (50:50) for OTA and T-2/HT-2 • H_2O for DON - Shaking 300 rpm 3 min - Centrifugation 5000 rpm 10 min (for DON only) - Filtration Whatman No. 1	AFs OTA ZEN FBs DON T-2/HT-2	0.5 1 5 200 100 10	1 2 25 1000 500 25	97.9–100.1	(Foerster et al. 2022)
Fermented forage and feeds	- Solvent MeOH:H_2O (70:30) - Shaking 30 min - Filtration Munktell-Ahlstrom 292 filter paper (5–8 µm) - pH 6.5	AFB_1	2.346–2.443	2.958–3.037	82.14–96.3	(Horváth et al. 2022)
Cereals	- Solvent MeOH:H_2O (8:2) - Shaking 5 min - Centrifugation 3000×g 3 min	DON	0.56	NA	NA	(Liu et al. 2022)
Corn and wheat	- Solvent MeOH:H_2O (70:30) - Shaking 5 min - Centrifugation 4000 rpm 10 min - Filtration PTEE membrane filter 0.45 µm - Clean-up BsMAb-IAC	AFB_1 OTA	0.032 1.008	NA	95.4–105	(Lu et al. 2022)

Table 1 (continued)

Materials	Extraction protocol	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Cereals	- Solvent MeOH:H$_2$O (70:30) - Blending 10 min - Vacuum filtration Whatman No. 1 - ELISA kits • AgraQuant 1/20 (kit 1) • BioShield ES Total AFs (kit 2) • Ridascreen Total AFs (kit 3)	AFB$_1$ AFB$_2$ AFG$_1$ AFG$_2$	Kit 1/kit 2/kit 3 1.59/1.04/1.4	2.47/1.8/3.15	79.64–101.66/72.51–96.55/61.41–93.45	(Maggira et al. 2022)
Maize	- Solvent MeOH:H$_2$O (70:30) - Shaking 3 min - Resting - Filtration Whatman No. 1	AFB$_1$ FBs	2 200	NA	NA	(Ochieng et al. 2022)

recovery rates (up to 96.3%) due to matrix swelling. The analysis showed acceptable accuracy and repeatability too (Horváth et al. 2022).

To compare the performance of three available ELISA kits for AFs, Maggira et al. conducted a study on spiked feedstuff samples at known concentrations. All three kits achieved high recovery rates and low LOD and LOQ values, but "BioShield ES" ELISA kit was considered as the best due to its lowest LOD and LOQ values. Both ELISA and HPLC-FLD methods were reliable and suitable for AFs detection (Maggira et al. 2022). In another study, ELISA and UPLC-FLD were used to detect T-2 and HT-2 mycotoxins in cereals, with both methods showing acceptable performance criteria such as, sensitivity, precision, selectivity, linearity, and ruggedness (D'Agnello et al. 2021).

The simultaneous detection of AFB$_1$, DON, and ZEN in cereal samples, soybeans, and peanuts has been conducted using C-ELISA (calibration curve implanted enzyme-linked immunosorbent assay). This new method is rapid, reliable, sensitive, and accurate for detecting multiple mycotoxins in food matrices, without requiring standard chemicals. The linear detection ranges, recovery rates, and low RSD values (< 5%) confirmed its suitability for use (Wu et al. 2020). FBs (the sum of FB$_1$ and FB$_2$) were analyzed in two additional studies with quantification rates between 250 and 5000 µg/kg in maize and 1970 µg/kg in breakfast cereals. The LOD of FBs was 200 µg/kg in both studies (Foerster et al. 2022; Ochieng et al. 2022). According to the Commission Regulation (EC) No 1881/2006 of 19 December 2006, these LOD values are not compatible with the maximum levels of FBs already set as 200 µg/kg in foodstuffs like processed maize-based foods and baby foods for infants and young children (Pereira et al. 2022). The recovery rates were in the range of 97.9 to 100.1%, and the RSD values ranged between 5.1 and

15.6% in breakfast cereals (Foerster et al. 2022). The high quantification ranges of FBs in maize in the study conducted by Ochieng et al. required the dilution of the samples with distilled water (ration 1:20) (Ochieng et al. 2022). It can be concluded from the results shown in Table 1 that there is no effect of the dilution on the sensitivity of the results.

The combination of indirect competitive ELISA (ic-ELISA) with immuno-affinity column (IAC) based on bispecific monoclonal antibody (BsMAb) has demonstrated high efficiency and reliability in the simultaneous detection of AFB$_1$ and OTA in wheat and corn. The detection results were strongly correlated with LC–MS results (correlation = 0.9). This developed technique achieved low LODs of 0.032 µg/Kg and 1.008 µg/Kg for AFB$_1$ and OTA, respectively. Additionally, it exhibited acceptable recovery rates ranging from 95.4 to 105% for both mycotoxins. However, further improvements are necessary to address the demonstrated cross-reactivities of the BsMAb to five toxins with identical structures (Lu et al. 2022).

High-performance liquid chromatography (HPLC)

Liquid chromatography (LC) is used for the separation of thermolabile, non-volatile compounds, and substances of different polarities. Structurally related substances can also be separated using LC. LC does not necessarily require a derivatization step which is required by gas chromatography (Santos et al. 2022). The use of derivatization depends on the specific mycotoxins to be analyzed and on the technique to be used. In some cases, derivatization may be required to improve the detection of extrinsically fluorescent analytes, to stabilize the samples, and to provide compatibility and

separation with selected analytical techniques (Muhammad et al. 2022; Zhang and Banerjee 2020). HPLC is the most widely used chromatographic method for the determination of organic compounds. HPLC coupled to ultraviolet (HPLC–UV) or fluorescence (HPLC-FLD) detectors are considered the most popular types (Abbas 2021; Iqbal 2021; Shanakhat et al. 2018). The presence of a chromophore in the molecules to be tested by HPLC–UV or HPLC-FLD is mandatory. AFs (AFB$_1$, AFB$_2$, AFG$_1$, and AFG$_2$) and OTA can be detected by HPLC-FLD thanks to their natural fluorescence that could be enhanced by derivatization. FBs are among the mycotoxins lacking chromophores in their structure, and they require a derivatization step to add chromophores or fluorescent moieties (Janik et al. 2021). Furthermore, the need of this derivatization step, expensive equipment, and skilled specialist to perform the analysis is the main limitation of HPLC. HPLC is favored owing to the high sensitivity, good selectivity, identification precision, and the short time required for analysis (Shanakhat et al. 2018). Electrochemical and photochemical principles can be followed for the derivatization of aflatoxins to increase their natural fluorescence. Trifluoroacetic acid (TFA), potassium bromide (KBr), or iodine are used to achieve the electrochemical derivatization (Miklós et al. 2020). Pre-column and post-column derivatizations (PCD) can be used where the latter is the prevalent type. Pre-column derivatization uses TFA as a reagent, and it is time-consuming because of the concentration process to be performed. Most studies utilized the post-column derivatization (PCD) by electrochemical and photochemical principles since it is easy to use and provides better sensitivity and a wider range of linearity. Iodine and KBr are usually used for the chemical derivatization in post-column type (Zhang et al. 2018).

High-performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) represents an alternative to the mostly used HPLC-FLD methods that require time-consuming derivatization in the absence of a UV chromophore in the mycotoxin to be analyzed (Bacha et al. 2023; Qin et al. 2020). Wu et al. highlighted the reliability and convenience of ELSD due to its simplicity, safety, rapidness, and cost-effective sample preparation procedures used prior to the detection of ZEN in barley (Qin et al. 2020; Wu et al. 2011). It is worth noting that many disadvantages hinder the use of ELSD such as the low sensitivity, the destructive nature of the analysis, and the non-linear response (Lucena et al. 2007).

Many authors shed light on the effect of mycotoxins sample preparation on the accuracy of various analytical methods (Aichinger et al. 2020; Assaf et al. 2018a). Assaf et al. showed that filtration was able to affect the analytical results of AFM$_1$. The margin of error has been increased as a result of the retention of the mycotoxin in the filter cavities and cake space (Assaf et al. 2018a). In another study, Aichinger

et al. used many syringe-type filters for the microfiltration of a complex of *Alternaria alternate* strains. The adsorptive phenomena of mycotoxins have been proved, and it was influenced by the membrane material and the mycotoxin nature. A complete loss of the analytes occurred in some cases, especially for alternariol (AOH) and its monomethyl ether (AME) (Aichinger et al. 2020).

