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# Review of deep eutectic solvents for microextraction of mycotoxins from foods

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ABSTRACT

Mycotoxins (MTs) are toxic secondary metabolites produced by certain fungi that commonly contaminate agricultural products. These compounds can cause serious effects on health, including hepatotoxicity, immunosuppression, and carcinogenic effects, particularly liver cancer. Therefore, accurate and efficient detection of MTs in food matrices, including cereals, fruit juices, and edible oils, is essential for ensuring the safety of food and protecting public health. In recent years, microextraction techniques have received considerable attention for the identification and quantification of MTs because of their simplicity, lesser solvent consumption, large preconcentration capacity, and environmental compatibility. Among the solvents used in these techniques, deep eutectic solvents (DESs) have emerged as promising alternatives to conventional organic solvents. DESs are considered a new generation of environmentally benign or "green" solvents because they generally offer lower toxicity, improved biodegradability, and easier preparation compared with many routinely used solvents. This review provides an overview of DES-based microextraction techniques for the detection and quantification of MTs in different food samples. The strengths, limitations, challenges, and analytical performance of these methods are critically discussed to guide future research and practical applications in food analysis.

## 1. Introduction

Mycotoxins (MTs) are naturally occurring compounds produced by certain fungi that, when present in food, pose significant risks to human health. These toxins have been associated with various adverse health effects, including cancer, liver damage, immunosuppression, respiratory issues, asthma, and chronic fatigue (Awuchi et al., 2022). In addition, MTs can interfere with metabolic processes in animals by affecting protein synthesis and hormonal balance, potentially leading to adverse reproductive outcomes and compromising overall health (Awuchi et al., 2022). Under favorable environmental conditions, particularly appropriate temperature and humidity, several fungal genera, including

*List of abbreviations and acronyms:* Explanations, Abbreviation Acronym; MTs, Mycotoxins; AFTs, Aflatoxins; OTA, Ochratoxin A; OTB, Ochratoxin B; ZEA, Zearalenone; PAT, Patulin; DON, Deoxynivalenol; AOH, Alternariol; AFB1, Aflatoxin B1; AFB2, Aflatoxin B2; AFG1, Aflatoxin G1; AFG2, Aflatoxin G2; AFM1, Aflatoxin M1; DESs, Deep eutectic solvents; ChCl, Choline chloride; EG, Ethylene Glycol; NaDES, Natural deep eutectic solvents; LPME, Liquid-phase microextraction; DLLME, Dispersive liquid–liquid microextraction; DEAC, N,N-diethanol ammonium chloride; EMAC, Ethyl methyl ammonium chloride; UA-LLME, Ultrasound-assisted liquid–liquid microextraction; VA-LLME, Vortex-assisted liquid-liquid microextraction; AA-LLME, Air assisted liquid-liquid microextraction; DecA, Decanoic acid; HBAs, Hydrogen bond acceptors; HBDs, Hydrogen bond donors; HPLC, High performance liquid chromatography; LLE, Liquid-liquid extraction; LOD, Limit of detection; RSDs, Relative standard deviations; SPE, Solid phase extraction; SPME, Solid phase microextraction; SFOD, Solidified floating organic drop; VA-LPME, Vortex-assisted liquid-phase microextraction; TBACl, Tertbutyl ammonium chloride; EA-ME, Effervescence-assisted microextraction; SFOD, Solidification of a floating organic drop; SPME, Solid-phase microextraction; D-μSPE, Dispersive micro-solid phase extraction; MOF, Metal organic framework.

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*Penicillium*, *Fusarium*, *Aspergillus*, and *Trichoderma,* can produce MTs (Abdelfattah, 2023). MTs have been detected in a wide range of agricultural and food products, including nuts, fresh and dried fruits, vegetables, cereals such as rice and wheat, and beverages and liquid products such as tea, coffee, wine, milk, and dairy products (Agriopoulou et al., 2016; Abdelfattah, 2023). Several naturally occurring MTs, such as aflatoxins (AFTs), ochratoxin A (OTA), zearalenone (ZEA), patulin (PAT), and alternariol (AOH), have been extensively reported in the literature (Fig. 1) (Rashad et al., 2023). AFTs are characterized by a difuranocoumarin skeleton and are produced through a polyketide biosynthetic pathway. They are fluorescent compounds with polycyclic aromatic structures and contain a delta-valerolactone ring attached to the coumarin portion of the difurocoumarin structure. OTA is a pentaketide derivative belonging to the dihydrocoumarin family and is linked to β-phenylalanine; it is synthesized by several *Aspergillus* and *Penicillium* species. PAT is a harmful unsaturated heterocyclic lactone derivative produced by *Aspergillus* species through a polyketide pathway. AOH is a benzochromenone-type MT produced by Alternaria fungi (Abdelfattah, 2023).

AFTs have been reported to exhibit carcinogenic, mutagenic, teratogenic, and immunosuppressive effects (Agriopoulou et al., 2016; Agriopoulou et al., 2020). In particular, aflatoxin B1 (AFB1) has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen, indicating that it is carcinogenic to humans. Aflatoxin M1 (AFM1), a hydroxylated derivative of AFB1, is commonly detected in milk and milk-based infant formula products (Loprieno, 1975). OTA is a dihydrocoumarin derivative produced by *Aspergillus and Penicillium* fungi and is coupled with β-phenylalanine. OTA has been reported in contaminated grains, coffee, dried grapes, and even breast milk (Mwabulili et al., 2023). Its chemical structure and properties have been characterized using UV–visible spectroscopy, fluorescence spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry (Zhang et al., 2023). ZEA is a non-steroidal estrogenic MT produced by various species of *Fusarium*. In animals, ZEA is metabolized into two major forms, alpha(α)-zearalenol and beta(β)-zearalenol (Lu et al., 2022). PAT, an unsaturated heterocyclic lactone, is produced by toxin-producing fungi, including species of *Aspergillus*, and *Penicillium expansuman*, and is commonly associated with decaying apples and apple-based commercial products (Bacha et al., 2023). AOH and AOH-monomethyl ether are benzochromenone MTs produced by *Alternaria* fungi. These toxins have been detected in several cereal crops and diseased fruits, including mandarins, melons, lemons, oranges, apples, and berries (Islam et al., 2023).

The presence of MTs in food and animal feed is a global concern, leading to the establishment of regulations that limit their concentrations in products intended for human and animal consumption. Monitoring and controlling MT contamination in agricultural commodities are essential for protecting public health and ensuring food safety (Mukhtar et al., 2023). Mitigation strategies include the implementation of appropriate agricultural practices, proper storage and handling conditions, and the use of MT-binding agents to reduce exposure risks (Khan, 2024).

In addition, ongoing research continues to explore new methods for detecting, monitoring, and managing MTs in food and feed to protect human health and animal welfare. Because MTs can cause adverse effects even at small concentrations, their monitoring requires appropriate

