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Review paper

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STATE-OF-THE-ART MYCOTOXIN ANALYSIS: INSIGHTS FROM LC/MS-MS METHOD

Radmila O. Radović*[1], Bojana Đ. Radić[1], Sanja V. Đekić[3], Sanja D. Belić[2], Jovana J. Kos[1]

[1]University of Novi Sad, Institute of Food Technology in Novi Sad, 21000 Novi Sad, Bulevar cara Lazara 1, Serbia
[2]University of Novi Sad, Faculty of Sciences, 21000 Novi Sad, Trg Dositeja Obradovića 3, Serbia
[3]Analysis d.o.o., 11070 Belgrade, Japanska 4, Serbia

Abstract: Mycotoxins are secondary metabolites produced by fungi, known for their chemical and thermal stability, which makes them resistant to common food and feed processing methods. These toxins can contaminate food and feed, and cause a range of toxic effects upon ingestion, including mutagenic, teratogenic, carcinogenic, immunotoxic, neurotoxic, hepatotoxic, and dermatotoxic effects. In recent years, the application of liquid chromatography combined with tandem mass spectrometry has grown significantly for mycotoxin analysis, owing to its remarkable sensitivity and specificity. Recent literature highlights using various liquid chromatography systems for mycotoxin analysis, typically integrated with octadecylsilane columns and employing gradient elution with mobile phases consisting of water, organic solvents, and appropriate optional modifiers. The studies reviewed predominantly utilized electrospray ionization in positive and negative modes and mass spectrometric analysis in multiple reactions monitoring mode to ensure precise multi-mycotoxin quantification. Specific configurations of liquid chromatography systems, such as ultra-high performance liquid chromatography with different column types, and mass spectrometers, including triple quadrupole and QTrap tandem mass spectrometry systems, were mainly used. These advancements underscore the ongoing refinement and standardization of methodologies for accurate and efficient mycotoxin analysis in food matrices.

Key words: *mycotoxins, LC/MS-MS, multi-analyte methods*

INTRODUCTION

Fungi are frequently encountered as contaminants during the production, storage, processing, and distribution of raw materials, finished food products, and animal feed. They produce a diverse range of secondary metabolites, numbering over 15,000, with mycotoxins being among the most important from the perspective of food and feed safety (Bills & Gloer, 2016; Zhgun, 2023). Mycotoxins are small molecular weight secondary metabolites synthesized by fungi, typically as a defence mechanism against abiotic and biotic stress factors (Matumba et al., 2021).

Despite variations in structure and biosynthetic pathways, most mycotoxins are chemically and thermally stable, making them resistant to common food and feed processing methods. In both humans and animals, mycotoxins can cause a

Corresponding author: Phone: +381611821974 Fax: +381214853770
E-mail address: radmila.radovic@fins.uns.ac.rs

variety of diseases known collectively as mycotoxicoses. When ingested through food, the toxic effects of mycotoxins can be acute or chronic, leading to numerous disorders and diseases. These harmful effects can be mutagenic, teratogenic, carcinogenic, immunotoxic, neurotoxic, hepatotoxic, and dermatotoxic in nature (Khaneghah, Moosavi, Oliveira, Vanin & Sant'Ana, 2020).

To date, several hundred mycotoxins have been identified, with the most concerning ones being produced by fungi from the *Aspergillus, Fusarium, Penicillium*, and *Alternaria* genera (Campagnollo et al., 2016; Escrivá, Oueslati, Font & Manyes, 2017; Khaneghah, Martins, von Hertwig, Bertoldo & Sant'Ana, 2018; Kagot, Okoth, De Boevre & De Saeger, 2019; Uka et al., 2019; Greeff-Laubscher, Beukes, Marais & Jacobs, 2020). The mycotoxins that draw the most attention can be categorized into three main groups based on substantial evidence of their harmful effects on human and animal health. The first group consists of so-called regulated mycotoxins, for which maximum level (ML) in certain types of food and feed are prescribed by appropriate regulations, and for which there is the most evidence regarding their health impact (European Commission, 2002a; European Commission, 2006; European Commission, 2023a). This group includes aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), and M1 (AFM1), deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A (OTA), patulin (PAT), and fumonisins B1 (FB1) and B2 (FB2). The second group consists of mycotoxins labelled as non-regulated mycotoxins, for which MLs are not yet prescribed by regulations, and their health impact is still under investigation. This group includes tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), and sterigmatocystin (STC). The third group comprises emerging mycotoxins, which have recently become the research subject and do not have regulatory control but are frequently detected in food and feed. This group includes moniliformin (MON), enniatins (ENNs), beauvericin (BEA), fusaproliferin (FP), and others (Kovalsky et al., 2016; Radić, Kos, Tanackov, Hajnal & Mandić, 2019).

Mycotoxins can be present in a wide range of agricultural products, including cereals, nuts, dried fruits, coffee, spices, oilseeds, beans, various fruits and vegetables. Additionally, they may contaminate different food products due to the use of contaminated raw materials in the production process. Mycotoxins can also enter the human food chain through milk and other animal products such as eggs and meat, as livestock consuming contaminated feed (Turner, Subrahmanyam & Piletsky, 2009; Pascari, Ramos, Marín & Sanchís, 2018).

Environmental factors such as humidity and temperature have a substantial influence on the production of mycotoxins by mycotoxigenic fungi. Effective storage procedures must carefully consider these factors, including temperature, relative humidity, and moisture content, within the warehouse environment, as they are pivotal for fungi proliferation and mycotoxin biosynthesis (Agriopoulou, Stamatelopoulou & Varzakas, 2020). Until recently, one of the most cited references regarding the occurrence of mycotoxins was published by the Food and Agriculture Organization (FAO), based on which about 25% of the world's total agricultural production is contaminated with at least one mycotoxin (FAOSTAT, 2020). However, more recent research indicates that in recent years, mycotoxin contamination exceeds the FAO's estimates, ranging between 60% and 80%, with some mycotoxins present in 100% of tested samples in certain raw materials. Modern scientific literature also highlights several factors contributing to the increasing occurrence of mycotoxins, with climate change and the application of increasingly sensitive analytical methods for determining mycotoxins in very low concentrations and detecting new mycotoxins being the most influential factors (Eskola et al., 2020; Kos, Janić Hajnal, Malachová, Krska & Sulyok, 2022; Kos et al., 2023; Kos et al., 2024; Casu, Camardo Leggieri, Toscano, & Battilani, 2024).

