

REVIEW

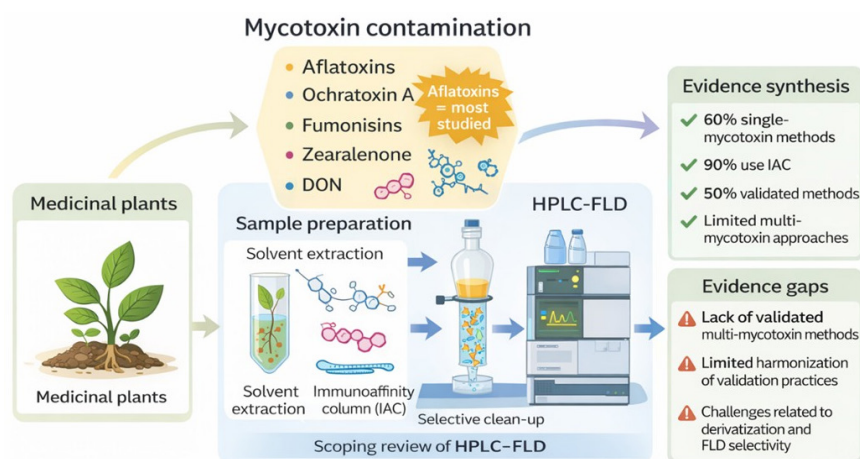
HPLC-Based Analytical Methods for the Determination of Mycotoxin Contaminants in Medicinal Plants: A Scoping Review

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Mycotoxins are toxic substances produced by fungi and can occur in medicinal plants. Among them are aflatoxins, ochratoxins, fumonisins, zearalenone, and deoxynivalenol, which can be quantified by HPLC analysis. The objective of this scoping review was to evaluate HPLC-based analytical methods using fluorescence detection and diode-array detection (HPLC-FLD and HPLC-DAD) for the quantification of mycotoxins in medicinal plants. The study was conducted in accordance with the Joanna Briggs Institute

recommendations. 3,939 articles were identified, of which 30 were selected for review. Among the selected papers, 27.6% were published in China, where the use of medicinal plants is common due to Traditional Chinese Medicine. Of the 30 articles, 23 investigated aflatoxins, a group of mycotoxins with the highest health risk. Furthermore, 60% of the publications mention the development of an analytical method for only a single group of mycotoxins, highlighting the difficulty of developing multi-mycotoxin methods with the detector used. Of the articles included in the scoping review, 90% involve solvent extraction followed by immunoaffinity columns. Mobile phases consist mostly of a composition of water, methanol, and acetonitrile in varying proportions. Half of the articles reported performing analytical method validation, with the main parameters evaluated being limit of detection, limit of quantification, precision, and linearity.

Keywords: fluorescence detection, immunoaffinity columns, sample preparation, chromatographic analysis, method validation

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INTRODUCTION

Mycotoxins are toxic substances produced by certain fungi, mainly species of *Aspergillus*, *Penicillium*, and *Fusarium*.¹ In medicinal herbs, the most important mycotoxins are aflatoxins, ochratoxins, fumonisins, trichothecene (e.g. deoxynivalenol, HT-2 toxin, T-2 toxin), and zearalenone.¹ These substances are often found and are highly toxic. Aflatoxins, such as AFB1, AFB2, AFG1, and AFG2, are produced by certain *Aspergillus* strains and are among the most potent natural carcinogens and mutagens.² Ochratoxins, produced by *Aspergillus* and *Penicillium*, come in three types: A, B, and C, with ochratoxin A being the most common and toxic, causing cancer and weakening the immune system.² Fumonisins, mainly produced by *Fusarium verticillioides*, are considered potential human carcinogens, with fumonisin B1 being the most toxic.³ Zearalenone is a mycotoxin that acts like estrogen and is stable at high temperatures, showing fluorescence under UV light.⁴ Overall, aflatoxin B1, ochratoxin A, and fumonisins are considered the most hazardous to human and animal health, causing cancer, genetic mutations, liver damage, birth defects, and weakened immune systems.⁵

Herbal medicines include various plant parts such as flowers, fruits, leaves, seeds, stems, and roots, which can be used fresh or dried.⁶ However, these raw materials are highly vulnerable to fungal contamination during cultivation, harvesting, processing, and storage.⁷ Fungal growth not only reduces yield and quality but also leads to the accumulation of mycotoxins, posing serious risks to safety and therapeutic effectiveness. Therefore, effective measures for prevention, monitoring, and quantifying mycotoxins in medicinal plants are crucial for maintaining product quality and consumer safety.⁷

Liquid chromatography (LC) has become the analytical technique of choice for mycotoxin determination in complex matrices, due to its sensitivity and selectivity.⁸ Common detectors include diode array (LC-DAD), fluorescence (LC-FLD), and mass spectrometry (LC-MS/MS), the latter being the current gold standard. Although LC-MS/MS is currently considered the gold standard for mycotoxin determination due to its high sensitivity, selectivity, and suitability for multi-mycotoxin analysis, this technique was not included in the scope of the present review. The focus was intentionally restricted to HPLC-based methods using fluorescence detection and diode-array detection. This decision was based on the continued relevance of HPLC-FLD and HPLC-DAD in routine quality control laboratories, particularly because these techniques are generally less expensive, more widely available, and operationally simpler than LC-MS/MS.⁸ Therefore, this review does not aim to replace or compare mass spectrometry-based methods, but rather to map the evidence on the use of HPLC-FLD and HPLC-DAD for mycotoxin determination in medicinal plant matrices.

Critical to all LC-based methods is sample preparation, which involves extraction and clean-up to eliminate matrix interferences.⁸ Solid-phase extraction (SPE), especially with immunoaffinity columns (IAC), is the most frequently reported clean-up strategy due to its high selectivity and specificity.⁹ Because some mycotoxins, such as fumonisins, lack natural chromophores or intrinsic fluorescence, derivatization is required to enhance FLD detection.⁹ Strategies include pre-column derivatization with trifluoroacetic acid (TFA), post-column chemical derivatization with iodine or bromide, and post-column photochemical derivatization (PCD).^{10,11} Differences in fluorescence intensity between aflatoxins (B1/G1 versus B2/G2) can also be optimized through these approaches, improving the sensitivity and reliability of quantification.

The presence of mycotoxins in medicinal herbs poses a significant safety concern, and regulatory agencies and pharmacopeias recognize the need to control them in herbal materials. In Brazil, RDC No. 26/2014 establishes that, for the registration of herbal medicines or the notification of traditional herbal products, mycotoxin determination is required when contamination is reported for the species or cited in technical-scientific documentation.^{12,13} In addition, pharmacopeial requirements establish specific limits mainly for aflatoxins.¹³ The European Pharmacopeia sets limits for herbal drugs of not more than 2 µg/kg for aflatoxin B1 and 4 µg/kg for the sum of aflatoxins B1, B2, G1, and G2.¹⁴ The United States Pharmacopeia and the Brazilian Pharmacopeia establish limits of 5 µg/kg for aflatoxin B1 and not more than 20 µg/kg for the sum of aflatoxins.^{13,15}

These regulatory references highlight the need for analytical methods with adequate sensitivity and selectivity for complex plant matrices. Although established limits are predominantly focused on aflatoxins, other mycotoxins remain relevant due to possible co-occurrence and variations associated with geographical

and climatic conditions. In this context, this scoping review provides a comprehensive overview of HPLC-FLD and HPLC-DAD methodologies applied to mycotoxin determination in medicinal plants, highlighting their relevance as routine analytical tools for quantitative quality control.

Methodology

The study was conducted following the Joanna Briggs Institute (JBI) recommendations, and the results were reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses in a scoping review extension (PRISMA-ScR).^{16,17} The study protocol was registered in the OSF platform .

Research methodology

The search was conducted across PubMed, Scopus, and Web of Science databases using terms related to developing a method for quantitatively determining mycotoxins in herbal medicines. Each term was adapted to the selected databases using the Boolean operators “AND” and “OR” (Appendix 1). The research was conducted until August 2025.

Eligibility criteria

The eligibility criteria were based on the PCC (Product, Concept, and Context) framework, as recommended by the Joanna Briggs Institute for scoping reviews. This framework guided the formulation of the following research question: “Which HPLC-FLD and HPLC-DAD quantitative methods have been reported for the determination of mycotoxins (aflatoxin B1, B2, G1, G2, ochratoxin A, fumonisins B1 and B2, trichothecene, deoxynivalenol, and zearalenone) in medicinal plants?” In this review, the product considered was medicinal plants, including fresh or dried materials used in herbal medicines. The concept refers to quantitative analytical methods for determining mycotoxins — specifically aflatoxins, ochratoxin A, fumonisins, trichothecenes, deoxynivalenol, and zearalenone — using HPLC-based approaches with fluorescence or diode-array detection. The context comprised analytical studies focused on the safety and quality control of herbal drugs.

Specifically, we sought scientific articles describing quantitative analytical methods for these mycotoxins in medicinal plants. Articles published at any time frame and in Roman characters were included. Exclusion criteria comprised scientific articles published in non-Roman characters, review papers, studies involving foodstuffs, edible oils, or non-plant material, and studies employing non-quantitative or immunoassay-based methods. Studies using LC-MS/MS or other mass spectrometry techniques were excluded because they fall outside this review’s predefined scope. The focus was specifically on evaluating HPLC-based methods with fluorescence or diode-array detection, due to their suitability for routine quality control and labs lacking mass spectrometry equipment.

Study selection and data extraction

All references were added independently to the Rayyan web app (free version; Rayyan Systems, Inc.) based on the search results.¹⁴ In this platform, the duplicates were identified and excluded. After removing duplicates, each study was independently reviewed by the two authors of this study (MAG and LMF), who assessed the titles and abstracts for inclusion. Studies that met the eligibility criteria were also independently analyzed in full text by two reviewers (MAG and GAMT). The studies that show discrepancies in the reviewers’ decisions were resolved by consensus, and an additional reviewer was consulted if necessary. Unsuitable articles were excluded, and the identification and exclusion reasons were recorded in a table. The inclusion process was documented using an adapted PRISMA flow for scoping review (PRISMA ScR).

Data extraction was performed by the same reviewers who assessed study eligibility. The objective of this stage was to systematically collect all relevant information to address the aims of the present review.

The extracted data were organized in Microsoft Excel® and structured according to the following categories:

- **Publication details:** article title, authors, and year of publication;
- **Study details:** type of mycotoxin analyzed and the medicinal plant matrix or species investigated;
- **Methodological details:** HPLC analysis parameters, limits of detection (LOD), limits of quantification (LOQ), analytical range, and procedures for sample and standard preparation.

The extracted information was synthesized into summary tables and further illustrated through descriptive statistics, charts, and graphs to provide both qualitative and quantitative insights.

