Chapter

Chromatographic Techniques for Estimation of Aflatoxins in Food Commodities

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Abstract

Aflatoxins, produced mainly by Aspergillus *flavus* Aspergillus *parasiticus*, have been documented as one of the major food contaminants throughout the world. Because of their toxic nature, these food contaminants have acknowledged considerable attention in recent years. Among the different types of Aflatoxins, the most prevalent and predominant Aflatoxins are AFB1, AFB2, AFG1, AFG2, AFM1, AFM2 which are considered the more lethal as compared to others. Several analytical and immunological methods are available for testing and estimating aflatoxins in different food commodities. However, chromatographic techniques have been considered superior regarding the estimation of aflatoxins both qualitatively and quantitatively. Chromatographic techniques have numerous applications for the separation and identification of chemical and biological compounds in food industry. It has grown to be the most popular and versatile of all analytical techniques in laboratories used for the analysis of multiple components in different matrices. For preliminary qualitative detection of Aflatoxins, Thin layer chromatography (TLC) is considered the best analytical technique which is being used broadly in food industry. However, liquid chromatographic techniques including High Performance Liquid Chromatography (HPLC) and Liquid chromatography-mass Spectrometry (LC–MS) are the best analytical techniques developed so far for the quantification of Aflatoxins in food commodities.

Keywords: Food, Aflatoxins; TLC, HPLC, LC–MS

1. Introduction

Aflatoxins are toxic substances formed by certain kind of fungi (molds) that have the potential to contaminate food, feed, crops and pose a serious health risk to humans and livestock. Aflatoxins are also assumed to be responsible for the annual loss of 25% or more of the world's food crops, which has significant economic implications. Various procedures for the detection and analysis of aflatoxins are available in feed and food, as they are highly specific, practical, and useful [1].

Aflatoxins are cancerous secondary metabolites produced primarily by Aspergillus *flavus* and Aspergillus *parasiticus* in foodstuff. Basically the chemical composition of aflatoxins contain the difurano-coumarin molecules which are synthesized following the polyketide pathway [2]. Eighteen different types of aflatoxins have been identified however six are well-known and recognized as B1, B2,

G1, G2, M1, and M2, respectively, [3]. Aflatoxins B1, B2, G1 & G2 found in different kinds of food & feed which are metabolized in animal body and then excreted mainly via milk as aflatoxins M1 and M2. All these aflatoxins have molecular differences; the aflatoxin B-group (B1 and B2) contains the cyclopentane ring which shows blue fluorescence under ultraviolet (UV) light whereas the aflatoxin G-group (G1 and G2) comprises the lactone ring and shows yellow-green fluorescence under UV light [4]. The different color fluorescence is important for identifying and differentiating between the aflatoxins B & G groups. Aflatoxin B1 is most commonly found in different kind of food matrixes [5] and widespread maximally [6, 7] in the world and accounts for 75% of all aflatoxins contamination in food commodities [8]. Aflatoxins M1 and M2 are hydroxylated products (metabolites) of aflatoxins B1 and B2, respectively, which are concomitant with animal milk upon ingestion of aflatoxins B1 and B2 contaminated feed. Furthermore, once converted from B1 and B2 forms, aflatoxins M1 and M2 remain stable during milk processing [9].

To protect consumer health, maximum levels (MLs) for mycotoxins in foodstuffs have been established worldwide. In particular, the European Union legislation (often considered as the most stringent one) has established MLs for aflatoxins [10].

International organizations for example AOAC (Association of Official Analytical Chemists), CEN (European Committee for Standardization) and ISO (International Organization for Standardization) have continued rendezvous experts over the years to develop internationally recognized analytical standards. The main objective is to evade the discrepancies in outcomes that may arise from the use of different analytical methodologies, with the risk to partial worldwide food trade. Currently, seventy-two official methods are offered from these organizations for scrutinizing the mycotoxins in food commodities.

A variety of methods to detect aflatoxins in food and feed are available for different needs and different techniques for their detection and analysis have been extensively researched to develop those that are highly specific, useful and practical.