Table 2 represents a detailed overview of many studies using HPLC to detect and quantify different mycotoxins in solid food and feed. After mycotoxins extraction using several solvents by mechanical means, a clean-up procedure followed such as conventional IAC (Gonçalves et al. 2020), automated IAC (Dhanshetty et al. 2021), or QuEChERS method (Wu et al. 2011). In addition to the LODs, the LOQs, and the recovery rates, HPLC details such as the derivatization reagents, the mobile phases, the flow rates, and the excitation/emission wavelengths are shown in Table 2.

DON has been extracted from cereals and cereal products mechanically using water and by a subsequent clean-up procedures using IAC (Golge and Kabak 2020; Gonçalves et al. 2020). A first study detected low LOD (3 µg/kg) and LOQ (10 µg/kg) of DON using hot water and in-line IAC clean-ups. The analysis was performed using HPLC coupled to post-column derivatization with a fluorescence detector (HPLC-PCD-FLD) technique without any organic solvents. The recovery rate was 95%, and the used method showed good precision with an RSD values ranging from 7.4 to 11.3%. The major DON conjugates (15-AcDON, 3-AcDON, and DON-3-G) were analyzed following the same procedure and the results were also satisfactory (Gonçalves et al. 2020). Golge and Kabak quantified DON in wheat and maize using reversed-phase HPLC coupled with photodiode array (HPLC–PDA). The LOD values were higher than those obtained in the above-mentioned study. Lower LOD and LOQ were achieved in wheat than in maize (Golge and Kabak 2020). Another study was carried out by Tan et al. to optimize the analysis of DON in cereal grains and feedstuffs. The best results were obtained using acetonitrile:water (84:16) mixture for extraction, the Supel™ Tox DON SPE for purification, and acetonitrile:water (1:9) mixture as the mobile phase to perform the HPLC–PDA analysis. The LOD and LOQ values were similar to those achieved in the previously discussed studies. The recovery rates were satisfactory in the range of 94.8 to 98.5%, and a high level of precision was obtained with RSD values between 2.5 and 3.3% (Tan et al. 2022). A mixture of phosphate-buffered saline (PBS) and water was used to analyze DON and other mycotoxins in wheat bran. This method resulted in higher LOD (12.58 µg/kg) and LOQ (38.11 µg/kg) of DON compared with the other studies. The recovery rates (87–100.8%) were acceptable with good repeatability values (RSDr < 7.2%) established at three spiking levels (Irakli et al. 2017).

Table 2 Comparative table of extraction protocols and HPLC analyses of mycotoxins in cereals and nuts

Materials	Extraction protocol	Analysis details	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Barley	- Solvent MeOH - Blending with NaCl – 5 min - Filtration filter paper - Clean-up QuECh-ERS method	**HPLC–ELSD** - Flow rate 1mL/min - Mobile phase ACN:H$_2$O:MeOH (46:46:8) - ELSD conditions – 50°C – nitrogen – 30psi	ZEN	1.56	NA	83–91.5	(Wu et al. 2011)
Cereals and cereal products	- Solvent H$_2$O:ACN:AA (20:70:10) - Shaking 1 min - Clean-up QuECh-ERS method - Centrifugation 400 rpm 5 min - Filtration syringe 0.45 µm	**HPLC-FLD** - Separation C18column - Flow rate 1mL/min - Mobile phase 6% AA in H$_2$O:ACN (25:75) - Excitation/emission λ 333/465 nm	OTA	0.18–0.62	0.6–2.08	85.2–109.8	(Sirhan et al. 2012)
Wheat bran	- Solvent PBS/MeOH - Shaking 5min - Centrifugation 4000 rpm 10min - Filtration - Shaking MeOH:PBS 10min - Centrifugation 12,000 rpm 10 min 4°C - Filtration glass microfiber filter - Clean-up multi-IAC	**HPLC–DAD-FLD** (diode-array detector) - Derivatization photochemical reactor - Flow rate 1mL/min - Mobile phase (A) H$_2$O, (B) ACN and (C) MeOH - Excitation/emission λ AFs 365/460nm – OTA 335/460 nm – ZEN 235/460 nm – DON 220 nm	AFB$_1$ AFB$_2$ AFG$_1$ AFG$_2$ OTA ZEN DON	0.36 0.16 0.14 0.12 0.40 6.74 12.58	1.08 0.48 0.42 0.38 1.20 20.43 38.11	70.3–101.4 70.2–105.8 88.2–103.3 89.7–99.7 92–100.9 84.5–97.3 87–100.8	(Irakli et al. 2017)
Cereals and cereal products	- Solvent H$_2$O - Blending 3 min - Filtration Whatman No. 4 - Clean-up IAC	**HPLC–PDA** - Flow rate 0.4mL/min - Mobile phase MeOH:H$_2$O (15:85) - Detection λ 220nm	DON	14.08–21.7	46.9–72.3	85.8–98.6	(Golge and Kabak 2020)
	- Solvent ACN:H$_2$O (75:25) - Blending - Clean-up by EASI-EXTRACT® ZEARALENONE	**RP-HPLC-FLD** - Flow rate 1 mL/min - Mobile phase ACN:H$_2$O (52:48) - Excitation/emission λ 274/455 nm	ZEN	1.06–1.12	3.5–3.7	97.4–105.1	
Cereals	- Solvent H$_2$O - Shaking 1 h - Centrifugation 3200×g 5 min - Filtration Whatman folded 113V - Clean-up IAC	**HPLC-PCD-FLD**	DON DON-3-G 15-AcDON 3-AcDON DON	3 3 3 3 3	10 10 10 10 10	95 102 96 92 94.8–98.5	(Gonçalves et al. 2020)

Table 2 (continued)

Materials	Extraction protocol	Analysis details	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Cereals and nuts	- Solvent MeOH:H$_2$O (80:20) - Shaking 200 rpm 30 min - Clean-up IAC - Double centrifugation 2800×g 5 min/5600×g 5 min - Clean-up IAC	**HPLC-FLD** - Post-column derivatization with Br - Flow rate 0.8 mL/min - Mobile phase (A) H$_2$O + ACN + MeOH + KBr + HNO$_3$ (4 M) and (B) MeOH + H$_2$O - Excitation/emission λ 365/456 nm	AFB$_1$ AFB$_2$ AFG$_1$ AFG$_2$	0.03 0.015 0.03 0.02	0.125–0.5	73–104.1	(Dhanshetty et al. 2021)
Maize	- Solvents MeOH:H$_2$O (80:20)/ACN:H$_2$O (75:25)/ ACN:MeOH:H$_2$O (25:25:50) - EASI-EXTRACT ®r-Biopharm - Clean-up IAC	**HPLC-FLD** - Derivatization of FBs O-phthalaldehyde solution and of AFs electrochemical cell (CoBrA cell) - Flow rate for AFs 0.8 mL/min— for FBs, OTA and ZEN 1 mL/min - Mobile phases: • MeOH:ACN:H$_2$O (20:20:60) + KBr + HNO3 (4 M) for AFs • MeOH:NaH$_2$PO$_4$ (80:20) for FBs • ACN:H$_2$O:AA (50:48:2) for OTA • ACN:H$_2$O (45:55) for ZEN - Excitation/emission λ AFs 365/440 nm – FBs 335/440 nm – OTA 336/465 nm – ZEN 274/418 nm	FB$_1$ FB$_2$ FBs AFB$_1$ AFB$_2$ AFG$_1$ AFG$_2$ AFs ZEN OTA	NA	NA	79–95	(Ekwomadu et al. 2021)
Corn-based foods	Using single-toxin IAC For AFs - Solvent MeOH:H$_2$O (70:30) + NaCl - Blending - Filtration fluted paper - Filtration micro-fiber filter paper For OTA: - Solvent ACN:H$_2$O (65:35) - Blending For ZEN: - Solvent ACN:H$_2$O (90:10) - Blending - Filtration - Clean-up IAC Using multi-toxin IAC - Solvent MeOH:H$_2$O (80:20) - Blending 2min - Filtration fluted filter - Clean-up IAC	**HPLC-FLD** - Separation reverse-phase C18 column - Flow rate 1 mL/min - Mobile phases: • H$_2$O:ACN:MeOH (600:350:50) for AFs • ACN:H$_2$O:AA (99:99:2) for OTA and ZEN - Excitation/emission λ AFs 360/440 nm—OTA 333/460 nm – ZEN 274/440 nm	AFB$_1$ AFB$_2$ AFG$_1$ AFG$_2$ OTA ZEN	Single-toxin/multi-toxin IAC Values 0.05/0.06 0.05/0.05 0.03/0.03 0.04/0.05 0.04/0.03 1.34/1.37	0.15/0.18 0.15/0.15 0.09/0.09 0.12/0.15 0.12/0.09 4.02/4.11	70.9–106.1	(Dimzoska et al. 2022)

