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Advances and Challenges in Multi-Mycotoxin Detection: Ensuring Safety in Herbal Medicinal Products

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Abstract

Herbal medicinal products have experienced a dramatic surge in global use, particularly in regions where they serve as the primary source of healthcare. Despite their long-standing history and perceived safety, these products are increasingly contaminated by mycotoxins, posing significant health risks due to their potent nephrotoxic, carcinogenic, and immunosuppressive properties. This review provides a comprehensive examination of mycotoxin contamination in herbal remedies, focusing on key toxins such as aflatoxins, ochratoxins, zearalenone, trichothecenes, and fumonisins. It critically assesses the strengths and limitations of current analytical methodologies for simultaneous detection of multiple mycotoxins. Advanced techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) and ultra-high performance liquid chromatography (UHPLC) offer high sensitivity and specificity, yet they require significant capital investment and technical expertise. Complementary methods, including immunochromatographic assays, surface plasmon resonance biosensors, and surface-enhanced Raman spectroscopy, provide rapid and cost-effective screening options but are challenged by issues like matrix interferences and limited quantification capabilities. Integrating these diverse approaches promises a more holistic strategy for robust multi-mycotoxin analysis. Future research should target methodological enhancements to overcome current limitations, thus ensuring comprehensive risk assessment and regulatory compliance in the herbal medicine sector. This review highlights the urgent need for continued innovation in detection technologies to safeguard public health and enhance food safety protocols.

Keyword: Mycotoxin; Herbal Medicine Safety; Multi-Residue Analysis

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Introduction

Over the past three decades, there has been a dramatic surge in the global utilization of herbal medicinal products, with estimates suggesting that up to 80% of the world's population now relies on these remedies for various aspects of primary healthcare. [1] This trend is especially pronounced in developing regions, where traditional herbal medicines often serve as the most accessible and cost-effective form of treatment in the absence of comprehensive modern healthcare infrastructures. The longstanding historical use of these products, coupled with a widespread perception of their inherent safety relative to synthetic pharmaceuticals, [2] has fuelled their popularity. However, this burgeoning reliance on herbal remedies is not without significant risk.

A growing body of evidence indicates that herbal medicinal products are particularly vulnerable to contamination by mycotoxins, secondary metabolites produced by fungi that can elicit a range of deleterious health effects in both humans and animals. Notably, aflatoxins and ochratoxin A have been frequently detected in medicinal herbs at concentrations that exceed the stringent regulatory limits established by bodies such as the European Union. This regulatory challenge represents a critical hurdle in safeguarding consumer health, as effective monitoring and control measures for mycotoxin levels in herbal preparations remain elusive. [3]

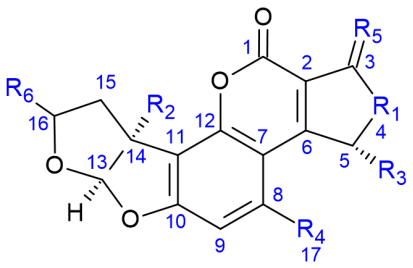
The scientific investigation into mycotoxins was notably galvanized following a series of severe poisoning incidents, including a catastrophic event in 1962 that resulted in the death of approximately 100,000 turkeys after the ingestion of contaminated peanut products. [4] Since then, over 300 distinct mycotoxins have been identified, though a select few including aflatoxins, trichothecenes, zearalenone, fumonisins, ochratoxins, and patulin, are most consistently implicated in contamination events. [5] Historical records further underscore the global impact of mycotoxin exposure, with documented poisoning events ranging from fumonisin-induced equine disease in 1891, to fatal aflatoxin outbreaks in Taiwan in 1967 and Kenya in 2004, which together account for significant morbidity and mortality. [6]

Given the potent nephrotoxic, carcinogenic, and mutagenic properties of mycotoxins, their presence in herbal medicinal products is a matter of both public health and economic concern. This review critically examines the current state of knowledge regarding mycotoxin contamination in herbal products, evaluates the efficacy of existing regulatory frameworks, and discusses emerging analytical methodologies aimed at mitigating this pervasive risk.

