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Miniaturized sample preparation strategies for the determination of N-nitrosamines in pharmaceutical products: A comprehensive review

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ABSTRACT

N-Nitrosamines (NAs) are classified as potent mutagenic impurities, raising substantial concerns due to their presence in various consumer products, including pharmaceuticals products. The detection of NAs in valsartan-containing medicines in 2018 led regulatory agencies to establish strict guidelines for permissible levels in drug formulations. The standard-gold analytical techniques for NAs determination have been chromatographic techniques (liquid and gas chromatography) coupled with mass spectrometry system, which present higher sensibility and accuracy for target NAs. However, accurate determination of NAs in medicines remains challenging due to their trace-level concentrations and susceptibility to matrix effects, carryover, and contamination of the analytical instrumentation, emphasizing the need for robust sample preparation strategies. Traditional sample preparation methods, although effective, often involve high consumption of solvents, samples, and extraction phases, along with substantial waste generation. In response, miniaturized sample preparation techniques have emerged as sustainable alternatives, offering reduced solvent usage, minimal sample requirements, and lower energy consumption, all while maintaining analytical performance. These green approaches not only align with sustainable analytical practices but also enhance efficiency and environmental compliance in pharmaceutical analysis. This review provides a comprehensive overview of recent advancements in miniaturized sample preparation strategies for the determination of NAs in pharmaceutical products, highlighting their analytical merits and potential for regulatory adoption.

1. Introduction

Over the last few years, the pharmaceutical industry has been concerned regarding the occurrence of N-nitrosamines (NAs) in a range of commercial medicines [1,2]. In 2018, this class of mutagenic impurities were found in sartan medicines such as valsartan and losartan, which is continuum use drugs for blood pressure regulation [3,4]. These findings have led regulatory agencies worldwide to establish guidelines for acceptable levels of NAs in these products. Given the threshold of toxicological concern of NAs and the maximum daily drug dosage, the permitted limits are expressed in nanograms of nitrosamine per milligram of the active pharmaceutical ingredient (API). Therefore, the determination of NAs in pharmaceutical products requires sensitive and accurate methods, capable of detecting these impurities at low concentrations [5–7].

The main strategies for determining NAs in these products lie in chromatography separation coupled with mass spectrometry (MS) [8]. Both gas chromatography (GC) and liquid chromatography (LC) are widely applied for NAs determination for their sensitivity and accurate results in separating these impurities. Due to the presence of API and excipients in high concentrations on the medicine's samples, the analysis of NAs via GC-MS and LC-MS may present some drawbacks when the step of extraction and sample clean-up is neglected [7–9]. The absence of a sample preparation step may induce matrix and carryover effects, which influences the chromatography separation and the behavior of the analytes, leading to errors in their quantification and identification [8,10]. Moreover, the lack of a sample preparation technique may allow unwanted substances to be introduced during injection, compromising the analytical system. Therefore, a sample preparation step is a key parameter to ensure reliable results in determining NAs in pharmaceutical products [8,10,11].

The literature presents a range of sample preparation methods for NAs determination in pharmaceutical products, most of them based on traditional methods such as solid-phase extraction (SPE) and liquid-liquid extraction (LLE) [12–15]. These methods are well established in the literature, presenting suitable precision and accuracy and great

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recuperation rates for determining a range of impurities in pharmaceutical products, such as NAs. Despite these advantages, traditional methods often involve labor-intensive and time-consuming procedures, as well as requiring higher sample volume and large amounts of extraction phase and/or organic solvents [10,16].

With the advent of green chemistry concepts in the 1980s, the reduction of organic solvent consumption, minimization of sample volume, and decrease in energy demand became key drivers in the development of miniaturized sample preparation techniques [17–19]. Therefore, in the mid-1990s, miniaturized sample preparation techniques arose as more sustainable alternatives, aligning with the principles of green chemistry [18,20]. In this context, solid phase microextraction (SPME) emerged as the first miniaturized sample preparation technique which was widely diffused, mainly due the prompt automation aggregating the possibility to extract, and pre-concentrate analytes using small amounts of extraction phase, solvent, and sample with higher precision, accuracy and reducing time analysis. Subsequently, several other miniaturized sample preparation techniques, based on SPE and LLE, gained prominence due to their ease of handling, speed, and compatibility with sustainable analytical methods [17,21,22].

Miniaturized sample preparation techniques have spread throughout analytical applications, such as bioanalysis [23,24], forensic studies [25,26], metabolomics [27,28] and pharmaceutical studies [10,29]. Hence, this review focuses on highlighting the applicability of different miniaturized sample preparation strategies for NAs determination in pharmaceutical products. The use of these techniques for assessing impurities in commercial pharmaceuticals marks a significant advancement in modern analytical chemistry, addressing the needs of the pharmaceutical industry, and enhancing safety and quality control in pharmaceutical products.

2. Case of N-nitrosamine's contamination in pharmaceutical products

NAs are characterized by a deprotonated amine linked to a nitroso group (R_2N_2O) and are formed by the reaction of secondary, or tertiary amines and nitrosating agents, as nitric acid or nitrate and nitrite salts (NOx), under acid conditions [7,30,31]. Studies suggest that the carcinogenic effect of NAs is due to the bioactivation of these molecules through oxidative metabolism catalyzed by enzymes such as cytochrome P450s (Fig. 1) [7,31,32]. This oxidation reaction produces a diazohydroxide intermediate and an aldehyde derived from the parent molecule. The decomposition of the diazohydroxide generates an N-alkyldiazonium ion, a highly reactive alkylating electrophile capable of interacting with proteins and DNA. Moreover, this reaction also forms an aldehyde as byproduct (such as formaldehyde), that can also react with the DNA to form additional adducts. Notably, formaldehyde is classified as a human carcinogen by both the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC). Thus, NAs are considered bidentate carcinogenic molecules [32–34].

The International Agency for Research on Cancer (IARC) classified NDMA and N-nitrosodiethylamine (NDEA) as possibly carcinogenic for humans (group 2 A) since 1987, and nowadays at least 24 NAs are presented in this category [7,35]. ICH guidelines also add NAs in a list of carcinogenic impurities as "cohort of concern" due to its high mutagenic potential. Hence, the presence of NAs has been monitored throughout the last years as impurities in a range of sources, such as drinking water [33,36], tobacco smoke [37–39], processed meats [40–42] and cosmetic products [43].

Tobacco-specific NAs are formed from tobacco alkaloids like nicotine through reactions with nitrosating agents [37–39]. These carcinogenic compounds are present in both traditional and electronic cigarettes [37]. In drinking water, NAs can be associated with physical and chemical water treatment process, with the use of chloramination and poly(diallyldimethylammonium chloride), the ionization treatment or the use of activated charcoal [33,44,45]. The literature also reports several occurrences of these mutagenic compounds in processed and fermented meats, being related to the use of nitrite and nitrate salts as additives is common which serves as preservatives with antioxidant and antimicrobial properties [42,46].

In June 2018, NAs were first detected in medicines containing valsartan, an API from the sartans class, which are used as antihypertensive agents [4,7,47]. In this class of medicines, the formation of NAs can be related to the synthesis of the tetrazole ring, a chemical structure present in most of the sartan drugs [35]. This step involves a biphenylnitrile reagent and an azide salt (usually NaN_3) which is usually added in excess. The unreacted azide salt must be removed due to its high toxicity and explosive nature, and one alternative is the addition of nitrite to promote the formation of nitrous oxides [34,35,48]. Consequently, NAs can be formed, since dialkylamines can be present in the reaction medium. According to the European Medicines Agency (EMA), other APIs from the sartans family that undergoes the same synthesis step, such as losartan, olmesartan and irbesartan, are potential sources of NAs [35,49]. Moreover, the occurrence of NAs can also be linked to the use of solvents such as dimethylformamide (DMF), that is precursor NDMA. In addition, reagents such as dimethylamine, triethylamine, and N-methyl-4-aminobutyric acid have also been identified as potential precursors of NAs in drug products [3,35,50].