Table 2 (continued)

Materials	Extraction protocol	Analysis details	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Cereals and feedstuffs	- Solvent ACN:H$_2$O (84:16) - Vortex 2 min - USAE 30 min - Centrifugation 2500×*g* 10 min - Purification supel™ Tox SPE - Washing ACN:H$_2$O - Filtration syringe 0.45 µm	**HPLC–PDA**	DON	3	10	94.8–98.5	(Tan et al. 2022)

HPLC-FLD was used to analyze AFs in different solid matrices using IAC after extraction by methanol:water (Dhanshetty et al. 2021; Dimzoska et al. 2022; Ekwomadu et al. 2021) and PBS:methanol (Irakli et al. 2017). The LOD and LOQ values of AFB$_1$, AFB$_2$, AFG$_1$, and AFG$_2$ were low enough to confirm the sensitivity of the used methods. In the study carried out by Dhanshetty et al. (2021), the LOD values of the different AFs ranged from 0.015 to 0.03 µg/kg. The use of the automated clean-up has been validated and optimized to the required time for HPLC-FLD analysis of AFs (Dhanshetty et al. 2021). Similar values were obtained in another study conducted on corn-based foods. The use of multi-toxin IAC procedure was confirmed. The possibility of using the same extraction method and one multi-toxin IAC clean-up for AFs, OTA, and ZEN was also proven by the low LOD and LOQ values of all concerned mycotoxins, with acceptable recovery rates (70.9–106.1%) and low RSD values (< 10.5%) (Dimzoska et al. 2022). Another study applied the multi-toxin IAC clean-up procedure after two rounds of extraction of AFs, OTA, and DON in wheat bran. PBS with a mixture of PBS:methanol were used in the first and the second extractions, respectively. This method proved its success to quantify mycotoxins in 34 wheat bran samples in Greece (Irakli et al. 2017).

Concerning the quantification of OTA in cereals and cereal products, in corn-based foods, and in wheat bran, similar and satisfactory results were achieved by using HPLC-FLD in many studies. Different extraction solvents were used for OTA, such as water:acetonitrile:acetic acid mixture (20:70:10), 70% methanol and NaCl, or PBS:methanol mixture. The clean-up was performed either by QuEChERS method, single-toxin IAC, or multi-toxin IAC. All of these studies demonstrated their sensitivity, accuracy, and precision in the analysis of OTA (Dimzoska et al. 2022; Irakli et al. 2017; Sirhan et al. 2012).

The comparison of the different studies shown in Table 2 reveals that the obtained LOD values using methanol or acetonitrile were lower than those achieved by PBS:methanol.

They showed also that QuEChERS method and IAC clean-up procedures could be used for sample preparation (Dimzoska et al. 2022; Golge and Kabak 2020; Irakli et al. 2017; Wu et al. 2011).

Liquid chromatography-tandem mass spectrometry (LC–MS/MS)

LC–MS/MS is essentially and broadly used to determine and quantify multiple mycotoxins in complex food matrices (De Santis et al. 2017). The high selectivity, the increased sensitivity, and the reliability of detection of the mycotoxins in different food and feed matrices were the result of the combination of LC with MS/MS, considered as a reference technique (Janik et al. 2021; Laganà 2017). LC–MS/MS is an effective and powerful technique suitable for the detection of new toxins and their metabolites in the tested food samples (Janik et al. 2021; Malachova et al. 2017). Multiple mycotoxins are usually produced in crops by the same mycotoxigenic fungus or by a simultaneous or subsequent invasion of these crops by different fungi (Iqbal 2021; Shi et al. 2018). Many detectors can be used for mycotoxins revelation such as triple quadrupole, ion trap, time-of-flight, and orbital ion trap mass analyzers. A combination of two analyzers could be alternatively used as a hybrid system. The available screening data shows that the triple quadrupole system (QqQ) with multiple reaction monitoring (MRM) modes is the most commonly used MS detector (De Girolamo et al. 2022; Malachova et al. 2017).

Many factors stand behind the successful usage of LC–MS/MS techniques for the simultaneous detection of mycotoxins. The food or feed matrices determine the set of mycotoxins to be detected or analyzed and the prioritization of the detection of mycotoxins to be quantified in each matrix and which ones are substantial if detected or quantified. On the other hand, it is important to determine the quantification levels to be achieved by LC–MS/MS, which may range from

remnant traces in baby food to higher levels in raw materials and feed. This could be important to define the concentration ranges, the procedures of sample preparation (dilution or concentration), and the equipment to be used (Krska et al. 2017). It is worth noting that in addition to the matrix effect that may affect the accuracy of the quantification, the diversity of the mycotoxins and the difference in their nature, which range from very polar (i.e., moniliformin and nivalenol) to non-polar (i.e., beauvericin and enniatins), will revoke the possibility of adapting unique extraction or clean-up procedure to be the master key for all mycotoxins (Krska et al. 2017; Murugesan et al. 2015). Mycotoxins are soluble to a great extent in polar and slightly polar solvents, but they are considered insoluble in non-polar solvents. Polar solvents such as acetonitrile, acetone, chloroform, dichloromethane, ethyl acetate, or methanol are the most commonly used separately or in combination with the addition of amounts of water or acids (Kappenberg and Juraschek 2021).

A representative sampling is a prerequisite for the accuracy and precision of LC–MS/MS techniques, followed by the size reduction and the homogenization of the solid food or feed to be tested. LC–MS/MS can be optimized by the adoption of suitable extraction solvents and clean-up methods according to the nature of the mycotoxins and the food matrices (Mushtaq et al. 2020).

The matrix effect is the most important challenge of LC–MS/MS analysis of multi-mycotoxins in complex food commodities such as cereals and nuts. The matrix-matched calibration and the isotope-labeled standards are two calibration methods that can be used to compensate but not to remove the matrix effects (Wu and Ordinario 2021). The drawbacks of the matrix-matched calibration and the isotope-labeled standards can be the lack of samples that can be used as a common true blank for all analytes and the non-availability of the labeled standards for all mycotoxins. The chemical structure and the chromatographic properties of the stable isotope-labeled internal standards are similar to those of the target toxins (Al-Taher et al. 2017; Fiby et al. 2021; Wu and Ordinario 2021).

Many recent LC–MS/MS studies are shown in Table 3. A methodical review has been conducted to screen the different used solvents, the extraction methods, the adopted clean-up procedures, the chromatography separation details, and the mass spectrometry parameters. To assess and compare the experimental validation of these techniques, several attributes are shown, including LOD, LOQ, accuracy or the recovery percentages (recovery rate), and precision under repeatability conditions (RSDr%). On the other hand, the advantages and the disadvantages of the LC–MS/MS method and its variations as mentioned in the studied experiments are summarized in Table 5.

According to the guidance document SANTE/2015/11945, the acceptable recovery percentages range between 70 and 120%. These latter do not require any adjustments. If the recovery values are outside this range, poor accuracy must be considered where recovery correction is decidedly recommended using isotopically labeled standards. The associated repeatability (RSDr) must be lower than 20% for the set of designated analytes. The effect of the matrix has been investigated by comparing the responses from solvent standards with the matrix-matched standards that must be in the range of $\pm 20\%$. The value of the LOQ should be below the maximum residue limit (MRL).