The increasing occurrence of mycotoxins is raising global concern, not only because of their negative impact on health, but also due to the significant economic losses they can cause in the food and feed industry (Khan, Anwar, & Ghazali, 2024). This heightened concern drives and intensifies research in mycotoxicology, focusing on understanding the prevalence and nature of mycotoxins, uncovering the causes of their appearance, and exploring their toxicity, as well as developing improved techniques for their control and reduction. Consequently, to protect human and animal health and ensure the

safety and economic viability of food and feed products, precise and sensitive instrumental methods are indispensable for detecting and quantifying mycotoxins (Picardo, Filatova, Nunez & Farré, 2019). These methods are essential for continuous monitoring of mycotoxin levels and prompt implementation of necessary interventions. However, from an analytical chemistry perspective, food and feed matrices present significant complexity due to their substantial content of both exogenous and endogenous compounds. These compounds can act as interferents, complicating the accurate determination of target mycotoxins (Mejía-Carmona, Maciel & Lanças, 2020). Hence, there is a critical imperative to develop rapid analytical techniques capable of effectively analysing mycotoxins within these complex matrices, with the choice of method largely depending on whether one or multiple mycotoxins are being targeted (Goud et al., 2018; Mejía-Carmona et al., 2020).

In this context, mycotoxin analysis in food and feed involves several complex procedures, including sampling, sample preparation, and instrumental analysis, each of which is a critical point in ensuring reliable, accurate, and precise quantification of mycotoxins in the analysed sample. First, sampling must yield a representative laboratory sample that accurately reflects the mycotoxin content of the original lot. Then, through sample preparation, it is essential to efficiently isolate and extract the mycotoxins from the sample matrix into an appropriate solvent, while minimizing interference from other compounds present in the sample. Lastly, the concentration of the targeted mycotoxins in the sample extract is determined using an appropriate instrumental method of analysis. Various detection methods can be used for mycotoxin quantification, ranging from traditional techniques like thin-layer chromatography (TLC) and enzyme-linked immunosorbent assay (ELISA) to more modern and widely used methods such as high-performance liquid chromatography (HPLC) coupled with diode array and fluorescence detectors, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The choice of the method depends on several factors, primarily on whether the analysis aims to detect a single mycotoxin, a few, or a larger number of different mycotoxins (Smaoui, Braïek & Hlima, 2020; Salvatore, Andolfi & Nicoletti, 2023). Regardless of the analytical method employed, it must be validated in accordance with the European Regulation (European Commission, 2002b) and the Technical Report of the European Committee for Standardization (Technical Committee, 2010).

This review paper aims to present the latest advancements related to the LC-MS/MS method for multi-mycotoxin analysis, including all necessary analytical steps and sample preparation techniques that typically precede this procedure.

Sampling

In mycotoxin analysis, sampling represents a key step in achieving accurate and reliable results, as it dictates the procedure for selecting a representative sample from a lot and determining its appropriate size for analysis. Errors introduced during the sampling process are often the most significant contributors to total uncertainty in the analytical workflow, frequently surpassing those associated with sample preparation or instrumental analysis. Consequently, even highly efficient extraction methods or advanced detection techniques cannot compensate for an unrepresentative sample which compromises the entire analysis. Notably, studies have shown that sampling error can account for more than 90% of the overall error in mycotoxin determination, underscoring the critical need for a rigorous sampling strategy (Spanjer et al., 2006; Whitaker, 2006).

Sampling involves transforming an initial large-scale sample, typically weighing tons, into a manageable laboratory sample of kilograms or grams, from which a smaller sub-sample (1-10g) is extracted for mycotoxin analysis (Köppen et al., 2010). This process is critical because it has greatly influenced how well the laboratory sample represents the initial lot. Since mycotoxins are often produced in localized areas and are typically not evenly distributed throughout stored commodities, only a small percentage of the sample-such as 0.1%-may be contaminated, while the rest remains uncontaminated. This inherent heterogeneity presents significant challenges for obtaining representative samples, making careful sampling essential to capture potential contamination and avoid errors from selecting non-representative portions of the lot. One of the key considerations in this process is sample size, as large

initial samples are recommended to enhance the probability of detecting mycotoxins. To address these challenges, the European Commission (European Commission, 2023b) has established specific guidelines for sampling and analytical performance, outlining structured sampling plans for various lot sizes. Incremental sampling is also crucial for addressing the heterogeneity of mycotoxin distribution. This process involves collecting numerous small increments from different areas of a lot to form an aggregate sample, which more accurately reflects the contamination level. These samples are then reduced to manageable laboratory samples, typically weighing kilograms or grams, for further analysis. It is recommended that a bulk sample consists of at least 100 incremental samples to ensure representativeness. The regulation (European Commission, 2023b) prescribes the mass and number of the incremental samples to be collected, which is determined by both the mass of the initial lot sample and the type of sample from which the sampling is conducted. For example, when sampling grains and grain products from lots weighing less than 50 tonnes, incremental samples must be collected, ranging from 10 to 100, depending on the lot's total weight, to form an aggregate sample of 1 to 10 kg (Whitaker, 2003; Elkenany & Awad, 2020; European Commission, 2023b). Typically, a laboratory sample weighing between 10 and 100 g is utilized for the extraction of the target toxin from the food matrix, ensuring that the mycotoxin content is accurately represented (Weaver, Adams & Yiannikouris, 2020; Iqbal, 2021). This structured approach helps ensure that the laboratory sample reflects the mycotoxin concentration of the entire lot, thereby enhancing the precision of the analysis (Shephard, 2016; Alshannaq & Yu, 2017).

Following the sampling procedure, the representative laboratory sample is processed using methods such as extraction, purification (clean-up), and preconcentration (De Girolamo, Lippolis & Pascale, 2022).

Sample preparation (extraction and purification)

In the initial phase of sample preparation, mycotoxins should be extracted from a representative homogenized and ground sample, followed by optional clean-up procedures designed to improve the specificity and sensitivity of the detection method (Leite, Freitas, Silva, Barbosa & Ramos, 2020). The choice of extraction and clean-up methods is generally guided by three primary considerations: the chemical properties of the mycotoxins, the composition of the matrix, and the detection technique intended for use (Ridgway, Smith & Lalljie, 2012). These methods aim to eliminate matrix interferences, preconcentrate the target analytes, and transfer them into a suitable solvent, considering the requirements of the subsequent analytical technique (de Toffoli, Maciel, Fumes, & Lanças, 2018). Furthermore, the clean-up step during mycotoxin extraction is critical for removing unwanted compounds and ensuring that only the target analytes proceed to analysis. Historically or previously, solid-phase extraction (SPE) and immunoaffinity columns (IAC) have been the most popular methods for this purpose (Munkvold, Arias, Taschl & Gruber-Dorninger, 2019; Singh & Mehta, 2020; Smaoui et al., 2020). However, with ad-vances in instrumental methods capable of simultaneously detecting multiple analytes, there has been a notable shift from traditional techniques like SPE and IAC, designed for the isolation and purification of one or a few analytes, towards more comprehensive methods that allow simultaneous extraction of multiple compounds. Since purification processes can lead to the loss of important compounds in multi-analyte methods, current trends emphasize sample preparation approaches that minimize sample treatment.