Initial findings in sartan-based medicines prompted investigations into the presence of NAs in other pharmaceutical products, leading to the inclusion of ranitidine, metformin, and nizatidine among the primary targets of these studies [51,52]. Ranitidine, an antiulcer agent, was withdrawn from the market worldwide due to the detection of NDMA in elevated concentration in several batches. In this case, although the exact pathway of NA formation remains uncertain, studies by the U.S. Food and Drug Administration (FDA) showed that NDMA levels increase over the product's shelf life, suggesting that ranitidine API itself acts as a precursor to NAs [53,54]. In metformin hydrochloride, the presence of NDMA is primarily associated with the reaction between the API and residual DMF, which is used as a solvent. However, other by-products from metformin synthesis, such as dimethylamine hydrochloride (a secondary amine) may also contribute to nitrosamine formation in the presence of nitrosating agents [55]. Recently, API specific NAs has been also reported. Rifampicin is an antibiotic used in tuberculosis treatment and became a source of NAs due to the detection of 1-methyl-4-nitroso

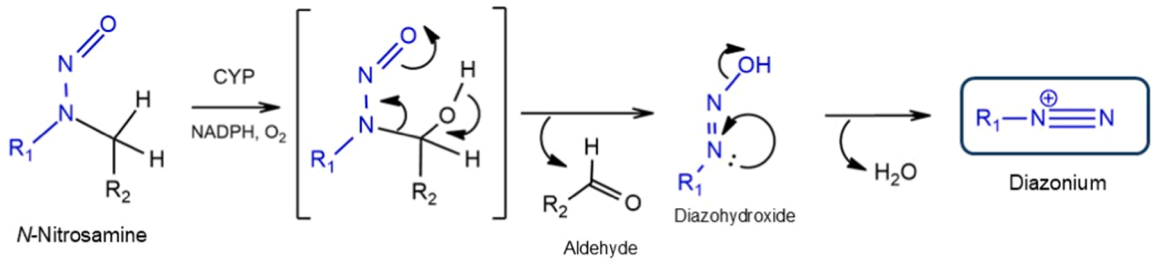


Fig. 1. Enzymatic oxidation metabolism of N-nitrosamines.

piperazine [56]. Also, risperidone, a medicine used in the treatment of bipolar disorder and schizophrenia, is another API that recently has been related to NAs occurrence [57].

Therefore, the presence of NAs in commercial medicines is primarily associated with the synthesis, degradation, and residues of solvents and reagents [7]. Additionally, contamination may result from interactions between the API and excipients. The presence of aldehydes can promote the formation of NAs, and these compounds can originate from excipients [3,58]. Furthermore, certain excipients can serve as sources of nitrates and nitrites, which also contribute to the formation of NAs. Furthermore, the packing material can also be one of the reasons for NAs occurrence in medicines. EMA reported that the use of nitrocellulose lidding foils and amines from the printing inks may promote the formation of NAs [35,50,59].

Given the widespread detection of NAs in various medicines, regulatory agencies have implemented strict controls based on risk assessment. As a result, all stages of drug manufacturing must be evaluated for potential NA formation. Identifying possible sources of contamination is essential to prevent their occurrence in pharmaceutical products [60–62]. Based on the daily dosage of each drug and the mutagenic potential of NAs, regulatory agencies such as the FDA and EMA have set acceptable intake limits for each NA, typically in the nanogram-per-milligram range of API (ppb level) [7,63]. Therefore, NAs are expected to be present only at trace levels in pharmaceutical products. To ensure accurate and reliable control, analytical protocols are provided in these regulatory guidelines, most of which rely on chromatographic separation coupled with mass spectrometry (LC-MS and GC-MS) [63–65]. In such trace-level analyses, the sample preparation step prior to instrumental analysis is particularly critical, as it aims to eliminate interferences, preconcentrate the analytes, and preserve instrument integrity [10,11].

3. Miniaturized sample preparation for N-nitrosamine's determination in pharmaceutical products

The need for an efficient sample preparation step prior to NAs determination via LC-MS or GC-MS is vital to lead to reliable results. Some regulations have provided standard protocols for the analysis of NAs in pharmaceuticals products [66–68]. However, many of these protocols lack a sample preparation step, which may lead to undesirable outcomes during analysis, such as contamination of the analytical system, clogging of lines and fittings, underestimated levels, as well as degradation and reduced efficiency of the chromatographic column. Additionally, analytical challenges such as matrix effects and carryover may arise and be negatively significant for method performance. These issues stem from the fact that NAs are typically present at trace levels in pharmaceutical formulations, which are composed of both the API and various excipients [8,69]. Some of these protocols have incorporated a sample preparation step, commonly employing traditional techniques.

With the crescent urgent to adhere as much as possible to the green chemistry paradigms [70], many traditional analytical protocols were found to be inconsistent with the more sustainable principles it promotes. In response, the concept of "green analytical chemistry" was introduced to align analytical methodologies with environmentally conscious practices. Based on green chemistry principles, Gałuszka et al. [71,72] proposed twelve principles of green analytical chemistry which were rapidly embraced by the scientific community. These foundational principles emphasize environmental stewardship and human safety in the development and implementation of analytical procedures. They aim to minimize waste generation, reduce the use and hazards of chemical reagents, promote energy efficiency, and limit derivatization steps [73,74]. Accordingly, these techniques have been implemented into NAs determination. Fig. 2 provides an overview of the application of these techniques in protocols aimed at analyzing NAs in medicines.

3.1. *Recent developments in solid phase microextraction for NAs determination in pharmaceutical products*

SPE is a well-established and effective sample preparation method that has demonstrated high efficiency across a wide range of analytical protocols. SPE is a technique originally developed in the 1940s, but it gained significant prominence after the 1970s [73,75]. This technique is based on the interaction between analytes and a solid sorbent phase. Since its incorporation into analytical protocols, SPE has proven to be