Owing to its common incidence and toxic nature, numerous analytical and immunological methods were developed. However, there are minor modifications actually in most of these analytical methods from the officially adopted basic methods for certain food commodities. They differ only in the analytical techniques used for assessing the strength of fluorescence of the analyzed mycotoxins and in the extraction solvents used to extract the mycotoxins from different food matrixes. A plethora of methods are available for different needs, ranging from techniques/methods for regulatory control in Official laboratories starting from simple rapid test kits (AgraStrip®, CHARM EZ-M) to advanced methods [including immunochemical methods comprises radioimmunoassay (RIA), Enzyme-Linked Immunosorbent Assay (ELISA), Immunoaffinity column assay (ICA), Immunodipstick and immunosensors; Spectroscopic methods including Fluorometer, Spectrophotometer, Fourier-transform infrared spectroscopy (FTIR), Quartz Crystal Microbalances (QCMs), Surface Plasmon resonance Spectroscopy (SPRS); and some Chromatographic methods such as Thin Layer Chromatography (TLC) with densitometer, High-performance Thin Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Liquid Chromatography-Mass Spectrometry (LC–MS)] for factories and grain silos (**Table 1**).

Quickness and effortlessness in analysis are the other features that have gain worldwide consideration recently. When a large number of samples have to be analyzed in a short time period then enzyme-linked immunosorbent assay (ELISA), mini-column quick methods, and radio-immunoassay (RIA) techniques may be used.

Method	Sample preparation	LOD	Field Applicable	Reference
AgraStrip®	Simple extraction with Methanol	4 ppb	Appropriate	[11]
Immunodipstick	Extraction only	5 μg/Kg	Appropriate	[12]
CHARM EZ-M	Water based extraction	1 ppb	Inappropriate	[13]
Fluorometer	IAC	5–5000 μg/Kg	Inappropriate	[14]
TLC densitometer	Liquid Extraction, SPE	1–20 ng/kg	Inappropriate	[15, 16]
HPTLC	Liquid Extraction	Pictogram	Inappropriate	[17]
RIA	Liquid Extraction	1 μg/Kg	Inappropriate	[18]
FTIR	Liquid Extraction	<10 μg/Kg	Inappropriate	[19]
ELISA	Liquid Extraction	1 μg/Kg	Inappropriate	[20]
HPLC	IAC or SPE	0.5 μg/Kg	Inappropriate	[21]
LC-MS	Liquid Extraction	0.1 μg/Kg	Inappropriate	[22]
QCMs	Liquid Extraction	0.01–10 ng/mL	Inappropriate	[23, 24]
SPRS	Liquid Extraction	3.0–98 ng/mL	Inappropriate	[25]
Electrochemical	Liquid Extraction	0.1–2 μg/Kg	Inappropriate	[26, 27]

Table 1.
Evaluation of different methods for analysis of aflatoxins in food and feed.

Potential innovative aflatoxins-detection techniques, based on the emerging techniques, include electronic noses, dip-stick kits, molecularly imprinted polymers, hyper-spectral imaging, and aptamer-based biosensors (small organic molecules that can bind specific target molecules). The latter techniques may have significance in remote areas because of their use, stability and ease of production. However, any method recommended for aflatoxin analysis should be economical and convenient to the handlers, taking into account their available laboratory facilities, as well as providing greater accuracy in the results.

All analytical methods for aflatoxins involve basically the same steps: sampling and sample extraction, clean-up, work-up, detection, and confirmation, as well as estimation of the toxin.

2. Sampling procedures are problematic

Adequate sampling techniques as well as appropriate sample preparation procedures are the most significant steps before performing the chemical analysis of aflatoxins. Aflatoxins are present in only a few grains and kernels obviously and have highly crooked distribution in food and feed commodities therefore, some variations in analytical results might be possible if the sample collected for analysis is not representative of the bulk [28–31].