Consequently, solid-liquid extraction (SLE), and dilute-and-shoot (DaS) have gained prominence in multi-mycotoxin analyses due to their simplicity and efficiency (González-Jartín et al., 2019; Abreu et al., 2020; Greer, Chevallier, Quinn, Botana & Elliott, 2021). In addition, techniques such as liquid-liquid extraction (LLE), QuEChERS (quick, easy, cheap, effective, rugged, and safe), ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE), and novel green methods such as pressurized liquid extraction (PLE), and eco-friendly solvents like deep eutectic solvents (DES) and natural deep eutectic solvents (NADES) have also been widely applied. The effectiveness of these methods largely depends on the physicochemical properties of the target mycotoxins and the specific matrix involved (Turner et al., 2009; Sulyok, Stadler, Steiner &

Krska, 2020; Leite, Freitas, Barbosa & Ramos, 2023).

In the subsequent sections of this paper, an overview of the aforementioned sample preparation methods is provided.

Liquid-liquid extraction (LLE)

LLE, also known as solvent extraction, relies on the differing solubilities of mycotoxins in aqueous and immiscible organic layers. It is commonly used for extracting mycotoxins from liquid samples, such as milk, wine, and juices, by utilizing organic solvents like methanol, acetonitrile, and chloroform to dissolve the mycotoxins from the food matrix (Turner et al., 2009; Rausch, Brockmeyer & Schwerdtle, 2021). Although the most commonly used organic solvents in the extraction and determination of mycotoxins offer certain advantages, such as excellent solvation and extraction capabilities, they do not align with the principles of green analytical chemistry due to several drawbacks. These include high environmental toxicity (non-biodegradability), risks to the health of analysts, and significant costs (Clarke, Tu, Levers, Brohl & Hallett, 2018; Dwamena, 2019). Consequently, there is a pressing need to improve analytical methods for the extraction and determination of mycotoxins, aiming to "green" these processes by reducing or eliminating their harmful impact on the environment and analyst health. Novel green LLE methods are emerging, utilizing aqueous biphasic systems based on polymers, ionic liquids, and eutectic mixtures, which offer significant potential in terms of extraction efficiency (Mazzola et al., 2008). Among these novel approaches, DES and NADES have garnered considerable attention as potential replacements for traditional organic solvents due to their numerous advantages, including biodegradability, recyclability, non-flammability, non-toxicity, negligible volatility, and low cost.

Moreover, DESs are easy to prepare without the need for further purification, remain liquid at room temperature, and have proven capabilities in dissolving complex mixtures/matrices (Elik, Unal & Altunay, 2019; Jeliński, Przybyłek & Cysewski, 2019; Makoś, Słupek & Gębicki, 2020). In this context, He, Zhou, Wan & Tan (2020) developed a straightforward method using a DES based on tetramethylammonium chloride and malonic acid (1:2 molar ratio) to extract AFs from rice samples. Following optimization, the method achieved high precision, with relative standard deviations (RSD) below 5.5%, and satisfactory recoveries ranging from 79% to 114%, demonstrating that DES could serve as an efficient and green extractant for AFs in rice. This study highlights the potential of DES in advancing the green chemistry principles in mycotoxin analysis.

Pressurized Liquid Extraction (PLE)

In addition to DES, other advanced green extraction techniques, such as PLE, also known as Accelerated Solvent Extraction (ASE), are employed for mycotoxin extraction. This technique typically utilizes green solvents like water, supplemented with a small amount of organic solvent, in lower quantities than traditional LLE methods. ASE operates under relatively high pressures and temperatures above the boiling point, thereby enhancing mycotoxin extraction efficiency from food matrices while simultaneously reducing the negative environmental impact by minimizing the use of organic solvents. This approach improves solvation and extraction kinetics by increasing pressure, which promotes better interaction between the solvent and analytes.

Additionally, the elevated temperatures help to disrupt the interactions between target compounds and the matrix, further increasing extraction efficiency (Iqbal, 2021).

Ultrasonic- and microwave-assisted extractions (UAE, MAE)

Moreover, UAE and MAE are increasingly employed for the extraction of mycotoxins from both liquid and solid samples, as they improve extraction efficiency and shorten extraction time. UAE uses ultrasonic waves to disrupt the food matrix, thereby improving solvent penetration and facilitating the release of mycotoxins. MAE utilizes microwaves for a similar purpose, rapidly heating the matrix to promote efficient extraction (Sartori, de Moraes, dos Santos, Souza & da Nóbrega, 2017; Wu, Sun, Pu & Wei, 2023). In one notable study, Sartori et al. (2017) developed a simple strategy based on UAE for extracting 14 mycotoxins from cereal-based porridge intended for infant consumption. After brief shaking followed by sonication for 10 minutes, without requiring additional clean-up steps, recoveries between 64% and 113% were achieved, demonstrating the method's efficacy in extracting

a wide range of mycotoxins quickly and efficiently. This approach underscores the growing preference for UAE in mycotoxin analysis due to its ability to deliver rapid, reliable results with minimal sample preparation.

Solid-liquid extraction (SLE)

The extraction of mycotoxins from solid matrices (cereals, dry fruits, spices, and feed) of varying consistencies can be effectively accomplished using the SLE technique. The simplicity and efficiency of SLE in handling a wide range of sample types, coupled with its demonstrated effectiveness in extracting mycotoxins from various solid matrices, have been established as a widely used technique in mycotoxin analysis. Moreover, the method's ability to accommodate different consistencies of solid samples further underscores its versatility in analytical procedures. This process typically involves the use of organic solvents such as methanol, acetonitrile, or chloroform to transfer mycotoxins from solid food samples into a liquid phase.