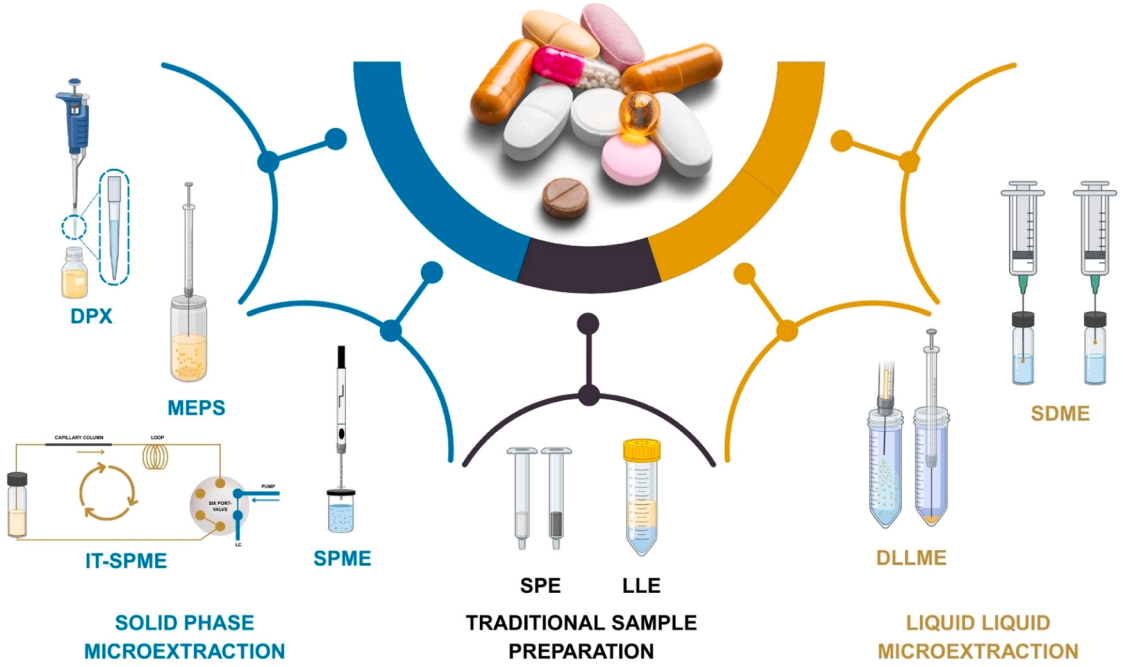


Fig. 2. Sample preparation techniques employed in determining NAs in medicines matrices (SPE: solid phase extraction; LLE: liquid-liquid extraction, SPME: solid phase microextraction; IT-SPME: in tube – solid phase microextraction; DPX: disposable pipette extraction; MEPS: microextraction by packed sorbent; DLLME: dispersive liquid liquid microextraction; SDME: single-drop microextraction).

highly effective for sample clean-up, extraction, and pre-concentration of analytes. SPE operates using a syringe-like cartridge that contains a specific amount of sorbent material packed between two frits [73,76]. Extraction is performed by applying a defined volume of the sample to the top of the cartridge, allowing it to pass through the sorbent phase. The analytes are retained on the solid phase based on their affinity between the liquid sample matrix and the sorbent [73,77–79]. Subsequently, the retained analytes are eluted with an appropriate solvent for analysis. Various parameters in this procedure can be optimized to enhance extraction efficiency, such as sample and solvent volumes, the nature of the sorbent phase, and pH adjustment [73,79]. Despite offering several advantages, such as high recovery rates and the potential for automation, SPE also presents certain drawbacks, including the relatively large amounts of sorbent material, sample, and solvent required, as well as being a time-consuming process [73,80,81]. With the introduction of green chemistry principles, miniaturized sample preparation techniques have emerged as sustainable alternatives to traditional methods [18,19,73].

3.1.1. Solid phase microextraction – SPME

Solid phase microextraction (SPME) emerged as an alternative technique that integrates sampling, concentration, and analysis into a single step, thereby reducing overall analysis time [82,83]. This technique utilizes a coated device (typically a fused silica fiber) containing a small amount of sorbent phase. Traditionally, materials such as polydimethylsiloxane (PDMS), carboxen and divinylbenzene (DVB) are largely employed as coating materials, but literature also presents alternative sorbent phases such as molecularly imprinting polymers (MIPs) and metal organic frameworks (MOFs) [16,21,84].

SPME is a non-exhaustive technique, and the efficiency of the extraction largely depends on the affinity and partitioning of the analyte between the sorbent material and the sample matrix. Thus, the choice of sorbent must be tailored to the physicochemical properties of the target analytes [73,83,85]. The extraction process can be performed either by immersing the SPME device directly into the liquid sample or via headspace sampling for volatile compounds. Subsequently, analyte desorption can be achieved using an appropriate solvent or through thermal desorption, depending on the analytical setup. This headspace setup is particularly advantageous for GC analysis once the use of solvents is minimized or unnecessary and desorption is performed by adding the fiber onto the GC injection port. Given its advantages over traditional sample preparation techniques, such as simplicity, speed, and reduced solvent consumption, SPME has become an attractive approach for a wide range of analytical applications [82,86]. Notably, its use in the determination of NAs in pharmaceuticals has already been reported [50,87,88].

Chang *et al.* [88] proposed the use of headspace-SPME for extraction of NAs across diverse pharmaceutical products followed by GC-MS analysis. This study encompasses the analysis of 14 drug-related NAs across 44 pharmaceutical products, including sartan medicines, antibiotics, metformin and ranitidine. The headspace-SPME procedure employed a fiber coated with DVB/carboxen/PDMS with condition temperature of 260 °C. For extraction, the samples of the pharmaceutical product (20 $mg.mL^{-1}$) were spiked with a pool or 14 NAs at 5 $ng.mL^{-1}$. The main parameters regarding extraction procedures were evaluated to their optimal values. The adsorption temperature was evaluated ranging from 50 to 90 °C, demonstrating a better efficiency at 80 °C. The desorption temperature was evaluated from 200 to 280 °C, and a better efficiency in desorbing NAs was reached applying 250 °C. The evaluation of adsorption/desorption temperature must be carefully considered taking account of the thermal stability of the analytes and the fiber. Extraction time was also evaluated, and the equilibrium between sample and the sorbent was achieved in 30 min. After evaluation, the fiber lifetime was set as 50 runs presenting great efficacy in extracting NAs. This protocol demonstrated great analytical performance, with limits of quantification (LOQ) of 0.05 $ng.mg^{-1}$ for the 14 NAs evaluated, with recoveries rates up to 100 %. The authors compared this methodology to direct injection (without sample preparation), which presented LOQs ranging from 0.05 – 0.25 $ng.mg^{-1}$. Therefore, the headspace-SPME demonstrated advantageous over traditional methodologies.

In 2020, a study was published by Alshehri *et al.* [87] regarding the use of headspace-SPME for NDMA extraction in ranitidine products for subsequent analysis via GC-MS. In the case of ranitidine, it is important to remove the API prior to analysis, as the pathways for NDMA formation in the medicine are still not fully understood in the literature. Consequently, the impurity content may increase during analysis due to degradation of the API. Therefore, the developed protocol employed a fiber coated with polyacrylate and the main parameters regarding extraction were optimized. A sample of ranitidine was added into a headspace vial with an aqueous solution of NaCl (36 %), which was submitted to constant stirring and adsorption temperature of 45 °C. The equilibrium was reached in 45 min, and after this time the fiber was conducted for thermal desorption in GC applying a temperature of 220 °C. The method demonstrated great linearity ranging from 5 – 100 $ng.mL^{-1}$, with LOQ of 5 $ng.mL^{-1}$ and great values of precision and recovery for NDMA. These results were compared to the LC-MS standard method proposed by Food and Drug Administration agency (FDA), which demonstrated the feasibility of headspace-SPME GC-MS method for determining NDMA in ranitidine medicines.

As mentioned, the packing material can be a source of NAs contamination in medicines. Golob *et al.* [50] demonstrated a sample preparation step based on SPME for NDMA and NDEA determination in nitrocellulose blisters used in pharmaceutical products. SPME fibers coated with DVB/Carboxen/PDMS were exposed for 10 min to the gas phase at specific positions within and near a blistering equipment. This setup allowed the extraction of volatile NAs present in the vapor phase during the blistering process. Following exposure, the fibers were immediately introduced into the injection port of a GC–MS system for thermal desorption and analysis. A blank sample was also prepared by exposing an additional SPME fiber for 10 min in a standard analytical laboratory environment to account for background contamination. The lidding foils used in the blistering equipment presented NDMA and NDEA. For comparison, the foils and the blister were also evaluated, adding TFA for NAs extraction, and the supernatant were collected for NAs evaluation. The SPME test was only qualitative, but for quantitative purposes, a TFA solution was used, demonstrating high amounts of these impurities in nitrocellulose lidding foils. Furthermore, this study elucidates the formation of NAs in this case once nitrocellulose acts as a nitrosating agent for secondary amines, as dimethylamine and diethylamine found in printing inks, leading to the formation of NAs in lidding foil.