As molds and aflatoxins aren't equally dispersed all through the bulk shipments and batches of stored grain, proper sampling is essential for obtaining a representative result. Proper protocols for sampling have been established, particularly in the perspective of regulatory control. For example, the Codex Alimentarius Commission has set the protocols to be used for various food commodities in setting maximum levels for aflatoxins. The United Nation's Food and Agriculture Organization (FAO) has established a mycotoxins sampling contrivance that is available on-line. The use of recommended sampling methods is a problem,

especially for subsistence farmers in rural areas who do not produce enough grain to allow for accurate testing. As a result, to improve surveillance and control in rural areas, low-cost, rapid and low-technology aflatoxins detection techniques are required. Food organizations trying their best to control aflatoxins in Africa and the World Food Programme's are also addressing these issues, for example, the World Food Programme has introduced the appropriate Purchase guidelines to ensure grain quality.

A precise and accurate sample can be selected by collecting a representative sample in large quantity and then dividing it into three equal parts. Differences in weight of selected samples may also be critical which depend on the regulations of a specific country. For example; the United Kingdom (UK) has proposed a sample weight of 10.5 kg, while the United States (US) has recommended the sample weight of 66 kg, greatly a larger amount. However, an average sample weight of 5–10 kg has been adopted by most of the countries. Precise grinding and sub-division of the sample would also be critical for accurate determination of aflatoxins. Spinning riffles, rotary sample divisors, and cascade samplers may also be used to select the representative sub-samples [31–33]. The size of the sub-samples may vary from 20 to 100 g. However, in most of the methods 50 g sample was used for analysis of aflatoxins, which looks to be the best in terms of economy in using costly extraction solvents.

3. Aflatoxins extraction and clean-up methods

The frequently used extraction and clean-up techniques for aflatoxins analysis are liquid–liquid extraction (LLE), solid-phase extraction (SPE) and "Quick, Easy, Cheap, Effective, Rugged, and Safe" (QuEChERS) methods. Furthermore, some other extraction methods are also offered in the literatures that are not commonly used in routine analysis at the moment.

3.1 Liquid–Liquid extraction (LLE)

Liquid–liquid extraction procedures are the simple, easy and cheap methods for the extraction of aflatoxins. It is based on the partition coefficient and different solubility properties of the mycotoxin in the organic or aqueous phase or in their combination mixtures. However, the shortcomings of these extraction techniques are that it does not provide appropriately clean analyte in all cases.

An efficient extraction method is required for the qualitative detection and quantification of aflatoxins in food and feed samples. Aflatoxins are commonly soluble in the polar-protic solvents like acetone, acetonitrile, chloroform and methanol. Hence, aflatoxins can be extracted by using either any of the mentioned pure solvents or in combination of these solvents as well as with small quantity of water [34, 35]. Several studies have been conducted on different food matrices to determine the extraction efficiency of various aqueous-organic solvents [36–38] and the different extraction recoveries have been reported. Since methanol has a minor negative effect on antibodies than other organic solvents like acetone and acetonitrile therefore aflatoxins extraction using a mixture of methanol with water (e.g.; 8 + 2 v/v) [37, 39] is required for determination of aflatoxins on immunoassay technique.

3.2 Liquid–Solid extraction (LSE)

Liquid–solid extraction technique is another simple and easy extraction method for the extraction of aflatoxins using solid matrices of different consistency.

Initially, the selection of an appropriate and the most effective extraction solvents is a crucial step to extract the component of interest. Most frequently used extraction solvents are mixtures of methanol/water or acetonitrile/water in different ratios [40]. For instance, the 80% methanol/water mixture proved to be the most optimal for extraction of aflatoxins in the case of nutmeg samples. The choice of methanol for further use is also preferable, because the antibodies better tolerate higher concentrations of methanol than acetonitrile. Methanol was also suitable for chromatographic separation, as aflatoxins were measurable without interference [41]. The extraction efficiency is significantly influenced by the composition of the extraction agents, the sample/solvent ratio, and the time of extraction. Sometimes, the use of only LSE method is inadequate to extract aflatoxins without interference and additionally some purification step(s) are required for proper extraction. The extraction process comprises the different steps including the weighing of homogenized sample which will be properly grind having appropriate particle size, addition of suitable extraction solvents and then dissolution or disintegrating the mixture applying, e.g., vortex, blender, shaker, or other approaches to extract the required components. After extraction, sample is filtered and cleaned prior to analysis.