RESULTS AND DISCUSSION

After removing 1,264 duplicates, the remaining 2,675 articles were screened by title and abstract according to the predefined eligibility criteria described in the Methodology section. At this first screening stage, the inclusion and exclusion criteria were applied based on the information available in the title and abstract, considering whether the studies addressed quantitative HPLC-FLD or HPLC-DAD methods for the determination of mycotoxins in medicinal plants. Studies that clearly did not meet these criteria were excluded. As a result, 97 manuscripts were selected for full-text assessment.

During the full-text assessment stage, the same eligibility criteria were reapplied in greater detail to confirm whether each article met the scope of the review. After complete reading, 30 articles were included for further discussion, while 67 were excluded. Among the excluded full-text articles, 28 were excluded because they investigated matrices other than medicinal plants, and 39 were excluded because they employed analytical techniques or detection systems outside the scope of this review, namely, techniques other than HPLC-FLD or HPLC-DAD. The process of identification of the studies is depicted in Figure 1.

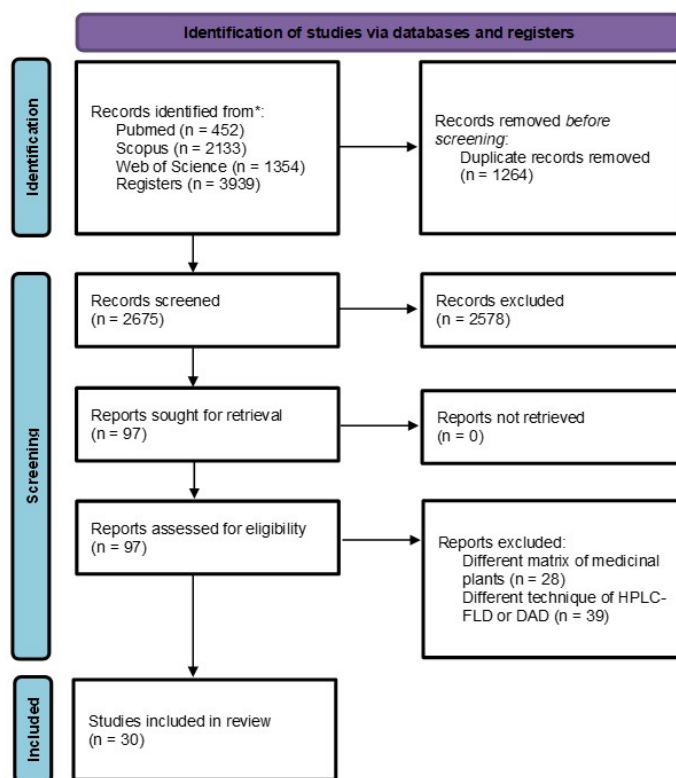


Figure 1. Flowchart of the selection process. Title and abstract screening were performed according to the predefined eligibility criteria described in the Methodology section. Full-text articles were subsequently assessed using the same criteria, and the specific reasons for exclusion were recorded.

The geographic distribution of studies shows a strong predominance of research originating in China (Figure 2A), accounting for nearly one-third of all included publications. This trend may be associated with the long-standing use of Traditional Chinese Medicine and the extensive scientific and regulatory interest in the quality control of traditional Chinese medicinal materials.¹⁹ A relevant contribution also came from European countries, such as Spain and Germany, as well as the United States, each of which contributed multiple studies to the field.

In contrast, fewer studies using HPLC-FLD or HPLC-DAD methods were identified from regions such as Brazil, Iran, and South Africa, despite their rich biodiversity and traditional use of medicinal plants. Within the scope of the present review, this imbalance suggests that fewer studies have reported these specific analytical approaches for the determination of mycotoxins in medicinal plants from these regions. Therefore, additional studies using validated HPLC-FLD and HPLC-DAD methods could help expand the evidence base for routine quality control, while studies employing other techniques, such as LC-MS/MS, may provide complementary information beyond the scope of this review.

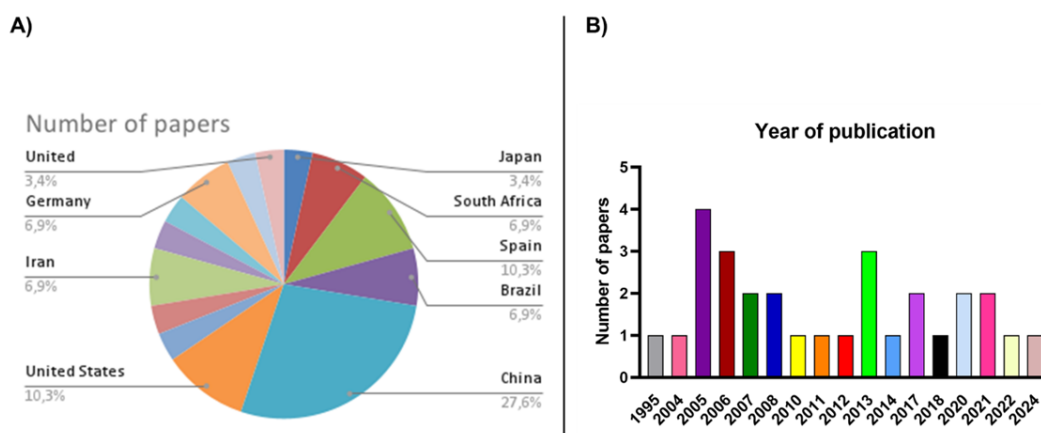


Figure 2. Distribution of the included studies by (A) country of origin and (B) year of publication.

The temporal distribution of publications reveals two distinct peaks of research activity (Figure 2B). The first peak occurred in the mid-2000s (2005–2007), coinciding with a period of increasing attention to mycotoxin control and the broader adoption of HPLC-based analytical methods. A second rise was observed in 2013–2014, which may reflect renewed methodological interest in improving analytical sensitivity and expanding the application of HPLC-FLD and HPLC-DAD methods to medicinal plant matrices.

Despite these peaks, the overall number of publications per year identified in this review remains low, indicating that studies employing HPLC-FLD and HPLC-DAD for mycotoxin determination in medicinal plants are still limited. After 2014, publications within this specific methodological scope became more sporadic, with only isolated contributions in the past five years. Therefore, although mycotoxin monitoring in medicinal plants remains analytically relevant, the evidence mapped in this review indicates that HPLC-FLD and HPLC-DAD approaches have not been consistently reported for this application, highlighting a specific methodological gap for future studies.

Mycotoxins and medicinal plants

Figure 3 shows the distribution of mycotoxins investigated in the studies included in this review. Aflatoxins clearly stand out as the most frequently analyzed group, followed by ochratoxin A and, to a lesser extent, zearalenone. This predominance reflects not only their widespread occurrence in herbal materials but also their well-established toxicological relevance, especially that of AFB1. These belong to Group I and are classified as potential carcinogens by the International Agency for Research on Cancer (2002). For health considerations, some countries and organizations have established reasonable regulatory limits.²⁰ According to the European Pharmacopoeia (2025), the limit for aflatoxin B1 in herbal drugs is not more than 2 µg/kg. A limit of 4 µg/kg is set for the sum of aflatoxins B1, B2, G1, and G2. The United States Pharmacopoeia (2025) has a limit of 5 µg/kg for aflatoxin B1 and a limit of not more than 20 µg/kg for the sum of aflatoxins.

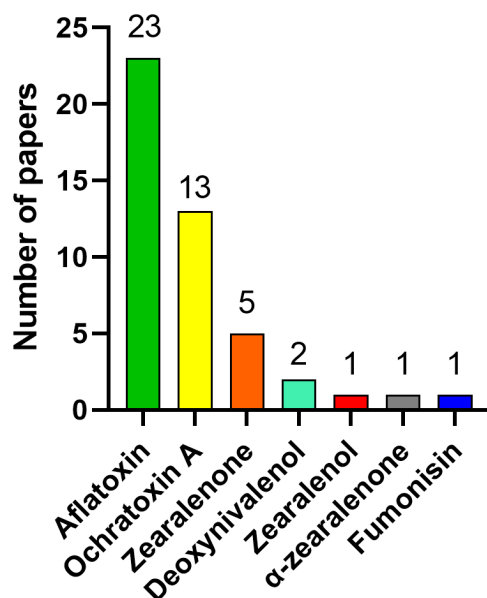


Figure 3. Number of studies reporting different groups of mycotoxins in medicinal plants.

The selected studies investigated between 1 and 4 mycotoxin groups; 60% (18 articles) focused on a single group, whereas 40% (12 articles) analyzed multiple mycotoxins. Table I summarizes the occurrence of different mycotoxins reported in medicinal plants, including contamination levels, plant species or matrix, and the specific plant parts analyzed when this information was available. It should be noted that 57% of the selected studies did not specify the plant part used, which limits a more detailed comparison among plant matrices.

Table I. Occurrence of mycotoxins in medicinal plants according to the included studies

Ref.	Mycotoxin	Contamination	Plant Species	Plant Part
21	AF	AFB1: 0.26 µg/kg AFB2: 0.07 µg/kg AFG1: 0.10 µg/kg	Medicinal plants	–
5	AF, OTA and ZEA	AF: 0.03 to 31.46 µg/kg OTA: 0.2 to 10.09 µg/kg ZEA: 0.1 to 23.35 µg/kg	Medicinal plants	Bark, root, leaves, stem, corm, bulbs, whole plants
22	OTA	1.4 to 252.8 ng/g	<i>Glycyrrhiza glabra</i> L.	Root
10	AF		<i>Monteverdia ilicifolia</i> (Mart. ex Reissek) Biral (sin. <i>Maytenus ilicifolia</i>)	
20	AF	0.26 a 27.52 µg/kg	Medicinal plants	
23	AF	16 ng/g	<i>Panax ginseng</i> C.A.Mey.	Root
24	AF	-	Medicinal plants	Aerial part and root
25	AF and OTA	-	<i>Cannabis sativa</i> L.	Flower
2	AF and OTA	AF: 205.1 to 4,704.94 ppb OTA: 0.11 to 0.53 ppb	Medicinal plants	–

(continued on next page)

Table I. Occurrence of mycotoxins in medicinal plants according to the included studies (continued)