Aflatoxin

Aflatoxins (AFs) are a group of highly toxic secondary metabolites predominantly produced by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus*. [7] These mycotoxins commonly contaminate agricultural commodities such as cereals, spices, and oil-rich crops, including tree nuts, peanuts, and oilseeds. [6] To date, over 18 different aflatoxin variants have been identified, with the primary types being B_1, B_2, G_1, and G_2. Among these, aflatoxin B_1 (AFB_1) is recognized as the most potent carcinogen, [7] exhibiting a carcinogenic potency that varies across species, with some, such as rats and monkeys, being particularly susceptible. The core structure of aflatoxins is characterized by a coumarin nucleus fused to a bifuran moiety. The structural variations among aflatoxin subtypes are summarized in Table 1.

Table 1 Structural variations among aflatoxin subtypes



Aflatoxin	R_1	R_2	R_3	R_4	R_5	R_6	15, 16 position[#]
B1	CH_2	H	H	OCH_3	=O	CH	Unsaturated bond
B2	CH_2	H	H	OCH_3	=O	CH_2	Saturated bond
B2a	CH_2	H	H	OCH_3	=O	OH	Saturated bond
G1	O	H	H	OCH_3	=O	CH	Unsaturated bond
G2	O	H	H	OCH_3	=O	CH_2	Saturated bond
M1	CH_2	H	H	OCH_3	=O	CH	Unsaturated bond
M2	CH_2	H	H	OCH_3	=O	CH_2	Saturated bond
P1	CH_2	H	H	OH	=O	CH	Unsaturated bond
Q1	CH_2	H	OH	OCH_3	=O	CH	Unsaturated bond
Q2	CH_2	H	OH	OCH_3	=O	CH_2	Saturated bond
AFL	CH_2	OH	H	OCH_3	OH	CH	Unsaturated bond

#The columns labeled R1–R6 indicate the specific substituents attached to the aflatoxin core, while the "15, 16 position" column shows whether the bond at that position is unsaturated or saturated.

Aflatoxins exhibit both acute and chronic toxicity. Acute aflatoxicosis, though less common, results from the ingestion of food highly contaminated with aflatoxins and can lead to severe health outcomes, including liver failure and death. [8] Chronic exposure, which is more prevalent, occurs through the consumption of food containing lower levels of aflatoxins over extended periods. This form of exposure has been strongly associated with hepatocellular carcinoma (HCC) in humans, particularly in regions with high aflatoxin contamination and prevalence of hepatitis B or C infections. [8]

Beyond carcinogenicity, aflatoxins have been implicated in immunosuppression, rendering individuals more susceptible to infections. [8] They also adversely affect reproductive health, leading to conditions such as testicular degeneration, delayed testicular development, and increased sperm abnormalities. [8] The International Agency for Research on Cancer (IARC) has classified aflatoxin B_1 as a Group 1 carcinogen, indicating its carcinogenicity to humans.

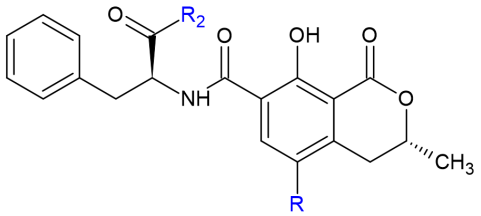
Ochratoxins

Ochratoxins represent a significant class of mycotoxins predominantly synthesized by certain species within the *Aspergillus* and *Penicillium* genera, notably *Aspergillus ochraceus*, *Aspergillus carbonarius*, and *Penicillium verrucosum*. Among the approximately 20 identified ochratoxin analogs, ochratoxin A (OTA) emerges as the most toxic and prevalent. [9]

Structurally, OTA is characterized by an amide linkage between an isocoumarin derivative and L-phenylalanine, with the presence of a chlorine atom in the dihydroisocoumarin moiety being pivotal to its high toxicity. [9] This chlorinated structure distinguishes OTA from its non-

chlorinated counterpart, ochratoxin B (OTB), and its ethyl ester derivative, ochratoxin C (OTC) (Table 2).

Table 2 Key structural distinctions among the major ochratoxin analogs



Ochratoxin	R	R_2
A	Cl	OH
B	H	OH
C	Cl	OCH_2CH_3

OTA contamination has been documented across a diverse array of agricultural commodities, including cereals, coffee, dried fruits, and red wine. [4] The toxin's stability under typical food processing conditions exacerbates its potential for human exposure. [8] Upon ingestion, OTA exhibits a high binding affinity to serum albumin, facilitating its systemic distribution and prolonged half-life. The primary toxicological manifestations of OTA involve nephrotoxicity, [10] with evidence linking it to Balkan endemic nephropathy and an elevated incidence of urinary tract tumors. Furthermore, OTA has been implicated in immunosuppression, teratogenicity, and carcinogenicity, underscoring its multifaceted threat to human health.