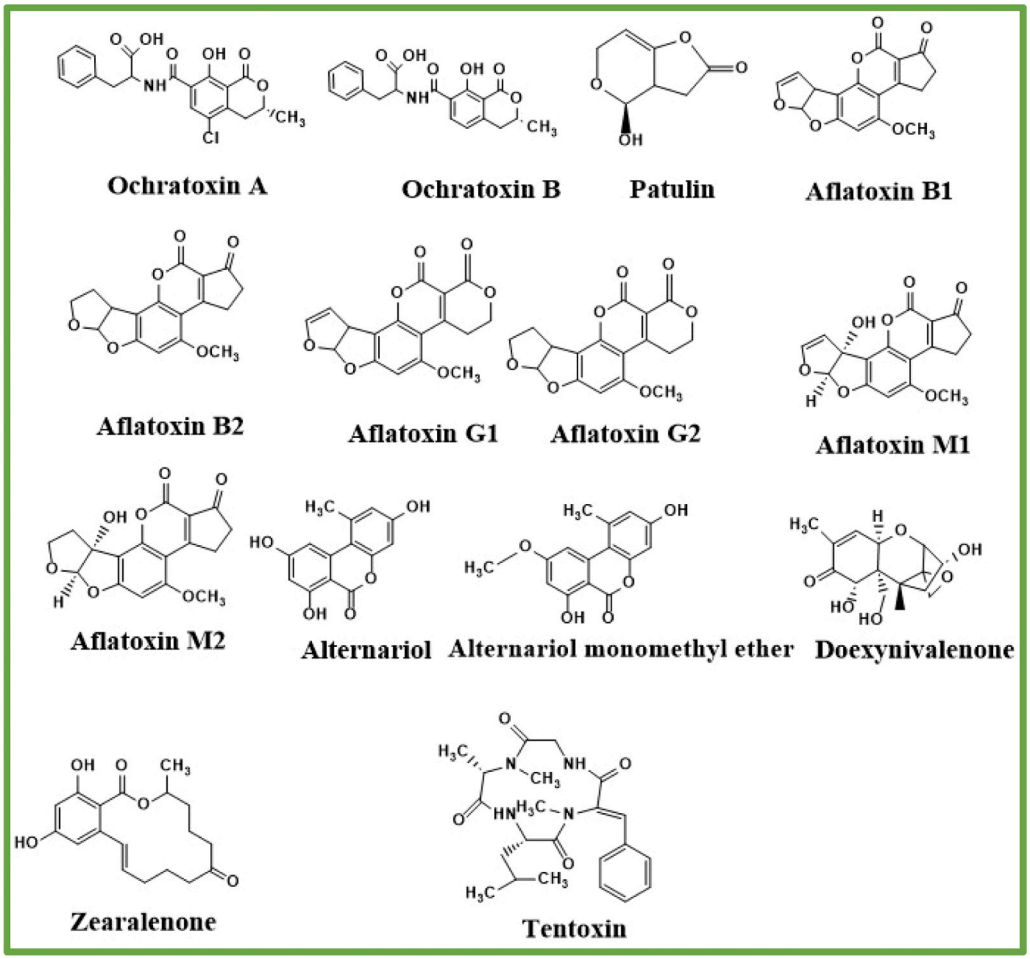



**Fig. 1.** Chemical structure of mycotoxins.

and validated methods for accurate identification and quantification. Reliable MT analysis must be both accurate and precise. Various methods have been used for MT extraction, including solid-phase extraction (SPE), liquid-liquid extraction (LLE), and vortex assisted low density solvent microextraction (VALDS-ME) (Agriopoulou et al., 2020; Al-Jaal et al., 2019; Alshannaq and Yu, 2017). In addition to extraction methods, several analytical techniques have been applied for MT quantification, including thin-layer chromatography (TLC), gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography with ultraviolet detection (HPLC-UV), and liquid chromatography–mass spectrometry (LC–MS) (Al-Jaal et al., 2019; Tittlemier et al., 2019).

Current sample-preparation efforts are focused on identifying environmentally friendly solvents, optimizing extraction procedures, and achieving rapid analytical results (Somsubsin et al., 2018). However, conventional methods for extracting MTs from food samples often rely on hazardous organic solvents, which pose risks to analysts and the environment. These methods commonly involve the extractions of MTs from solid matrices using organic solvents extraction (OSE), in which analytes are separated based on their differential solubility in two immiscible liquids (Cigić and Prosen, 2009; Janik et al., 2021).

Solvents commonly used to extract MTs include acetonitrile, chloroform, dichloromethane, methanol, ethanol, ethyl acetate, and isooctane (Turner et al., 2015). The extensive use of OSE raises environmental and safety concerns. Moreover, solvent extraction can be time-consuming, often requiring 24 h or longer. Another challenge associated with conventional OSE methods is the costs of high-purity solvents and waste disposal (Janik et al., 2021; Khodadadi et al., 2024). Therefore, analytical chemists are increasingly exploring greener extraction techniques to reduce the use of toxic chemicals and minimize environmental impact (Khodadadi et al., 2025). These efforts aim to develop more sustainable and safer sample-preparation methods for quantification of MTs.

The objective of this review is to provide a critical evaluation of recent advances in the extraction of MTs using DESs. Various experimental methodologies are considered to assess their efficiency, effectiveness, and practical feasibility. In addition, the advantages and limitations of microextraction strategies for MT analysis are discussed. By taking advantage of the unique properties of DESs, greener and more efficient extraction approaches can be developed to support food safety, human health, and environmental protection.

## 2. Deep eutectic solvents

Recently, DESs have received significant attention (Florindo et al., 2017; Hansen et al., 2020). These innovative, more environmentally

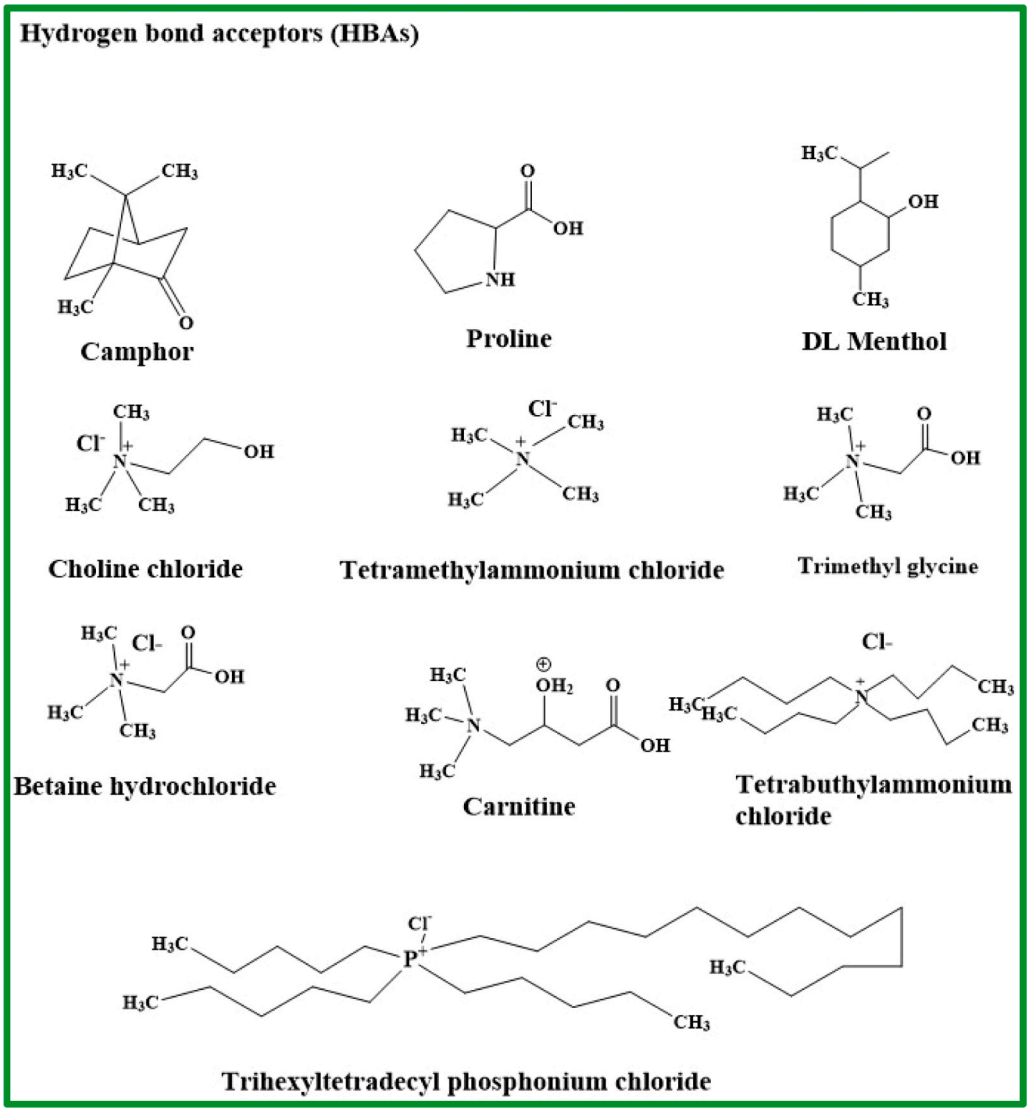



**Fig. 2.** Chemical structure of Hydrogen Bond Acceptors.

benign, "green" solvents can be readily prepared by combining two environmentally friendly components, typically a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) (Figs. 2 and 3). This combination forms a eutectic mixture with a depressed melting point. These chemicals offer several advantages, including cost-effectiveness, chemical stability in aqueous solutions, and ease of preparation. Furthermore, many DESs are biodegradable and generally exhibit lower toxicity than other organic solvents (Radošević et al., 2016).

DESs are categorized into four main types based on their composition. Types 1, 2, 3, and 4 consist of a quaternary ammonium salt combined with either a metal chloride (type 1), a metal chloride hydrate (type 2), or a hydrogen bond donor (type 3) (Jablonský et al., 2018; Smith et al., 2014). Type 4 DESs are composed of a metal chloride hydrate and an HBD (Smith et al., 2014). DESs can be prepared using three main methods: thermal mixing, vacuum evaporation, and freeze-drying. The heating time and temperature are typically adjusted according to the nature of the selected components (Shoshtari-Yeganeh et al., 2024). Because of its cost-effectiveness, biodegradability, and less potent toxicity, choline chloride (ChCl) is one of the most widely used components in the production of DESs. When combined with suitable hydrogen bond donors, such as urea, ethylene glycol (EG), carboxylic acids (e.g., oxalic acid, citric acid), amino acids, and carbohydrates, ChCl readily forms DESs (Zhang et al., 2012).