3.1.2. In tube solid phase microextraction – IT-SPME

The advancements of SPME led the development of SPME-related technologies, such as in tube SPME (IT-SPME). This approach was designed to overcome certain limitations associated with conventional fiber-based SPME, including limited fiber stability over some organic solvents, low sorption capacity, and reduced efficiency for weakly volatile compounds [17,22,89]. In contrast to traditional SPME fibers, classical IT-SPME utilizes an open tubular fused silica capillary coated on its inner surface with a suitable stationary phase. Additionally, IT-SPME enables the online coupling of the sample preparation step with LC analysis, enhancing automation and reducing sample handling. Traditionally, IT-SPME can operate in two main configurations: I - The draw/eject mode is based on the repeated cycles of aspiration and ejection of the sample into the capillary until equilibrium is reached; II – The flow-through mode based on the continuous passage of the sample through the capillary, allowing sustained interaction with the stationary phase until the limit of quantitation is achieved (Fig. 3) [89–92].

The earliest capillary columns employed in IT-SPME methods were based on internal coated open tubular columns and porous layers open tubular columns. Both configurations exhibited certain limitations: the

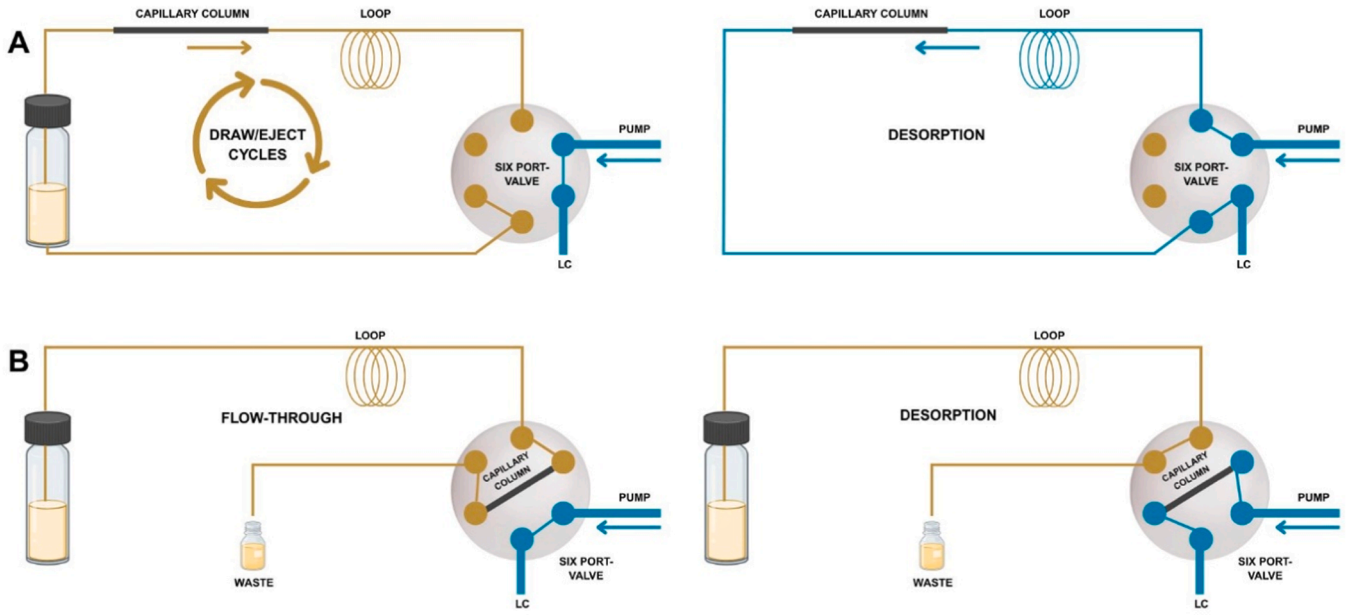


Fig. 3. In-tube SPME configurations: **A**: Draw/eject cycles system; **B**: Flow-through system.

internally coated capillaries may present restricted extraction capacity, while the porous layer columns, although offering high extraction efficiency, often demonstrated limited stability under solvent flushing conditions [89,92]. Particle-packed and monolith capillary columns were also applied to IT-SPME, demonstrating advantages such as reduced void volume and higher extraction capacity. As with other SPE-based techniques, the extraction efficiency is intrinsically dependent on the nature of the sorbent phase employed. Traditional sorbents such as diphenyl-polydimethylsiloxane, porous DVB, and Carboxen molecular sieves have been widely used in IT-SPME. However, ongoing advancements in sorbent materials have expanded the range of options to include MIPs, MOFs, magnetic nanoparticles, boron-affinity materials, and biologically derived ligands such as lectins, aptamers, and antibodies [22,93–95].

Throughout IT-SPME applications, this technique has been successfully applied to determination of NAs in medicines. Ishizaki *et al.* [96] demonstrated the performance of IT-SPME for determination of NAs present in metformin commercial medicines followed by LC-MS analysis. The authors employed a GC porous layer open tubular column (60 cm×0.32 mm i.d.), which was placed between the injection loop and the injection needle of the autosampler in an LC instrumentation, and a draw/eject mode was employed. The study used a six-port valve that allowed the injection of the sample consecutively, followed by the elution of the analytes by the mobile phase. For NAs extraction, metformin tablet solution (100 mg.mL^{-1}) was prepared, adding a pool of seven NAs at 100 ng.mL^{-1}. The main parameters regarding extraction procedure were evaluated to enhance the NAs extraction. Three GC capillary columns with the same diameters were tested for the extraction of NAs (Carboxen 1006, Carboxen 1010 and DVB), and the most suitable was Carboxen 1006, attributed to its high adsorption surface. The number of draw/eject cycles sample was also evaluated, and the equilibrium was archived using 20 cycles when 40 μL of sample was injected into the capillary column, in a flow rate of 0.2 mL.min^{-1}. Sample pH was evaluated, and it was found to have little effect on the NAs recovery, therefore pH was not adjusted. This optimized method was validated, demonstrated great linearity (0.2 – 50 ng.mL^{-1}), LOQ ranging from 0.1 – 5 pg.mg^{-1}, and recoveries up to 102 %. Compared with conventional SPE methods, the use of IT-SPME demonstrated advantages regarding enhanced sensitivity and lower limits of detection (LOD) and LOQ, which highlights the outstanding performance of IT-SPME in determining seven NAs simultaneously in metformin tablets.

3.1.3. *Disposable pipette extraction – DPX*

Disposable Pipette Extraction (DPX) is another miniaturized sample preparation technique based on SPE principles, which employes a pipette tip containing a small amount (≈20 mg) of dispersive sorbent phase [97–99]. Therefore, its apparatus consists of a micropipette tip (1 or 5 mL) with an upper and a lower filter that traps the sorbent phase. The bottom filter can be composed of various materials such as glass wool and serves as a permeable barrier to fluids during the extraction process while maintaining the sorbent phase into the pipette. The upper filter is employed to avoid the entrance of contaminants into the pipette tip. DPX procedure involves repeated cycles of suction and disposal of the sample until extraction equilibrium is achieved. Following extraction, analytes are desorbed through sequential suction and disposal of an appropriate solvent (Fig. 4). Depending on the sample matrix, conditioning and clean-up steps may be necessary [99–102].