Aflatoxins showed low water solubility, but they showed great solubility in moderately polar solvents such as chloroform, methanol, and dimethyl sulfoxide (DMSO) (Tahir et al. 2018). Many mycotoxins are soluble in acetonitrile, and an improvement of extraction can be achieved at higher ACN percentages in the used ACN-water extraction mixtures. OTA, ZEN, and AFs are soluble in polar solvents such as methanol and acetonitrile (polar organic solvents), and the hydrophilicity of FBs enables them to be soluble in water and the aforementioned organic solvents too. The water solubility of DON, T-2, and HT-2 is enhanced by the use of a mixture of water with other polar solvents such as acetonitrile and methanol (Pantano et al. 2021). Table 3 shows the solvents used for the extraction of mycotoxins: acetonitrile:water:methanol (79:20:1) (Houissa et al. 2019), water:methanol:acetonitrile (2:1:1) (Ndoro et al. 2022), acetonitrile:water:acetic acid or formic acid (79:20:1) (André et al. 2022; Eyring et al. 2021; Kovač et al. 2021; Mohammed et al. 2022), acetonitrile:H_2O_2:formic acid (84:15.8:0.2) (Liang et al. 2022), methanol (Nguyen et al. 2018), water:methanol:acetonitrile (2:1:1), or specific supplied analysis kits (Er Demirhan and Demirhan 2021).

The detection of aflatoxins in corn or maize was conducted using different mixtures of acetonitrile and water with the addition of acetic or formic acids (Bessaire et al. 2019; Eyring et al. 2021; Pantano et al. 2021). The extraction solution composed of water and a mixture of acetonitrile:formic acid (80:20) leads the best performance after the application of the QuEChERS clean-up method, especially that acetonitrile can reduce the extraction of fat and lipophilic materials with the great capability of acetonitrile to extract molecules of different polarities. This method was able to achieve acceptable recovery rates and repeatability values (RSDr%) of aflatoxins with ranges varying from 75 to 117% and 0.057 to 0.95%, respectively. The drawback of this method is the strong matrix effect in the case of AFs in maize (-20.18 to $+43.74\%$) and black pepper (-26.17 to -0.89%). Based on the data included in Table 3, it is obvious that the QuEChERS method was not successful for DON, FB_1, and FB_2 which showed the strongest matrix effect values (66.82% for FB_2), the lowest recovery rates (60% for FB_1 and FB_2), and the highest RSD (141% for FB_2 and 223% for FB_3) (Pantano et al. 2021). This drawback has been resolved by

Table 3 Comparative table of extraction protocols and LC–MS/MS analyses of mycotoxins in cereals and nuts

Materials	Extraction protocol	Analysis details	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Rice	- Solvent	- Separation	ALT ATX-I	0.13 4	0.25 8	83.5–99.2	(Nguyen et al. 2018)
Cereals and nuts	- Solvent ACN + AA (1%) - Shaking 300 rpm 10 min - Centrifugation 4000×g 10 min - Clean-up QuEChERS (for all mycotoxins) and IAC (for AFs and OTA)	- Separation C18 column 50°C - Flow rate 0.4 mL/min - Mobile phase	AFB_1 AFB_2 AFG_1 AFG_2 AFs OTA ZEN DON T-2 HT-2 T-2 + HT-2 FB_1 FB_2 FBs	NA	NA	91–116 96–109 98–108 84–109 94–112 97–123 103–129 91–111 94–107 83–113 88–110 103–111 93–115 101–113	(Bessaire et al. 2019)
Pearl millet	- Solvent ACN: H_2O:MeOH (79:20:1) - Shaking 90 min - Clean-up dilute and shoot	- Separation C18 column 25°C - Analyzer Qtrap - Ionization ESI+/ESI– - Mode MRM	FB1 OTA ZEN Fusarium MTX Alternaria MTX	NA	NA	94	(Houissa et al. 2019)
Wheat, corn, and soybean	- Solvent ACN:H_2O (80:20) - Vortex 2800 rpm - Soaking 10 min - Shaking 30 min - Centrifugation 3000 rpm 3 min - Filtration polyetetrafluoroethylene filter 0.2 µm	- Separation C8 column (1st dimension) C18 column (2nd dimension) 30°C - Flow rate 0.2 mL/min - Mobile Phases: • 1st dimension (A) H_2O + NH_4HCO_2 + FA and (B) ACN:H_2O (90:10) + NH_4HCO_2 + FA • 2nd dimension (A) H_2O + NH_4HCO_2 + FA and (B) MeOH + NH_4HCO_2 + FA - Ionization ESI + - Mode sMRM	AFB_1 AFB_2 AFG_1 AFG_2 OTA DON ZEN HT-2 T-2 FB_1	NA	0.001 0.001 0.001 0.001 0.001 0.2 0.05 0.05 0.05 0.05	80–112 93–110 89–118 84–103 82–102 82–107 76–94 70–86 78–115 87–109	(Kresse et al. 2019)

Table 3 (continued)

Materials	Extraction protocol	Analysis details	MTX	LOD μg/kg	LOQ μg/kg	Recovery %	Reference
Cereal-based baby foods	- Solvent Jasem analysis kit reagent 1 - Shaking 15 min - Centrifugation 3000 rpm 5 min - Filtration nylon filter 0.45 μm	- Separation multi-mycotoxin analytical column - Flow rate 0.5 mL/min - Analyzer QqQ - Ionization ESI	AFB_1	0.01	0.02	98	(Er Demirhan and Demirhan [2021])
			AFB_2	0.02	0.07	102	
			AFG_1	0.04	0.12	102.34	
			AFG_2	0.04	0.15	96.3	
			FB_1	0.58	1.93	82.53	
			FB_2	0.24	0.8	92.97	
			OTA	0.01	0.03	91.17	
			STE	0.02	0.07	100.27	
			DON	2.4	8.01	108.67	
			ZEN	0.13	0.45	107.61	
			HT-2	0.4	1.35	97.94	
			T-2	0.15	0.49	96.47	
Maize	- Solvent acidified ACN:H_2O and heptane - Shaking 1 h (WAHSPE procedure) - Filtration filter vial 0.2 μm	NA	AFB_1	NA	NA	100–102	(Eyring et al. [2021])
			AFB_2			92–98	
			AFG_1			107–103	
			AFG_2			111–94	
			OTA			73–77	
			FB_1			112	
			FB_2			51	
			ZEN			63	
			DON			72	
			HT-2			103–72	
			DAS			93–116	

Table 3 (continued)

Materials	Extraction protocol	Analysis details	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Cereals	- Solvent ACN:H$_2$O:FA (79:20:1) - Shaking 90 min - Filtration nylon filter 0.22 µm	- Separation HSS T3 column 40°C - Flow rate 0.3 mL/min - Mobile phase (A) NH$_4$ HCO$_2$ aqueous solution and (B) MeOH - Analyzer QqQ - Ionization ESI+/ESI− - Mode MRM	AFB$_1$	0.3	1	99.1	(Kovač et al. 2021)
			AFB$_2$	0.15	0.5	101.2	
			AFG$_1$	0.3	1	104.9	
			AFG$_2$	0.15	0.5	96.9	
			DON	61	200	111.1	
			FB$_1$	46	150	101.4	
			FB$_2$	46	150	100.4	
			ZEN	9	30	97.2	
			T-2	3	10	97.3	
			HT-2	3	10	93.5	
			OTA	0.3	1	94.3	
Maize	- Solvents H$_2$O/ACN:FA (80:20) - Vortex 15 min - Resting 15 min – 20°C - Clean-up QuEChERS method	- Separation Thermo Scientific Hypersil Gold reversed-phase UHPLC column - Mobile phase (A) H$_2$O and (B) MeOH - Analyzer QqQ - Ionization ESI + - Mode MRM	OTA	NA	NA	73	(Pantano et al. 2021)
			ZEN			65	
			AFB$_1$			79	
			AFB$_2$			117	
			AFG$_1$			75	
			AFG$_2$			77	
			DON			120	
			FB$_1$			60	
			FB$_2$			60	
			FB$_3$			92	
			T-2			99	
			HT-2			120	
Wheat	- Solvent	- Separation C18 column for method A and YMC CHIRAa ART Cellulose-sc column for Method B and C - Flow rate		Methods A-B-C			(Wang et al. 2021)
			DON	NA-4-4	5-8-8	83.5–118.8	
			15-AcDON	6-4-4	10-8-8		
			3-AcDON	12-4-4	40-8-8		