The addition of water to these organic solvents increases their polarity, enhancing their penetration into the food matrix and improving the extraction process. Furthermore, incorporating an acidic buffer into the extraction mixture aids in breaking the strong bonds between the analytes and food components like proteins and sugars, significantly boosting extraction efficiency (Xie, Chen & Ying, 2016; Boshra, El-Housseiny, Farag & Aboshanab, 2024).

A critical aspect of the procedure is the selection of the extraction solvent, as the chemical diversity of mycotoxins necessitates different solvents for optimal extraction. For instance, high organic solvents are particularly effective for extracting hydrophobic compounds such as AFs, OTA, and ZEN, while aqueous or acidic solvents are more suitable for polar compounds like FBs and mycophenolic acid (MPA) (Sulyok, Berthiller, Krska & Schuhmacher, 2006; Lattanzio, Solfrizzo, Powers & Visconti, 2007). In recent years, a similar shift towards the use of green solvents in SLE has occurred, aiming to enhance sustainability and mitigate environmental and health impacts. These green solvents include those used in green LLE, such as water-based systems, supercritical carbon dioxide, DES, and ionic liquids (Abdelfattah, 2023).

QuEChERS

The QuEChERS method is another solvent extraction technique that incorporates a dispersive solid-phase extraction (dSPE) step for enhanced extract purification. It is currently the increasingly favored clean-up approach due to its speed, simplicity, cost-effectiveness, robustness, safety, and suitability for multi-analyte determination. Originally developed for pesticide analysis, the QuEChERS method involves solvent extraction (LLE or SLE) with acetonitrile and water mixture and a salting-out step induced by adding inorganic salts (magnesium sulfate and sodium chloride) to remove water. The subsequent dSPE with salts and sorbent materials further purifies the compounds. Its ability to perform rapid extraction, efficient purification, and multi-analyte extraction has led to its increasing adaptation for mycotoxin analysis in recent years (González-Jartín et al., 2019).

Dilute-and-shoot

However, some of the aforementioned traditional methods may face challenges with co-extraction, resulting in significant matrix interference (Greer et al., 2021). The DaS method in mycotoxin extraction simplifies sample preparation by directly diluting the sample extract with a suitable solvent before subjecting it to instrumental analysis, such as LC-MS/MS. This dilution reduces the concentration of interfering matrix components, thus mitigating matrix effects (MEs), which can otherwise distort analytical results by causing signal suppression or enhancement (Sulyok et al., 2020). By minimizing these matrix interferences, the DaS method also reduces the loss of analytes commonly observed in more complex sample preparation procedures, making it particularly well-suited for multi-analyte analyses in complex matrices such as food and feed samples (González-Jartín et al., 2019). A crucial factor in the success of the DaS method is the balance between reducing matrix effects and maintaining analytical sensitivity, with the choice of solvent and dilution factor playing a pivotal role in optimizing performance. This technique offers advantages in terms of simplicity and faster workflows, as it eliminates the need for extensive clean-up steps, which are often time-

consuming and prone to analyte loss during extraction (Malachová et al., 2018).

For instance, Sulyok et al. (2020) extensively applied the DaS method in their mycotoxin analysis research, highlighting its efficiency in handling a broad range of mycotoxins, including both regulated and emerging contaminants, across diverse matrices while ensuring minimal matrix interference. The method's capacity to simplify sample preparation while maintaining accuracy and reliability in quantification underscores its value in modern analytical laboratories.

In summary, the increasing demand for comprehensive mycotoxin analysis across diverse food and feed matrices has sustained the popularity of pretreatment methods such as SLE and QuEChERS, largely due to their commercial availability and ease of implementation (Bian, Zhang, Zhou, Wei & Feng, 2023). Additionally, the DaS method has also gained wider acceptance in recent years, further enhancing the versatility and efficiency of multi-mycotoxin analyses by minimizing analyte loss during sample preparation (Malachová et al., 2018). Furthermore, green solvents, including DES and NADES, are increasingly considered for environmental benefits, but their adoption in mycotoxin analysis remains limited. Most studies utilizing these solvents have been restricted to the extraction of a limited number of mycotoxins. However, the growing emphasis on green chemistry suggests a promising shift toward more sustainable and environmentally friendly extraction methods, with ongoing research aiming to expand their applicability (Pradanas-González et al., 2021). Future advancements in sample preparation are likely to focus on the broader integration of these greener alternatives, balancing analytical efficiency with sustainability. Building on these advances in sample preparation, the next section will address analytical methods that enable precise and accurate quantification of mycotoxins in food and feed matrices, with a primary focus on the increasingly popular, efficient, accurate and selective instrumental method, LC-MS/MS.

Analytical methods in mycotoxin analysis

To illustrate advancements in mycotoxin analysis, it is important to primarily mention the traditionally used methods, particularly ELISA and TLC, which were the primary analytical techniques applied in various food samples, as documented in earlier studies (Shephard, 2008; Alshannaq & Yu, 2017). ELISA techniques have been extensively validated across diverse food matrices (Pereira, Fernandes & Cunha, 2014; Rahmani, Jinap & Soleimany, 2009), contributing to their wide-spread adoption in mycotoxin analysis due to their rapidity, user-friendliness, and cost-effectiveness. Furthermore, the availability of kits from various manufacturers for detecting all regulated mycotoxins enhances their accessibility. However, its limitations include the potential for false-positive results due to cross-reactivity with structurally similar toxins or matrix components, the need for an equilibrium phase that prolongs the analysis time, and the capability to detect primarily individual mycotoxins rather than multiple toxins simultaneously (Akiyama, Goda, Tanaka & Toyoda, 2001; Agriopoulou et al., 2020; Tittlemier et al., 2021). Similarly, while TLC was once a standard screening method and remains an economical option for mycotoxin analysis in various food and feed samples, its use has diminished due to its lower sensitivity and reproducibility compared to more advanced techniques. Advancements in analytical technology have led to the increasing prevalence of HPLC, especially when coupled with diode array detectors (DAD) and fluorescence detectors (FLD), which offer significant improvements in sensitivity and specificity. HPLC allows for the reliable determination of one or a few mycotoxins simultaneously (Iqbal, 2021). Nevertheless, the complexity of food matrices and the need for comprehensive mycotoxin profiling have driven the development of more sophisticated techniques such as LC-MS/MS, which can detect multiple mycotoxins in a single analytical run (Malachová et al., 2014; Sulyok et al., 2020).