3.3 Ultrasound extraction

Liquid–solid extraction efficiency can substantially be improved with the use of ultrasound. In the ultrasound extraction process the container (e.g., flask, centrifuge tube or vials) containing the sample to be extracted and the extraction solvent is most often immersed into an ultrasonic bath that contains water. After a few minutes, the acoustic cavitation under the influence of ultrasonic field in liquids significantly increases the transfer of the analytes and matrix components from the sample to the extraction solvent, thereby increasing the recovery and efficiency of extraction [42].

3.4 Supercritical fluid extraction (SFE)

Supercritical fluid extraction uses a supercritical fluid for the extraction of the required compound from the matrix. The SFE procedure is mainly used efficiently for the extraction of apolar organic molecules [43]. During the extraction of polar aflatoxins with SFE a number of problems have arisen, e.g., low recoveries and high concentrations of co-extracts. Furthermore, lipids may cause difficulties during further cleanup and chromatographic separation [44].

3.5 Solid phase extraction (SPE)

Solid phase extraction techniques are considered the most accurate and reliable approaches to clean-up the mixtures before qualitative and quantitative estimations. With the help of SPE, required analyte can be separated and unwanted components which may interfere during analysis can be removed accurately. Two types of SPE are used.

SPE is a multi-step process, starting from the conditioning then followed by the sample loading, washing and at the end elution of required analyte. In the SPE, the required analyte either bound to the matrix component(s) or removed from the sample [45]. Various extenders are used in the SPE columns. Aflatoxins are often analyzed by using C-18 (octadecylsilane) column. A specific application of SPE is the so-called immunoaffinity clean-up columns (IAC) and Multi-functional clean-up columns (MFC) including MultiSep®, MycoSep®, and Myco6in1 column [46]. The extraction of aflatoxins is usually followed by a cleanup step. The common

cleanup technique used is immunoaffinity column (IAC) chromatography and Mycosep multi-functional cleanup (MFC) columns [47]. These purification techniques are considered the best choice for isolation of target analyte (like aflatoxins) and to clean-up or remove the unwanted components before their quantitative estmation using HPLC [48].

Immunoaffinity chromatographic technique proved to be the accurate and highly specific which reversibility of binding between an antigen and antibody to isolate, purify and separate the target molecule from matrices [49]. During the cleanup process, the extracted liquid sample is applied to the IAC which holding the specific antibodies to bind with aflatoxins that immobilized on a solid surface such as silica or agarose. As the extracted sample moves down the IAC column, the aflatoxins bind to the antibodies and are retained onto the column. To remove the unbound proteins and impurities washing step is generally required using appropriate ionic strength buffers or distilled water. Thereafter, the aflatoxins are recovered or removed from the IAC by using pure solvents like acetonitrile or methanol which breaks the bond between the antibody and the aflatoxins.

Mycosep multi-functional cleanup (MFC) columns are also recognized a best approach for purification of aflatoxins. It is simple, easy, handy to use and a rapid one-step purification technique. These columns are designed to retain certain groups of basic compounds that may create interferences in HPLC analysis. On the other hand, MFC purification columns allow the molecules of interest to pass through the columns. During the MFC cleanup procedure, after extraction of aflatoxins using suitable solvents a portion of the extract is passed through an MFC column designed particularly for aflatoxins analysis. Compounds that may create interferences are retained in MFC, whereas aflatoxins pass through the column. Ideal recovery (> 95%), precision and coefficient of variation (< 3%) of aflatoxins were observed by these columns [50].

4. Work-up

After the clean-up step, the extract must be worked up to make it suitable for the estimations. The purified pooled extract can be treated with sodium sulphate (anhydrous) to remove the moisture if present in the extract. To concentrate the extracted solvent evaporated it to dryness using nitrogen stream or in a rotary evaporator at 50°C. On the other hand, evaporation of solvents can be achieved with the help of steam bath under the nitrogen stream preferably. Finally, reconstituted the residues using pure organic solvents like acetonitrile or methanol and used for estimations.