Ref.	Mycotoxin	Contamination	Plant Species	Plant Part
26	AF	–	Medicinal plants	–
27	AF	0.2 to 57.5 µg/kg	Herbs and spices	–
4	ZEA	ZEA: 5.3 to 295.8 µg/kg	Medicinal plants	–
28	ZEA, ZOL, and DON	ZEA 0.52 to 14.51 µg/g ZOL 1.28 µg/g DON 5.60 to 14.45 µg/g	Medicinal plants	Rhizome and root tubers
29	OTA	Extracts: 26 to 141 µg/kg Root material: 8 to 52 µg/kg	<i>Glycyrrhiza glabra</i> L.	Root powder and extracts paste, and powder
30	AF	–	<i>Hibiscus sabdariffa</i> L.	Calyx and bract
31	AF and OTA	AF: 0.67–2.81 µg/kg OTA: 0.54–30.86 µg/kg	<i>Camellia sinensis</i> (L.) Kuntze / Black and green tea	–
32	AF, OTA, ZEA, and DON	AF, DON, and ZEA of 2.364 to 5.714 µg/kg	Medicinal plants	–
33	AFB1	< 1.0 µg/kg	Medicinal plants	–
34	OTA	2.82 – 208.70 µg/kg	<i>Glycyrrhiza glabra</i> L.	–
35	AF	B1: 1.780 ppb B2: 1.333 ppb G1: 1.444 ppb G2: 0.659 ppb	Medicinal plants	–
36	Fumonisin B1 and AFB1	AFB1: not contaminated Fumonisin B1: 8 to 1553 µg/kg	Medicinal plants	–
37	AF	1.7 to 14.3 ng/g	Medicinal plants	–
38	AF and OTA	AF: 0.25 to 16.0 µg/kg OTA: 0.25 to 8.0 µg/kg	<i>Panax ginseng</i> C.A.Mey. and <i>Zingiber officinale</i> Roscoe	–
39	AF and OTA	AF: 1 to 31 ng/g OTA: 1 to 10 ng/g	<i>Panax ginseng</i> C.A.Mey. and <i>Zingiber officinale</i> Roscoe	Root
11	AF and OTA	AF: 3.37 - 5.76 µg/kg OTA: 1.05 - 1.19 µg/kg	<i>Zingiber officinale</i> Roscoe	–
40	AF and OTA	1 to 10 µg/kg	<i>Cannabis sativa</i> L.	Leaves and flowers
41	AF	2.90 to 32.18 µg/kg	Medicinal plants	–
42	AF	6.75 µg/kg	Medicinal plants	Fructus, cortex, radix, and rhizome
43	ZEA	18.7 to 211.4 µg/kg	Medicinal plants	Mature seed

Notes: AF: Aflatoxins; DON: Deoxynivalenol; OTA: Ochratoxin A; ZEA: Zearalenone; ZOL: Zearalenol.

Among all the plants evaluated, contamination levels ranged from 0 to 4,704.94 µg/kg. The highest contamination level was reported by Haq et al. (2024)² for *Justicia adhatoda*. Furthermore, contamination rates varied among studies: Areo et al. (2020)⁵ reported that 86% of the plants examined were contaminated with aflatoxin B1; according to Trucksess et al. (2007),³⁹ 67% of the samples were contaminated; and Pakshir et al. (2020)³¹ reported that 40% of the samples contained aflatoxins. Of the medicinal plants evaluated, 74% of the samples tested positive for ochratoxin A, according to Trucksess et al. (2007).³⁹ The highest contamination level reported was 252.8 µg/kg by Ariño et al. (2007).²² The mycotoxin zearalenone was

detected in 55.62% of the plants surveyed by Zhang et al. (2011),⁴³ with the highest contamination level of 295.8 µg/kg reported by Kong et al. (2013).⁴ Based on the articles reviewed, fumonisins were detected in the tested samples at levels up to 1,553 µg/kg. Furthermore, deoxynivalenol was detected in 6.92% of the medicinal plants evaluated, with a maximum contamination level of 14.45 µg/g.

These findings highlight both the diversity of mycotoxins contaminating medicinal plants and the variability in their distribution across different plant parts. The frequently detected plant matrices include roots, leaves, flowers, bark, and whole plants. Roots and rhizomes are highly susceptible to fungal colonization due to their nutrient composition, which may explain the frequent detection of these toxins in such tissues.²⁸ Underground rhizomes and root tubers are susceptible to mycotoxin contamination during harvesting and storage. In these plant tissues, the production of these toxins can be activated by the high concentration of mineral salts and carbohydrates, including simple sugars.²⁸

The contamination of herbal drugs results from improper cultivation, harvesting, drying, and storage practices. The growth of fungi and the subsequent production of mycotoxins in medicinal plants are substantially affected by environmental factors. Elevated levels of humidity and temperature during both the cultivation and storage periods are particularly conducive to fungal proliferation and the resultant mycotoxin formation.²⁴

It is noteworthy that several studies did not provide quantitative levels of contamination, indicating variability in reporting practices. Nevertheless, the consistent detection of aflatoxins and ochratoxin A across different studies underscores their importance as major contaminants in herbal materials, reinforcing the need for reliable analytical methods for routine monitoring.

Extraction method

Plant samples, both solid and liquid, must undergo solvent extraction, followed by a clean-up step. In the reviewed articles, 10% reported using only Liquid Liquid Extraction (LLE), which employed immiscible liquid phases such as acetonitrile, iso-octane, and chloroform²⁸ or dichloromethane.⁵ In 90% of the articles reviewed, LLE and Solid Phase Extraction (SPE) were employed. The SPE typically involved an immunoaffinity column (IAC). This method is often chosen for aflatoxin analysis due to its operational simplicity and reduced solvent consumption.²⁰

Pre-concentration and sample cleanup are critical steps. They serve to remove matrix compounds and eliminate potential interference from co-extractives,²⁵ thereby ensuring the method's high specificity and efficiency.⁴² Immunoaffinity extraction is a highly selective cleanup method that uses columns packed with a gel matrix. This matrix has antibodies against mycotoxins immobilized on it. When a sample passes through the column, the mycotoxins bind to these antibodies, allowing for a very specific and effective separation process.¹⁰ According to Zhang et al. (2017),⁴² the use of IAC can result in low recoveries when applied to complex herbal matrices.

High acidity in an herbal extract can reduce the binding affinity of a column for aflatoxins, a problem reported by Ip and Che (2006).²⁶ However, this affinity can be improved through several sample preparation methods, including reducing the sample amount, diluting the sample, and adding additives such as PBS and Tween, as noted by Wen et al. (2013).¹¹

In conclusion, the extraction/clean-up strategies proposed above are intended to yield purified extracts with minimum matrix effects, thus facilitating the chromatographic analysis. The quality of the preparative step is a critical step, as it will have a direct effect on the results obtained in the analysis. In the above approach, the choice of chromatographic systems/conditions will be the next step in the approach, as discussed in the *Analytical method* section of this review.

Analytical method

Based on the articles reviewed and summarized in Table II, reversed-phase C18 columns were the most frequently employed stationary phases for mycotoxin determination in medicinal plants. This predominance is consistent with the physicochemical characteristics of several target mycotoxins, which generally present low to moderate polarity and can be retained under reversed-phase conditions. C18 columns provide hydrophobic interactions that allow the separation of structurally related compounds, such as aflatoxins

B1, B2, G1, and G2, while also being compatible with aqueous-organic mobile phases commonly used in routine HPLC-FLD and HPLC-DAD method.⁴⁴

The mobile phases reported in the reviewed studies were predominantly composed of water, methanol, and/or acetonitrile in different proportions.^{2,5,10,20,21,23,24,26,27,30,31,33,39,41,42,45} The selection of these organic solvents is not merely related to cost, but also to chromatographic selectivity, elution strength, viscosity, pressure, baseline stability, and detector compatibility.⁴⁶⁻⁴⁸ Acetonitrile generally provides stronger elution strength in reversed-phase chromatography, lower viscosity, and reduced system backpressure, which may favor sharper peaks and shorter analysis times.^{49,50} Methanol, in contrast, can provide different selectivity due to its protic character and hydrogen-bonding capacity, which may improve the resolution of some analytes depending on the stationary phase and mobile-phase composition.^{49,51} Therefore, the choice between methanol and acetonitrile should be understood as a chromatographic optimization variable rather than only as an economic consideration.

For aflatoxins, the most frequently reported mobile phases consisted of mixtures of water, methanol, and acetonitrile. This composition allows adequate retention and separation of aflatoxins with different fluorescence responses. In some methods, potassium bromide and nitric acid were added to the mobile phase to support post-column derivatization, improving the fluorescence response of aflatoxins B1 and G1.^{25,35,38,40} Such derivatization strategies are particularly relevant in HPLC-FLD methods because fluorescence intensity differs among aflatoxin analogs, and signal enhancement may be necessary to achieve adequate sensitivity at regulatory concentration levels.^{52,53}

Derivatization is therefore a critical analytical strategy in fluorescence-based mycotoxin determination.⁵³ Its main purpose is to enhance the fluorescence response, improve detectability at low concentration levels, and enable the quantification of analytes with weak or absent native fluorescence.⁵³ In the case of aflatoxins, post-column derivatization is commonly used to increase the fluorescence of AFB1 and AFG1, which generally show lower native fluorescence responses than AFB2 and AFG2.^{53,54} Chemical derivatization with bromide or iodine, as well as photochemical derivatization, can improve sensitivity and enable quantification at concentrations compatible with regulatory requirements.^{30,52-54}

For other mycotoxins, different chromatographic adjustments were required. In the case of ochratoxin A, acidic mobile phases containing acetic acid were commonly employed.^{11,32} This condition helps control the ionization of acidic functional groups, thereby improving retention, peak shape, and reproducibility.⁴⁴ Similarly, fumonisins, which contain multiple carboxylic groups and are more polar than aflatoxins, require specific chromatographic conditions and, frequently, derivatization to enable fluorescence detection.³⁶ For fumonisins, pre-column derivatization is usually required because these compounds do not present adequate native fluorescence. This approach allows the formation of fluorescent derivatives suitable for HPLC-FLD analysis, but it introduces additional analytical complexity, since reaction time, pH, reagent stability, and derivative stability may affect precision, accuracy, and robustness.