The term, "natural deep eutectic solvents" (NaDES) has recently been introduced to describe liquids formed by combining various naturally occurring molecules. They are termed "natural" because they can significantly facilitate the solubilization, storage, or transport of water-insoluble metabolites in living cells and organisms (Ruesgas-Ramón et al., 2017). NaDESs are generally considered a subclass of type 4 DESs and are commonly composed of sugars, alcohols, amines, amino

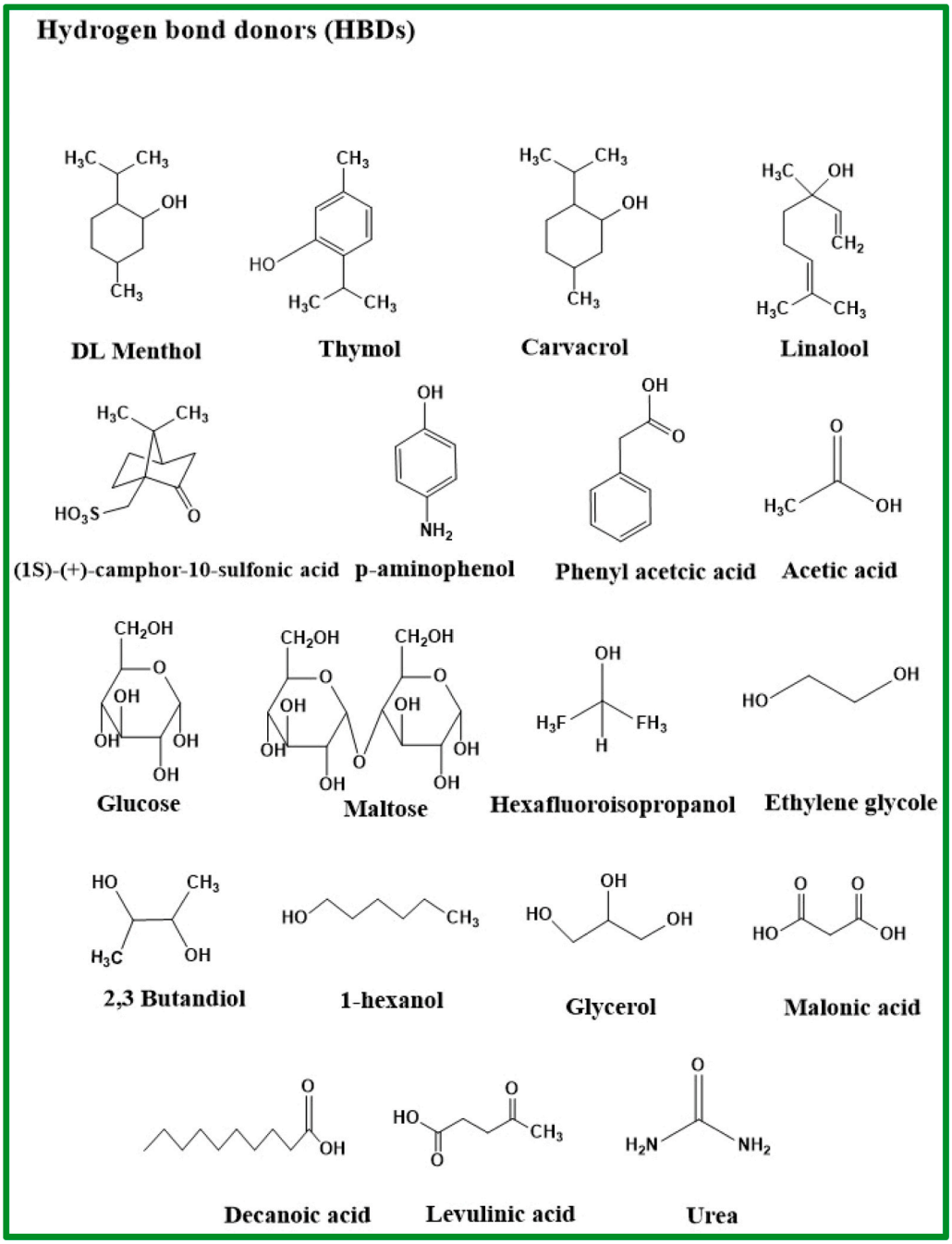



**Fig. 3.** Chemical structure of hydrogen bond donors.

acids, and organic acids (Yang et al., 2017). Consequently, DESs and NADESs share similar physicochemical properties, including strong solubilization capacity for protic molecules, low vapor pressure, and water miscibility (Ruesgas-Ramón et al., 2017).

The effectiveness of DESs as extractants for MTs in complex food matrices is mainly governed by the combined effects of their physicochemical properties (Omar & Sadeghi, 2022; Płotka-Wasylka et al., 2020). Rather than acting independently, these properties work together to influence dispersion efficiency, mass-transfer kinetics, and final phase separation. The extraction process begins with the dispersion behavior of the DES within the sample matrix, which is primarily controlled by its viscosity and polarity. High-viscosity DESs, often formed because of strong hydrogen-bonding networks, require optimized mechanical energy, such as vortex mixing or ultrasound, to achieve homogeneous dispersion. Once dispersed, the hydrogen-bonding capacity of the selected HBD and HBA components can create a high-affinity environment that retains MT molecules (Yang et al., 2024). This affinity can be further influenced by the acidity or basicity of the DES components. By adjusting the pH, the ionization state of the target MT can be modified to improve its partitioning into the DES phase and enhance extraction kinetics.

As extractions proceed, the thermodynamic preference of the analyte for the DES phase competes with its affinity for the food matrix. In this stage, the polarity and density of the DES play important roles. A properly selected polarity can improve the selective solubility of MTs over matrix interferences, while an appropriate density difference between the DES-rich phase and the aqueous matrix can support efficient phase separation (Tang and Row, 2020). Unlike conventional volatile organic solvents, DESs have negligible volatility, which helps maintain extractant stability during extraction, prevents solvent loss, and supports a consistent, safer, and greener microextraction protocol (Gurkan et al., 2020; Perna et al., 2020).

Consequently, the rational design of DESs requires a balanced selection of components that provide sufficient polarity and hydrogen-bonding capacity to promote extraction, while maintaining suitable density and viscosity for rapid and clean phase separation from complex food-derived extracts. To date, DESs have been combined with several analytical methods for the extraction of target analytes (Semysim et al., 2025). For example, an air-assisted rapid synergistic cloud-point extraction method using DESs was developed for the determination of zinc in blood and food samples (Al-Murshedi et al., 2025). In addition, several Liquid-liquid microextraction techniques have been developed based on in-situ formation/decomposition of DESs (Ahmadi et al., 2023).

## 3. DESs-based extraction methodologies of MTs

One of the crucial steps before the identification and quantification of MTs is the extraction. The choice of extraction solvent and the extraction method strongly influence extraction efficiency. Ideally, an extraction solvent should selectively remove MTs from the solid matrix, be cost-effective, ensure user safety, and minimize matrix effects (Agriopoulou et al., 2020). In this context, DESs have recently been employed as innovative green solvents for the effective extraction of MTs from food and feed samples (Ameen and Al-Masri, 2023; Carbonell-Rozas et al., 2023; Altunay et al., 2019) (Table 1).

### 3.1. Liquid-phase microextraction techniques

Liquid-phase microextraction (LPME) is a miniaturized extraction technique derived from liquid-liquid extraction (LLE). It uses microliter volumes of solvent instead of the milliliter quantities commonly required in conventional LLE. This approach enables the efficient extraction of a wide range of analytes from various matrices before instrumental analysis, while emphasizing simplicity, speed, and environmental sustainability (Lambropoulou and Albanis, 2007). Environmentally friendly or "green" methods have been integrated with LPME to improve the sustainability of sample preparation (García-Atienza et al., 2024).

A DES based on LPME was developed for the direct analysis of AFTs, including AFB1, AFB2, AFG1, AFG2, in edible oil (He et al., 2020). Due to fluorescence quenching of AFG1 and AFB1, pre-column derivatization is typically required for AFTs quantification. In this study, an acidic DES comprised of ChCl and malonic acid provided an extraction media that enhanced the fluorescence intensity of AFTs, particularly AFB1 and AFG1. This DES not only provided high extraction efficiency but also served as a derivatization agent (Makoś et al., 2018). The analytes were extracted by the addition of 30 μL of DES to 5 g diluted oil, followed by vigorous ultrasonic extraction for 50 min. Then, 5 μL of the resulting DES extract was directly injected into an HPLC-FLD system for analysis. Under optimized conditions, the LODs and LOQs were below 0.003 μg $kg^{-1}$ and 0.01 μg $kg^{-1}$, respectively.

#### 3.1.1. Dispersive liquid-liquid microextraction

A dispersive liquid–liquid microextraction (DLLME) technique was developed for the determination of OTA and Ochratoxin B (OTB) in capsicum. In this method, a DES synthesized from thymol and decanoic acid at a 1:1 molar ratio was used to extract OTA and OTB. An LC-MS/MS system was employed for the quantifications of these compounds. The method showed LODs and LOQs were in the ranges of 0.14–0.45 μg/kg and 0.45–1.45 μg/kg, respectively (Yang et al., 2023).

A simple and environmentally friendly DLLME method was introduced for quantifications of four MTs, namely deoxynivalenol (DON), AOH, OTA and ZEA in edible vegetable oils. Under optimized conditions, 0.17 g of oil sample was mixed with 320 μL of a DES (ChCl: urea with a molar ratio of 1:2) and stirred for 31 min at 32 °C. The DES phase containing the MTs was then separated and analyzed by HPLC-FLD. The LODs ranged from 0.07 to 300 μg $kg^{-1}$, and recoveries were close to 100%, except for ZEA, which showed a recovery of 35%. The relative standard deviations (RSDs) were below 9% (Pradanas-González et al., 2023).