Regardless of the analytical method employed for mycotoxin determination, validation according to relevant guidelines is essential. For LC-MS/MS methods, the key validation parameters outlined in international guidelines include linearity, limits of detection (LOD), limits of quantification (LOQ), trueness, recovery rates, precision, (both repeatability and reproducibility), sensitivity, specificity, robustness, and matrix effects (European Commission, 2021a,

European Commission, 2021b). These parameters ensure the method is reliable, reproducible, and suitable for application across diverse food and feed matrices. Recovery experiments are essential for evaluating a method's effectiveness in extracting and quantifying multiple mycotoxins under controlled conditions, with recovery rates reflecting the proportion of the analyte successfully extracted and quantified relative to its actual concentration in the sample. In multi-mycotoxin methods, the objective is to optimize these recovery rates across a broad spectrum of mycotoxins by implementing uniform sample preparation and chromatographic conditions, ensuring consistency and analytical reliability. Moreover, matrix effects, which describe the impact of co-extracted substances on the detection and quantification of target analytes, represent a critical consideration in method validation, as they can significantly influence both the accuracy and reproducibility of the analytical method.

This approach distinguishes multi-mycotoxin methods from single-mycotoxin methods, which typically focus on maximizing recovery for one or a few specific mycotoxins. Multi-mycotoxin methods further emphasize balancing recovery rates for a wide range of analytes while maintaining sensitivity and specificity. This complexity underscores the necessity for robust validation practices and innovative analytical strategies to address the unique challenges posed by these methods (Vidal, Meng-Reiterer, Kunz-Vekiru, De Saeger & Schuhmacher, 2018; Sulyok et al., 2020; European Commission, 2021a).

Given these requirements, LC-MS/MS has emerged as one of the most robust techniques for the simultaneous detection and quantification of multiple mycotoxins in complex matrices, as will be presented in greater detail in the following section.

Liquid chromatography-tandem mass spectrometry

Over the last decade, LC-MS/MS has become the predominant method for the quantitative determination of mycotoxins and other low-molecular-weight contaminants in food and feed samples, owing to its unmatched capacity to manage complex matrices while delivering precise and reliable results. LC-MS/MS is particularly effective for separating compounds with high polarity, thermal instability, and non-volatility. One of the major advantages of this technique is its high sensitivity and selectivity, allowing the detection of analytes without the need for derivatization. This technique, which combines the separation capabilities of liquid chromatography with the analytical precision of tandem mass spectrometry, enables the simultaneous monitoring of multiple ion fragments during analyte elution, making it highly effective for the detailed identification and quantification of multi-mycotoxins in complex food matrices, even at trace levels (Sulyok et al., 2020; Zhang, Flannery & Zhang, 2024).

The LC-MS/MS method first utilizes liquid chromatography to separate mycotoxins based on their chemical properties as they pass through a chromatographic column. This step is essential for isolating individual mycotoxins from complex matrices. Once separated, the eluted compounds enter the mass spectrometer, where they undergo fragmentation in the tandem mass spectrometry phase. This process, when combined with multiple reaction monitoring (MRM) - a technique that selectively monitors specific ion transitions of target mycotoxins in tandem mass spectrometry - yields detailed molecular information, facilitating accurate identification and quantification of mycotoxins (Greer et al., 2021). Moreover, its integration with advanced sample preparation techniques further enhances its effectiveness, making it an indispensable tool for ensuring food safety and regulatory compliance (Iqbal, 2021). The increased use of LC-MS/MS reflects its ability to provide detailed, precise, and simultaneous detection of mycotoxins, addressing the limitations of earlier techniques and meeting the high standards set by regulatory bodies like the Association of Official Analytical Chemists (AOAC) and European Standardization (Agriopoulou et al., 2020; Tittlemier et al., 2021).

Table 1 presents an overview of selected recent studies, along with relevant details on sample preparation and key achieved analytical parameters, where mycotoxin analysis was performed using the LC-MS/MS method. The LC-MS/MS method is particularly valued for its robustness and reliability, as demonstrated by the comprehensive approach developed by Sulyok, Suman and Krska (2024), which represents the most extensive and advanced method for mycotoxin detection currently available. The study by Sulyok et al. (2024) de-

tailed the validation of this method for the simultaneous quantification of over 700 mycotoxins, secondary fungal metabolites, and plant toxins in various food matrices, including pasta, biscuits, crackers, and muesli. Utilizing a DaS sample preparation approach with a solvent mixture of acetonitrile, water, and acetic acid in a ratio of 79:20:1 (v/v/v), the method demonstrated minimal matrix effects, with only 7-14% of analytes significantly impacted. Most analytes achieved recovery rates within the target range of 70-120%, with only 26 compounds falling outside this range. It also exhibited high repeatability and intermediate precision, meeting the <20% criterion for 95-98% and 99% of analytes, respectively. The application of this method, which covers the largest number of mycotoxins among published methods, indicates that analysis of 157 samples from the European market revealed frequent detection of ENNs and DON, with no regulatory limits exceeded, except in a few cases where the sum of ergot alkaloids surpassed the forthcoming limit of 50-150 µg/kg.

The method described by Sulyok et al. (2024) represents a significant enhancement of earlier methodologies. Initially, Sulyok et al. (2006) validated a method for the detection of 39 mycotoxins in wheat and maize. Over time, the method was significantly expanded, both in terms of the number of analytes and the complexity of the matrices used for validation. By 2014 (Malachová, Sulyok, Beltrán, Berthiller, & Krska, 2014), it was adapted to quantify 295 secondary metabolites across diverse matrices, including apple puree (high water content), hazelnuts (high fat content), maize (high starch, low fat), and green pepper (a challenging matrix). In 2020 (Sulyok et al., 2020), the method was further extended to cover over 500 compounds, with validation in wheat, maize, figs, dried grapes, walnuts, pistachios, and almonds. Ultimately, the method evolved into the 2024 version (Sulyok et al., 2024), which is capable of quantifying 730 mycotoxins and secondary fungal metabolites in more complex matrices, as previously described. Despite these advancements, the sample preparation method remained consistent throughout, relying on the DaS technique without additional purification steps, using the same extraction solvent as in the 2024 method. Instrumentation improvements also contributed to the method's evolution. Initially, the method employed the QTrap 4000 LC-MS/MS system (Applied Biosystems), but in 2014 (Malachová et al., 2014), it was upgraded to the QTrap 5500 sys-tem, which offered greater sensitivity and faster acquisition speeds. Notably, despite the up-grade in instrumentation, the same Gemini C18 chromatographic column (150 × 4.6 mm i.d., 5 µm particle size), equipped with a C18 security guard cartridge (4 × 3 mm i.d., all from Phenomenex, Torrance, CA, US), was retained due to its robustness, particularly when handling turbid samples. Additionally, the mobile phase composition (5 mM ammonium acetate in both mobile phases, consisting of methanol/water/acetic acid in ratios of 10:89:1 (v/v/v) for eluent A and 97:2:1 (v/v/v) for eluent B) and injection volume (5 µL) remained unchanged across all studies. Further refinements introduced in 2020 (Sulyok et al., 2020), such as narrowing the retention window width in sMRM mode, enhanced the method's performance without compromising sensitivity, enabling robust detection in even more complex sample matrices.