Although derivatization can substantially improve the sensitivity of HPLC-FLD methods, it also has limitations.^{55,56} Pre-column derivatization may be affected by incomplete reaction, derivative instability, matrix interference, and increased sample preparation time.^{55,57} Post-column derivatization reduces some sample-handling limitations but requires additional instrumentation, reagent control, and optimization of reaction conditions.^{57,58} Photochemical derivatization avoids the continuous use of chemical reagents but still requires specific equipment and validation of irradiation conditions.⁵⁶ Therefore, derivatization should be considered a critical method variable and should be adequately optimized and validated, especially when applied to complex medicinal plant matrices.^{56,57}

Zearalenone, due to its intrinsic fluorescence and more hydrophobic character, can be separated using aqueous-organic reversed-phase systems, although matrix interferences may still affect selectivity and quantification.^{4,5,28,32,43}

Table II. Summary of HPLC-based methods for the determination of mycotoxins in medicinal plants

Ref.	Mycotoxin	Column	Detector	Mobile Phase	Sample preparation	LOD	LOQ	Validation*	Parameters	Key Results
21	AF	Capcell Pak C18 UG120 and Shim-pack CLC-ODS	FLD	Water, methanol, and acetonitrile (70:20:10, v/v)	LE/SPE	0,01 ug/kg	–	0	–	Contamination is relatively low.
5	AF, OTA, and ZEA	Nova-Pak C18 Waters Symmetry C18	FLD	AFs: Water, methanol, and acetonitrile (60:20:20, v/v) OTA: Acetonitrile, water, and acetic acid (50:48:2, v/v) ZEA: Acetonitrile, water, and methanol (46:46:8, v/v)	LLE	AFB1: 0,03 µg/kg AFB2: 0,01 µg/kg AFG1: 0,05 µg/kg AFG2: 0,02 µg/kg OTA: 0,05 µg/kg ZEA: 0,03 µg/kg	AFB1: 0,1 µg/kg AFB2: 0,03 µg/kg AFG1: 0,2 µg/kg AFG2: 0,1 µg/kg OTA: 0,2 µg/kg ZEA: 0,1 µg/kg	1	Recovery, LOD, and LOQ	Of the 36 samples analyzed, 86% were contaminated with AF. OTA occurred in 61% of samples. 39% (<i>n</i> =36) of the samples contained ZEA.
22	OTA	Ace 5 C18 250 x 4.6 mm	FLD	Water, acetonitrile, and acetic acid (51:48:1, v/v)	LE/SPE	–	0.5 ng/g	0	–	The concentration was unaffected by sorting or washing, but it was much reduced by peeling (a 53% reduction).
22	OTA	Kromasil RP C18	FLD	Water, acetonitrile, and acetic acid (51:48:1, v/v)	LE/SPE	–	0.5 ng/g	0	–	
10	AF	Shim-pack CLC-ODS	FLD	Water, methanol, and acetonitrile (56:29:17, v/v)	LE/SPE	B1 and G1: 3,5 ng/g B2 and G2: 0,1 ng/g	B1, B2, and G2: 4 ng/g G1: 7 ng/g	1	Selectivity, recovery, accuracy, precision, linearity	No aflatoxins were detected for these samples.
20	AF	Capcell Pak C18	FLD	Methanol and water (50:50, v/v)	LE/SPE	AFB1: 0.08 ng/mL AFB2: 0.02 ng/mL AFG1: 0.06 ng/mL AFG2: 0.02 ng/mL	AFB1: 0.20 ng/mL AFB2: 0.05 ng/mL AFG1: 0.20 ng/mL AFG2: 0.08 ng/mL	1	Linearity, LOD, LOQ, precision, recovery, and repeatability	Ten out of 11 samples were contaminated by AFB1, and the levels of 3 samples exceeded 5 ug/kg.
23	AF	YMC ODS-AQ S-3 C18	FLD	Methanol, acetonitrile, and water (25:15:60, v/v)	LE/SPE	0.1 ng/g	–	0	–	–
24	AF	Scharlau Chemie C18	FLD	Methanol and water (52:48, v/v)	LE/SPE	–	0.05 - 0.1 ng/g	0	–	If the levels of aflatoxins are over 1-2 ng/g, can be allowed a reliable identification.
25	AF and OTA	Inertsil ODS-3V C18	FLD	A: Water and methanol (55:45, v/v) containing 90 mL/L of 4 N nitric acid and 119 mg/L of potassium bromide B: Water and methanol (80:20, v/v) containing 90 mL/L of 4 N nitric acid and 119 mg/L of potassium bromide	LE/SPE	aflatoxin B1: 0.2 ug/kg in all samples Total aflatoxins: 0.5 ug/kg to 0.7 ug/kg OTA: 1.8 ug/kg to 2.0 ug/kg	aflatoxin B1: 0.6 ug/kg to 0.7 ug/kg Total aflatoxins: 1.6 ug/kg to 2.4 ug/kg OTA: 5.9 ug/kg to 6.5 ug/kg	1	Repeatability, recoveries, LOQ	Recoveries were in the range 77 to 99% for aflatoxin B1. Similarly, ochratoxin A recoveries were from 64 to 94%.

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Table II. Summary of HPLC-based methods for the determination of mycotoxins in medicinal plants (continued)

Ref.	Mycotoxin	Column	Detector	Mobile Phase	Sample preparation	LOD	LOQ	Validation*	Parameters	Key Results
2	AF and OTA		FLD	Acetonitrile, methanol, and water (8:27:65, v/v)	LE/SPE	–	–	0	–	26.6% herbal plants were contaminated by various types of aflatoxins and 13.3% by ochratoxin A.
26	AF	Alltima C18	FLD	Water, methanol, and acetonitrile (3:1:1, v/v)	LE/SPE	0.06 ug/kg	0.3 ug/kg	1	linearity, recovery, precision, LOQ, LOD	–
27	AF	C18	FLD	Water, acetonitrile, and methanol (60:20:30, v/v)	LE/SPE	AFB1: 0.033 ppb	AFB1: 0.1ppb	0	–	The highest prevalence of aflatoxin was attributed to aflatoxin B1 (30.8%).
4	ZEA	Ultimate XB-C18	FLD	Acetonitrile and water (50:50, v/v)	LE/SPE	ZON: 4 ug/kg α -ZOL: 2.5 ug/kg	not included	1	linearity, LOD, precision, reproducibility, accuracy	12% of these tested samples were contaminated with ZON and, no samples were detected with α -zearalenone.
28	ZEA, ZOL and DON	ODS, RP-C18	FLD	Acetonitrile and water (55:45, v/v)	LLE	–	–	0	–	Contamination of ZEN was detected in 13.07% samples, DON was detected in 6.92% samples, and only one sample was contaminated with ZOL.
29	OTA	Zorbax SB-C18 XTerra RP18 Lichrospher C18 endcapped ZORBAX eclipse XDB-C18 Zorbax SB-C18 ODS2 Alltech Alltima C18 SphereClone ODS (2)	FLD	A: Methanol and water–acetic acid solution (70:30, v/v; water:acetic acid 3:1) B: Methanol (100%, v/v)	LE/SPE	–	–	0	–	–
30	AF	CAPCELL PAK C18	FLD	Methanol and acetonitrile (40:18, v/v) with water as the aqueous phase	LE/SPE	AFB1: 0.18 μ g/kg AFB2: 0.15 μ g/kg AFG1: 0.65 μ g/kg AFG2: 0.41 μ g/kg	AFB1: 0.60 μ g/kg AFB2: 0.53 μ g/kg AFG1: 2.18 μ g/kg AFG2: 1.22 μ g/kg	1	linearity, LOD, LOQ, accuracy, precision, stability, selectivity and robustness	–

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Table II. Summary of HPLC-based methods for the determination of mycotoxins in medicinal plants (continued)

Ref.	Mycotoxin	Column	Detector	Mobile Phase	Sample preparation	LOD	LOQ	Validation*	Parameters	Key Results
31	AF and OTA	ZORBAX Eclipse XDB C18	FLD	Water, methanol, and acetonitrile (55:15:30, v/v)	LE/SPE	AFB1: 0,1 µg/kg AFB2: 0,2 µg/kg AFG1: 0,3 µg/kg AFG2: 0,2 µg/kg OTA: 0,47 µg/kg	AFB1: 0,4 µg/kg AFB2: 0,7 µg/kg AFG1: 0,9 µg/kg AFG2: 0,6 µg/kg OTA: 1,23 µg/kg	1	linearity, LOD, LOQ, accuracy,	40% of samples were contaminated with AFs. All of the samples were contaminated with OTA.
32	AF, OTA, ZEA and DON	Phenomenex Gemini C18	DAD	A: Water containing 0.5% acetic acid B: Methanol and acetonitrile (50:50, v/v)	LLE	0.01563 – 0.5161 ng/mL	0.05210 – 1.720 ng/mL	1	Linearity, LOD, LOQ, precision and recovery	–
33	AFB1	Shim-Pack CLC – ODS	FLD	Water, acetonitrile, and methanol (60:20:20, v/v)	LE/SPE	0.6 µg/kg	1.0 µg/kg	0	–	Aflatoxin B1 was noticed in a single sample (< 1.0 µg/kg).
34	OTA	RP C-18 ODS	FLD	Water, acetonitrile, and acetic acid (26:70:4, v/v)	LE/SPE	–	–	1	Repeatability, Reproducibility and Recovery	–
35	AF	Radial-Pak cartridge, Type 8 NVC C-18	FLD	Water and methanol (58:42, v/v) containing 119 mg/L of potassium bromide and 100 µL of nitric acid (65%) per liter	LE/SPE	0.05 ppb	–	1	Specificity, precision, accuracy and linearity	–
36	Fumonisin B1 and AFB1	ODS Luna C18	FLD	Monopotassium phosphate buffer (0.01 M), acetonitrile, and methanol (690:220:75, v/v)	LE/SPE	–	–	0	–	None of the plant extracts contained detectable levels of aflatoxin B1; however, eight plants, four dietary and four medicinal, were positive for fumonisin B1.
45	AF	Licrospher 100 RP18	FLD	Water, methanol, and acetonitrile (620:220:160, v/v)	LE/SPE	0.04 ng/g	–	1	Linearity, LOD, precision, reproducibility, accuracy,	No samples used in study had levels above of 20 ng/g. The contaminated products were those in tablet and capsule dosage forms.
38	AF and OTA	Aflatoxins: YMC ODS-AQ S-3 Ochratoxin A: Ultrasphere	FLD	Aflatoxins: Water, methanol, and acetonitrile (600:250:150, v/v) containing 4 M nitric acid and potassium bromide Ochratoxin A: Acetonitrile, water, and acetic acid (500:500:10, v/v)	LE/SPE	–	–	0	–	–

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Table II. Summary of HPLC-based methods for the determination of mycotoxins in medicinal plants (continued)