A DLLME method was designed for the determination of ZEA in bread, wheat, and corn flakes. In this approach, a DES composed of a terpenoid and a long-chain alcohol was used to extract ZEA, while acetonitrile served as the dispersant. For this purpose, 0.5 g of the sample was added to 1.5 mL of DES consisting of DL-menthol and 1-hexanol at a 2:1 molar ratio in acetonitrile. ZEA was separated into the DES phase through sonication and centrifugation. For the DLLME, 1.0 mL of the supernatant was injected into 4 mL of ultra-pure water and mixed for 1 min. Phase separation was attained by centrifugation (8000 ×g) for 3 min. ZEA was then quantified by HPLC-FLD. The reported LOD and LOQ were 2 and 5 μg $kg^{-1}$, respectively. The RSDs were below 12%, and mean recoveries as great as 94% (Pochivalov et al., 2022).

A combination of DES-based pressurized liquid extraction (PLE) and DLLME was used to extract AFTs from rice (Lesan et al., 2023). Water-miscible DESs served as the solvent for extraction in both micro-PLE (MPLE) and DLLME, while water-immiscible DESs were used exclusively in the DLLME phase. The water-miscible DESs were prepared by mixing ChCl as the HBA with EG, dicarboxylic acid (DCA), and phenol as HBDs. In contrast, the water-immiscible DESs were created by combining ethyl methyl ammonium chloride (EMAC) as an HBD with carvacrol and thymol as HBAs. In the MPLE-DLLME process, the extractant used in the MPLE step also functioned as the dispersive solvent. The analytes were extracted with a water-miscible DES (ChCl:EG) under elevated pressure and temperature. The obtained supernatant was mixed with 90 μL of a water-immiscible DES (EMAC: carvacrol) and centrifuged. The analytes were then determined using HPLC-FLD. Under optimized conditions, the LODs and LOQs were 0.02–0.07 ng $g^{-1}$ and 0.07–0.23 ng $g^{-1}$, respectively.

Solvent extraction was combined with DLLME for the determination of AFM1 in cheese (Masrouri et al., 2021). A novel water-immiscible DES composed of N,N-diethanol ammonium chloride (DEAC) and

**Table 1**
DES-based extraction procedure for quantification of mycotoxins (MTs).

| Analyte | Matrix | Micro-extraction method | Analyzing Method | Comments | Linear range | LOD | ref |
|---|---|---|---|---|---|---|---|
| AFB1<br>AFG1<br>AFB2<br>AFG2 | Edible oils | LPME | HPLC-FLD | **DES:** ChCl + Malonic acid, 1:2 (Molar ratio).<br>**Pretreatment:** None.<br>**Procedure:** 30 μL DES + 5 g diluted oil sample. Sonication: 50 min at 55°C. DES phase collected and analyzed.<br>**Note:**<br>Optimization by BBD. | AFB1, AFG1<br>0.01–0.75<br>μg $Kg^{-1}$<br>AFB2, AFG2<br>0.003–0.25<br>μg $Kg^{-1}$ | 0.0005–0.003 μg $Kg^{-1}$ | (He et al., 2020) |
| OTA<br>OTB | Capsicum | DLLME | LC-MS/MS | **DES:** Thymol + Decanoic acid, 1:1<br>**Pretreatment:** None.<br>**Procedure:**<br>1 g powdered sample + 5 mL purified water, vortex:2 min, left to equilibrate for 30 min. + 5 mL acetonitrile (containing 1% formic acid), shaking and vortex: 5 min, + 4 g anhydrous magnesium sulphate + 1 g sodium chloride, vortex:2 min, centrifugation: 10 min at 5000 rpm. Clarified liquid was separated. 1 mL extract capsicum + 3 mL pure water + 100 μL DES, hand shaking:30 s, upper DES phase was collected and analyzed.<br>**Note:** none. | 0.5–250<br>μg $kg^{-1}$ | 0.14–0.45<br>μg $kg^{-1}$ | (Yang et al., 2023) |
| DON<br>AOH<br>ZEA<br>OTA | Edible vegetable oils | DLLME | HPLC-FLD/DAD | **DES**: ChCl + Urea, 1:2<br>**Procedure**: 0.17 g of spiked sample + 0.32 mL of DES. Shaking: 31 min at 32°C, stayed at room temperature for 10 min, 50 μL supernatant diluted with Milli-Q water, analyzed by HPLC–FLD/DAD.<br>**Note**: optimization of the extraction conditions by using a FFD | 0.9–9400<br>μg $kg^{-1}$ | 0.07–300<br>μg $kg^{-1}$ | (Pradanas-González et al., 2023) |
| ZEA | Cereal | DLLME | HPLC-FLD | **DES:** DL-menthol + 1-hexanol, 2:1.<br>**Sample preparation:** homogenization of wheat, corn flakes or bread sample.<br>**Procedure:** homogenized sample + 1.5 mL of DES, shaking:1 min, ultrasonication:15 min at 75°C, centrifugation: 3 min at 8000 g. 1 mL supernatant injected into 2 mL ultra-pure water, shaking: 1 min, centrifugation: 3 min at 8000 g. 30 μL of terpenoid-rich phase diluted twice with methanol and analyzed by HPLC.<br>**Note:**<br>Hydrophobic DES based on terpenoids and long-chain alcohols were used in the DLLME process for the first time. | 5–500<br>μg $Kg^{-1}$ | 2 μg $Kg^{-1}$ | (Pochivalov et al., 2022) |
| AFB1<br>AFG1<br>AFB2<br>AFG2 | Rice | DES based PLE combined with DLLME | HPLC-FLD | **DES:** DES1: ChCl + EG, 1:2; (employed for PLE);<br>DES2: EMAC: Carvacrol, 1:2 (employed for DLLME).<br>**Sample preparation:** 2 g rice and 1 g sieved sand mixed and blended into a mortar for 5 min.<br>**Procedure:** In the first step, PLE was used for extraction of AFTs from rice sample. Then, DLLME employed for preconcentration of analytes.<br>**Note:** Combination of PLE and DLLME removed difficulties of each method (when used alone). | 0.07–1000 μg $Kg^{-1}$ | 0.02–0.07 μg $Kg^{-1}$ | (Lesan et al., 2023) |
| AFM1 | Cheese | TPSE-DES-DLLME | HPLC-FLD | **DES**: DEAC + Carvacrol, 1:2.<br>**Procedure**: 1 g homogenized sample + 2 mL deionized water + 2.1 mL ACN, shaking 2 min, adding 0.78 g sodium chloride and 1.0 mL n-hexane, vortex: 3.6 min, centrifugation: 5 min at 4000 rpm, 1.3 mL of the ACN phase + 64 μL DES, injected to 5 mL deionized water, centrifugation: 5 min at | 2.56–2000<br>ng $kg^{-1}$ | 0.74 ng $kg^{-1}$ | (Masrouri et al., 2021) |

(*continued on next page*)

**Table 1** (*continued*)

| Analyte | Matrix | Micro-extraction method | Analyzing Method | Comments | Linear range | LOD | ref |
|---|---|---|---|---|---|---|---|
| | | | | 4000 rpm, the sediment phase mixed by 5 mL ACN and analyzed.<br>**Note:**<br>Optimization by CCD. | | | |
| AFM1 | Milk-based athlete sports supplements | pH switchable DES combined with DLLME | HPLC-FLD | **DES**: L-Menthol + Salicylic acid, 4:1.<br>**Sample preparation:** 100.0 mg of solid sample were added to 10 mL of methanol–water (2:3 v/v) and sonicated.<br>**Procedure**: 10 mL of pre-prepared sample was mixed with 50 μL of DES. 100 μL of 6 molar KOH was added to the above solution. Then, 100 μL of 6 molar HCl was added. The separation of DES phase was performed by solidification of organic phase in ice bath. HPLC-FLD was employed for analysis.<br>N**ote:** pH switchable DES simplified extraction of AFM1. | 0.015-50 μg $kg^{-1}$ | 0.005 μg $kg^{-1}$ | (Tong and Yang, 2025) |
| OTA | Dried fruit samples | HLLE-VA-DLLME | UV–VIS spectrophotometer | **DES:** HFI + Carnitine, 1:3.<br>**Pretreatment**: None.<br>**Procedure:** 10 g dried fruit sample + 7 mL HCl (1.5 mol $L^{-1}$) + 2 mL THF, sonication: 2 min at room temperature, centrifugation: 3 min at 3500 rpm, pH was adjusted to 5.6 by phosphate buffer solution, + 485 μL DES + NaCl (4.3 w/v). vortex: 3.5 min, DES phase was analyzed by spectrophotometer.<br>**Note:** The HLLE step was implemented to mitigate the matrix interference and enhance OTA extraction. | 0.4–300 ng $mL^{-1}$ | 0.21 ng $mL^{-1}$ | (Elik et al., 2023) |
| PAT | Fruits and fruit juices | VA-magnetic DES-LPME | UV-Vis spectrophotometer | **DES:** Trihexyltetradecylphosphonium chloride + $MnCl_2$ + Octanoic acid, 1:2:2.<br>**Pretreatment**: None.<br>**Procedure:**<br>**Note:** | 2–300 ng $mL^{-1}$ | 0.37 ng $mL^{-1}$ | (Haq et al., 2025) |
| AFB1 | Cereal products | DES-UAEME | DPV | **DES:** ChCl + Urea, 1:2.<br>**Pretreatment**: None.<br>**Pretreatment:** 2.5 g cereal sample + 15 mL of a mixture of ACN–phosphate buffer solution (80: 20 v/v, pH 7), vortex:3 min, centrifugation: 5 min at 4000 rpm, the supernatant was filtered. the filtrate was diluted at 1–2 with the extraction solvent.<br>**Procedure:** 4 mL sample + ACN–phosphate buffer (90: 10 v/v, pH 7) + 150 mL of the DES, sonication: 40 s with a frequency of 28 kHz, centrifugation: 10 min at 4000 rpm, 100 μL DES phase was withdrawn and was analyzed.<br>**Note:**None. | 0.2–80.0 mg $L^{-1}$ | 0.05 mg $L^{-1}$ | (Zohdijamil et al., 2021) |
| PAT | Fruit juices | UA-ELPME | UV–VIS spectrophotometery | **DES**: TBACl + 2,3-butanediol, 1:2.<br>**Procedure:** 3 mg of sample including 100 μg $L^{-1}$ of PAT, pH adjusted to 8 by tris buffer, 75 μmol $L^{-1}$ Mg(II) solution, 500 μL of DES + 250 μL of acetone, sonicated for 12 min at room temperature, centrifugation: 5 min at 4000 rpm.<br>**Note:** Mg (II) solution was used to form the PAT-metal complex. | 7.5–420 μg $L^{-1}$ | 2.2 μg $L^{-1}$ | (Altunay et al., 2019) |
| ZEN | Grains | UA-ME | UV-Vis spectrophotometer | **DES**: Trimethyl glycine + Phenyl acetic acid, 1:2.<br>**Pretreatment**: 10 g or 10 mL sample + 40 mL methanol + 10 mL water. sonication: 30 min at room temperature, the mixture was filtered.<br>**Procedure**: 10 mL sample solution + 40 ng $mL^{-1}$ ZEN, pH was adjusted to 3.8 with sodium acetate buffer solution, + 175 μL DES, sonication: 11 min at 33°C, centrifugation: 2 min at 4000 rpm. | 3–800 ng $mL^{-1}$ | 1.6 ng $mL^{-1}$ | (Elik & Altunay., 2024) |