5. Methods for detection and quantification of aflatoxins

The most commonly used chromatography techniques for analysis of aflatoxins are Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Gas chromatography (GC) and Liquid Chromatography-Mass Spectrometry (LC–MS). Although many of the chromatographic techniques are very sensitive, they require trained skilled technician, cumbersome pretreatment of sample, and expensive apparatus/equipment [51].

5.1 Thin-layer chromatography (TLC)

Thin-layer chromatography is one of the most widely used separation techniques for detection of aflatoxins. TLC has been regarded by the Association of Official

Analytical Chemist (AOAC) as the method of choice since 1990. It consists of a solid immobilized stationary phase may contain either alumina or cellulose or silica on an inert material such as plastic or glass, called the matrix. The mobile phase is contained of acetonitrile: methanol: water mixture [52], which brings the sample along as it moves through the stationary phase. In TLC, aflatoxins are distributed between the mobile phase and stationary phases on the basis of partition coefficient or differences in solubility of the analytes in the two phases. Different types of aflatoxins (B1, B2, G1 & G2), according to their interaction with the stationary and mobile phases as well as due to the different molecular structures, either adhere to the solid surface of stationary phase more or remain in the mobile phase, thus allowing for effective and quick separation. TLC technique has been commonly used in food industry for the determination of aflatoxins [53–55] and detection limit of 1–20 ppb of different types of aflatoxins has been reported. The major advantage the TLC is that it can detect different types of mycotoxins with good resolution and excellent sensitivities [56]. It also requires pre-treatment of sample, skilled and trained technician, and expensive equipment as well [57]. In addition, there are also some drawbacks of TLC which may probably be occurring during spotting, TLC plate development, and interpretation.

Quantification of aflatoxins on TLC plates using fluoro-densitometer is considered to be a more precise and accurate method than visual estimates [58] with the minimum limit of detection (LOD) is 1 μg/kg. Although fluoro-densitometers are commercially available, but not commonly used due to its high cost and visual fluorescence identification method is still to be continue for identification of aflatoxins [59].

Attempts to improve TLC have led to the development of automated form of TLC, called the high-performance thin-layer chromatography (HPTLC).

5.2 High performance thin layer chromatography (HPTLC)

The conventional TLC method has improved through the automation of sample spotting, plate development and interpretation in HPTLC. Currently, HPTLC is one of the best analytical methods for estimation of aflatoxins [60, 61].

Automated sample applicator, digital scanner, and a computing integrator, lead to improve the sensitivity and precision in the quantification of aflatoxins. The other benefit of HPTLC method is the use of minimum amount (only 1 μl) for sample spotting, instead of 10-20 μl used for the conventional TLC method. With the use of HPTLC minimum concentrations of aflatoxins (5 pg) can be possibly detected [59].

However, the costly equipment, extensive sample treatment procedure and the requirement for skilled researcher, limit the HPTLC technique to the laboratory and thus it is inapplicable in field situations.

5.3 High-performance liquid chromatography (HPLC)

The most commonly used chromatographic technique for separation and determination of organic compounds is High Performance Liquid Chromatography (HPLC). Worldwide, approximately 80% of all organic compounds are estimated using HPLC [62].

The HPLC technique for estimation of aflatoxins has high automation, high sensitivity and high precision. There are two types of phase systems comprising normal phase (wherein mobile phase: non-polar & stationary phase: polar) and reverse phase (wherein mobile phase: polar & stationary phase: non-polar)) in combination with UV/VIS absorption and fluorescence detection. Reverse phase HPLC is broadly used for estimation of aflatoxins [59].

In HPLC, the stationary phase is confined to either a plastic or glass tube and the mobile phase containing the organic/aqueous solvents that pass through the solid adsorbent. The sample to be examined is introduced on top of the column which passes through and distributes between both the stationary and mobile and phases.