Zearalenone

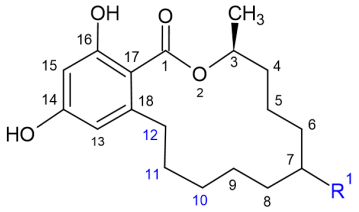
Zearalenone (ZEA) is a mycotoxin predominantly synthesized by various species of the *Fusarium* genus, notably *F. graminearum*, *F. culmorum*, *F. cerealis*, and *F. equiseti.* [11] These fungi thrive in cool, moist environments and can infect crops during pre-harvest, post-harvest, and under suboptimal storage conditions. Crops such as maize, rice, millet, oats, barley, wheat, and sorghum are particularly susceptible to ZEA contamination. [6]

Structurally, ZEA is a non-steroidal mycoestrogen that mimics natural estrogens due to its resorcylic acid lactone structure [11] (Table 3). This structural similarity enables ZEA to bind to estrogen receptors, eliciting estrogenic responses in various organisms. [12]

Upon entering an organism, ZEA undergoes metabolic transformations, primarily through the action of 3α- and 3β-hydroxysteroid dehydrogenases (HSDs). [13] This enzymatic activity reduces ZEA to two principal stereoisomeric metabolites: α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL). [13] Among these, α-ZEL exhibits a higher binding affinity to estrogen receptors, resulting in more pronounced estrogenic effects compared to both β-ZEL and the parent compound ZEA. [11] Further metabolic derivatives include zearalanone (ZAN), α-zearalanol (α-ZAL), and β-zearalanol (β-ZAL).

The estrogenic properties of ZEA and its metabolites have been implicated in reproductive disorders across various species. In livestock, exposure to ZEA has been associated with infertility, reduced litter sizes, and other reproductive anomalies. In humans, chronic dietary intake of ZEA-contaminated products has raised concerns due to reports linking ZEA exposure to precocious puberty and other hormonal disturbances. [14,15] Additionally, studies have demonstrated that ZEA can induce DNA damage in gametes, leading to compromised embryonic development and reduced offspring viability. [12]

Table 3 Summary of structure of ZEA and its derivatives



Zearalenone	R_1#	10, 11	11, 12
ZEA	=O	Saturated bond	Unsaturated bond
α-ZEL	····OH	Unsaturated bond	Saturated bond
β-ZEL	◄OH	Unsaturated bond	Saturated bond
ZAN	=O	Saturated bond	Saturated bond
α-ZAL	····OH	Saturated bond	Saturated bond
β-ZAL	◄OH	Saturated bond	Saturated bond

ketone (=O) or hydroxyl (–OH) group

Trichothecenes

Trichothecenes are a diverse group of sesquiterpenoid mycotoxins predominantly synthesized by various *Fusarium* species, notably *F. graminearum* and *F. serrata.* [16] These mycotoxins are implicated in significant agricultural losses worldwide due to their contamination of essential cereal crops. [17] Structurally, trichothecenes are categorized into four primary types: Type A: Characterized by the absence of a carbonyl group at the C-8 position, with prominent toxins including T-2 toxin (T2) and HT-2 toxin (HT2); Type B: Distinguished by a carbonyl group at C-8, with deoxynivalenol (DON) and nivalenol (NIV) being notable representatives; Type C: Identified by an additional epoxy group, and; Type D: Defined by a macrocyclic ring structure. [8] Figure 1 summarizes the structures of various trichothecene subtypes.