(*continued on next page*)

**Table 1** (*continued*)

| Analyte | Matrix | Micro-extraction method | Analyzing Method | Comments | Linear range | LOD | ref |
|---|---|---|---|---|---|---|---|
| | | | | The DES phase was analysed by UV–VIS spectrophotometer. **Note:** None. | | | |
| AFM1 | Milk | UA-DLLME | UV–Vis | **DES**: Betaine chloride + Maltose, 1:2. **Pretreatment**: 5 mL of milk sample incubated at 5 ∘C for 30 min, centrifugation: 10 min at 3500 rpm. 4 mL of milk serum + 1 mL of 100% methanol, loaded into immunoaffinity column, washing column with 2 mL of deionized water + washing with 2 mL of 10% methanol. the AFM1 from samples was eluted with 1 mL of 100% methanol. **Procedure**: 5 mL of milk sample, pH adjusted to 3.8. + 1 mL of 200 mmol $L^{-1}$ pyrogallol + 170 μL of sugar DES. Sonication: 8 min at room temperature, centrifugation: 3 min at 4000 rpm. The sediment phase was analyzed by UV–VIS. **Note:** CCD, 0.2 mol $L^{-1}$ sodium acetate buffer solution was uses to adjust the pH. | 0.02–300 μg $L^{-1}$ | 6.1 ng $L^{-1}$ | (Gürsoy et el., 2022) |
| AFB1 AFB2 AFG1 AFG2 AFM | Cheese | DES-MAE–IL-TC–LPME | HPLC-FLD | **DES:** ChCl + EG, 1:2. **Pretreatment:** None. **Procedure:** 3 g cheese + 3.5 mL deionized water + 1.5 mL DES. microwave: 60 s at 180 W, centrifugation: 2 min at 5000 rpm, supernatant + 55 μL [$C_6$MIM] [$PF_6$] IL, centrifugation: 7 min at 5000 rpm and −4°C, e IL phase collected + 50 μL mobile phase and was analyzed by HPLC. **Note:** None. | 1–30 × $10^6$ ng $kg^{-1}$ | 9–23 ng $kg^{-1}$ | (Kaboodi et al., 2023) |
| OTA | Cereal | EA-ME | ELISA | **DES:** Glucose + Levulinic acid, 1:4. **Pretreatment:** None. **Procedure:** 1 g cereal samples + 1.25 mL NADESs, vortex:1 min, 200 μL supernatant + g 2 mg modified lignin, vortex:1 min, extraction solution was obtained. 100 μL extraction solution + 100 μL MB-Ag probes + 100 μL of Ab-AuNP-HRP conjugates + 200 μL $NaHCO_3$ (2 M). washing tube with PBST (three times), + 200 μL TMB, incubation: 10 min at 37°C, + 100 μL HCl (1 M), 200 μL of the chromogenic solution was transferred to a 96-well plate, the absorbance was recorded at 450 nm. **Note:** Green NADES are produced from glucose and levulinic acid, derived from cellulose and hemicellulose. | 3–100 μg $kg^{-1}$ | 0.68–0.83 μg $kg^{-1}$ | (Zhao et al., 2024) |
| PAT | Fruit juice dried fruit | AA-NADES-SH-LPME | UV-Vis | **DES:** L-proline + Glycerol, 3:1. **Pretreatment**: 10 mL the water + 75 μL pectinase + 5 mL fruit juice. Sonication: 1 h at 30°C, + 20 mL acetonitrile, vortex:2 min, centrifugation: 5 min at 4000 rpm. Supernatant + 1.5 g of N-propylethylenediamine + 0.5 g of $MgSO_4$, dried at 45°C under nitrogen flow a rotary evaporator. resulting residues + 2 mL acetonitrile–water (1:1). **Procedure**: 2 mL sample solution, pH was adjusted to 5.6 + 5.1 mg/L Zn(II) solution + 410 μL of NADES. Kept in ice bath for 7 min, the solidified phase was separated, after melting was quantified by UV–VIS. **Note:** Optimization by CCD. | 10–750 μg $L^{-1}$ | 3.5 μg $L^{-1}$ | (Taşpınar et al., 2021) |
| AFM1 AFM2 | Milk | SFOD-DLLME | HPLC-FLD | **DES**: Menthol + Phenylacetic acid, 3:1. **Procedure**: 5 mL raw cow milk sample + 1 mL zinc sulfate solution (25% v/w), vortex: 3 min, centrifugation: 5 min at 5000 rpm, upper phase + 65 μL DES + 110 μL chloroform. $N_2$ gas (20 mL/min) was flown for 3 min, small droplets of | AFM1: 4.83–$10^6$ ng $L^{-1}$ AFM2: 6.21–$10^6$ ng $L^{-1}$ | AFM1: 1.45 ng $L^{-1}$ AFM2: 1.86 ng $L^{-1}$ | (Rabie et al., 2024) |

(*continued on next page*)

**Table 1** (*continued*)

| Analyte | Matrix | Micro-extraction method | Analyzing Method | Comments | Linear range | LOD | ref |
|---|---|---|---|---|---|---|---|
| | | | | DES were released and analytes extracted into its droplets. After 3 min ice bath, a solidified drop was obtained and analyzed by HPLC.<br>**Note:** Zinc sulfate was employed to precipitate the proteins and chloroform was used as a density modifier. | | | |
| AOH<br>OTA<br>AME<br>TEN | Apple juices<br>Apple purees | DLLME-SFO | HPLC-MS/MS | **DES**: Acetic acid: Glucose: water, 5:1:3.<br>**Pretreatment:** None.<br>**Procedure**: 1 mL or 1 g portion of apple juice + 4 mL of acidified water (0.5% formic acid), Vortex: 10 s. 500 μL DES + 200 μL 1-dodecanol. Shaking: 10 min, centrifugation: 5 min at 4500 rpm and 30°C. ice bath for 5 min, the residue was reconstituted with 300 μL of EtOH and analyzed by HPLC-MS/MS.<br>**Note:** DES as a dispersive solvent and 1-dodecanol used as an extraction solvent. | AOH: 0.1–50 μg $Kg^{-1}$<br>AOM: 0.2–50 μg $Kg^{-1}$<br>TEN: 0.03–50 μg $Kg^{-1}$<br>OTA: 0.1–50 μg $Kg^{-1}$ | 0.25 μg $Kg^{-1}$ | (Carbonell-Rozas et al., 2023) |
| AFB1 | Cereal (rise, wheat, peanut) | pH-switchable HDESs combined with SFO-LPME | HPLC-FLD | **DES:** L-menthol + (1S)-(+)-camphor-10-sulfonic acid, 5:1.<br>**Pretreatment:** 10 g of each cereal sample was completely homogenized. 0.20 g of the homogenized sample + 1.0 mL of a methanol: water mixture (70: 30, v/v), sonicated for 20 min, centrifugation: 5 min at 4000 rpm, filtered the supernatant.<br>**Procedure:** 10 mL of the sample solution + 70 mL of DES + 100 μL KOH (5 mol $L^{-1}$), shaking solution + 100 m of HCl (5 mol $L^{-1}$ ), in an ice bath the organic phase was separated, and after melting was analyzed.<br>**Note:** KOH was used as a disperser and HCl was added for phase separation. | 0.007–20 μg $Kg^{-1}$ | 0.002 μg $Kg^{-1}$ | (Ameen and Al-Masri, 2023) |
| PAT<br>OTA | Fruit juices | DμSPE | HPLC–MS/MS | **DES:** ChCl + EG, 1:2.<br>**Pretreatment:**<br>***Synthesis of MOF***:<br>0.562 g vitamin B3 + 1.09 g CoCl2·6H2O + 15 mL deionized water. NaOH solution (1 M) was added dropwise to reach pH 7. Autoclave: 180°C for 72 h. dried precipitate at 70°C for 4 h.<br>**Procedure:** 5 mL of sample + 15 mg MOF, vortex: 2.5 min, centrifugation: 3 min at 5000 rpm, the upper phase was discarded. The MOF particles were eluted with 140 μL ChCl: EG DES, vortex: 2.5 min, centrifugation: 3 min at 5000 rpm, the supernatant was analysed.<br>**Note:** For the first time, a vitamin-based MOF was used as a green and efficient sorbent. | PAT: 131.7–1 × $10^6$ ng $L^{-1}$<br>OCT A: 82.6–1 × $10^6$ ng $L^{-1}$ | PAT: 39.6 ng $L^{-1}$<br>OCT A: 24.8 ng $L^{-1}$ | (Mohebbi et al., 2022) |
| AFM1<br>OTA | Milk | D-μSPE was combined with MOFs and DLLME | HPLC-FLD | **DES:** ChCl: EG, 1:2; Menthol: Decanoic acid, 1:2.<br>**Pretreatment**: None.<br>**Procedure:**.7 mL cow milk + 125 mg TCA, vortex 2 min, centrifugation: 3 min at 5000 rpm, supernatant mixed with 50 mg of synthesized sorbent. Vortex: 3 min, centrifugation: 3 min at 5000 rpm, 1 mL ChCl: EG, vortex: 3 min, 60 μL menthol: decanoic acid, ice bath for 2 min, drop was separated + 10 μL ACN was analyzed.<br>**Note:** MOF used as a sorbent. ChCl: EG DES used as elution and dispersive solvent. menthol: decanoic acid DES was extracted analytes.<br>ACN was used for dissolving of final droplet of menthol: decanoic acid DES containing the extracted analytes. | 1.23–$10^5$ ng $L^{-1}$<br>0.83–$10^5$ ng L-1 | 0.37 ng $L^{-1}$<br>0.25 ng $L^{-1}$ | (Badali et al., 2023) |