The components present in the sample move through the column with the mobile phase at different speeds because of their different relative affinities and interactions. Separate fractions containing individual components in the sample elute from the HPLC column at different rates. The HPLC technique involves the use of a stationary phase (polar or non-polar columns), a pump that moves the mobile phase(s) through the column or other parts of HPLC at constant flow rate, a degasser to remove the trapped gases or air bubbles in the mobile phase, a detector to quantify the analytes and read out device to display the retention times of individual components.

Reversed phase chromatographic mode is most commonly used in HPLC for the identification and quantification of aflatoxins. Chemical derivatization of aflatoxins B1 and G1 typically required to improve the sensitivity because the natural fluorescence of aflatoxins B1 and G1 may be inadequate to meet the necessary detection limit [63]. **Figure 1** depicts derivatization reactions of aflatoxin B1 with the acid and halogens. In the first reaction, Trifluoro Acetic Acid (TFA) hydrolyzes the second furan ring of aflatoxin B1 to produce highly fluorescent aflatoxin B2a, while bromine and iodine are used as chemical reagents in the second and third derivatization reactions, respectively. When these halogens react with aflatoxin B1, they produced highly fluorescent aflatoxin B1 derivatives.

HPLC provides quick, accurate and reliable aflatoxins results within a short time. FLD has been presented an excellent sensitivity of 0.1 ng/kg [65]. However, the shortcoming of using HPLC to analyze the aflatoxins is the requirement of laborious purification columns to clean-up the sample. Furthermore, HPLC involves the tedious pre-column or post-column derivatization processes to improve the sensitivity of aflatoxins [62]. To overcome the challenges of derivatization processes

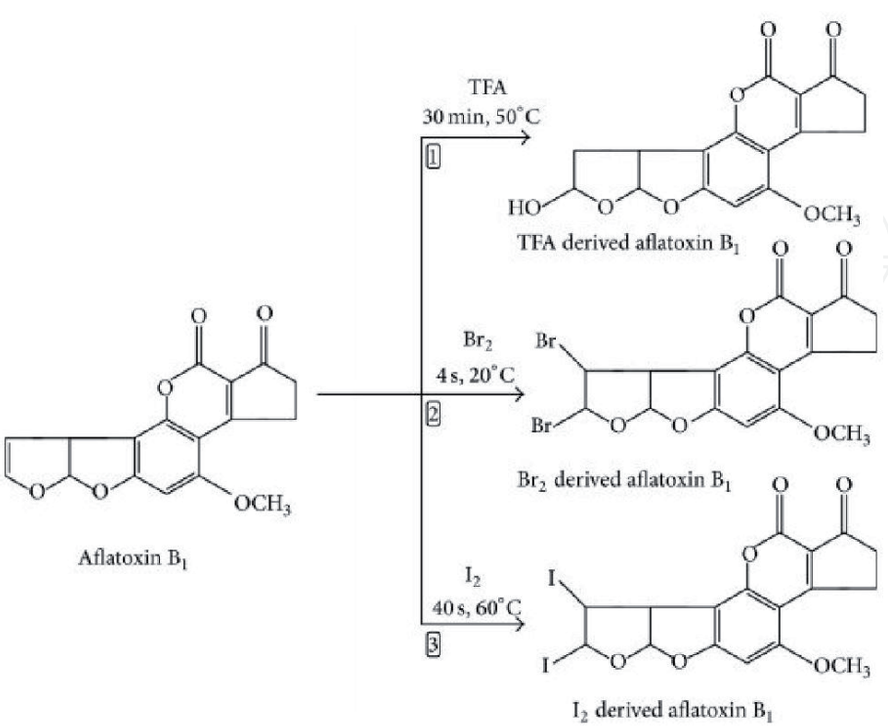

Figure 1.
Derivatization of aflatoxin B1 with trifluoroacetic acid, bromine (Br_2) and iodine (I_2) [64].

in aflatoxins testing, a modification of the HPLC protocol in which the HPLC is coupled to mass spectrometry has been developed and is currently used in aflatoxin determination [66].