Ref.	Mycotoxin	Column	Detector	Mobile Phase	Sample preparation	LOD	LOQ	Validation*	Parameters	Key Results
39	AF and OTA	Aflatoxins: YMC ODS-AQ S-3 Ochratoxin A: Ultrasphere	FLD	AF: Water, methanol, and acetonitrile (600:250:150, v/v) OTA: Acetonitrile, water, and acetic acid (50:50:1, v/v)	LE/SPE	AFB1: 0.1 ng/g OTA: 0.1 ng/g	AFB1: 1 ng/g OTA: 1 ng/g	0	–	39 commercially samples were analyzed (26 with AF 67%, 29 with OTA 74%, and 10 no AF or OTA 26%)
11	AF and OTA	ACCHROM Unitary C18	FLD	Acetic acid (0.5%) and methanol (48:52, v/v)	LE/SPE	0.03 - 0.3 ug/kg	0.1 - 0.9 ug/kg	1	Linearity, LOD, LOQ, recovery, reproducibility and precision	–
40	AF and OTA	Manual IAC: Inertsil ODS-3V In-line IAC: InertSustain AQ C18	FLD	A: Methanol and water (45:55, v/v) containing 350 µL of 4 N nitric acid and 119 mg/L of potassium bromide B: Methanol and water (80:20, v/v) containing 350 µL of 4 N nitric acid and 119 mg/L of potassium bromide	LE/SPE	Manual IAC method Aflatoxins: 0.2 to 0.5 µg/kg Ochratoxin A: 0.1 to 0.3 µg/kg In-line IAC method Aflatoxins: 0.01 to 0.2 µg/kg Ochratoxin A: 0.10 to 0.2 µg/kg	not included	0	–	–
41	AF	LiChrospher 100 RP-18	FLD	Acetonitrile, methanol, and water (1:1:4, v/v)	LE/SPE	B1: 0.72 ug/kg B2: 0.22 ug/kg G1: 0.75 ug/kg G2: 0.30 ug/kg	–	1	Linearity, LOD, precision, reproducibility and accuracy	Aflatoxins were detected in 15.78% of the samples
42	AF	Venusil MP C18	FLD	Methanol, acetonitrile, and water (30:18:52, v/v)	LE/SPE	0.04–0.2 ug/kg	0.25–1.0 ug/kg	1	selectivity, stability, linearity, LOD, LOQ, precision, accuracy, and robustness	–
43	ZEA	Eclipse XDB-C18	FLD	Water, acetonitrile, and methanol (46:46:8, v/v)	LE/SPE	9.5 ug/kg	–	0	–	55.6% of samples were highly contaminated with ZON (>60 ug/kg)

Note: AF: Aflatoxins; DON: Deoxynivalenol; OTA: Ochratoxin A; ZEA: Zearalenone; ZOL: Zearalenol; LOD: Limit of Detection; LOQ: Limit of Quantification; *Validation 0: no validation; *Validation 1: validation realized; LLE: Liquid Liquid Extraction; LE/SPE: Liquid Extraction/ Solid Phase Extraction

Based on the results for aflatoxins, the Limit of Quantification (LOQ) for roots—the most frequently cited part of the plant—ranged from 0.03 $\mu\text{g}/\text{kg}$ ⁵ to 1.0 $\mu\text{g}/\text{kg}$.^{39,42} For other, less commonly cited matrices, the LOQ varied between 0.03 $\mu\text{g}/\text{kg}$ ⁵ and 7.0 $\mu\text{g}/\text{kg}$.¹⁰ The Limit of Detection (LOD) values for aflatoxins in roots ranged from 0.04 to 0.2 $\mu\text{g}/\text{kg}$,⁴² while other matrices showed LODs from 0.01 $\mu\text{g}/\text{kg}$ (Ali et al., 2005)²¹ to 3.5 $\mu\text{g}/\text{kg}$.¹⁰

The LOQ for ochratoxin A in the studies ranged from 0.2 $\mu\text{g}/\text{kg}$ ⁵ to 6.3 $\mu\text{g}/\text{kg}$,²⁵ while the LOD ranged from 0.05 to 2.0 $\mu\text{g}/\text{kg}$, also reported by Areo et al. (2020)⁵ and Greaves et al. (2021),²⁵ respectively. For zearalenone, the LOD values across studies ranged from 0.03 $\mu\text{g}/\text{kg}$ to 9.5 $\mu\text{g}/\text{kg}$. The only LOQ cited was 0.03 $\mu\text{g}/\text{kg}$.

In addition, the reliability of the analytical results depends on proper method validation. Analytical validation is the evaluation of an analytical procedure through experimental testing, with the purpose of providing objective evidence that the procedure is suitable for its intended use. In an international context, the ICH Q2(R2)⁶⁰ guideline provides recommendations for the selection and evaluation of validation tests for analytical procedures, including parameters such as specificity/selectivity, linearity, range, accuracy, precision, detection limit, quantitation limit, and robustness.^{59,60} Among the reviewed articles, 50% reported analytical validation data (Figure 4A). However, this result should be interpreted in light of the different objectives of the included studies, since some articles focused mainly on contamination/occurrence assessment, while others were specifically designed for analytical method development and validation.

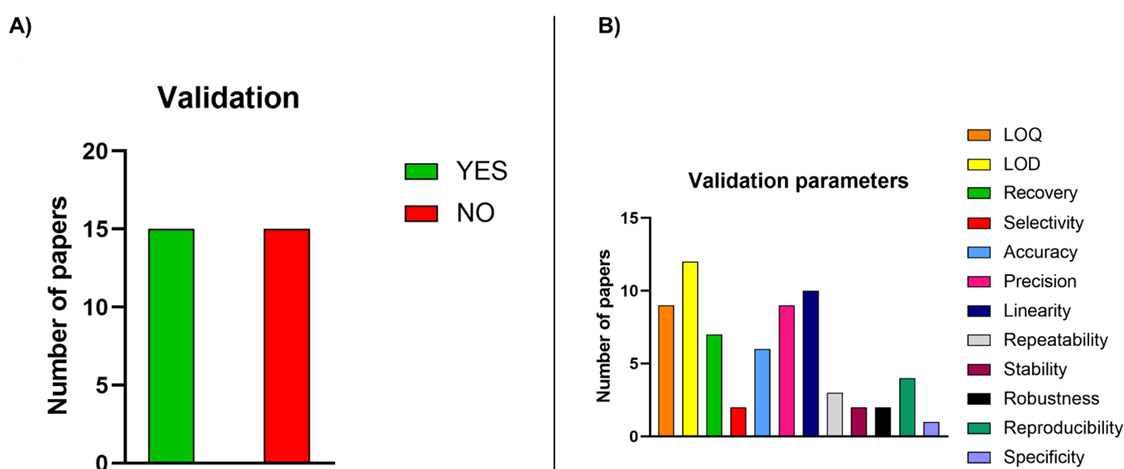


Figure 4. A) Number of papers that reported validation, and B) Validation parameters evaluated in the papers.

The main parameters evaluated were LOD, LOQ, linearity, and precision (Figure 4B). Selectivity was evaluated in 20% of the reviewed articles. It refers to a method's ability to identify an analyte in a matrix without interference from other substances, such as impurities, diluents, or matrix components.⁵⁹

Linearity was assessed in 80% of the reviewed articles, and this concerns the ability to obtain analytical responses that are directly proportional to the analyte concentration.⁵⁹ The range is the interval spanning from the lowest to the highest concentration values where the method remains precise, accurate, and linear.⁶¹

Precision was assessed in 93% of the reviewed articles and relates to the proximity between measured results. It is usually expressed as the variance, standard deviation, or coefficient of variation of a series of measurements. There are two types of precision: repeatability expresses precision under the same operating conditions over a short interval of time, and intermediate precision expresses variations within laboratories, such as those caused by different days, different analysts, different equipment, and so on.⁵⁹ The accuracy was assessed in 53% of the reviewed articles and refers to the degree of agreement between individual results of the method under study and a value accepted as the true value.⁵⁹

Articles reporting LOD and LOQ were assessed at 87% and 73%, respectively. The LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value. The LOQ is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy.⁶¹ Reporting these limits is essential in analytical methods for mycotoxin determination, as they are key parameters evaluated by regulatory agencies to ensure method suitability and compliance with established safety standards. Low LOD and LOQ values are particularly important for detecting trace levels of mycotoxins, which may pose significant health risks even at very low concentrations and are therefore required to meet current regulatory limits for contaminants in herbal and medicinal plant materials.

Robustness was assessed in 13% of the reviewed articles, typically during analytical method development. It refers to the method's ability to resist deliberate variations in parameters such as flow rate, column temperature, injection volume, mobile phase composition (in liquid chromatography), or any other variable inherent to the method of analysis (e.g., stability of analytical solutions, extraction time, etc.).⁶¹

Matrix effects are another important aspect to be considered in the validation and application of analytical methods for medicinal plants. Herbal matrices are chemically complex and may contain alkaloids, polyphenols, flavonoids, pigments, tannins, essential oils, sugars, organic acids, and other secondary metabolites.⁶²⁻⁶⁵ These compounds may interfere with extraction efficiency, clean-up selectivity, chromatographic separation, fluorescence response, and analyte recovery.^{62,64,65} In the studies included in this scoping review, matrix effects were not systematically evaluated as an independent parameter. This limited reporting should be considered a methodological gap, particularly because matrix composition may vary substantially according to plant species, plant part, cultivation conditions, processing, and storage.

Strategies to reduce or compensate for matrix effects include optimized extraction conditions, efficient clean-up procedures such as solid-phase extraction and immunoaffinity columns, dilution of the extract, adjustment of pH, reduction of sample mass, matrix-matched calibration, recovery studies at different concentration levels, and selectivity assessment in representative herbal matrices.^{64,66,68} For HPLC-FLD methods, these precautions are particularly relevant because co-extracted fluorescent compounds may affect the analytical response.^{30,67} For HPLC-DAD methods, spectral comparison and peak purity assessment may help detect co-elution, although these approaches do not provide the same confirmatory capability as mass spectrometry.^{67,69} Therefore, future studies using HPLC-FLD and HPLC-DAD for mycotoxin determination in medicinal plants should more explicitly evaluate and report matrix effects to improve method reliability and comparability.

The fact that 50% of the articles did not report analytical validation data should be interpreted with caution. The studies included in this scoping review had different objectives: while some were specifically focused on the development, optimization, and/or validation of analytical methods, others primarily investigated the occurrence or contamination levels of mycotoxins in medicinal plant samples, sometimes using previously established methods. Therefore, the absence of complete validation data does not necessarily indicate inadequate methodological quality but may reflect the study's aims, journal reporting practices, or the regulatory context at the time of publication. This aspect is particularly relevant because no publication period restriction was applied in the present review, and older studies may have been conducted under different validation requirements. Nevertheless, for current applications in routine quality control and regulatory assessment, analytical validation remains essential. In addition to national regulatory requirements, international guidelines such as ICH Q2(R2)⁶² underscore the importance of validation to demonstrate the reliability of analytical procedures. Thus, reporting parameters such as selectivity/specificity, linearity, range, accuracy, precision, detection limit, quantitation limit, and robustness is important to support comparability, regulatory acceptance, and confidence in analytical results obtained from complex matrices such as medicinal plants.^{60,61}

Taken together, the validation data reported in the included studies should be interpreted not only as isolated analytical parameters, but also in relation to the intended use of each method. For routine quality control and regulatory applications, the reliability of HPLC-FLD and HPLC-DAD methods depends on the combined evaluation of selectivity/specificity, linearity, range, accuracy, precision, recovery, detection and quantitation limits, robustness, and potential matrix effects.^{12,60,67,70} This is particularly relevant for medicinal

plant matrices, whose chemical complexity may affect extraction efficiency, chromatographic separation, detector response, and analyte recovery. Therefore, future studies should provide more complete validation reports aligned with international guidelines, such as ICH Q2(R2),⁶⁰ especially when methods are proposed for routine monitoring or regulatory decision-making.