carvacrol at a 2:1 ratio was used as the extraction solvent in DLLME. In the first step, AFM1 was extracted using an acetonitrile: water: n-hexane mixture. Efficient and selective extraction of the analyte occurred in the acetonitrile phase. After centrifugation, the acetonitrile phase was collected and mixed with the DEAC: Carvacrol DES. The DES was then injected into deionized water, forming a cloudy solution. Finally, an aliquot of the organic phase was introduced into the HPLC-FLD. Under optimized conditions, the LOD and LOQ were 0.74 and 2.56 ng $kg^{-1}$, respectively.

An eco-friendly and simple DLLME was developed for the quantification of AFM1 in milk-based sports supplements. A pH-variable DES was used for extraction and preconcentration without vortex mixing, dispersant addition, or ultrasonic treatment. A 10 mL portion of the sample was combined with 50 μL of a DES comprised of L-menthol and salicylic acid (4:1 molar ratio). The extractive phase was dispersed into the matrix using KOH and subsequently separated using HCl. The DES phase was then solidified and separated in an ice bath. HPLC-FLD was used for quantification of AFM1, with an LOD of 0.005 μg $kg^{-1}$ and recoveries ranging from 92.0% to 105.0% (Tong & Yang, 2025).

### 3.1.2. vortex-assisted liquid-phase microextraction

The dispersive, liquid-liquid microextraction method uses a dispersive solvent to enhance dispersion and increase the interfacial area between the mass transfer organic phase and the aqueous donor phase, which is essential for rapid extraction. However, the use of a dispersant can reduce mass transfer and partitioning of the analytes into the donor phase, thus reducing the effect on efficiency. In addition, two-phase extraction methods typically require a time-consuming evaporation step when HPLC or capillary electrophoresis (CE) is used for analysis (Makahleh et al., 2015). These inherent limitations of DLLME have been partially addressed through the use of vortex mixing, sonication, and magnetic stirring (Farajzadeh et al., 2020).

Vortex-assisted liquid-liquid microextraction (VA-LLME), first introduced in 2010, uses vortex mixing to disperse the extraction solvent (Machado et al., 2019). Unlike conventional DLLME, VA-LLME can be performed without a dispersive solvent and, in some cases, without a centrifugation step (Hosseini et al., 2014).

The potential of combining DESs with VA-DLLME for the quantification of OTA in dried fruit was assessed (Elik et al., 2023). The DES was composed of hexafluoroisopropanol (HFI) and Carnitine. The researchers employed a combination of homogeneous liquid–liquid extraction (HLLE) and VA-DLLME for the extraction process. In the first step, 10 g of homogeneous matrix was dissolved in 7 mL of HCl solution and 2.0 mL of tetrahydrofuran (THF). OTA was extracted by sonication and centrifugation, then mixed with 485 μL of DES at pH 5.6. Finally, the tube was vortexed for 2 min. The DES phase containing OTA was collected from the top of tube and transferred to a microcuvette. The absorbance of the analyte was recorded at 355 nm using a spectrophotometer. The results demonstrated that OTA was extractable using HFI: Carnitine at a molar ratio of 1:3. Validation of the developed method yielded a LOD of 0.12 ng $mL^{-1}$, precision ranging from 3.4% to 4.1%, and accuracy between 92.1% and 99.2%.

More recently, a vortex-assisted magnetic DES-based LPME (VA-MDES-LPME) method was applied for the extraction of PAT. The MDES was synthesized from trihexyltetradecylphosphonium chloride, $MnCl_2$, and octanoic acid (molar ratio 1:2:2). This technique provided a high enrichment factor greater than 169 and a recovery greater than 96.3% (Haq et al., 2025).

### 3.1.3. Ultrasound /microwave-assisted liquid-liquid microextraction

Ultrasound-assisted liquid–liquid microextraction (UA-LLME) has been presented as an alternative to the use of dispersive solvents. In UA-LLME, ultrasound is used to disperse the extraction solvent and enhance mass transfer (Zohdijamil et al., 2021). This technique offers several advantages, including high extraction efficiency and high recovery. However, it also has some limitations, such as the possibility of sample degradation and the need for operator control during the extraction process (Shoshtari-Yeganeh et al., 2024).

A DES-based ultrasound-assisted emulsification microextraction (DES-UAEME) method was introduced for the quantification of AFB1 in cereals. In this method, samples were diluted with 8 mL of acetonitrile–phosphate buffer, followed by the addition of 150 μL of DES. The mixture was then sonicated in an ultrasonic bath for 40 s. A DES synthesized from ChCl- urea acted as both the extraction solvent and the electrolyte for differential pulse voltammetry (DPV) detection. After centrifugation, the DES phase was separated and transferred to a microcell for voltametric detection. The proposed method showed a linear range of 0.2–80.0 mg $L^{-1}$ and an LOD of 0.05 mg $L^{-1}$. The RSDs (%) for the determination of 5.0 mg $L^{-1}$ AFB1 ranged from 3.4 to 3.9 (Zohdijamil et al., 2021).

A new and environmentally friendly approach for determining PAT in fruit juices was developed using ultrasound-assisted emulsification liquid-phase microextraction (UA-ELPME) (Altunay et al., 2019). Researchers used a method that involves the formation of a chelate complex between PAT and magnesium (Mg (II)) in the presence of a tris-buffer, a nucleophilic metal chelating agent, at pH 8.0. The resulting complex is then extracted into micro-drops of a DES via a donor-acceptor mechanism. The best extraction results were obtained using 500 μL of an alcohol-based DES composed of Tertbutyl ammonium chloride (TBACl), and 2,3-butanediol. Optimal conditions were 75 μmol $L^{-1}$ of Mg (II) solution, pH-8, and 12-min of ultrasound time. The LOD of the method was calculated to be 2 μg $L^{-1}$. Recoveries ranged from 90% to 106%, and the RSD (%) were less than 4.2%.

ZEA in grains and foods was determined using DES-combined ultrasound-assisted microextraction (UA-ME) (Elik et al., 2024). A DES composed of phenyl acetic acid and trimethyl glycine at a 2:1 molar ratio revealed the highest extraction efficiency. The Box–Behnken design (BBD) was applied for the identification and optimization of the critical parameters. Under optimized conditions, the LOD and LOQ were 1.6 ng $mL^{-1}$ and 5.0 ng $mL^{-1}$ , respectively.

A sugar-based DES-UA-DLLME method was applied for the extraction of AFM1 from milk samples (Gürsoy et al., 2022). In this approach, ChCl and betaine chloride functioned as HBDs, whereas sugars such as xylose, fructose, sucrose, and maltose were used as HBAs. The best yield during extraction among the synthesized DESs was obtained with a combination of betaine chloride and maltose at a 1:2 molar ratio. The extraction method was optimized using the following parameters: 170 μL of DES, pH 3.8, 13.6 mmol $L^{-1}$ pyrogallol, and 8 min of ultrasound treatment. Under these optimized conditions, the LOD of the developed method was 6.1 ng $L^{-1}$.