The DaS extraction method was also employed in the study by Rausch et al. (2021) which analyzed 42 mycotoxins in beer samples. In this study, acetonitrile served as the extraction solvent, with recovery rates for all analytes ranging from 79% to 100% and LOQ spanning from 0.04 to 75 µg/L.

Ning et al. (2024) developed and validated a UHPLC-MS/MS method for the analysis of 67 mycotoxins in human plasma. The sample preparation employed the LLE approach, classifying analytes into three groups based on their physicochemical properties. Group A compounds were extracted using acetonitrile acidified with 0.1% formic acid, without further purification. For Group B compounds, the extraction solvent was acetonitrile/water (84:16, v/v), and the supernatant was purified using Captiva EMR-Lipid cartridges. Group C compounds followed a similar procedure to Group B but employed PRiME-HLB cartridges for purification. The method achieved intra-day precision ranging from 1.8% to 11.9% RSD and intra-day trueness from 82.7% to 116.6% for most mycotoxins, except for certain compounds that exhibited trueness values between 66.4% and 129.8%. A total of 40 mycotoxins, including 24 emerging ones, were detected in 184 plasma samples (89 from infertile males and 95 from healthy males), demonstrating widespread human exposure to both traditional

and emerging mycotoxins. Notably, the incidence of exposure to multiple mycotoxins was significantly higher in infertile males, with elevated levels of specific compounds. These findings highlight the necessity for extensive biomonitoring to explore the potential relationship between mycotoxin exposure and male infertility.

The QuEChERS extraction method is prominently featured in several studies for its effecttiveness in analyzing mycotoxins across various matrices. Laouni et al. (2024) utilized the QuEChERS method to extract five mycotoxins from chicken feed and, less commonly in-vestigated matrices, such as eggs. Their protocol involved using acetonitrile with 5% formic acid for the extraction, followed by the addition of salts such as magnesium sulfate, sodium chloride, sodium citrate, and disodium hydrogen citrate sesquihydrate to facilitate phase separation and clean-up.

In a separate study, Rodríguez-Cañas et al. (2023) applied the QuEChERS method to analyze 32 mycotoxins in cheese. They used a 2% formic acid aqueous solution combined with acetonitrile as the extraction solvent. The process also involved the use of salts like magnesium sulfate and sodium chloride to ensure efficient extraction and clean-up of the cheese matrix.

Further, Castilla-Fernández, Rocío-Bautista, Moreno-González, García-Reyes, and Molina-Díaz (2022) tested various solid-phase extraction clean-up methods-PRiME HLB, EMR-Lipid, molecularly imprinted polymers (MIPs), Zsep+, C18, and PSA-each selected for their potential to improve extract cleanliness in fatty matrices. Although these sorbents were rigorously evaluated, none significantly minimized matrix effects. Instead, the DaS method demonstrated superior performance, achieving matrix effects below 20% with a 1:100 dilution factor, emphasizing its efficacy in multi-analyte extractions. The sample preparation was grounded in a citrate-buffered QuEChERS protocol for the analysis of 5 mycotoxins in walnuts, incurporating acetonitrile and formic acid in a 99:0.1 (v/v) ratio and supplemented with salts such as anhydrous magnesium sulfate, sodium chloride, sodium citrate, and citric acid. Compared to traditional clean-up approaches, DaS delivered cleaner extracts, more reliable recoveries, and higher throughput, establishing it as an optimal choice for complex matrices where controlling matrix interferences is essential.

In addition to the widely used QuEChERS method, the studies also employed other extraction techniques, such as SLE and NADES, to target specific mycotoxins in various food matrices. SLE was utilized in several studies with tailored protocols depending on the matrix. For example, Liang, York, Konschnik, Majer and Steimling (2023) applied SLE to analyze 37 mycotoxins in baby wheat cereal, peanuts, and tomato puree. Their extraction involved using acetonitrile/water (4:1, v/v) with 0.5% formic acid for baby wheat cereal and peanuts, whe--reas tomato puree samples were extracted without formic acid. The method demonstrated effective recovery rates ranging from 72 to 117% for different mycotoxins, with lower recoveries observed in tomato puree where citrinin was an exception.

Similarly, Er Demirhan and Demirhan (2021) employed SLE for the analysis of 13 mycotoxins in cereal-based baby foods, using a Jasm analysis kit, which included a reagent mixture of acetonitrile/water and additional salts, achieving recovery rates between 83% and 109%.

NADES, the innovative extraction technique, was applied by Pradanas-González et al. (2021) for the analysis of 6 mycotoxins in edible insects. This method is particularly notable for its use of environmentally friendly solvents. The NADES they used consisted of a mixture of choline chloride and urea in a 1:2 molar ratio, with 15% water added to the solution. This approach yielded high recovery rates, ranging from 70% to 104%, except for the T-2 toxin, which had a lower recovery of around 50%. NADES is gaining attention due to its non-toxic nature and efficiency in extracting bioactive compounds from various complex matrices (Pradanas-González et al., 2021).These alternative extraction methods, SLE and NADES, complement the QuEChERS approach, offering versatility in handling different food matrices and mycotoxins, with each method tailored to optimize recovery and minimize matrix effects.

While Table 1 provides specific parameters regarding the matrix types, the number of targeted mycotoxins, extraction techniques, clean-up procedures, recovery rates, and LOQ from the referenced studies, it is also important to consider the chromatography systems utilized in these analyses.