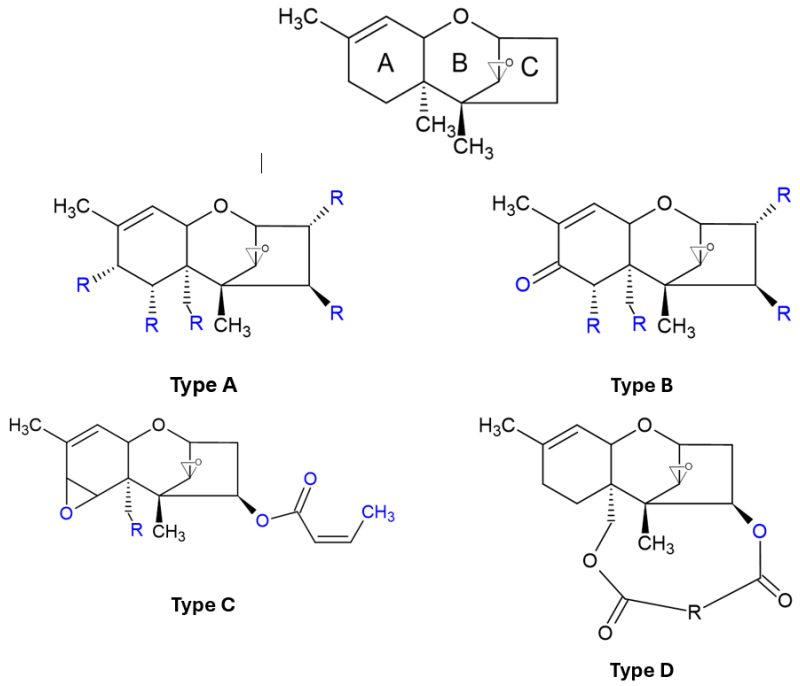


Figure 1 Classification of trichothecene structures

The presence of a 12,13-epoxy ring is a critical structural feature contributing to the biological activity of trichothecenes. This functional group is pivotal in their interaction with cellular components, leading to their cytotoxic effects. Trichothecenes exert their toxicity through the inhibition of protein synthesis, [8] affecting rapidly dividing cells and leading to immunosuppressive effects. Clinical manifestations in humans and animals include gastrointestinal disturbances, dermal irritation, [6] and hematological disorders. The severity of these symptoms is dose-dependent and varies based on the specific trichothecene involved.

Fumonisins

Fumonisins represent a critical group of mycotoxins with significant implications for both agriculture and public health. Maize and maize-derived products, staples in the global food supply are particularly vulnerable to fumonisin contamination. [18] These toxins are predominantly synthesized by *Fusarium* species, especially *F. verticillioides* and *F. proliferatum*, [19] although they have also been detected in other cereal grains including barley, wheat, oats, rye, and millet. [20]

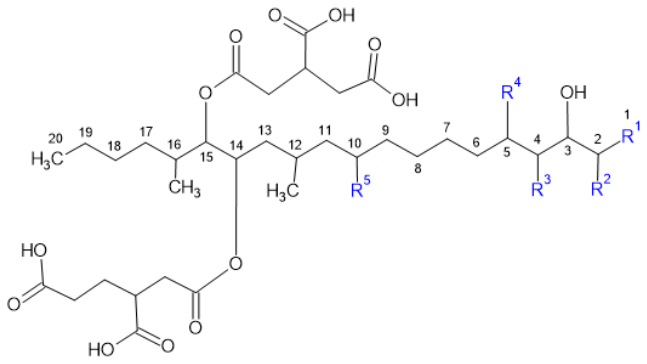
Structurally, fumonisins share remarkable similarities with sphingolipids, a characteristic that underpins their mechanism of toxicity. Their hydrophilic nature differentiates them from many other mycotoxins that are typically soluble in organic solvents, [4] and they exhibit considerable thermal stability, with significant degradation observed only at temperatures above 150°C. [8]

To date, at least twelve fumonisin analogues have been identified, which are classified into four principal series: fumonisin A, B, C, and P, with the fumonisin B series being the most prevalent [8] (Table 4.0). Within this group, fumonisin B_1 (FB_1) is recognized as the most toxic. [20] FB_1 exerts its deleterious effects primarily by inhibiting ceramide synthase, a key enzyme in the biosynthesis of sphingolipids. This inhibition results in the accumulation of free sphingoid

bases, such as sphingosine and sphinganine, which can disrupt cellular homeostasis and ultimately induce cell death. [6]

The health implications of fumonisin exposure are extensive. Beyond its role in cellular toxicity, FB_1 is implicated as a cancer promoter, particularly in liver and esophageal cancers, despite being non-genotoxic. [20] Moreover, fumonisins have been linked to neural tube defects and are a well-established cause of porcine pulmonary edema, [20] further emphasizing the urgent need for effective monitoring and control strategies in the food supply chain.