As with other SPE-based techniques, the extraction efficiency of DPX depends on the affinity between the analytes and the sorbent phase, as well as the subsequent affinity of the analytes for the desorption solvent [99,103,104]. Therefore, the sorbent phase must be carefully selected to ensure effective removal of analytes from the matrix, along with an appropriate choice of desorption solvent. In addition to the chemical nature of the sorbent and solvent, their quantities must also be optimized [103,105,106]. Other parameters should be considered in DPX extraction procedures, such as the number of extraction and desorption cycles required to reach equilibrium between phases, and typically, three to seven cycles are sufficient. The equilibration time per cycle (usually ranging from 10 to 60 s) can also significantly impact analyte extraction. Moreover, factors such as sample pH and the salting-out effect may influence extraction efficiency and should be evaluated during method development [99,103].

DPX exhibits a high analyte transfer rate and rapid equilibrium between the solid phase and the sample, characteristics that are intrinsically linked to the dispersive nature of the system, which enhances contact between the sample and the extraction phase. Moreover, the aspiration of sample or solvent introduces air bubbles into the pipette tip, further promoting mixing and phase dispersion [99,107,108]. Given the wide range of DPX applications, various sorbent phases are available and should be selected according to the specific analytical purpose. Commonly used phases include commercial sorbents (e.g., C8, C18, ion-exchange), biosorbents, restricted access materials (RAM), MIPs and MOFs [103,104,109]. This technique offers notable advantages,

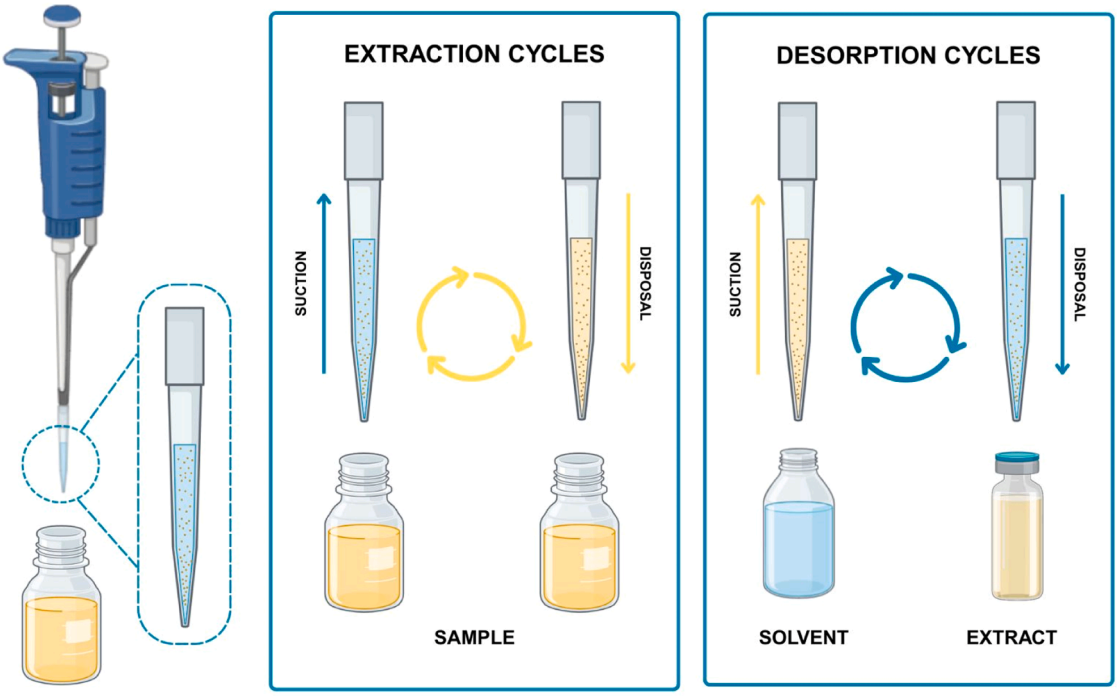


Fig. 4. Schematic representation of DPX extraction procedure (created with BioRender.com, 2025).

including reduced consumption of sorbent material, minimal sample and solvent volumes, easy manipulation and the possibility for automation, enabling the simultaneous extraction of multiple samples and thereby increasing analytical throughput [99,103].

Due to its outstanding performance, DPX has been applied in a range of analytical protocols, such as bioanalysis, forensic investigations, environmental analysis, and pharmaceutical studies. Batista Junior et al. [10] demonstrated the development of a DPX protocol for simultaneous determination of six NAs in sartan medicines followed by LC-MS analysis. The sartan samples were prepared in water at 30 mg.mL^{-1} and spiked with a pool of six NAs ranging from 0.5 – 150 ng.mL^{-1}. The authors employed a pipette tip of 5 mL with a glass wool bottom filter and the main parameters regarding DPX procedure were evaluated by univariate and multivariate approaches. The authors evaluated eight commercial sorbent phases, and NAs demonstrated affinity with C18 sorbent phase. Methanol, acetonitrile and isopropanol were evaluated as desorption solvents, once these solvents are compatible with LC-MS system, and methanol demonstrated efficiency in desorbing the analytes. Multivariate optimization set pH at 7, equilibrium time of 30 s, 2 extraction cycles and 5 desorption cycles. The optimized method enabled rapid extraction of NAs using minimal amounts of sorbent, sample, and desorption solvent in approximately 4 min. Analytical performance acknowledged the excellency of the method achieving recoveries of up to 98 %, with precision and accuracy below 15 % and LOQ of 1 ng mL^{-1} with no significant matrix effect.

3.1.4. Microextraction by packed sorbent – MEPS

Another notable microextraction technique based on SPE principles is microextraction by packed sorbent, also commonly referred to as microextraction in packed syringe (MEPS) [17,110]. This approach utilizes a small amount of sorbent material (usually 2–5 mg) packed into a syringe (100–1000 μL), which can be positioned either within the syringe barrel between the needle and barrel, or as a cartridge integrated into the syringe. Extraction procedure is analogous to DPX, as MEPS undergoes several cycles of aspiration and disposal of the sample until equilibrium between the sorbent and the sample is reached. Similarly, analytes desorption is performed in sequential cycles using an appropriate solvent, capable of elute the analytes extracted by the sorbent phase. Therefore, a typical MEPS workflow includes a conditioning step, the sampling (extraction) cycles, an optional washing step, and the final elution (desorption) cycles. Parameters such as number of cycles, sample and solvent volume and the nature of solvent and sorbent can be optimized to reach its optimal conditions, contributing to higher recoveries [111,112]. MEPS became an attractive technique due to its greener characteristics such as reduced sample and solvent volumes, low amount of sorbent and high throughput, besides its possibility of automation. Among its advantages, the MEPS setup offers reusability for up to approximately 100 cycles, depending on the application, and allows for easy replacement of the sorbent phase once it reaches the end of its usable lifespan, contributing to its cost-effectiveness [111,113].

MEPS has been successfully employed in a range of analytical protocols, including evaluation of NAs in sartan medicines by a study of dos Santos et al. [11]. In their work, the authors developed a simple and effective sample preparation strategy for the simultaneous determination of four NAs in losartan tablets using an automated MEPS followed by LC-MS analysis. Automation was achieved through the construction of a custom multi-syringe robotic system employing a 1 mL syringe. Within the syringe, 5 mg of sorbent material was packed between two frits, and critical MEPS parameters were optimized using both univariate and multivariate approaches. Seven different sorbent phases—comprising reversed-phase and hydrophilic-lipophilic balance materials—were tested, with the carboxylic acid-modified polystyrene-divinylbenzene copolymer showing superior retention of the four NAs. This enhanced performance was attributed to van der Waals and π–π interactions between the sorbent and the analytes. Although the conditioning step was also assessed, it did not improve analytical performance due to the intrinsic properties of the selected sorbent. The sampling and elution (number of cycles and sample/solvent volume) were evaluated by multivariate approaches, demonstrating optimal condition employing 500 μL of sample using 15 cycles for sampling and 50 μL of elution solvent using 12 cycles for elution. This automated protocol allowed the evaluation of six samples in 20 min, demonstrating the method high throughput. Analytical performance highlighted MEPS efficiency in evaluating NAs in losartan medicines,

demonstrated LOD of 50 ng.g^{-1}, linearity on the studied range and high recoveries. Thus, this study supports previous research demonstrating the high efficiency of solid-phase microextraction techniques for the analysis of NAs in medicines, contributing to the development of increasingly sustainable analytical protocols while maintaining robust analytical performance.