Table 3 (continued)

Materials	Extraction protocol	Analysis details	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Wheat grains and flours	- Solvent ACN:H$_2$O:AA (79:20:1) - Shaking 90 min - Centrifugation 4400 rpm 10 min - Clean-up Oasis® Prime HLC cartridge	- Separation C18 column 35°C - Flow rate 0.28 mL/min for ZEN and 0.25 mL/min for ENB - Mobile phases: • (A) H$_2$O+AA (0.1%) and (B) MeOH+AA (0.1%) for ZEN • (A) H$_2$O+FA (0.1%)+NH$_4$HCO$_2$ (5 mmol) and (B) MeOH+FA (0.1%)+NH$_4$HCO$_2$ (5 mmol) for ENB - Analyzer Q-TOF (quadrupole time-of-flight) – ionization ESI –	ZEN ENB	7.5 1.5	15 3	93 NA	(André et al. 2022)
Walnuts	- Solvent ACN:FA (99.9:0.1)+H$_2$O - Shaking 1 min - Addition of 4g anhydrous MgSO$_4$+1 g NaCl+1g Na3C$_6$H$_5$O$_7$·2H$_2$O,+0.5g C$_6$H$_6$Na$_2$O$_7$·1.5H$_2$O - Shaking 1 min - Centrifugation 2655×g 5min - Clean-up • Pass-through SPE using PRiME HLB cartridge • dSPE using EMR-lipid sorbent • SPE using MIP cartridges • dSPE using Z-sep+ sorbent • dSPE using C18 • dSPE using PSA	- Separation C18 column 40 °C - Flow rate 0.4 mL/min - Mobile phase (A) H$_2$O:FA (99.9:0.1) and (B) MeOH:FA (99.9:0.1) - Analyzer QqQ-Linear Ion Trap - Ionization ESI + - Mode sMRM	OTA AFB$_1$ AFB$_2$ AFG$_1$ AFG$_2$	NA	1.81±0.06 1.94±0.06 1.84±0.13 1.75±0.11 1.86±0.13	95.4–110.4 95.1–105.9 96.9–108.8 103.8–109.2 102.6–107.8	(Castilla-Fernández et al. 2022)

Table 3 (continued)

Materials	Extraction protocol	Analysis details	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Arecae semen and its processed products	- Solvent ACN:H$_2$O$_2$:FA (84:15.8:0.2) - Vortex mixing 1 min - Ultrasonic extraction 10 min at RT - Centrifugation 4000 rpm 10 min - Addition of AA - Clean-up MycoSpinTM 400 column - Filtration syringe 0.22 µm	- Separation C18 column 40°C - Flow rate 0.3 mL/min - Mobile phase (A) MeOH+FA (0.1%) and (B) NH$_4$HCO$_2$ aqueous solution - Analyzer QqQ - Ionization ESI+/ESI− - Mode optimized MRM	AFB$_1$	0.04	0.1	95–103.46	(Liang et al. 2022)
			AFB$_2$	0.05	0.1	92.18–98.84	
			AFM$_1$	0.04	0.1	85.66–93.57	
			AFM$_2$	0.04	0.1	85.96–95.5	
			AFG$_1$	0.04	0.1	73.65–79.82	
			AFG$_2$	0.05	0.1	86.44–88.98	
			15-AcDON	0.8	2.5	81.73–86.2	
			3-AcDON	0.8	2.5	84.97–88.24	
			DAS	0.8	2.5	103.24–113.21	
			HT-2	0.4	1	83.55–90.68	
			T-2	0.4	1	97.49–99.53	
			DOM-1	1	2.5	62.07–76.68	
			DON	1	2.5	59.52–75.38	
			NEO	0.8	2.5	109.77–117.28	
			ZEN	0.4	1	84.72–96.68	
			ST	0.3	1	93.62–105.24	
			OTA	0.1	0.25	83.87–106.85	
			OTB	0.1	0.25	94.9–102.54	
			OTC	0.1	0.25	98.21–105.57	
			FB$_1$	1.5	5	83.54–95.98	
			FB$_2$	1.5	5	94.39–107.77	
			FB$_3$	1.5	5	90.26–106.3	

Eyring et al. through the adoption of the WAHSPE (water acetonitrile heptane solid phase extraction) method which was able to achieve higher recovery rates of polar and non-polar mycotoxins in fatty matrices. The recovery rates of FB_1 and DON increased to reach 112 and 72%, respectively. In this study, the authors analyzed 11 mycotoxins (AFs, OTA, FBs, ZEN, DON, HT-2, and DAS) using the WAHSPE method and filtration without any additional clean-up procedures. The detection of aflatoxins was conducted on the acetonitrile phase. Satisfactory recovery values (92 to 111%) were achieved with acceptable RSDr ≤ 8% and adequate ME. These outcomes demonstrate the fitness of this method to test mycotoxins in cereals in conformity with the current EC regulations (EC 2006/1881). LC–MS was used to analyze the water phase, and both LC–MS and GC–MS were used to analyze the mycotoxins in the acetonitrile phase according to the analyte nature (Eyring et al. 2021).

Many mycotoxins were investigated by Kovač et al. in Croatian cereals using acetonitrile:ultrapure water:formic acid (79:20:1) as extraction solvent along with the dilution and filtration of the aliquot without any clean-up procedure. The recovery rates and the RSD values were acceptable. High LOD and LOQ for DON, FB_1, and FB_2 were obtained (Kovač et al. 2021). Another study conducted in Turkey used LC–MS/MS JASEM analysis kit for 12 mycotoxins cereal-based baby foods and showed very good accuracy and repeatability. The recovery rates ranged from 82.53 to 108.67%. The RSD values were below 8% with low LOD and LOQ for all mycotoxins. Additionally, satisfying results were obtained for DON and FBs (Er Demirhan and Demirhan 2021). LC–MS/MS coupled with a chiral column has been used to determine DON, 3-AcDON, and 15-AcDON simultaneously in wheat. Acceptable recoveries (80–120%) were realized with high stability confirmed by low RSD values (below 20%). This method showed better accuracy and efficiency compared to other methods using C18 column (Wang et al. 2021). QuEChERS method has been also used to analyze AFs, DON, ZEN, FB_1, FB_2, T-2, and HT-2 in a wide variety of samples after extraction by mechanical shaking of the sample and the addition of an equal volume of acetonitrile:acetic acid (995:5) and water. Most RSDr values were below 20% suggesting acceptable repeatability with recovery rates ranging between 70 and 130% (Bessaire et al. 2019). Ten mycotoxins were analyzed by Kresse et al. in 3 food matrices (corn, wheat, and soybean). Corn and wheat have similar starch content (60%), with 8% gluten in wheat and higher fat content in corn (3.6%) than wheat (1.8%). Soybean is considered as non-cereal food that is rich in protein (36%) and fat (20%) with low carbohydrates (30%). In this study, acetonitrile:water (80:20) was used as extraction solvent with an integrated online clean-up protocol adopted without any additional clean-up steps to prevent mycotoxins loss during sample

preparation. The results were satisfactory according to SANTE guideline document with low LOQ (0.01 μg/Kg for AFs to 0.2 μg/kg for DON), acceptable recovery rates (70–120%), and low RSD below 20%. This method has been considered as robust and precise even in protein-rich and fat-rich food matrices (Kresse et al. 2019).