Microwave-assisted extraction (MAE) uses microwave energy to promote the transfer of analytes from the sample matrix into the acceptor phase. This technique can improve extraction efficiency, reproducibility, and sample throughput (Eskilsson and Björklund, 2000). MAE method was combined with LPME for the quantification of AFTs in cheese samples (Kaboodi et al., 2023). In that study, two miscible DESs, ChCl:EG and ChCl:phenol, were used. These extractants had high boiling points that prevented evaporation during extraction. The results indicated that ChCl:EG provided higher extraction rates. When microwave power was varied from 90 W to 360 W, the optimal yield was achieved at 180 W. Notably, protein denaturation at power levels above 180 W hinders the release of AFTs into the liquid solvent, leading to decreased extraction rates. Under the best extraction conditions, the LOD and LOQ ranged from 9 to 23 and from 30 to 77 ng $kg^{-1}$, respectively.

### 3.1.4. Effervescence-assisted emulsification microextraction

In microextraction techniques, dispersing the acceptor phase as fine droplets within the donor phase increases the interfacial area between the two phases, thereby promoting mass transfer. Formation of this dispersion can be achieved via external energy input or the use of chemical substances. Effervescence-assisted microextraction (EA-ME)

was introduced in 2011 as a novel technique in this field. In EA-ME, carbon dioxide facilitates the dispersion of extractants into the sample solution (Lasarte-Aragonés et al., 2020).

A novel approach was developed to determine OTA in cereals by combining the merits of EA-ME and ELISA. A NaDES composed of glucose and levulinic acid was used as the extraction solvent. Modified lignin was also used to remove matrix interferences. The $NaHCO_3$ solution displayed a dual purpose in the ELISA process. It not only preserved the activity of HRP but also exhibited dispersive properties. Magnetic beads (MBs) were employed to decrease the duration of incubation and washing. The effervescent reaction in the MB-based ELISA promoted a faster immune competitive process. The LODs were 0.68–0.83 μg $kg^{-1}$ (Zhao et al., 2024).

#### 3.1.5. Floating organic drop solidification approach

Organic solvents typically employed in DLLME are denser than water, causing them to migrate to the bottom of the centrifugation tube. Collecting the organic solvent from the bottom of the tube is a challenging step. In contrast, low-density organic extractants float on top of the solid matrix. If the selected solvent has a freezing point between 10 and 25 °C, the floating droplet can be solidified by use of an ice bath. Solidification of a floating organic drop (SFOD) employs low-density solvents with freezing points near room temperature. Suitable solvents are typically immiscible with water, exhibit low volatility, and show a high partition coefficient (Mansour and Danielson, 2017).

An eco-friendly and fast analytical procedure was designed for the determination of PAT in dried fruit and fruit juice samples using air-assisted NaDES-based SFO. This method primarily depends on several key factors: NaDES volume, concentrations of Zn (II), pH, and cooling duration. Within the synthesized DESs, the best extraction efficiency was obtained using L-proline and glycerol mixed at a molar ratio of 3:1. Effective complexation is necessary to ensure the extraction of PAT from the matrix, with the goal of complexing PAT with Zn (II) ions. Determination of PAT was conducted using a spectrophotometer, yielding a method's LOD of 3.5 μg $L^{-1}$ and a preconcentration factor of 150 (Taşpınar et al., 2021).

The DLLME-SFOD is simple, enhances extraction kinetics and efficiency, and requires small volumes of organic solvent (Mansour and Danielson, 2017). An SFOD-based DLLME procedure was designed for the extraction of AFM1 and AFM2 in milk. For this purpose, after treatment with aqueous zinc sulfate to precipitate milk proteins, the clear supernatant was collected in a glass syringe. Chloroform and a DES composed of menthol and phenyl acetic acid were then added to the syringe, with chloroform serving as a density modifier. With the help of $N_2$ flow, the analytes were extracted into DES, and the syringe was subsequently immersed in an ice-water bath. The solidified drop was collected into a microtube. The determination of analytes was performed using HPLC-FLD, with the proposed method providing RSDs below 4.8% and limit of detections and limit of quantifications in the range of 1.45–1.86 ng $L^{-1}$ and 4.83–6.21 ng $L^{-1}$, respectively (Rabie et al., 2024).

Four MTs, including AOH, AOH-monomethyl ether (AME), tentoxin (TEN), and OTA, were extracted from apple-based products using DLLME and DLLME-SFO methods (Carbonell-Rozas et al., 2023). These procedures were optimized using a CCD design to account for interactions within the ternary system of dispersive solvents, extraction solvents, and salt quantities. A comparative analysis of environmental impact confirmed that the DLLME-SFO procedure is the more environmentally friendly alternative. The combination of 1-dodecanol and NaDES was employed in the DLLME-SFO procedures, achieving the highest extraction yield with 100 and 500 μL of extraction and dispersive solvents, respectively. Under optimized conditions, the LOD and LOQ did not exceed 0.25 and 0.8 μg $Kg^{-1}$, respectively.

SFO-LPME was used in conjunction with a pH-variable, low-polarity DES to isolate AFB1 in cereal samples. A DES composed of L-menthol: camphor-10-sulfonic acid was used for this purpose. In this method, the extractant phase (DES) was dispersed within the aqueous solution upon the addition of KOH (5 mol $L^{-1}$). Simultaneous extraction and phase separation were accomplished using HCl (5 mol $L^{-1}$). The container was immersed in an ice-water bath for several minutes, allowing the organic phase to solidify, which was then collected with a spatula.

The container was placed in an ice bath for several minutes, and the solidified organic phase was separated with a spatula. After melting at room temperature, the sample was analyzed using HPLC-FLD (Ameen and Al-Masri, 2023).

### 3.2. Sorbent-based microextraction techniques

In comparison with LPME, solid-phase microextraction (SPME) offers several advantages, including the reusability of the sorbent, minimization or even complete elimination of organic solvents, a high degree of greenness, and tunable selectivity (Trujillo-Rodríguez et al., 2020). As a versatile and high-throughput sample preparation technique, SPME integrates all steps of extraction (Jalili et al., 2020). Despite the benefits, SPME has inherent limitations, such as carryover and memory effects, longer extraction times, and a limited volume of extractant phase (fiber coating) (Hosseini et al., 2014).

#### 3.2.1. Dispersive micro solid-phase extraction method

Due to its simplicity and effectiveness, dispersive micro–solid phase extraction (D-μSPE) has attracted considerable attention as a sample preparation technique. Developed to overcome the inherent drawbacks of SPME, this method disperses the sorbent directly into the sample matrix rather than immobilizing it on a fiber. Such dispersion increases the contact area between the sorbent (acceptor) and the sample solution (donor), thereby reducing extraction and desorption times (Ghorbani et al., 2019). The versatility of D-μSPE lies in the broad range of sorbents that can be employed (Ghorbani et al., 2019). Moreover, coupling this method with green sorbents further improves its environmental compatibility (Sorouraddin et al., 2024).

A comparable technique was developed for the extraction of PAT and OTA from fruit juice samples. In this approach, a vitamin-based metal–organic framework (MOF) sorbent was synthesized using vitamin B3 as the bio-linker and $CoCl_2{\cdot}6H_2O$ as the metal source. For the extraction process, 5 mL of HPLC-grade water was added to the samples in glass tubes, followed by the addition of 15 mg of MOF. The mixture was then vortexed for 2.5 min to achieve uniform sorbent dispersion. After centrifugation, the bio-MOFs were collected, and analytes were desorbed with a ChCl–EG DES (1:2 molar ratio). HPLC-MS/MS was used for detection, giving LODs of 4.4 ng $L^{-1}$ for PAT and 3.9 ng $L^{-1}$ for OTA. LOQs were 39.6 and 24.8 ng $L^{-1}$ , respectively, with RSDs under 4.4% (Mohebbi et al., 2022).

According to Badali et al. (2023), D-μSPE was combined with MOFs and DLLME to extract AFM1 and OTA from milk matrices. The method utilized water-miscible and water-immiscible DESs in a 1:2 molar proportion, functioning as the elution/dispersive and extraction phases. In addition, MIL-88B (Fe) was synthesized and directly introduced into the sample solution to act as the sorbent. The highest yield was obtained by employing 50 mg of sorbent with an adsorption period of 3 min, using 1 mL of ChCl\EG DES as the elution/dispersive medium, a desorption time of 3 min, and 60 μL of a menthol\decanoic acid mixture as the extraction solvent, followed by microwave irradiation for 90 s. Under these optimized conditions, the LOD and LOQ were determined to be 0.25 and 0.37 ng $L^{-1}$ , respectively, and 0.83 and 1.23 ng $L^{-1}$ for the two MTs.