Final considerations and evidence gaps

Based on the selected articles addressing analytical methods for mycotoxin determination in medicinal plants using HPLC with fluorescence detection, it is evident that aflatoxins are the most extensively investigated group, reflecting their well-established health risks and regulatory relevance. Despite this focus, the review highlights important methodological limitations, particularly regarding the development of multi-mycotoxin analytical methods. Most of the studies included evaluated only a single group of mycotoxins, indicating challenges associated with simultaneous determination when using fluorescence detection.

These limitations are likely related to differences in the physicochemical properties of mycotoxins, including molecular structure, polarity, and chromatographic behavior under varying mobile phase compositions. In addition, the requirement for specific derivatization steps, especially for aflatoxins and fumonisins, further complicates the development of robust multi-analyte methods using HPLC-FLD.

It should be emphasized that the exclusion of LC-MS/MS was a methodological choice based on the predefined scope of this scoping review. LC-MS/MS remains the gold standard for mycotoxin determination, especially for simultaneous multi-mycotoxin analysis, because of its superior sensitivity, selectivity, and confirmatory capability. However, HPLC-FLD and HPLC-DAD continue to play an important role in routine quality control due to their lower cost, broader availability, and established use for regulated mycotoxins such as aflatoxins and ochratoxin A. In this sense, the evidence gaps identified in the present review should be interpreted specifically within the context of HPLC-FLD and HPLC-DAD methods, and not as a limitation of the entire field of mycotoxin analysis in medicinal plants.

From an evidence gap perspective, there is a clear lack of standardized and validated multi-mycotoxin methods applicable to complex medicinal plant matrices using fluorescence detection. Furthermore, a considerable proportion of studies did not report full analytical validation, which limits the comparability and regulatory applicability of the reported methods. Future research should therefore focus on the development and validation of comprehensive multi-mycotoxin analytical approaches, as well as on harmonizing validation practices to meet regulatory requirements and improve the reliability of mycotoxin monitoring in medicinal plant materials.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Use of artificial intelligence tools

The authors declare that ChatGPT (OpenAI), through its integrated image-generation tool, was used solely to create the graphical abstract. No artificial intelligence tools were used in any other aspect of the preparation of this article.

REFERENCES

- (1) Elamin, A.; Enomoto, H.; Watanabe, M.; Sakuda, S. The Mechanism of Ochratoxin Contamination of Artificially Inoculated Licorice Roots. *Toxins (Basel)*. **2023**, *15* (3), 219. <https://doi.org/10.3390/toxins15030219>

- (2) Haq, I. ul; Taj, R.; Nafees, M.; Hussain, A. Mycotoxin Detection in Selected Medicinal Plants Using Chromatographic Techniques. *Biomed. Chromatogr.* **2024**, *38* (4). <https://doi.org/10.1002/bmc.5831>
- (3) Han, Z.; Ren, Y.; Liu, X.; Luan, L.; Wu, Y. A Reliable Isotope Dilution Method for Simultaneous Determination of Fumonisin B1, B2 and B3 in Traditional Chinese Medicines by Ultra-high performance Liquid Chromatography–tandem Mass Spectrometry. *J. Sep. Sci.* **2010**, *33* (17–18), 2723–2733. <https://doi.org/10.1002/jssc.201000423>
- (4) Kong, W.; Shen, H.; Zhang, X.; Yang, X.; Qiu, F.; Ou-yang, Z.; Yang, M. Analysis of Zearalenone and α -zearalenol in 100 Foods and Medicinal Plants Determined by HPLC-FLD and Positive Confirmation by LC-MS-MS. *J. Sci. Food Agric.* **2013**, *93* (7), 1584–1590. <https://doi.org/10.1002/jsfa.5926>
- (5) Areo, O. M.; Phoku, J. Z.; Gbashi, S.; Njobeh, P. B. A Preliminary Study of Multi-Mycotoxins Contamination in Some Selected South Africa Medicinal Plants. *Emir. J. Food Agric.* **2020**, *32* (6), 426–433. <https://doi.org/10.9755/ejfa.2020.v32.i6.2113>
- (6) Muyumba, N. W.; Mutombo, S. C.; Sheridan, H.; Nachtergaeel, A.; Duez, P. Quality Control of Herbal Drugs and Preparations: The Methods of Analysis, Their Relevance and Applications. *Talanta Open* **2021**, *4*, 100070. <https://doi.org/10.1016/j.talo.2021.100070>
- (7) Stevic, T.; Pavlovic, S.; Stankovic, S.; Savikin, K. Pathogenic Microorganisms of Medicinal Herbal Drugs. *Arch. Biol. Sci.* **2012**, *64* (1), 49–58. <https://doi.org/10.2298/ABS1201049S>
- (8) Gholizadeh, S.; Mirzaei, H.; Khandaghi, J.; Afshar Mogaddam, M. R.; Javadi, A. Ultrasound–Assisted Solvent Extraction Combined with Magnetic Ionic Liquid Based-Dispersive Liquid–Liquid Microextraction for the Extraction of Mycotoxins from Tea Samples. *J. Food Compos. Anal.* **2022**, *114*, 104831. <https://doi.org/10.1016/j.jfca.2022.104831>
- (9) Kong, W.; Xie, T.; Li, J.; Wei, J.; Qiu, F.; Qi, A.; Zheng, Y.; Yang, M. Analysis of Fumonisin B1 and B2 in Spices and Aromatic and Medicinal Herbs by HPLC-FLD with on-Line Post-Column Derivatization and Positive Confirmation by LC-MS/MS. *Analyst* **2012**, *137* (13), 3166. <https://doi.org/10.1039/c2an35164a>
- (10) Braga, S. M. L. F. M.; de Medeiros, F. D.; de Jesus Oliveira, E.; Macedo, R. O. Development and Validation of a Method for the Quantitative Determination of Aflatoxin Contaminants in *Maytenus ilicifolia* by HPLC with Fluorescence Detection. *Phytochem. Anal.* **2005**, *16* (4), 267–271. <https://doi.org/10.1002/pca.837>
- (11) Wen, J.; Kong, W.; Wang, J.; Yang, M. Simultaneous Determination of Four Aflatoxins and Ochratoxin A in Ginger and Related Products by HPLC with Fluorescence Detection after Immunoaffinity Column Clean-up and Postcolumn Photochemical Derivatization. *J. Sep. Sci.* **2013**, *36* (23), 3709–3716. <https://doi.org/10.1002/jssc.201300885>
- (12) Agência Nacional de Vigilância Sanitária (ANVISA). *Resolução Da Diretoria Colegiada – RDC No. 26, de 13 de maio de 2014. Dispõe Sobre o Registro de Medicamentos Fitoterápicos e o Registro e a Notificação de Produtos Tradicionais Fitoterápicos*. Brazil, 2014.
- (13) Agência Nacional de Vigilância Sanitária (ANVISA). *Farmacopeia Brasileira, 7th Ed. Volume II: Plantas Medicinais*. ANVISA, Brasília, 2024, Vol. 2.
- (14) Council of Europe. *European Pharmacopoeia*, 11th ed. European Directorate for the Quality of Medicines & HealthCare, 2025.
- (15) United States Pharmacopeial Convention. *United States Pharmacopeia and National Formulary (USP–NF)*. Rockville, 2026.
- (16) Peters, M. D. J.; Godfrey, C. M.; Khalil, H.; McInerney, P.; Parker, D.; Soares, C. B. Guidance for Conducting Systematic Scoping Reviews. *Int. J. Evid. Based. Healthc.* **2015**, *13* (3), 141–146. <https://pubmed.ncbi.nlm.nih.gov/26134548/>
- (17) Tricco, A. C.; Lillie, E.; Zarin, W.; O'Brien, K. K.; Colquhoun, H.; Levac, D.; Moher, D.; Peters, M. D. J.; Horsley, T.; Weeks, L.; et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann. Intern. Med.* **2018**, *169* (7), 467–473. <https://doi.org/10.7326/M18-0850>