Acceptable AFs and OTA recovery percentages (95.1–110.4%) coupled to RSDr values ranging between 2.2 and 7.7%, with low LODs (< 2 μg/kg), were fulfilled at 3 different spiking levels (low level: 2 μg/kg, medium level: 5 μg/kg, and high level: 20 μg/kg) in walnuts using acetonitrile:formic acid (99.9:0.1) and water as extraction solvent along with container's shaking. This study proposed the use of the 1:100 dilute-and-shoot method as a validated method according to the EU regulations. The strong matrix effect while analyzing the nuts is considered as a main limitation due to the fatty matrix. Six clean-up procedures (PRiME HLB, EMR-Lipid, AFFINIMIP cartridges, Z-sep+, C18, and PSA) were utilized in this study to overcome the matrix effect. All of these methods failed to reduce the matrix effect and did not show any advantage against conducting the simple SLE, in addition to increasing the time, the cost, and the generation of residues (Castilla-Fernández et al. 2022). Another study conducted by Liang et al. revealed the high efficiency and convenience of SPE (MycoSpin™ 400) as a clean-up method when applied after ultrasonic extraction of mycotoxins from Arecae semen and its processed products using acetonitrile:H_2O_2:formic acid (84:15.8:0.2) at room temperature for 10 min. In this study, low LOD (0.04–0.05 μg/Kg) and LOQ (0.1 μg/Kg) values of AFs were attained, and satisfactory recovery percentages were carried out at 3 spiking levels. The recovery percentages of all mycotoxins ranged from 70 to 120%, and good repeatability (RSDr < 15%) has been also accomplished (Liang et al. 2022). Increasing the acidity of the acetonitrile-based solvents differently affects the recovery of mycotoxins. Higher recovery rates of FBs, OTA, OTB, OTC, AFM2, and AFG2 were attained by decreasing the pH of the extraction solvent to 4.6 with the addition of 15% formic acid. This protocol, with the mentioned attributes, was optimal to obtain the best recoveries. Additional acid percentage (> 15%) had no impact and did not affect the recovery values. On the other hand, the acidic pH negatively affected the recovery of DON, DOM-1, 15-AcDON, DAS, ZEN, and ST with a significant decrease in their expected values. These recoveries were not influenced by the rotation speed and the centrifugation time performed during extraction (Liang et al. 2022).

The occurrence of ZEN and enniatin B (ENB) in Swiss wheat grains and wheat flours has been investigated using LC–MS/MS too. In this study, the mycotoxins ZEN and ENB were extracted by shaking the sample using acetonitrile:water:acetic acid (79:20:1). To decrease the matrix effect, clean-up Oasis® Prime HLC cartridge was used to eliminate the fatty acids and phospholipids. The recovery of ZEN (93%) was acceptable, and the RSD values were satisfactory

ranging between 1 and 13.3% (André et al. 2022). Compared with other studies, the LOD and LOQ of ZEN executed by André and collaborators were high, recording values of 7.5 μg/ kg and 15 μg/kg, respectively. In other studies, LOD values of ZEN ranged between 0.13 and 0.45 μg/kg, while LOQ values ranged from 0.045 to 1 μg/kg (Er Demirhan and Demirhan 2021; Kresse et al. 2019; Liang et al. 2022). Alternatively, the LOD and LOQ of ENB were 1.5 and 3 μg/kg, respectively; the RSD values (5.8–16.3%) were satisfactory, but it was not possible to evaluate the recovery of ENB because no certified reference materials were available (André et al. 2022). Alternaria mycotoxins (ALT, ATX, AOH, AME, and TeA) in rice medium have been analyzed using LC–MS/MS method; methanol was used for extraction, and after filtration, a solution of 20% ammonium sulfate, partitioned into methylene chloride, was added to the filtrate for clarification. The recorded LOD values varied between 0.13 and 4 μg/kg, the LOQ values ranged from 0.25 and 8 μg/kg, and acceptable recoveries (83.5–99.2%) and RSD (0.59–12.06%) have been achieved (Nguyen et al. 2018).

The choice of the appropriate LC column is a prerequisite step for the success of LC separation. The retention time of the analyte must be at least twice that of the dead volume of the used column. It has to match the calibration standard too. The resolving power of the chromatographic system in charge is defined by the width of the retention time window (Malachova et al. 2017). The most widely adapted technique for LC analysis of mycotoxins refers to reversed-phase elution mode, using C8 or C18 as stationary phases (Vargas Medina et al. 2021). Most studies shown in Table 3 use a triple quadrupole (QqQ) mass spectrometer. These systems are essentially operating in multiple reaction monitoring (MRM) or selected reaction monitoring (SRM). To meet EU criteria, two MRM transitions must be monitored for each mycotoxin (1 parent and 2 product ions) (De Girolamo et al. 2022). Full scan mode could result in poor performance. Meanwhile, the adoption of MRM for data acquisition increases significantly the sensitivity and the selectivity of the method. Operating QqQ analyzer in SRM achieved optimal sensitivity, allowing for the attainment of low LODs (Malachova et al. 2017). The most commonly used ionization modes for mycotoxins in LC–MS/MS method are atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). The latter mode could lead to higher sensitivity and is used in the majority of the studies included in this review although the former shows better efficiency for A- and B-trichothecenes (Vargas Medina et al. 2021).

Gas chromatography-tandem mass spectrometry (GC–MS/MS)

Gas chromatography (GC) coupled to mass spectrometry (MS) has been used for the analysis of many contaminants in food matrices since 1970s. Recently, a shift from the use of GC–MS

to GC–MS/MS has been noticed in most analytical laboratories in order to increase the sensitivity, improve the accuracy, and decrease the interference caused by the matrix effect. The coincidence of certain compounds with selected ions in GC–MS hampers their separation by single-step MS (Xu et al. 2021).

Low volatility and high polarity of mycotoxins are the main reasons that stand behind the infrequent use of GC for their analysis in foods or feeds. A derivatization step must follow the clean-up procedure by silylation or acylation in order to decrease polarity and to convert these mycotoxins into volatile derivatives. Many detectors could be coupled to GC but the most widely used one is the MS detector (Agriopoulou et al. 2020; Janik et al. 2021; Yang et al. 2020). Table 4 represents an overview of two studies using GC–MS to analyze mycotoxins in breakfast cereals and flours (Cunha and Fernandes 2010) and in sorghum (McMaster et al. 2019), with two other studies using GC–MS/MS to detect the mycotoxins in Romanian wheat (Stanciu et al. 2019) and during the pre- and post-fermented silage (Juan et al. 2020).

GC–MS has been used for the detection of ZEN, DON, FUS-X, 15-AcDON, and NIV in breakfast cereals and flours. After humidification of the sample and washing with n-hexane, acetonitrile was used to extract the mycotoxins. QuEChERS method has been applied for clean-up, then the sample was evaporated to dryness, and the residues were silylated by the addition of BSA + TMCS + TMSI. Two capillary columns linked to microfluidic Dean Switch device were used to ensure the fast GC analysis and GC–MS was operated in selected ion monitoring (SIM) mode. In this study, LOD values and LOQ values were is the range of 2 to 15 μg/kg and 5 to 50 μg/kg, respectively. The recovery rates were acceptable for many mycotoxins except for FUS and NIV which achieved recoveries below 70%. RSDr was acceptable with values below 21% (Cunha and Fernandes 2010). In another study conducted by McMaster et al., GC–MS was used to determine DON in sorghum samples by the application of two methods, the traditional method using solid phase extraction (SPE) chromatography with C18 and the stable isotope dilution assay (SIDA). Uncertain DON values were achieved by the traditional methods, while SIDA method was able to reduce the matrix effect and increase the accuracy in the determination of DON in sorghum using deoxynivalenol-d1 (d1-DON) (McMaster et al. 2019).