**Abbreviations:** Liquid-phase microextraction (LPME); Choline chloride (ChCl); Box-Behnken Design (BBD); Dispersive liquid–liquid microextraction (DLLME); Fractional Factorial Design (FFD); pressurized liquid extraction (PLE); Ethylene Glycol (EG); Ethyl methyl ammonium chloride (EMAC); Ternary phases solvent extraction (TPSE); Hexafluoroisopropanol (HFI); DES-based ultrasound-assisted emulsification microextraction (DES-UAEME); Differential pulse voltammetry

(DPV); Ultrasound-assisted emulsification liquid phase microextraction (UA-ELPME); ultrasonic assisted microextraction (UA-ME); effervescence-assisted microextraction (EA-ME); air-assisted natural deep eutectic solvent-based solidified homogeneous liquid phase microextraction (AA-NADES-SH-LPME); Solidification of a floating organic droplet-based dispersive liquid–liquid microextraction (SFOD-DLLME); pH-switchable hydrophobic deep eutectic solvents (HDESs) combined with SFO-LPME.

## 4. Green analytical chemistry metrics

Green analytical chemistry (GAC) aims to develop environmentally benign methods that are safe for both humans and the environment. Several criteria have been introduced to assess the greenness of analytical methods, including miniaturization, reagent toxicity, waste generation, and energy requirements (Pena-Pereira et al., 2020). In this context, various greenness metrics have been established, including the Green Analytical Procedure Index (GAPI), the National Environmental Methods Index (NEMI), the Analytical Eco-Scale, and, more recently, AGREE (Analytical GREEnness) (Kaya et al., 2024). GAPI uses a pictogram and color scale to represent the greenness of each step of an analytical procedure. In this model, instrumentation, reagents, and procedural steps are evaluated. The GAPI pictogram allows researchers to compare different analytical procedures and select the most environmentally favorable method. It also clearly identifies the weakest points of an analytical technique in terms of greenness (Płotka-Wasylka and Wojnowski, 2021).

NEMI, introduced in 2002, is one of the simpler greenness metric systems. Its pictogram is circular and consists of four criteria related to waste generation, corrosiveness, hazardousness, and toxicity. Each section of the NEMI pictogram is colored green when the corresponding criterion is met; otherwise, that section remains uncolored. However, this system has several limitations, including its qualitative nature, time-consuming evaluation process, and limited level of detail (Yin et al., 2024).

The Analytical Eco-Scale is another widely used GAC metric. This system is based on assigning a total score of 100 points to an ideal green analytical method. Penalty points are then deducted based on waste generation, energy consumption, solvent or reagent amounts, and associated hazards. The Analytical Eco-Scale offers advantages such as quantitative assessment and simplicity. However, it also has limitations, including the lack of detailed information about the nature of hazards and the subjectivity of penalty-point assignment (Yin et al., 2024). As a more standardized GAC assessment system, the AGREE metric was developed to address limitations of earlier approaches, such as NEMI and the Analytical Eco-Scale. AGREE evaluation criteria are based on twelve principles of green analytical chemistry (Kaya et al., 2024).

The greenness score of a DES-based EA-ME method was assessed using GAPI and AGREE. The GAPI results showed that seven factors, including amount, health hazard, transport, safety hazard, preservation, storage, and additional treatments, were classified as green, whereas two parameters, sample collection and type of method, were classified as red. This pictogram confirmed the overall greenness of the proposed technique. In addition, the relatively high AGREE score of 0.72 further confirmed the environmentally friendly nature of the method (Zhao et al., 2024).

In 2022, AGREEprep was introduced to assess the greenness of sample-preparation techniques. It is the first metric specifically developed to evaluate the environmental impact of sample preparation. This metric includes ten steps corresponding to the ten principles of green sample preparation and uses open-source software to visualize the results (Pena-Pereira et al., 2022). Based on this system, the greenness of a DES-based DLLME method was evaluated and confirmed. In comparison with conventional DLLME, DLLME combined with solidification of a floating organic drop (DLLME-SFO) achieved a higher AGREEprep score (Carbonell-Rozas et al., 2023).

## 5. Challenges and limitations of DES-based microextraction

DESs have been introduced as alternatives to toxic organic solvents. However, recent studies have reported several challenges associated with their use. For example, the greenness and sustainability of some DESs remain uncertain and require further investigation (Zaib et al., 2022). Certain DESs, such as ChCl:malonic acid, can be thermally unstable because of esterification reactions and the decomposition of malonic acid into acetic acid and carbon dioxide (Marchel et al., 2022). High viscosity is also one of the main challenges encountered when DESs are used as mobile phases in HPLC (Heck et al., 2024).

When used as extraction solvents, the high viscosity of DESs can reduce the diffusion coefficients of analytes, leading to longer extraction times and potentially altering the solubility of target compounds. In organic synthesis, viscosity can limit mass and energy transfer during chemical reactions, thereby affecting reaction yields. The viscosity of DESs is mainly attributed to extensive hydrogen-bonding networks and van der Waals interactions (Chevé-Kools et al., 2025). Conventional solvents are typically separated from target compounds by evaporation. However, the low volatility and high viscosity of some DESs limit their evaporation and might affect extraction recovery (Chevé-Kools et al., 2025). Viscosity of DES can also hinder mass transfer and restrict industrial applications that involve stirring, pumping, and filtration. Other notable limitations include higher cost compared with some conventional solvents, high density, and limited recyclability (Svigelj et al., 2021).

Although DES components are often considered safe, natural compounds are not necessarily free of toxicity. Moreover, the toxicity of the final eutectic mixture may differ from that of its individual components. Therefore, not all DESs can be classified as rapidly biodegradable (Sharma and Lee, 2024). DESs have also been reported to exhibit hygroscopic behavior. They can absorb a few weight percent of water after exposure to open air, which may alter their final molarity (Green and Roy, 2025). They may also show thermal and chemical instability under certain conditions, especially during prolonged heating or exposure to high-ionic-strength environments (Soylak et al., 2025). Another limitation is that DESs do not evaporate easily; therefore, they can adhere to the GC liner and contaminate the mass spectrometer (MS) ion source. This contamination can reduce MS performance and shorten the lifetime of the instrument (Cunha et al., 2024).

Matrix effects are among the main challenges associated with LPME, MSPE, and LLME methods. In addition, these techniques are generally not suitable for large sample volumes. Emulsion formation is another major limitation in LLME and requires careful control. Sample degradation may also occur in microextraction methods that use microwave or ultrasound energy (Shoshtari-Yeganeh et al., 2024).

## 6. Conclusions and future perspectives

MTs continue to represent a major global challenge due to their prevalence in food and feed chains, pronounced chemical stability, and diverse toxicological impacts. As emphasized throughout this review, reliable MT detection critically depends on efficient sample preparation strategies capable of mitigating complex matrix interferences and achieving low detection limits. In this context, microextraction techniques—particularly those employing DESs have rapidly emerged as promising alternatives that overcome numerous limitations of conventional extraction and preparation methods.

This review demonstrates that DES-based microextraction approaches are not only aligned with the fundamental principles of GAC but also offer highly tunable physicochemical properties. Such tunability is central to achieving selective extraction of structurally diverse MTs. The inherent adaptability, low toxicity, and cost-effective synthesis of DES formulations further position them as attractive substitutes for traditional organic solvents and ionic liquids. Collectively, the reported studies demonstrate that DES-assisted extraction

techniques yield higher enrichment factors, require smaller solvent volumes, and integrate effectively with miniaturized and automated extraction platforms.

Despite these advantages, several research gaps remain. Most studies have been confined to laboratory-spiked samples, with limited validation using real-world, heavily contaminated matrices where matrix effects may substantially affect extraction efficiency. Although DESs are generally regarded as environmentally benign, comprehensive assessments of their long-term environmental behavior, biodegradability, and potential toxicity of constituent components are still lacking. Additionally, viscosity-induced mass-transfer limitations can constrain extraction kinetics, particularly in viscous or particulate-rich samples. To address these challenges, future research should prioritize several directions. The rational, computational design of task-specific DESs with optimized polarity, viscosity, and binding affinity can greatly enhance both selectivity and extraction efficiency. Extending DES-based techniques to multi-mycotoxin workflows, in combination with advanced analytical platforms such as LC–MS/MS and high-resolution mass spectrometry, would expand analytical coverage and improve throughput. Large-scale interlaboratory validation is urgently needed to standardize extraction protocols and establish methodological robustness in alignment with international regulatory requirements. Furthermore, integrating DES-microextraction with automated, portable, or sensor-based detection systems could enable rapid, on-site monitoring of MT contamination. Finally, comprehensive life-cycle and cost–benefit analyses will be essential to support the transition of DES-assisted microextraction from laboratory research into routine industrial and regulatory use. In conclusion, DES-based microextraction represents a promising, evolving approach for the determination of MTs in a sustainable, sensitive, and selective manner. Continued innovation in solvent design, methodological integration, and real-matrix validation will be pivotal to advancing this field and ensuring safer global food and feed systems.

## CRediT authorship contribution statement

**Bahareh Shoshtari-Yeganeh:** Writing – original draft, Methodology, Conceptualization. **Soheila Davoodi:** Writing – original draft, Methodology, Conceptualization. **John P. Giesy:** Writing – original draft, Methodology, Conceptualization. **Javad Feizy:** Writing – original draft, Methodology, Investigation, Conceptualization. **Rokhsareh Meamar:** Writing – original draft, Methodology, Conceptualization. **Hasan Badibostan:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hasan Badibostan reports a relationship with Isfahan University of Medical Sciences that includes: employment and funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data Availability

No data was used for the research described in the article. All chemical structures were designed in Chem Draw professional software version of 22.2.0.

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