Since the mass spectrometry does not requires the use of UV/VIS fluorescence or absorbance of analyte, thus chemical derivatization of compounds is no longer required. HPLC–MS/MS produces structural information using small amount of sample and has low detection limits developed up to now [21]. On the other hand, HPLC–MS/MS is costly equipment that can only be handled by trained, qualified and professional person. Furthermore, this also restricts its use to only well-equipped laboratory environment and not field conditions.

5.4 Liquid chromatography-mass spectrometry (LC: MS)

Although different HPLC methods are available for quantitative determination of aflatoxins with selective sample clean-up techniques, still the methods are required to confirm the identity of the substances. A method other than the commonly used UV/VIS and fluorescent methods, for the confirmation is mass spectrometry method that coupled with HPLC.

LC–MS technique has become the fastest growing technique available for analysis of mycotoxins. The potential benefits of LC–MS technique for mycotoxin analysis have long been recognized and exploited. Simultaneous determination of multi-mycotoxins can be possible with LC–MS according to the mass to charge ratio (m/z) of analytes, an intrinsic property that provides more specific identification based on molecular weight of the target analyte. The impact of modern LC–MS technique has been signified by the unmatchable sensitivity in quantitation, specificity in identification and number of mycotoxins that could be analyzed in one analysis [67].

A modern LC–MS instrument, particularly LC–MS-triple quadrupole (LC–MS-QQQ), has been developed and introduced with increasing sensitivity for quantitative analysis of mycotoxins. Despite high capital costs of LC–MS instruments, many efforts have been exerted to quantitate aflatoxins using this technique [68].

5.5 Gas chromatography (GC)

In gas chromatography, an inert gas is used as the mobile phase instead of liquid and the stationary phase may be a liquid coated onto inert solid particles or solid. GC analysis, like other chromatographic approaches, is based on the differential partitioning of analytes between the two phases. The stationary process is made up of inert particles covered with a liquid layer that confined in a long stainless steel or glass tube known as a column fixed in oven to maintain the specific temperature. The sample to be tested is vaporized into a gaseous form and transported by a carrier gas into the stationary phase.

The different chemical components within the sample will distribute themselves between the stationary phase and mobile phase. Components of the sample mixture with a higher affinity for the stationary phase travel through the column more slowly, while those with a lower affinity move through the column faster. Each portion of the analyte should, in reality, have its own partition coefficient, which will dictate how quickly it passes through the column [69]. After the separation of volatile compounds, these are detected using a universal GC detector known as Flame Ionization Detector (FID) or an Electron Capture Detector (ECD) and the most recent and advanced mass spectrometer (MS) detector [70].

Since aflatoxins are non-volatile, thus derivatization will be required to be detected [71]. However, GC is not commonly used in commercial analysis of aflatoxins because some other cheaper and simple chromatographic techniques are existed [72]. Furthermore, gas chromatography is limited to the analysis of a few mycotoxins, such as A-trichothecenes and B-trichothecenes, due to the requirement of preliminary cleanup step prior to analysis. GC technique has some other disadvantages including drifting responses, non-linearity of calibration curves, memory effects from previous samples, and high variation in repeatability and reproducibility [73].

Earlier, gas chromatography mass spectrometry with negative ion chemical ionization has been used for confirmation of aflatoxin B1 [74], injection was applied using an on-column injector, which is necessary because of the thermos-lability of the aflatoxins. Gas chromatography mass spectrometry have also been used with electron impact for aflatoxins B1, B2, G1 and G2 [75].

6. Conclusions

Several qualitative as well as quantitative methods have been explored for analysis of aflatoxins in food commodities, crops and feeds. Among all the different developed methods, chromatographic techniques are considered the most appropriate methods in aflatoxins analysis. Analytical methods based on immunochemistry and spectroscopy have also been added to the chromatographic methods, some of which emerged as better alternatives for routine and on-site estimation of aflatoxins. Although a large number of analytical techniques are constantly being optimized, the LC/MS/MS technique is considered the most valuable confirmation technique for analyzing multiple mycotoxins as it is high specific, sensitive, accurate and reliable.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication.

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