On the other hand, GC–MS/MS was used and proved its sensitivity, precision, and reproducibility in the determination of trichothecene and zearalenone in Romanian wheat. The samples were extracted by shaking using acetonitrile:water mixture (84:16); subsequently, clean-up procedure has been conducted using liquid–liquid extraction with hexane to eliminate hydrophobic compounds and decrease interference caused by the matrix effect (Stanciu et al. 2019). The derivatization was applied by the addition of BSA reagent to the dry extract. This study

Table 4 Comparative table of extraction protocols and GC–MS analyses of mycotoxins in cereals

Materials	Extraction protocol	Analysis details	MTX	LOD µg/kg	LOQ µg/kg	Recovery %	Reference
Breakfast cereals and flours	- Modified QuEChERS procedure - Addition of H_2O - Mixing 15 min - Washing twice n-hexane - Solvent $ACN + MgSO_4 + NaCl$ - Mixing 2 min - Centrifugation 5000 rpm 3 min - Addition of $MgSO_4 + C18$ to the extract - Vortex 30 s - Centrifugation 1500 rpm 1 min - Clean-up dispersive SPE	- Derivatization BSTFA (N,O-bis-trimethyl-silyltrifluoroacet-amide) or mixture BSA + TMCS + TMSI 20 min 80°C - Separation film DB-5HT primary analytical column and film thickness DB-5 ms secondary analytical column - Splitless injection mode - Electron impact ionization 70 eV - Quadrupole MS - Mode SIM	Breakfast cereals				(Cunha and Fernandes 2010)
			DON	11	37	83	
			FUS	9	30	67	
			15-AcDON	8	25	75	
			NIV	15	50	101	
			ZEN	2	7	96	
			Flours				
			DON	8	25	103	
			FUS	9	30	52	
			15-AcDON	8	25	93	
			NIV	12	40	61	
			ZEN	2	5	84	
Sorghum	- Solvent $ACN:H_2O$ (86:14) - Vortex - Shaking 1 h - Clean-up SPE column, C18 silica gel:aluminum oxide (1:3)	- Derivatization TMSI + TMCS 15 min RT - Traditional method: • Separation HP-5MS column • Splitless injection mode - SIDA method: • d1-DON - Carrier gas helium - Mode SIM	DON	0.2	NA	NA	(McMaster et al. 2019)
Wheat	- Solvent $ACN:H_2O$ (84:16) - Shaking 300 shakes/min 1 h - Centrifugation 4500 rpm 5 min 5°C - Filtration Whatman No. 4 - Evaporation 35°C - Reconstitution with $MeOH:H_2O$ (70:30) - Filtration syringe nylon filter - Drying nitrogen flow - Clean-up liquid–liquid extraction	- Derivatization BSA 30min RT - Separation HP-5MS capillary column - Splitless injection mode - Electron impact ionization 70 eV - Carrier gas helium - QqQ MS - Mode SRM	DON	0.5	1	95–127	(Stanciu et al. 2019)
			3AcDON	1.25	2.5	84–117	
			15AcDON	2.5	5	82–108	
			FUS-X	2.5	5	73–97	
			DAS	7.5	15	77–118	
			NIV	10	20	78–117	
			NEO	10	20	75–86	
			HT-2	7.5	15	84–100	
			T-2	2.5	5	103–113	
			ZEN	5	10	69–106	
Pre- and post-fermented cereals	- Solvent $ACN:H_2O:AA$ (79:20:1) - Shaking 15 min - Centrifugation $1792 \times g$ 5 min 5°C - Clean-up QuEChERS method - Filtration syringe nylon filter	- Derivatization BSA + TMCS + TMSL 30 min RT - Separation HP-5MS capillary column - Splitless injection mode - Electron impact ionization 70eV - Carrier gas helium - QqQ MS	DON	1.31	4.38	$(67 \pm 7)\%$ ZEN— $(108 \pm 9)\%$ BEA	(Juan et al. 2020)
			15-AcDON	1.12	3.73		
			3-ADON	1.86	6.21		
			NIV	1.55	5.15		
			NEO	2.17	4.63		
			DAS	1.7	5.66		
			T-2	4.36	8.53		
			HT-2	1.97	6.55		

accomplished the lowest LOD and LOQ, especially for DON, 3-AcDON, 15-AcDON, and NIV. Good recovery rates have been achieved at 3 spiking levels (LOQ, 2xLOQ, and 10xLOQ), and the RSDr values were acceptable too (≤ 19%) delivering satisfactory repeatability (Stanciu et al. 2019). GC–MS/MS was used by Juan et al. to analyze DON, 3-AcDON, 15-AcDON, NIV, DAS, NEO, T-2, and HT-2 in pre- and post-fermented silage. Acetonitrile/water/ acetic acid mixture (79:20:1) was employed as extraction solvent, and QuEChERS method was adopted for clean-up. The derivatization has been executed by the addition of the BSA + TMCS + TMSL (3:2:3) to the dry extract. The limit of detection (LOD) values were between 1.2 and 4.36 µg/kg, and the recovery ranged from $67 \pm 7\%$ for ZEN in triticale to $108 \pm 9\%$ for BEA in rayegrass, and RDSr values were below 15% (Juan et al. 2020). The results presented in Table 4 confirm the high sensitivity and precision of GC–MS/MS over GC–MS in the detection and quantification of mycotoxins by achieving lower limits of detection (LOD) and limits of quantification (LOQ).

Comparison between the different detection and quantification techniques of mycotoxins

The detection techniques discussed and compared in the previous sections can be classified according to their performance criteria such as sensitivity, accuracy, precision, required analysis time, and the complexity of preparing the required sample. Table 5 represents a summary of the advantages and disadvantages of each technique.

TLC is considered an old-fashioned method with limited analysis capabilities. The quantification through TLC is a challenging issue because it lacks sensitivity and accuracy (Aasa et al. 2022; Pernica et al. 2019). ELISA test kits represent a simple, rapid, and sensitive technique. It is widely used for the quick detection of mycotoxins in solid foods and feeds, but it lacks validation. Due to its rapidness and simplicity, it could be considered as a prescreening method that can be adopted when a large number of sample testing are required. The confirmation of the results can be conducted through another robust method as a second step (HPLC-FLD or LC–MS/MS). The requirements for a low volume and prompt sample preparation and clean-up favor the use of ELISA compared to TLC and HPLC (Nesic et al. 2017; Omar et al. 2020).

HPLC is more accurate and more specific than ELISA. It is cheaper than LC–MS/MS at the same time. The limitation of HPLC is related to the fact that ideal chromatography requires a well-designed sample preparation and an effective clean-up procedure such as QuEChERS method or IAC. The latter can be performed in the conventional form as single-toxin IAC or as multi-toxin IAC to adopt a common sample preparation

procedure for the multiple mycotoxins to be analyzed. The automation and the in-line IAC decreased the analysis time required for HPLC. In-line IAC using hot water for elution does not require the use of any chemical or organic solvents which increases the simplicity of HPLC and renders it eco-friendly. The automated IAC and the in-line HPLC-FLD have been validated for the analysis of aflatoxins achieving good linearity and reproducibility, acceptable precision and recoveries, and low LOD and LOQ values. A derivatization step is necessary to increase the fluorescence of AFB_1 and AFG_1 required before HPLC-FLD analysis. This derivatization could affect the sensitivity of ZEN which is turned in a less fluorescent compound. HPLC-ELSD is a good and rapid alternative to HPLC-FLD for ZEN analysis (Dhanshetty et al. 2021; Dimzoska et al. 2022; Gonçalves et al. 2020; Irakli et al. 2017; Omar et al. 2020; Wu et al. 2011).

LC–MS/MS is considered as the state-of-the-art due to its high performance in the simultaneous detection and quantification of the multiple mycotoxins in solid food and feed. As mentioned before, the sample preparation details such as the solvent, the extraction method, and the clean-up procedures are essential to achieve high sensitivity, acceptable recovery rates, accuracy, and repeatability. Dilute and shoot, QuEChERS method, and WAH-SPE methods are among the used clean-up alternatives before LC–MS/MS analysis. The majority of mycotoxins showed good solubility in acetonitrile–water mixtures, and the recoveries were commonly improved by increasing acetonitrile percentage in these extraction mixtures. The recovery rates were improved by decreasing the pH of the mobile phase. The optimal recoveries have been achieved by the addition of 15% of acetic acid. The dilute-and-shoot method has been validated according to the EU regulations and represents a simple, rapid, and cheap method. Its implementation in the ratio of 1:100 dilution of SLE extract and in combination with acceptable resolution and good instruments sensitivity could eliminate or reduce the matrix effect to be negligible. QuEChERS method is suitable and efficient to extract moderately polar mycotoxins. Low recovery rates of FB_1, FB_2, and DON are achieved by QuEChERS. Meanwhile, WAHSPE method was favored because it is can achieve higher recoveries of the polar and non-polar mycotoxins in high-lipid matrices. An adjustment or modification in pH or SPE sorbent will extend the use of WAHSPE method to other challenging matrices. On the other hand, LC–MS/MS showed many drawbacks. It is expensive due to the high cost of equipment to be used, the cost of maintenance, and the need for expertise and personal training. It is worth noting that results showing high LOD and LOQ could not be neglected (Bessaire et al. 2019; Castilla-Fernández et al. 2022; Dimzoska et al. 2022; Eyring et al. 2021; Irakli et al. 2017; Liang et al. 2022; Pantano et al. 2021; Wang et al. 2021).