- (18) White, H.; Albers, B.; Gaarder, M.; Kornør, H.; Littell, J.; Marshall, Z.; Mathew, C.; Pigott, T.; Snilstveit, B.; Waddington, H.; Welch, V. Guidance for Producing a Campbell Evidence and Gap Map. *Campbell Systematic Reviews* **2020**, *16* (4). <https://doi.org/10.1002/cl2.1125>
- (19) Song, X.-Y.; Li, Y.-D.; Shi, Y.-P.; Jin, L.; Chen, J. Quality Control of Traditional Chinese Medicines: A Review. *Chin. J. Nat. Med.* **2013**, *11* (6), 596–607. [https://doi.org/10.1016/S1875-5364\(13\)60069-2](https://doi.org/10.1016/S1875-5364(13)60069-2)
- (20) Cao, J.; Zhou, S.; Kong, W.; Ma, X.; Yang, M.; Wan, L.; Yang, S. Simultaneous Determination of Aflatoxins B₁, B₂, G₁, G₂ in *Fructus Bruceae* by High-performance Liquid Chromatography with Online Postcolumn Photochemical Derivatization. *J. Sep. Sci.* **2014**, *37* (19), 2771–2778. <https://doi.org/10.1002/jssc.201400501>
- (21) Ali, N.; Hashim, N. H.; Saad, B.; Safan, K.; Nakajima, M.; Yoshizawa, T. Evaluation of a Method to Determine the Natural Occurrence of Aflatoxins in Commercial Traditional Herbal Medicines from Malaysia and Indonesia. *Food Chem. Toxicol.* **2005**, *43* (12), 1763–1772. <https://doi.org/10.1016/j.fct.2005.05.019>
- (22) Ariño, A.; Herrera, M.; Estopañan, G.; Juan, T. High Levels of Ochratoxin A in Licorice and Derived Products. *Int. J. Food Microbiol.* **2007**, *114* (3), 366–369. <https://doi.org/10.1016/j.ijfoodmicro.2006.08.005>
- (23) D'Ovidio, K.; Trucksess, M.; Weaver, C.; Horn, E.; McIntosh, M.; Bean, G. Aflatoxins in Ginseng Roots. *Food Addit. Contam.* **2006**, *23* (2), 174–180. <https://doi.org/10.1080/02652030500442524>
- (24) Gómez-Catalán, J.; Piqué, E.; Falcó, G.; Borrego, N.; Rodamilans, M.; Llobet, J. M. Determination of Aflatoxins in Medicinal Herbs by HPLC. An Efficient Method for Routine Analysis. *Phytochem. Anal.* **2005**, *16* (3), 196–204. <https://doi.org/10.1002/pca.845>
- (25) Greaves, A.; Maddison, K.; Doran, M.; Lin, S.; Geiling, B. Single-Laboratory Validation of an Immunoaffinity Column Cleanup LC Method for the Analysis of Aflatoxins and Ochratoxin A in Cannabis Plant Material, Resins, Vapes, Isolates, and Edible Products. *J. AOAC Int.* **2021**, *104* (5), 1264–1271. <https://doi.org/10.1093/jaoacint/qsab057>
- (26) Ip, S.-P.; Che, C.-T. Determination of Aflatoxins in Chinese Medicinal Herbs by High-Performance Liquid Chromatography Using Immunoaffinity Column Cleanup. *J. Chromatogr. A* **2006**, *1135* (2), 241–244. <https://doi.org/10.1016/j.chroma.2006.10.025>
- (27) Khazaeli, P.; Mehrabani, M.; Heidari, M. R.; Asadikaram, G.; Lari Najafi, M. Prevalence of Aflatoxin Contamination in Herbs and Spices in Different Regions of Iran. *Iran. J. Public Health* **2017**, *46* (11), 1540–1545.
- (28) Koul, A.; Sumbali, G. Detection of Zearalenone, Zearalenol and Deoxynivalenol from Medicinally Important Dried Rhizomes and Root Tubers. *Afr. J. Biotechnol.* **2008**, *7* (22), 4136–4139. Available at: <http://academicjournals.org/journal/AJB/article-abstract/77041658352> (accessed: July 2026).
- (29) Lerda, D.; Ambrosio, M.; Kunsagi, Z.; Stroka, J.; Cea, J.; Cepurnieks, G.; De Girolamo, A.; De Rechter, P.; Gambin, D.; Ioannou-Kakouri, E.; et al. Determination of Ochratoxin A in Licorice and Licorice Extracts by High-Performance Liquid Chromatography Coupled with Fluorescence Detection: Collaborative Study. *J. AOAC Int.* **2013**, *96* (2), 331–340. <https://doi.org/10.5740/jaoacint.12-251>
- (30) Liu, X.; Ying, G.; Sun, C.; Yang, M.; Zhang, L.; Zhang, S.; Xing, X.; Li, Q.; Kong, W. Development of an Ultrasonication-Assisted Extraction Based HPLC With a Fluorescence Method for Sensitive Determination of Aflatoxins in Highly Acidic *Hibiscus sabdariffa*. *Front. Pharmacol.* **2018**, *9*. <https://doi.org/10.3389/fphar.2018.00284>
- (31) Pakshir, K.; Mirshekari, Z.; Nouraei, H.; Zareshahrabadi, Z.; Zomorodian, K.; Khodadadi, H.; Hadaegh, A. Mycotoxins Detection and Fungal Contamination in Black and Green Tea by HPLC-Based Method. *J. Toxicol.* **2020**, *2020*, 1–7. <https://doi.org/10.1155/2020/2456210>
- (32) Pi, J.; Jin, P.; Zhou, S.; Wang, L.; Wang, H.; Huang, J.; Gan, L.; Yuan, T.; Fan, H. Combination of Ultrasonic-Assisted Aqueous Two-Phase Extraction with Solidifying Organic Drop-Dispersive Liquid–Liquid Microextraction for Simultaneous Determination of Nine Mycotoxins in Medicinal and Edible Foods by HPLC with In-Series DAD and FLD. *Food Anal. Methods* **2022**, *15* (2), 428–439. <https://doi.org/10.1007/s12161-021-02134-w>

- (33) Prado, G.; Altoé, A. F.; Gomes, T. C. B.; Leal, A. S.; Morais, V. A. D.; Oliveira, M. S.; Ferreira, M. B.; Gomes, M. B.; Paschoal, F. N.; Souza, R. von S.; et al. Occurrence of Aflatoxin B1 in Natural Products. *Braz. J. Microbiol.* **2012**, *43* (4), 1428–1435. <https://doi.org/10.1590/S1517-83822012000400026>
- (34) Raters, M.; Matissek, R.; van Haren, W.; Fledderus, K. Determination of Ochratoxin A in Liquorice Products Using HPLC-Based Analytical Methods. Part II: Harmonised Method and Method Validation Study. *Mycotoxin Res.* **2010**, *26* (2), 101–108. <https://doi.org/10.1007/s12550-010-0044-9>
- (35) Reif, K.; Metzger, W. Determination of Aflatoxins in Medicinal Herbs and Plant Extracts. *J. Chromatogr. A* **1995**, *692* (1–2), 131–136. [https://doi.org/10.1016/0021-9673\(94\)00841-V](https://doi.org/10.1016/0021-9673(94)00841-V)
- (36) Sewram, V.; Shephard, G. S.; van der Merwe, L.; Jacobs, T. V. Mycotoxin Contamination of Dietary and Medicinal Wild Plants in the Eastern Cape Province of South Africa. *J. Agric. Food Chem.* **2006**, *54* (15), 5688–5693. <https://doi.org/10.1021/jf060483b>
- (37) Tassaneeyakul, W.; Razzazi-Fazeli, E.; Porasuphatana, S.; Bohm, J. Contamination of Aflatoxins in Herbal Medicinal Products in Thailand. *Mycopathologia* **2004**, *158* (2), 239–244. <https://doi.org/10.1023/B:MYCO.0000041892.26907.b4>
- (38) Trucksess, M. W.; Weaver, C. M.; Oles, C. J.; Fry, F. S.; Noonan, G. O.; Betz, J. M.; Rader, J. I. Determination of Aflatoxins B1, B2, G1, and G2 and Ochratoxin A in Ginseng and Ginger by Multitoxin Immunoaffinity Column Cleanup and Liquid Chromatographic Quantitation: Collaborative Study. *J. AOAC Int.* **2008**, *91* (3), 511–523. <https://doi.org/10.1093/jaoac/91.3.511>
- (39) Trucksess, M. W.; Weaver, C. M.; Oles, C. J.; Rump, L. V.; White, K. D.; Betz, J. M.; Rader, J. I. Use of Multitoxin Immunoaffinity Columns for Determination of Aflatoxins and Ochratoxin A in Ginseng and Ginger. *J. AOAC Int.* **2007**, *90* (4), 1042–1049. <https://doi.org/10.1093/jaoac/90.4.1042>
- (40) Wilcox, J.; Pazdanska, M.; Milligan, C.; Chan, D.; MacDonald, S. J.; Donnelly, C. Analysis of Aflatoxins and Ochratoxin A in Cannabis and Cannabis Products by LC–Fluorescence Detection Using Cleanup with Either Multiantibody Immunoaffinity Columns or an Automated System with In-Line Reusable Immunoaffinity Cartridges. *J. AOAC Int.* **2020**, *103* (2), 494–503. <https://doi.org/10.5740/jaoacint.19-0176>
- (41) Yang, M.-H.; Chen, J.-M.; Zhang, X.-H. Immunoaffinity Column Clean-Up and Liquid Chromatography with Post-Column Derivatization for Analysis of Aflatoxins in Traditional Chinese Medicine. *Chromatographia* **2005**, *62* (9–10), 499–504. <https://doi.org/10.1365/s10337-005-0647-z>
- (42) Zhang, L.; Dou, X.; Kong, W.; Liu, C.; Han, X.; Yang, M. Assessment of Critical Points and Development of a Practical Strategy to Extend the Applicable Scope of Immunoaffinity Column Cleanup for Aflatoxin Detection in Medicinal Herbs. *J. Chromatogr. A* **2017**, *1483*, 56–63. <https://doi.org/10.1016/j.chroma.2016.12.079>
- (43) Zhang, X.; Liu, W.; Logrieco, A. F.; Yang, M.; Ou-yang, Z.; Wang, X.; Guo, Q. Determination of Zearalenone in Traditional Chinese Medicinal Plants and Related Products by HPLC–FLD. *Food Addit. Contam. Part A* **2011**, *28* (7), 885–893. <https://doi.org/10.1080/19440049.2011.563429>
- (44) Kralj Cigić, I.; Prosen, H. An Overview of Conventional and Emerging Analytical Methods for the Determination of Mycotoxins. *Int. J. Mol. Sci.* **2009**, *10* (1), 62–115. <https://doi.org/10.3390/ijms10010062>
- (45) Tassaneeyakul, W.; Razzazi-Fazeli, E.; Porasuphatana, S.; Bohm, J. Contamination of Aflatoxins in Herbal Medicinal Products in Thailand. *Mycopathologia* **2004**, *158* (2), 239–244. <https://doi.org/10.1023/B:MYCO.0000041892.26907.b4>
- (46) Poole, C. F.; Atapattu, S. N. Study of System Properties in Reversed-Phase Liquid Chromatography for Binary and Ternary Solvent Mobile Phase Compositions Using the Solvation Parameter Model. *J. Chromatogr. Open* **2022**, *2*, 100039. <https://doi.org/10.1016/j.jcoa.2022.100039>
- (47) Poole, C.; Atapattu, S. N. Analysis of the Solvent Strength Parameter (Linear Solvent Strength Model) for Isocratic Separations in Reversed-Phase Liquid Chromatography. *J. Chromatogr. A* **2022**, *1675*, 463153. <https://doi.org/10.1016/j.chroma.2022.463153>