Table 4 Summary of different structures of fumonisins derivatives



Fumonisin	R_1	R_2	R_3	R_4
B1	CH_3	NH_2	H	OH
B2	CH_3	NH_2	H	OH
B3	CH_3	NH_2	H	H
B4	CH_3	NH_2	H	H
B6	CH_3	NH_2	OH	OH
C1	H	NH_2	H	OH
C3	H	NH_2	H	H
C4	H	NH_2	H	H
A1	CH_3	$NHCOCH_3$	H	OH
A2	CH_3	$NHCOCH_3$	H	OH
A3	CH_3	$NHCOCH_3$	H	H
A4	CH_3	$NHCOCH_3$	H	H
P1	H	3-hydroxypyridinium	H	OH
P2	H	3-hydroxypyridinium	H	OH
P3	H	3-hydroxypyridinium	H	H

Simultaneous Detection Methods for Multiple Mycotoxins

Multiple mycotoxins often occur together in contaminated food, leading to additive or synergistic toxic effects. [21] Given the complexity and diversity of food matrices, as well as the strict regulatory limits set by food safety authorities, the use of analytical instruments capable of simultaneously detecting and quantifying multiple mycotoxins in food is extremely crucial. Therefore, due to the co-occurring nature of mycotoxin contamination, reliable and

comprehensive multi-mycotoxin analysis becomes essential for precise risk assessment, food safety, legal compliance, and the preservation of public health.

Liquid Chromatography Coupled with Tandem Mass Spectrometry (LC-MS/MS) for Multi-Mycotoxin Analysis

LC-MS/MS has emerged as the gold standard for simultaneous detection of multiple mycotoxins in complex food matrices. This technique leverages the robust separation capabilities of liquid chromatography to resolve co-eluting mycotoxins based on subtle differences in their physicochemical properties, while tandem mass spectrometry delivers high sensitivity and selectivity. In practice, analytes are ionized—typically via electrospray ionization (ESI) and subjected to a two-stage mass analysis (MS1 followed by MS2), which provides detailed fragmentation patterns for unambiguous identification and quantification.

This approach is particularly advantageous when analyzing challenging matrices such as medicinal herbs, cereals, and processed foods, where multiple mycotoxins often co-occur and may interact synergistically to enhance toxicity. [22,23,24] By enabling simultaneous detection of a broad spectrum of mycotoxins in a single analytical run, LC-MS/MS facilitates comprehensive risk assessment and ensures compliance with stringent regulatory limits.

However, the technique is not without its limitations. LC-MS/MS systems require high capital investment, sophisticated maintenance, and highly skilled operators. Additionally, matrix effects inherent to complex food samples can compromise sensitivity and necessitate rigorous sample preparation and method validation protocols. [25]

High-Performance and Ultra-High Performance Liquid Chromatography (HPLC/UHPLC) for Multi-Mycotoxin Screening

HPLC and its advanced counterpart, UHPLC, have significantly enhanced multi-mycotoxin analysis by offering superior resolution, reduced run times, and increased sensitivity. UHPLC, in particular, utilizes sub-2 μm particle columns under elevated pressures, which improves separation efficiency while reducing solvent consumption and analysis time—a critical advantage for high-throughput laboratories.

When coupled with high-resolution detectors, such as Time-of-Flight MS (ToF-MS) or high-resolution mass spectrometry (HRMS), both HPLC and UHPLC provide robust, accurate quantification of mycotoxins even at trace levels. [26,27] These systems markedly reduce the risk of false positives by delivering precise mass measurements that help differentiate closely related compounds. Nonetheless, the expense of UHPLC systems, coupled with the need for specialized technical expertise, remains a barrier for routine implementation in less-equipped settings.

Immunochromatographic Assays (ICA)

Immunochromatographic assays, commonly known as lateral flow assays (LFAs) or rapid test strips, have gained significant traction for preliminary mycotoxin screening in field settings due to their cost-effectiveness, simplicity, and rapid turnaround. These assays exploit the high

specificity of antigen-antibody interactions, enabling the detection of target mycotoxins, such as AFB_1, OTA, and ZEA, in as little as a few minutes. [28] Recent technological advancements have further expanded their utility by developing multiplex platforms that can simultaneously screen for several mycotoxins within a single assay. [29]

Despite their advantages, ICAs are inherently qualitative or semi-quantitative, limiting their ability to provide the rigorous quantification required for regulatory compliance. Their sensitivity is generally lower compared to advanced chromatographic or mass spectrometric techniques, and issues such as antibody cross-reactivity and matrix interferences can compromise assay specificity and accuracy. Thus, while ICAs serve as valuable tools for rapid, on-site screening and preliminary decision-making, confirmatory analysis using more sophisticated methods is often necessary to ensure comprehensive risk assessment and definitive quantification.