Table 5 Advantages and disadvantages of the detection and quantification techniques discussed in this review

Technique	Advantages	Disadvantages	References
TLC	- Specific for initial screening	- Challenging quantification due to lack of sensitivity	(Aasa et al. 2022)
ELISA	- High throughput assays with low sample volume requirements - Fewer clean-up procedures compared to TLC and HPLC - Rapid, simple, specific and sensitive technique - The most common method for the detection of mycotoxins in food and feeds	- Commonly occurring matrix effect causing underestimation or overestimation in mycotoxins concentration - Insufficient validation - Used routinely only for analysis of matrices extensively tested - Confirmatory analyses by more robust methods are required for contamination levels that approach the legal limit	(Nesic et al. 2017; Omar et al. 2020)
HPLC	- More accurate that ELISA - HPLC-FLD is cheaper than LC–MS/MS - Multi-toxin IAC for analysis of many mycotoxins in one run - In-line IAC renders the HPLC technique simple, rapid, and eco-friendly - In-line IAC clean-ups do not require any organic solvents for sample preparation - IAC elution using hot water results in low LOQ values - Automated IAC and in-line HPLC-FLD for AFs analysis are validated - HPLC-ELSD provides a convenient and reliable alternative to common HPLC-FLD methods for rapid determination of ZEN content	- Clean-up necessary for an ideal chromatography figure - Decreased sensitivity of ZEN caused by the derivatization of AFB_1 and AFG_1. This derivatization will turn ZEN into a less fluorescent compound	(Dhanshetty et al. 2021; Dimzoska et al. 2022; Gonçalves et al. 2020; Irakli et al. 2017; Omar et al. 2020; Wu et al. 2011)
LC–MS/MS	- Very good performance (low LOD and LOQ values) - Bias from matrix effect is compensated using isotopically labeled standards - "Dilute-and-Shoot" is a cheap, simple, and fast method validated by EU regulations - High dilution factor recommended to eliminate matrix effect (if resolution and sensitivity of instrumentation are acceptable) - Coupling with chiral column is suitable for the simultaneous determination of DON, 3- AcDON and 15-AcDON - Coupling with chiral column achieved better accuracy and efficiency as compared with the method using a C18 column - ESI+ gives a better response for the overall of mycotoxins - Advantages of WAHSPE vs. QuEChERS extraction procedures include higher rates of recovery of both highly-polar and non-polar mycotoxins in lipid-rich matrices - Facilitated screening of multiple mycotoxins using WAHSPE method in a single sample and collection of more data with less effort in sample preparation - WAHSPE protocol achieves recovery rates of 70 to 120%	- High cost of equipment, maintenance and personal trainings or expertise - No significant effect of centrifugation on mycotoxins recovery - AA concentration increased the purification efficiency but caused darkening of the purified extract - The concentration of acid affected the binding ability to adsorb and the co-extractive components in the matrix - Fatty matrices like nuts limit mycotoxins analysis through the strong matrix effect - None of the following clean-ups (PRiME HLB, EMR-Lipid, AFFINIMIP cartridges, Z-sep+, C18, and PSA) effectively reduced the strong matrix effect encountered by the SLE - The strong matrix effect in the mycotoxin analysis was caused by the lipid/water/protein content of matrices analyzed - Strong matrix effect for DON and FBs in Maize - QuEChERS is more efficient for moderate polarity compounds - Some of the recoveries are challenging	(Bessaire et al. 2019; Castilla-Fernández et al. 2022; Eyring et al. 2021; Liang et al. 2022; Pantano et al. 2021; Wang et al. 2021)

Table 5 (continued)

Technique	Advantages	Disadvantages	References
GC–MS	- Low LOQs and good recovery rates	- Derivatization is required and it generates chemical wastes	(Cunha and Fernandes 2010; Eyring et al. 2021; McMaster et al. 2019; Stanciu et al. 2019)
GC–MS/MS	- Good performance criteria: acceptable sensitivity, precision, and reproducibility, and satisfactory recovery rates	- Required ACN phase to undergo clean-up to remove lipids and water before analysis by GC–MS	
	- Stable isotope to overcome potential matrix effect	- The original QuEChERS method has been applied almost exclusively in matrixes with high water content	
	- SIDA provides accurate determination of DON levels in sorghum samples	- The traditional method produced inconsistent DON values following controlled spiking and recovery experiments with different sorghum accessions	
	- Small quantities of reagents are used		
	- The possibility of getting the smallest detection limits for some mycotoxins (DON, 3AcDON, 15AcDON and NIV) below the legislated levels in cereals		

GC–MS is not frequently used for the determination of mycotoxins due to their limited volatility and low polarity. The acetonitrile phase to be tested requires a clean procedures to remove lipids and water before GC–MS analysis. A derivatization step is mandatory to turn the mycotoxins into volatile compounds suitable for GC. The adoption of GC–MS/MS instead of GC–MS offered many insights by achieving low LODs of mycotoxins (below the legislation levels) in solid food and feed and by improving the recovery rates (Cunha and Fernandes 2010; Eyring et al. 2021; McMaster et al. 2019; Stanciu et al. 2019).

Analytical issues concerning modified mycotoxins

Mycotoxins may be subjected to metabolism or modification reactions by organisms such as plants, animals, or microbes or by food processing steps, causing their chemical alteration and the formation of the so-called modified mycotoxins (Kovač et al. 2018; Rychlik et al. 2014). These modified forms of mycotoxins are not detectable by routine and conventional analysis methods such as ELISA, and the established legislation did not determine their maximum limits in food and feed (Berthiller et al. 2013; Leite et al. 2021). Modified mycotoxins are generated in free (extractable conjugates) and bound (non-extractable conjugates) forms by binding on food components such as proteins or carbohydrates (Berthiller et al. 2013; Mousavi Khaneghah et al. 2019). The concern of these mycotoxins lies in their potential to be converted into the parent mycotoxins when digested by humans or animals causing an adverse health effects (Freire and Sant'Ana, 2018; Gratz 2017; Zhang et al. 2020). The modification of these mycotoxins affects their chromatographic properties and extraction efficiency (Kovalsky et al. 2016). LC–MS/MS is the reference method for the detection and quantification of modified mycotoxins thanks to its selectivity and sensitivity, in addition to the capability of LC–MS/MS method to elicit their structure. The prerequisite of this analysis is the setup of the extraction and clean-up steps (Freire and Sant'Ana 2018).

In this critical review, we screened, evaluated, and discussed many studies focused on the most commonly used analytical methods of mycotoxins in solid food and feed. In conclusion, TLC and ELISA are considered as rapid and simple screening methods, but ELISA showed better sensitivity and accuracy than TLC. When these two methods were used, they required further analysis using robust and more sensitive techniques for validation. HPLC-FLD and HPLC–UV are the most commonly used in the analysis of mycotoxins containing chromophores in their structure such as AFs and OTA. Nevertheless, a derivatization step must be adopted like the case of FBs. FLD-ELSD is an efficient alternative of HPLC-FLD because it requires a simple, fast, and cost-effective sample

pretreatment. GC–MS and GC–MS/MS are rarely used in the case of mycotoxins analysis as a result of their high polarities and limited volatilities. A derivatization step by silylation or acylation could be performed in order to decrease mycotoxins polarities and generate volatile derivatives suitable for GC. This step requires the use of organic solvents with an increase in the chemical waste. LC–MS/MS is the avant-garde technique due to its ability to detect multiple mycotoxins in food and feed matrices. Its high performance has been optimized by the selection of suitable extraction solvents and methods, and the adoption of the appropriate clean-up procedures, in addition to the setting of MS/MS parameters. The acidification of the acetonitrile/water mixture showed good capabilities for many mycotoxins with different polarities. According to the mycotoxins to be tested, different procedures have been used such as dilute-and-shoot, QuEChERS method, and WAHSPE methods and IAC. The majority of studies used the triple quadrupole (QqQ) analyzer operated in multiple reaction monitoring (MRM) or selected reaction monitoring (SRM) and ESI as ionization mode. Both modes increased the sensitivity of the analysis and achieved low LODs.

Declarations

Conflict of interest The authors declare no conflict of interests.

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