Table 1.
Overview of selected recent studies on mycotoxin analysis using LC-MS/MS, including sample preparation and key analytical parameters (LOQ and recovery values)

Matrix	Number of analytes	Extraction protocol	Clean-up	Recovery (%)	LOQ	Reference
Pasta **Biscuits** **Crackers** **Musli**	700 mycotoxins and other secondary metabolites	Dilute and shoot - acetonitrile/water/acetic acid 79:20:1, v/v/v	/	70-120 (except for 26 analytes)	Biscuits: $7.2\cdot10^{-4}$-$4.9\cdot10^{2}$ µg/kg Musli: $1.2\cdot10^{-3}$-$5.4\cdot10^{2}$ µg/kg	Sulyok et al., 2024
Human plasma	67 mycotoxins	LLE - Group A compounds: acetonitrile with 0.1% formic acid - Group B compounds: acetonitrile/water 84:16, v/v - Group C compounds: acetonitrile/water 84:16, v/v	Group A compounds: / Group B compounds: SPE-Captiva, EMR-Lipid cartridges Group C compounds: PRiME-HLB cartridges	60-146	$0.2\cdot10^{-2}$-1.0 µg/L	Ning et al., 2024
Chicken feed **Eggs**	5 mycotoxins	Feed (QuEChERS): - acetonitrile with 5% formic acid - salt: magnesium sulfate, sodium chloride, sodium citrate, and disodium hydrogen citrate sesquihydrate Eggs (QuEChERS): - methanol/water 80:20, v/v with 1% acetic acid - salt: sodium sulfate anhydrous and sodium acetate anhydrous	/	Feed: 88-103 Eggs: 92-108	Feed: 0.4-1.1 µg/kg Eggs: 0.3-0.8 µg/kg	Laouni et al., 2024
Maize **Sorghum**	10 mycotoxins	QuEChERS: - acetonitrile/water 80:20 v/v with 0.1% formic acid; - salts: sodium chloride and/or magnesium sulfate	/	88-110	0.5-89.3 µg/kg	Mbisana, Rebagamang, Mogopodi & Chibua, 2023
Cocoa beans	34 mycotoxins	Modified SLE: - acetonitrile/water/ acetic acid 7:2.5:0.5, v/v/v - salt: NaCl	/	70-130	1-75.0 µg/kg	Abreu et al., 2020

Baby wheat cereal **Peanut** **Tomato puree** **Blended flour**	37 mycotoxins	SLE: -0.5% formic acid* acidified acetonitrile/water 4:1, v/v *no formic acid was used for tomato puree samples	/	72–112 (except citrinin: in all samples≈30, except in tomato puree where it was≈75)	Tomato puree $0.5 \cdot 10^{-1}$-2.5 µg/L The remaining three matrices 0.05-1 µg/L	Liang et al., 2023
Cheese	32 mycotoxins	QuEChERS: -2 % formic acid aqueous solution and acetonitrile -salts: magnesium sulfate and sodium chloride	/	above 70 for most mycotoxins	$0.6 \cdot 10^{-1}$-9.7 µg/L	Rodríguez-Cañás et al., 2023
Walnuts	5 mycotoxins	The citrate-buffered QuEChERS: -acetonitrile/formic acid 99.9:0.1, v/v -salts: anhydrous magnesium sulfate, sodium chloride, sodium citrate dihydrate, and sodium hydrogencitrate sesquihydrate	A: / B: 6 Clean-up approaches (PRiME HLB cartridge, EMR-Lipid sorbent, AFFINIMIP, Z-sep$^+$ sorbent, C18, PSA)	95-110	1.8-1.9 µg/kg	Castilla-Fernandez et al., 2022
Cereal-based baby foods	13 mycotoxins	SLE: -Jasem analysis kit, reagent 1 (JSM FO 9704 and JSM FO 1503)	/	83-109	$0.1 \cdot 10^{-1}$-8.0 µg/kg	Er Demirhan & Demirhan, 2021
Edible insects	6 mycotoxins	NADES: - choline chloride/urea, 1:2 mole ratio, with 15% water	/	70-104 except for T-2 toxin (50)	40-$3.7 \cdot 10^{2}$ µg/kg	Pradanas-González et al., 2021
Milk	40 mycotoxins	QuEChERS: -0.5% formic acid in acetonitrile -salts: magnesium sulfate and sodium chloride	A: / B: d-SPE clean-up using magnesium sulfate and C18	61-120	$0.7 \cdot 10^{-2}$-14.6 µg/L	González-Jartín et al., 2021
Beer	41 free and modified mycotoxins	Dilute and shoot: acetonitrile	/	79-100	$0.4 \cdot 10^{-1}$-75.0 µg/L	Rausch et al., 2021
Oats	42 mycotoxins	QuEChERS: -1% aqueous acetic acid solution and acetonitrile -salts: anhydrous magnesium sulfate and sodium chloride	/	80-120	0.5-100 µg/kg	De Colli et al., 2020

For multi-analyte methods in mycotoxin analysis, C18 columns are commonly employed due to their versatility in separating a wide range of analytes.

These columns, sourced from various manufacturers, differ in particle size and length, providing flexibility in optimizing separation conditions based on specific analytical requirements. Notably, Castilla-Fernandez et al. (2022), Pradanas-González et al. (2021), Mbisana et al. (2023), and Laouni et al. (2024) favoured UHPLC systems equipped with Zorbax Eclipse Plus C18 columns. In contrast, De Colli et al. (2020) and Abreu et al. (2020) selected UHPLC systems with Acquity BEH C18 columns, while Rodríguez-Cañás et al. (2023) employed an Acquity HSS T3 column for their analyses. Rausch et al. (2021) utilized a combination of Raptor Fluoro Phenyl and Raptor Biphenyl columns in series, along with a Fluoro Phenyl guard column cartridge for most analytes, except for zearalenone 14, 16-disulfate (ZENdiS), which was separated using a Raptor HILIC-Si column in a subsequent chromatographic run. Liang et al. (2023) implemented a UPLC system with a Raptor Biphenyl column, along with a Raptor Biphenyl EXP guard column. Similarly, Ning et al. (2024) opted for a Poroshell 120 EC C18 column for chromatographic separation.

In the reviewed studies, various mobile phases were utilized, demonstrating different combinations of solvents and additives to achieve optimal chromatographic separation. All studies operated in gradient elution mode, with mobile phases generally consisting of water combined with either methanol and/or acetonitrile, along with additives such as formic acid, acetic acid, or ammonium formate. Se-veral authors utilized water and acetonitrile as mobile phases, with varying additives. Mbisana et al. (2023) employed water with 0.1% formic acid and a 50:50 (v/v) mixture of water and acetonitrile with 0.1% FA. Abreu et al. (2020) used aqueous phase A with 0.1% formic acid, 5 mmol/L ammonium formate, and 2% methanol, while phase B consisted of acetonitrile with 0.1% formic acid. Ning et al. (2024) implemented an eluent composed of 0.1% formic acid with 1 mM ammonium formate in phase A and acetonitrile in phase B. Pradanas-González et al. (2021) and Rausch et al. (2021) used a si-milar combination with ammonium formate as an additive. In contrast, De Colli et al. (2020) utilized water and methanol, adding ammonium acetate and/or acetic acid. Laouni et al. (2024), Castilla-Fernandez et al. (2022), and Liang et al. (2023) also employed water and methanol with 0.1% formic acid as an additive. Rodríguez-Cañás et al. (2023) combined methanol and water with formic acid and ammonium formate in both phases.