3.2. Enhanced methods for NAs determination in pharmaceutical products based on liquid-liquid microextraction

In addition to SPE techniques, LLE represents an alternative strategy in sample preparation. This classical technique enables the separation of analytes from complex liquid matrices by exploiting the differential solubility and interactions of compounds in two immiscible or partially immiscible liquid phases [114]. Typically, an organic extractant is added to an aqueous sample, and upon vigorous mixing, a cloudy emulsion forms due to the dispersion of fine organic droplets throughout the aqueous phase [114,115]. This process facilitates the mass transfer of the target analyte into the organic phase, primarily driven by polarity compatibility. Following extraction, the mixture is centrifuged to disrupt the emulsion and achieve phase separation, with the organic layer being collected with the target analyte for subsequent analysis [116–118].

Aiming to be in accordance with green chemistry principles, Liu and Dasgupta (1996) [119] were among the first to propose the miniaturization of the conventional LLE technique, introducing the concept of single-drop microextraction (SDME), which represents one of the earliest developments in liquid-liquid microextraction (LLME) methodologies [120,121]. Subsequently, dispersive liquid-liquid microextraction (DLLME), low-temperature partition liquid-liquid microextraction (LLME-LTP), vortex assisted liquid liquid microextraction (VALLME) and hollow-fiber liquid-liquid microextraction (HF-LLME) were also developed based on LLE principles [118,122]. These miniaturized systems represent a significant advancement over traditional LLE-based approaches, as they drastically reduce solvent and sample consumption, while also minimizing analysis time. Moreover, their inherent simplicity and adaptability facilitate integration into automated, on-line analytical platforms, thereby enhancing throughput and analytical efficiency [121–123]. These techniques have been of great importance in a range of analytical protocols, such as the pharmaceutical field, being successfully applied to trace-level determination of impurities in pharmaceutical products.

3.2.1. Single-drop microextraction – SDME

SDME was one of the first miniaturized LLE techniques to be established in literature, introduced by Dasgupta's team in the mid-1990's. In the same period, Jeannot and Cantwell [124] extended the application of SDME to analytical chromatography systems, by immersing a single drop (8 μL) of n-octane into an aqueous sample to extract 4-methylacetophenone under stirring, for 5 min, later, 1 μL of the organic solvent was injected into GC system to analytical determination. Overall, the method involves suspending a single microdrop of organic solvent (< 10 μL) at the tip of a needle or capillary, which acts as the extraction phase in a biphasic system. Depending on the nature of the analytes, the solvent drop can be directly immersed into the sample solution (DI-SDME) or exposed to the headspace above the sample (HS-SDME) (Fig. 5) [120,125].

During extraction, analytes diffuse from the sample matrix into the solvent drop until dynamic equilibrium is reached. After an appropriate extraction time, the drop is retracted and subjected to analysis. The efficiency of extraction of the process is influenced by various parameters (e.g., solvent type, drop volume, extraction time, stirring rate, temperature, *etc.*) that are normally evaluated in SDME optimization [125–127]. Although SDME has been widely reported in the literature for the isolation of active pharmaceutical ingredients from complex drug matrices, its application for the extraction of impurities remains scarcely explored [121].

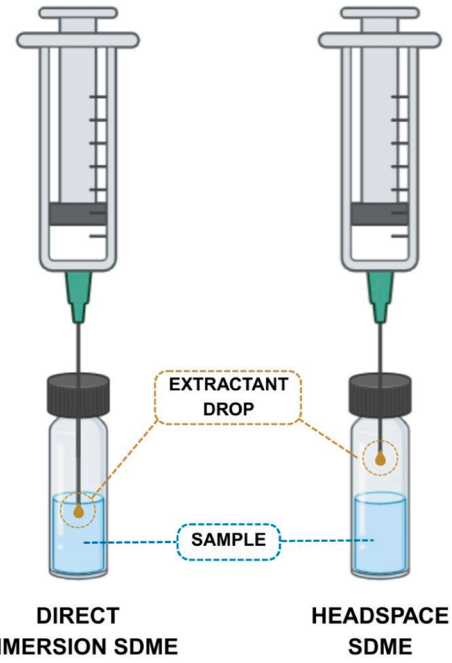


Fig. 5. Schematic representation of the SDME conventional extraction procedures. A) Direct Immersion SDME and B) Headspace SDME (created with BioRender.com, 2025).

In this context, Santos et al. [128] were the first to report the use of SDME for the extraction of NAs in pharmaceutical products. This study employed the HS-SDME approach to isolate four NAs impurities - NDMA, NDEA, N-nitrosoethylisopropylamine (EIPNA), and N-nitrosodiisopropylamine (NDIPA) - from losartan tablets. Aiming to enhance the environmental compatibility of the method, the authors proposed the use of water as a green extractant solvent, effectively reducing matrix interferences. An automated extraction system was developed, in which 600 mg of powdered losartan was placed in headspace vials and heated at 75 °C. The system automatically handled the water drop (50 μL), maintaining it in static exposure for 45 min, after which the enriched extract was directed to LC–UV-Vis analysis. Extraction parameters were optimized, such as sample mass, equilibrium time, drop volume, and extraction temperature. The optimized method demonstrated LOD compliant with current regulatory thresholds for NAs in medicines (50–80 ng.g^{-1}), a linear range of 100–2400 ng g^{-1}, and satisfactory recovery and precision.

Despite the limited number of studies reporting the application of SDME in pharmaceutical analysis, its effectiveness demonstrates a huge potential in this branch, especially to explore impurities in medicine contents. Its ability to accommodate a wide range of extractant solvents allows for effective minimization of matrix interferences, thereby enhancing selectivity. Nonetheless, the technique still faces challenges regarding reproducibility, primarily due to the instability of the single drop of solvent [125]. However, recent studies have already begun integrating this technique into automated systems, paving the way for its application in routine quality control and high-throughput analytical workflows [129,130].