- (48) Kalisz, O.; Hulicka, G.; Tobiszewski, M.; Bocian, S. Performance Evaluation of Green and Conventional Solvents in Reversed-Phase Liquid Chromatography Based on the Separation of Non-Polar and Polar Substances. *Green Chemistry* **2025**, 27 (11), 3020–3031. <https://doi.org/10.1039/D4GC05737F>
- (49) Steinhoff, A.; Hölzel, A.; Tallarek, U. Mobile-Phase Contributions to Analyte Retention and Selectivity in Reversed-Phase Liquid Chromatography: 1. General Effects. *J. Phys. Chem. B* **2025**, 129 (25), 6385–6400. <https://doi.org/10.1021/acs.jpcc.5c01695>
- (50) Yabré, M.; Ferey, L.; Somé, I. T.; Gaudin, K. Greening Reversed-Phase Liquid Chromatography Methods Using Alternative Solvents for Pharmaceutical Analysis. *Molecules* **2018**, 23 (5), 1065. <https://doi.org/10.3390/molecules23051065>
- (51) Lafossas, C.; Benoit-Marquié, F.; Garrigues, J. C. Analysis of the Retention of Tetracyclines on Reversed-Phase Columns: Chemometrics, Design of Experiments and Quantitative Structure-Property Relationship (QSPR) Study for Interpretation and Optimization. *Talanta* **2019**, 198, 550–559. <https://doi.org/10.1016/j.talanta.2019.02.051>
- (52) Huertas-Pérez, J. F.; Arroyo-Manzanares, N.; Hitzler, D.; Castro-Guerrero, F. G.; Gámiz-Gracia, L.; García-Campaña, A. M. Simple Determination of Aflatoxins in Rice by Ultra-High Performance Liquid Chromatography Coupled to Chemical Post-Column Derivatization and Fluorescence Detection. *Food Chem.* **2018**, 245, 189–195. <https://doi.org/10.1016/j.foodchem.2017.10.041>
- (53) Asghar, M. A.; Iqbal, J.; Ahmed, A.; Khan, M. A.; Shamsuddin, Z. A.; Jamil, K. Development and Validation of a High-Performance Liquid Chromatography Method with Post-Column Derivatization for the Detection of Aflatoxins in Cereals and Grains. *Toxicol. Ind. Health* **2016**, 32 (6), 1122–1134. <https://doi.org/10.1177/0748233714547732>
- (54) Rahmani, A.; Jinap, S.; Khatib, A.; Tan, C. P. Simultaneous determination of aflatoxins, ochratoxin A, and zearalenone in cereals using a validated RP-HPLC method and phred derivatization system. *J. Liq. Chromatogr. Relat. Technol.* **2013**, 36 (5), 600–617. <https://doi.org/10.1080/10826076.2012.670182>
- (55) Dogra, R.; Kumar, M.; Kumar, A.; Roverso, M.; Bogialli, S.; Pastore, P.; Mandal, U. K. Derivatization, an Applicable Asset for Conventional HPLC Systems without MS Detection in Food and Miscellaneous Analysis. *Crit. Rev. Anal. Chem.* **2023**, 53 (8), 1807–1827. <https://doi.org/10.1080/10408347.2022.2042671>
- (56) Hameedat, F.; Hawamdeh, S.; Alnabulsi, S.; Zayed, A. High Performance Liquid Chromatography (HPLC) with Fluorescence Detection for Quantification of Steroids in Clinical, Pharmaceutical, and Environmental Samples: A Review. *Molecules* **2022**, 27 (6), 1807. <https://doi.org/10.3390/molecules27061807>
- (57) Kamaris, G.; Tsami, M.; Lotca, G.-R.; Almpiani, S.; Markopoulou, C. K. A Pre-Column Derivatization Method for the HPLC-FLD Determination of Dimethyl and Diethyl Amine in Pharmaceuticals. *Molecules* **2024**, 29 (23), 5535. <https://doi.org/10.3390/molecules29235535>
- (58) Muhammad, N.; Hussain, I.; Fu, X.; Ali, A.; Guo, D.; Noureen, L.; Subhani, Q.; Ahmad, N.; Zhu, Q.; Cui, H.; Feng, Y. A Comprehensive Review of Instrumentation and Applications in Post-Column and In-Source Derivatization for LC-MS. *Mass Spectrom. Rev.* **2025**. <https://doi.org/10.1002/mas.21930>
- (59) Agência Nacional de Vigilância Sanitária (ANVISA). *Validação de Métodos Analíticos*. Resolução RDC No. 166. Brasília, July 25, 2017.
- (60) International Council for Harmonisation (ICH). *ICH Q2(R2) Guideline on Validation of Analytical Procedures*. Amsterdam, 2023.
- (61) Rambla-Alegre, M.; Esteve-Romero, J.; Carda-Broch, S. Is It Really Necessary to Validate an Analytical Method or Not? That Is the Question. *J. Chromatogr. A* **2012**, 1232, 101–109. <https://doi.org/10.1016/j.chroma.2011.10.050>
- (62) Fitzgerald, M.; Heinrich, M.; Booker, A. Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques. *Front. Pharmacol.* **2020**, 10. <https://doi.org/10.3389/fphar.2019.01480>

- (63) Barthwal, R.; Mahar, R. Exploring the Significance, Extraction, and Characterization of Plant-Derived Secondary Metabolites in Complex Mixtures. *Metabolites* **2024**, *14* (2), 119. <https://doi.org/10.3390/metabo14020119>
- (64) Wu, X.; Ding, Z. Evaluation of Matrix Effects for Pesticide Residue Analysis by QuEChERs Coupled with UHPLC-MS/MS in Complex Herbal Matrix. *Food Chem.* **2023**, *405*, 134755. <https://doi.org/10.1016/j.foodchem.2022.134755>
- (65) Williams, M. L.; Olomukoro, A. A.; Emmons, R. V.; Godage, N. H.; Gionfriddo, E. Matrix Effects Demystified: Strategies for Resolving Challenges in Analytical Separations of Complex Samples. *J. Sep. Sci.* **2023**, *46* (23). <https://doi.org/10.1002/jssc.202300571>
- (66) Kong, W.-J.; Li, J.-Y.; Qiu, F.; Wei, J.-H.; Xiao, X.-H.; Zheng, Y.; Yang, M.-H. Development of a Sensitive and Reliable High Performance Liquid Chromatography Method with Fluorescence Detection for High-Throughput Analysis of Multi-Class Mycotoxins in Coix Seed. *Anal. Chim. Acta* **2013**, *799*, 68–76. <https://doi.org/10.1016/j.aca.2013.08.042>
- (67) Zhang, L.; Dou, X.-W.; Zhang, C.; Logrieco, A.; Yang, M.-H. A Review of Current Methods for Analysis of Mycotoxins in Herbal Medicines. *Toxins (Basel)* **2018**, *10* (2), 65. <https://doi.org/10.3390/toxins10020065>
- (68) Sulyok, M.; Suman, M.; Krska, R. Quantification of 700 Mycotoxins and Other Secondary Metabolites of Fungi and Plants in Grain Products. *NPJ Sci. Food* **2024**, *8* (1), 49. <https://doi.org/10.1038/s41538-024-00294-7>
- (69) Preda, D.-M.; Comănescu, M.-A.; Voicu, V.; Grinberg, N.; Medvedovici, A.-V. Peak Spectral Homogeneity in LC/DAD: Exploring Alternatives in Peak Purity Evaluations. *Processes* **2024**, *12* (12), 2887. <https://doi.org/10.3390/pr12122887>
- (70) Elumalai, S.; Dantinapalli, V. L. S.; Palanisamy, M. Comparative Analysis of Analytical Method Validation Requirements Across ICH, USP, ChP and ANVISA: A Review. *J. Pharm. Res. Int.* **2024**, *36* (12), 54–71. <https://doi.org/10.9734/jpri/2024/v36i127628>

Appendix 1. Complete search strategies used in PubMed, Scopus, and Web of Science

Database	Search queries
Pubmed	(((((((mycotoxin[Title/Abstract]) OR (aflatoxin[Title/Abstract])) OR (ochratoxin[Title/Abstract])) OR (fumonisin[Title/Abstract])) OR (trichothecene[Title/Abstract])) OR (deoxynivalenol[Title/Abstract])) OR (zearalenone[Title/Abstract]))
	(((((((HPLC[Title/Abstract]) OR ("high performance liquid chromatography"[Title/Abstract])) OR (fluorescence[Title/Abstract])) OR (dad[Title/Abstract])) OR ("2-mercaptoethanol"[Title/Abstract])) OR ("o-phthaldialdehyde"[Title/Abstract]))
	(((("herbal drug"[Title/Abstract]) OR ("dried extract"[Title/Abstract])) OR (extract[Title/Abstract])) OR (oil[Title/Abstract]))
	(((((((((((mycotoxin[Title/Abstract]) OR (aflatoxin[Title/Abstract])) OR (ochratoxin[Title/Abstract])) OR (fumonisin[Title/Abstract])) OR (trichothecene[Title/Abstract])) OR (deoxynivalenol[Title/Abstract])) OR (zearalenone[Title/Abstract])) AND ((((((HPLC[Title/Abstract]) OR ("high performance liquid chromatography"[Title/Abstract])) OR (fluorescence[Title/Abstract])) OR (dad[Title/Abstract])) OR ("2-mercaptoethanol"[Title/Abstract])) OR ("o-phthaldialdehyde"[Title/Abstract])) AND (((("herbal drug"[Title/Abstract]) OR ("dried extract"[Title/Abstract])) OR (extract[Title/Abstract])) OR (oil[Title/Abstract]))
Scopus	(TITLE-ABS-KEY (mycotoxin) OR TITLE-ABS-KEY (aflatoxin) OR TITLE-ABS-KEY (ochratoxin) OR TITLE-ABS-KEY (fumonisin) OR TITLE-ABS-KEY (trichothecene) OR TITLE-ABS-KEY (deoxynivalenol) OR TITLE-ABS-KEY (zearalenone))
	(TITLE-ABS-KEY (hplc) OR TITLE-ABS-KEY ("high performance liquid chromatography") OR TITLE-ABS-KEY (fluorescence) OR TITLE-ABS-KEY (dad) OR TITLE-ABS-KEY ("2-mercaptoethanol") OR TITLE-ABS-KEY ("o-phthaldialdehyde"))
	(TITLE-ABS-KEY ("herbal drug") OR TITLE-ABS-KEY ("dried extract") OR TITLE-ABS-KEY (extract) OR TITLE-ABS-KEY (oil))
	((TITLE-ABS-KEY (mycotoxin) OR TITLE-ABS-KEY (aflatoxin) OR TITLE-ABS-KEY (ochratoxin) OR TITLE-ABS-KEY (fumonisin) OR TITLE-ABS-KEY (trichothecene) OR TITLE-ABS-KEY (deoxynivalenol) OR TITLE-ABS-KEY (zearalenone))) AND ((TITLE-ABS-KEY (hplc) OR TITLE-ABS-KEY ("high performance liquid chromatography") OR TITLE-ABS-KEY (fluorescence) OR TITLE-ABS-KEY (dad) OR TITLE-ABS-KEY ("2-mercaptoethanol") OR TITLE-ABS-KEY ("o-phthaldialdehyde"))) AND ((TITLE-ABS-KEY ("herbal drug") OR TITLE-ABS-KEY ("dried extract") OR TITLE-ABS-KEY (extract) OR TITLE-ABS-KEY (oil)))

(continued on next page)

Appendix 1. Complete search strategies used in PubMed, Scopus, and Web of Science (continued)

Database	Search queries
Web of Science	mycotoxin (Topic) or aflatoxin (Topic) or ochratoxin (Topic) or fumonisin (Topic) or trichothecene (Topic) or deoxynivalenol (Topic) or zearalenone (Topic)
	HPLC (Topic) or “high performance liquid chromatography” (Topic) or fluorescence (Topic) or dad (Topic) or “2-mercaptoethanol” (Topic) or “o-phathaldialdehyde” (Topic)
	“herbal drug” (Topic) or “dried extract” (Topic) or extract (Topic) or oil (Topic)
	mycotoxin (Topic) or aflatoxin (Topic) or ochratoxin (Topic) or fumonisin (Topic) or trichothecene (Topic) or deoxynivalenol (Topic) or zearalenone (Topic) AND HPLC (Topic) or “high performance liquid chromatography” (Topic) or fluorescence (Topic) or dad (Topic) or “2-mercaptoethanol” (Topic) or “o-phathaldialdehyde” (Topic) AND “herbal drug” (Topic) or “dried extract” (Topic) or extract (Topic) or oil (Topic)