Furthermore, as the sensitivity and robustness of the LC-MS/MS method are significantly influenced by the type of mass analyzer, it is essential to provide details regarding the specific instruments used in each study. Mass spectrometric analysis predominantly applied triple quadrupole mass analyzers (Malachová et al. 2018; Sulyok et al., 2020). Er Demirhan & Demirhan (2021) and Pradanas-González et al. (2021) employed a triple quadrupole mass spectrometer, specifically the Agilent 6470, while De Colli et al. (2020) used the Waters Quattro Premier XE. Laouni et al. (2024) utilized the triple Quad API 3200, and Liang et al. (2023) applied the Xevo TQ-S. Ning et al. (2024) conducted their analyses using a triple Quad™ 7500 mass spectrometry system.

The QTrap MS/MS system is also widely used across numerous studies, with Mbisana et al. (2023) and Abreu et al. (2020) utilizing the QTrap 6500+ in their research, while Rausch et al. (2021) employed an LC-MS/MS system equipped with a QTrap 5500 mass analyzer. In contrast, Castilla-Fernandez et al. (2022) used a more advanced mass analyzer, the Triple Quadrupole-Linear Ion Trap Mass Spectrometer.

Electrospray ionization interface (ESI) was uniformly applied across all studies, primarily in positive mode. However, Pradanas-González et al. (2021), Rausch et al. (2021), Abreu et al. (2020), Ning et al. (2024) and Sulyok et al. (2024) employed both positive and negative modes, and across all studies, analysis was operated in MRM data acquisition mode to enhance the precision and selectivity of mycotoxins detection.

In summary, these studies utilized a variety of LC systems, typically coupled with C18 or specialized columns, under gradient elution with carefully optimized mobile phases. The consistent application of mass spectrometers, including the QTrap MS/MS and triple quadrupole instruments, along with ESI in both positive and/or negative modes and the MRM acquisition method, underscores the robust methodologies developed for precise mycotoxin quantification.

CONCLUSIONS

In response to increasing consumer demands, modern trends in food and feed production are increasingly oriented toward meeting high standards in both nutritional composition and food and feed safety. However, despite the rapid technological advancements and the implementation of new technologies aimed at ensuring health safety and maintaining the nutritional and technological quality of products, the presence of fungi and mycotoxins in food products continues to pose a growing and significant challenge. LC-MS/MS has become the dominant analytical technique, recognized for its exceptional sensitivity, specificity, and capacity to detect multiple mycotoxins simultaneously. The trend in the development of multicomponent methods has accelerated, raising questions about the future limits and scope of these methods, particularly concerning selectivity, accuracy, equipment availability, and the cost of analysis. Despite the introduction of green mycotoxin extraction solvents, their application remains limited, with conventional methods like SLE and QuEChERS still prevalently employed. The ongoing refinement of LC-MS/MS, coupled with the advancement of more sustainable extraction methods such as DaS, holds promise for enhancing the accuracy, efficiency, and environmental sustainability of multiple mycotoxin analyses across various matrices. As these developments progress, they are expected to play a crucial role in addressing the persistent challenges associated with mycotoxin contamination, ultimately contributing to the production of safer and higher-quality food and feed products.

AUTHOR CONTRIBUTIONS

Conceptualization, R.O.R. and J.J.K; Methodology, R.O.R. and J.J.K.; Investigation, formal analysis, writing-original draft preparation, R.O.R.; Writing-review and editing, J.J.K., S.V.Đ, S.D.B and B.Đ.R; Supervision, J.J.K., S.D.B and S.V.Đ.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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NAPREDAK U DETEKCIJI MIKOTOKSINA: ULOGA LC/MS-MS METODE

Radmila O. Radović*[1], Bojana Đ. Radić[1], Sanja V. Đekić[3], Sanja D. Belić[2], Jovana J. Kos[1]

[1]Univerzitet u Novom Sadu, Naučni institut za prehrambene tehnologije u Novom Sadu, 21000 Novi Sad, Bulevar cara Lazara br. 1, Srbija
[2]Univerzitet u Novom Sadu, Prirodno-matematički fakultet, 21000 Novi Sad, Trg Dositeja Obradovića 3, Srbija
[3]Analysis d.o.o., 11070 Beograd, Japanska 4, Srbija

Sažetak: Mikotoksini su sekundarni metaboliti plesni i poznati su po svojoj hemijskoj i termičkoj stabilnosti, što ih čini otpornim na uobičajene metode obrade hrane i hrane za životinje. Mogu kontaminirati hranu i hranu za životinje, izazivajući niz toksičnih efekata prilikom unosa u organizam, uključujući mutagene, teratogene, kancerogene, imunotoksične, neurotoksicne, hepatotoksične i dermatotoksične efekte. Tečna hromatografija u kombinaciji sa tandem masenom spektrometrijom (LC-MS/MS) postala je dominantna analitička metoda za detekciju mikotoksina zbog svoje izuzetne osetljivosti i specifičnosti. Savremena literatura ukazuje na upotrebu različitih tečno-hromatografskih sistema, obično integrisanih sa oktadecilsilan kolonama, uz primenu gradijentnog načina eluiranja sa mobilnim fazama koje se najčešće sastoje od vode, organskih rastvarača i odgovarajućih modifikatora. U pregledanim studijama pretežno je korišćena elektrosprej jonizacija u pozitivnom i negativnom režimu i maseno-spektrometrijska analiza u režimu višestruke reakcije praćenja kako bi se obezbedila precizna kvantifikacija. Specifične konfiguracije tečno-hromatografskih sistema, kao što su ultra visokopritisna tečna hromatografija sa različitim vrstama kolona i masenim spektrometrima, uključujući trostruke kvadrupole i QTrap tandem masene sisteme, najčešće su korišćene. Ova dostignuća naglašavaju kontinuirani napredak i standardizaciju metodologija za tačnu i efikasnu analizu mikotoksina u prehrambenim uzorcima

.**Ključne reči:** *mikotoksini, LC/MS-MS, višekomponentne metode*

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