3.2.2. Dispersive liquid liquid microextraction (DLLME)

Another extensively employed LLME technique is DLLME. Basically, this technique consists in injecting a mixture of extracting and dispersing solvents into an aqueous sample. A dispersion is formed and facilitates fast extraction of analytes. The dispersion is removed by centrifugation and the extracting solvent containing analytes is taken for analysis with a syringe (Fig. 6). This technique was introduced in 2006

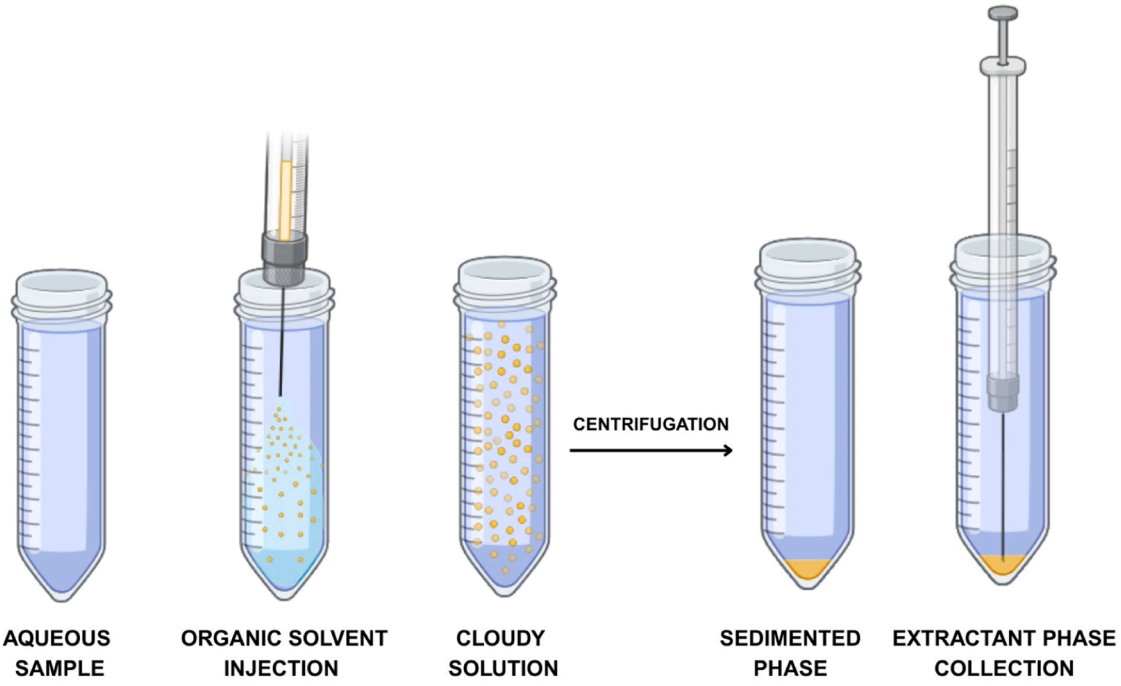


Fig. 6. Schematic representation of the DLLME extraction procedure. (created with BioRender.com, 2025).

by Berijani's group [131], demonstrating its applicability to extract organic pollutants from aqueous matrices. In their study, 8 μL of tetrachloroethylene and 1 mL of acetone were used to extract polycyclic aromatic hydrocarbons from a 5 mL water sample, followed by GC analysis. Since this pioneering work, numerous studies have incorporated this technique as a sample preparation step across diverse fields, including pharmaceutical and biomedical analysis [117]. A common modification in DLLME procedures involves the addition of salts to induce the salting-out effect, which enhances the ionic strength of the aqueous phase and reduces the solubility of target analytes, thereby improving their partitioning into the extractant phase. This strategy has proven particularly effective for increasing extraction efficiency in complex matrices. Notably, the method has also been adapted for the extraction of impurities in drug products [132,133].

Aiming to achieve NAs impurities in ranitidine medicines, Campillo et al. [54] developed a method based in DLLME, as a simple but effective technique to pre concentrate these impurities. Samples commercial ranitidine tablets were prepared in water (100 mg mL^{-1}), adding 0.5 g of NaCl, corresponding to a 5 % (m/v) salt concentration. Subsequently, a binary mixture of 0.5 mL methanol (dispersive solvent) and 150 μL chloroform (extractant solvent) was injected into the aqueous phase, promoting the formation of a fine dispersion of the extractant droplets. After centrifugation, the lower phase (chloroform) was collected and introduced into a GC-MS system. Key parameters, including the volumes of extractant and dispersive solvents and salt concentration, were optimized using multivariate approaches to enhance extraction efficiency. The method demonstrated excellent linearity for the nine targeted NAs, with LOQs ranging from 0.21 to 21 ng g^{-1} . Recovery studies yielded satisfactory results, with values ranging from 80.2 % to 102 % at the lowest concentration level, and 94.7–105 % at the highest. This study reinforces DLLME as a practical and highly effective approach for the extraction and quantification of NAs contaminants in pharmaceutical formulations.

Although the study conducted by Campillo et al. demonstrated promising results, Tay et al. [134] proposed an alternative approach aiming to target NAs compounds not previously detected, while also pursuing a lower LOQ to comply with current regulatory requirements. In this context, the authors introduced a significant modification to the conventional DLLME technique by incorporating inclusion complexation mechanisms. This modified approach, termed dispersive inclusion complex microextraction (DICM), relies on host–guest interactions rather than traditional solvent polarity as the primary extraction driving force. This strategic modification enhances the extraction of low hydrophobicity compounds from aqueous matrices, which is a limitation of conventional DLLME methods [29,134].

Thus, Tay et al. [134] applied the DICM methodology to determine five NAs impurities in pharmaceutical formulations containing ranitidine, losartan, and valsartan, followed by analysis via LC-MS/MS. The extraction process involved dissolving the sample in a diluent composed of 5 % (m/v) NaCl, 1.5 mM β-cyclodextrin (inclusion complexing agent), and 20 mM sodium dodecyl sulfate (as dispersant) in a 20 % (v/v) methanol aqueous solution. Subsequently, dichloromethane was rapidly injected into the mixture as the extracting solvent. After phase separation, the enriched organic phase was collected for instrumental analysis. All extraction parameters, including the choice of extractant, dispersant, and complexing agent, were optimized in preliminary experiments. The proposed DICM-LC-MS/MS method demonstrated effective extraction of NAs from pharmaceutical matrices, yielding satisfactory analytical performance. The method exhibited good linearity, satisfactory recoveries, and, notably, low LOQs (0.036–0.060 mg L^{-1}), fully compliant with current regulatory standards. Therefore, the proposed incorporation of an inclusion complex formed between β-CD and targeted analytes during extraction successfully overcomes the limitations of conventional DLLME technique, whereby the overall extraction efficiency has been improved especially when dealing with less hydrophobic analytes of interest, been especially good for analysis of NAs in medicines.

Other modifications of conventional DLLME were proposed in literature for NAs determination, such as Géhin et al. work [135], which changes solvent order addition, termed dispersant-first DLLME (DF-DLLME). This approach involves dissolving the sample in a moderately polar dispersant solvent instead of a polar aqueous phase at the initial step, favoring quantification of NDMA extraction from metformin medicines. Moreover, Schettino's et al. [43] proposes another variant, termed vortex assisted DLLME (VA-DLLME), that unlike conventional DLLME procedure where an organic solvent is used as

disperser solvent, the vortex agitation assists the formation of the cloudy solution. This modification significantly reduces the use of auxiliary organic solvents, relying solely on the extractant. These methodological advances underscore the versatility of DLLME and its applicability for the determination of NAs in pharmaceutical products.

3.3. Assessment of miniaturized sample preparation technique on NAs analysis

SPE is a well-established and effective sample preparation technique for the analysis of NAs in pharmaceuticals, offering high recovery, precision, and accuracy. Its standardized, commercially available formats enhance reproducibility and support routine analytical workflows. As a well-established technique used in a wide range of applications, SPE benefits from commercially available cartridges packed with various sorbent phases tailored to specific needs. The standardized nature of these cartridges enhances method reproducibility and facilitates implementation in routine analysis. The introduction of green analytical chemistry principles revealed the drawbacks related to SPE procedures. The development of the miniaturized sample preparation techniques presented here was gradual, with many of them remaining lab-made for a considerable period. Nowadays, many of the apparatus for these techniques are commercially available, enabling their broader dissemination across various analytical applications. Furthermore, the standardization of these devices has contributed to the reproducibility of miniaturized sample preparation techniques.

In the context of analytical procedures, both the literature and the studies presented here clearly demonstrate that miniaturized sample preparation techniques offer effective analytical performance while complying with current international regulatory requirements. Table 1 summarizes the main characteristics of these techniques employed for NAs determination.