Surface Plasmon Resonance (SPR) Biosensors

Surface Plasmon Resonance (SPR) is an advanced, label-free optical sensing technology that enables real-time monitoring of biomolecular interactions by detecting minute changes in the refractive index at the interface of a metal-coated sensor surface. In the realm of mycotoxin detection, SPR biosensors have been effectively employed to simultaneously monitor multiple targets in complex matrices such as corn and wheat. [30,31] This technique offers significant advantages by eliminating the need for additional labelling reagents, thereby streamlining sample preparation and reducing the potential for assay variability.

SPR biosensors function by immobilizing specific bioreceptors, typically antibodies or aptamers, onto a sensor chip. When a sample containing mycotoxins is introduced, binding events at the sensor surface induce measurable shifts in the resonance angle, allowing for the rapid and direct quantification of the analytes. This real-time detection capability is particularly valuable for high-throughput screening and on-site analysis, where speed and efficiency are critical.

However, the application of SPR in mycotoxin analysis is not without challenges. Matrix effects from complex food samples can introduce non-specific binding, potentially leading to false-positive signals or diminished assay sensitivity. Additionally, antibody cross-reactivity remains a concern, necessitating meticulous optimization of the sensor surface chemistry and rigorous validation protocols to ensure specificity and accuracy. Future advancements in sensor design, such as enhanced surface functionalization and integration with microfluidic systems are expected to mitigate these limitations, further bolstering the utility of SPR in comprehensive, multi-analyte detection.

Surface-Enhanced Raman Spectroscopy (SERS)

Surface-Enhanced Raman Spectroscopy (SERS) synergistically integrates the molecular fingerprinting capability of conventional Raman spectroscopy with the remarkable signal amplification provided by metallic nanostructures, typically gold or silver nanoparticles. This enhancement enables the detection of mycotoxins at ultra-low concentrations, making SERS a highly promising tool for food safety applications. Recent studies have demonstrated its

efficacy in the simultaneous detection of mycotoxins such as AFB_1, ZEA, and OTA in corn, underscoring its potential for rapid, multiplexed analysis. [32]

One of the primary advantages of SERS is its rapid analysis time, often yielding results within seconds to minutes while preserving the integrity of samples due to the use of low-power lasers. These features are particularly valuable in scenarios where high throughput and minimal sample processing are critical.

However, the performance of SERS is highly contingent on the reproducibility of nanoparticle synthesis and the uniformity of the substrate. Variability in nanoparticle size, shape, and distribution can lead to inconsistent signal enhancements, posing challenges for quantitative analysis. Moreover, the complexity of food matrices may impede effective adsorption of analytes onto the enhancing surface, potentially resulting in matrix interferences and reduced sensitivity. [33,34]

Future advancements in substrate fabrication such as the development of highly reproducible, engineered nanostructures and optimized sample preparation protocols are essential to overcome these limitations. Such improvements will further enhance the robustness, reliability, and quantitative capabilities of SERS for comprehensive multi-mycotoxin screening in diverse food matrices.

Conclusion

This review underscores the critical public health challenges posed by mycotoxin contamination in herbal medicinal products. The evidence reveals that mycotoxin such as aflatoxins, ochratoxins, zearalenone, trichothecenes, and fumonisins present significant risks due to their potent toxicological effects and frequent co-occurrence in food matrices. Advanced analytical techniques, notably LC-MS/MS and UHPLC coupled with high-resolution detectors, have revolutionized multi-mycotoxin detection, providing unparalleled sensitivity and specificity. Complementary methods, including immunochromatographic assays, SPR biosensors, and SERS, offer rapid and cost-effective screening alternatives, although each method carries inherent limitations such as matrix interferences, high operational costs, or the need for extensive sample preparation. Integrating these methodologies could mitigate individual shortcomings, enabling comprehensive risk assessment and more robust regulatory compliance. Future research should focus on optimizing sample preparation, enhancing detector performance, and developing cost-effective platforms to broaden the accessibility of high-level multi-mycotoxin analysis. These efforts are essential for safeguarding public health and ensuring the safety of herbal medicinal products.

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