These data clearly demonstrate that the advancement of miniaturized sample preparation techniques, both solid-phase and liquid-phase, has maintained the high recovery rates and low LOD traditionally achieved by conventional methods such as SPE. Notably, these techniques have significantly improved in terms of sample volume requirements, as well as the amount of solvent needed for cleaning and/or elution during the extraction process. Furthermore, these methods offer considerable reductions in extraction time, thereby increasing the analytical throughput. For techniques based on phase equilibrium as these miniaturized techniques, a critical parameter to consider is the time required to reach equilibrium and/or the number of extraction cycles needed to achieve it [99,136]. Depending on the technique and its intended application, this equilibrium time can be relatively long, ultimately limiting the method's analytical throughput. For instance, in the analysis of NAs, techniques such as SPME and SDME typically require around 30 min to reach equilibrium. In contrast, methods like DPX and DLLME achieve equilibrium in less than 5 min. This marked difference is primarily attributed to the dispersion of the extractant phase in DPX and, which significantly enhances mass transfer between phases and accelerates equilibrium [10,11,135].

An additional advantage is the potential for automation, as exemplified by Santos et al., [11] who demonstrated an automated MEPS procedure capable of simultaneously extracting six samples. As widely reported in the literature, the sample preparation step remains one of the most common sources of error in analytical workflows, largely due to manual handling and human-related variability. Automating this step, whether in conventional or miniaturized formats, can significantly reduce such issues and enhance method reliability. In this context, SPE has traditionally served as a robust and scalable platform for automated workflows, allowing for the simultaneous processing of multiple samples with high precision. Additionally, several miniaturized techniques have been adapted for automation as well, with commercially available systems now supporting SPME, DPX and MEPS further expanding their applicability in high-throughput and routine analysis [111,137]. In this scenario, the possibility of automating miniaturized sample preparation techniques further strengthens their applicability in routine pharmaceutical analysis. By minimizing human error, reducing analysis time, and enabling high-throughput workflows, these techniques align well with current demands for efficiency, reproducibility, and regulatory compliance. The availability of commercial systems for methods reflects the growing maturity and reliability of these approaches.

Related to the experimental procedure, Table 2 summarizes the configuration, advantages and possible drawbacks related to these miniaturizes sample preparation techniques applied to NAs determination in pharmaceutical products. These techniques present a range of advantages in terms of efficiency, selectivity, automation, reduced costs and high throughput. However, miniaturized sample preparation techniques may present limitations related to solvent usage, the need for complex optimization, and potential operational variability. Overall, the integration of miniaturized sample preparation strategies represents a powerful tool for improving the detection of trace-level impurities such as NAs, reinforcing the commitment to ensuring the safety and quality of pharmaceutical products.

4. Greener assesment of miniaturized sample preparation for NAs analysis

Throughout this review, a range of sample preparation techniques has been presented as effective alternatives for the determination of NAs in pharmaceutical products, with a consistent emphasis on their potential to enhance the environmental profile of analytical methods [19,138,139]. To demonstrate the alignment of these techniques with the principles of green analytical chemistry, several metrics have been proposed, such as the Green Analytical Procedure Index (GAPI) and Analytical GREEnness (AGREE). These tools evaluate the impact of various parameters on the overall sustainability of analytical methods

Table 1
Methods reported on the literature for NAs determination in medicine samples.

Extraction technique	Sample Amount	Solvent Amount*	LOD / LOQ	Extraction Time	Recovery (%)	Ref.
SPE	50 mL	31 mL	LOD: 0.07 – 0.30 $\mu g.kg^{-1}$	60 min	95–105	[52]
HS-SPME	5 mL	-	LOD: 1.0 $\mu g.L^{-1}$	45 min	-	[87]
HS-SPME	1 mL	-	LOQ: 0.05 $\mu g.g^{-1}$	30 min	95–102	[88]
IT-SPME	40 μL	80 μL	LOD: 3.0 – 112.0 $pg.mL^{-1}$	5 min	93–117	. [96]
MEPS	500 μL	350 μL	LOD: 50.0 $ng.g^{-1}$	20 min	80–136	[11]
DPX	1 mL	1 mL	LOD: 0.5 $ng.mL^{-1}$	4 min	63–107	[10]
DLLME	10 mL	650 μL	LOD: 0.07 – 6.6 $ng.g^{-1}$	3 min	80 – 105	[54]
HS-SDME	600 mg	75 μL	LOD: 50 – 80 $ng.g^{-1}$	45 min	79–145	[128]
DICM	10 mL	300 μL	LOD: 0.011 –0.018 $ng.mL^{-1}$	≈ 6 min	96–105	[134]

SPE: Solid phase extraction; **HS-SPME:** Headspace solid phase microextraction; **IT-SPME:** In-tube solid phase microextraction; **MEPS:** Microextraction by packed sorbent; **DPX:** Disposable pipette extraction; **DLLME:** Dispersive liquid liquid microextraction; **HS-SDME:** Headspace single-drop microextraction; **DICM**: Dispersive inclusion complex microextraction.
*Amount of solvent used on the entire extraction procedure.

Table 2
Comparative overview of miniaturized sample preparation techniques for NAs determination in pharmaceutical products.

Technique	Configuration	Sorbent Amount	Advantages	Drawbacks
HS-SPME	Fiber coated	≈ 50 – 85 μm	Solvent-free in HS configuration; simple; high sensitivity	Limited fiber lifetime; high cost related to fiber; limited analyte range; longer equilibration
IT-SPME	Capillary column in a LC autosampler	60 cm × 0.32 mm i.d.	Fully automated; reusable; integration with LC systems; high sensitivity	Instrument modification needed; optimization complexity.
DPX	Sorbent dispersed in pipette tip with bottom filter	≈ 20 mg	Fast equilibrium; low sample/solvent consumption; adaptable sorbents; potential automation	Manual steps may reduce reproducibility
MEPS	Sorbent packed inside syringe barrel	≈ 5 mg	Automatable; low sample/solvent consumption; high throughput	Potential clogging
HS-SDME	Suspended aqueous drop in vial headspace	50 μL	Greener solvent (uses water); low cost; simple setup	Drop instability; limited automation; longer equilibration
DLLME	Dispersive solvent	75 μL	High enrichment; fast; low solvent/sample volumes	Use of toxic solvents
DICM	Dispersive system	300 μL	Enhances extraction of hydrophilic analytes; compatible with LC-MS/MS	More complex process; need for optimization of inclusion/dispersive system

HS-SPME: Headspace solid phase microextraction; **IT-SPME:** In-tube solid phase microextraction; **MEPS**: Microextraction by packed sorbent; **DPX:** Disposable pipette extraction; **DLLME:** Dispersive liquid liquid microextraction; **HS-SDME:** Headspace single-drop microextraction; **DICM:** Dispersive inclusion complex microextraction

[138,139].

To assess the specifically environmental impact of sample preparation methods, the use of the AGREEprep metric has been encouraged, as it offers appropriate levels of accuracy and specificity for evaluating the greenness these procedures [140,141]. AGREEprep evaluates sample preparation processes based on ten principles of green sample preparation (Fig. 7), with each principle scored on a scale from 0 (not meeting the criterion) to 1 (fully meeting the criterion). A color-coded pictogram is generated to visualize the final score, with the score itself displayed at the center; in this scheme, red indicates an environmentally unfriendly procedure, whereas green signifies a more sustainable and environmentally benign method. The surrounding circular diagram is divided into ten segments, each representing a specific performance criterion. The length of each segment reflects the weight assigned to that criterion, while the color indicates the level of performance achieved. This visual

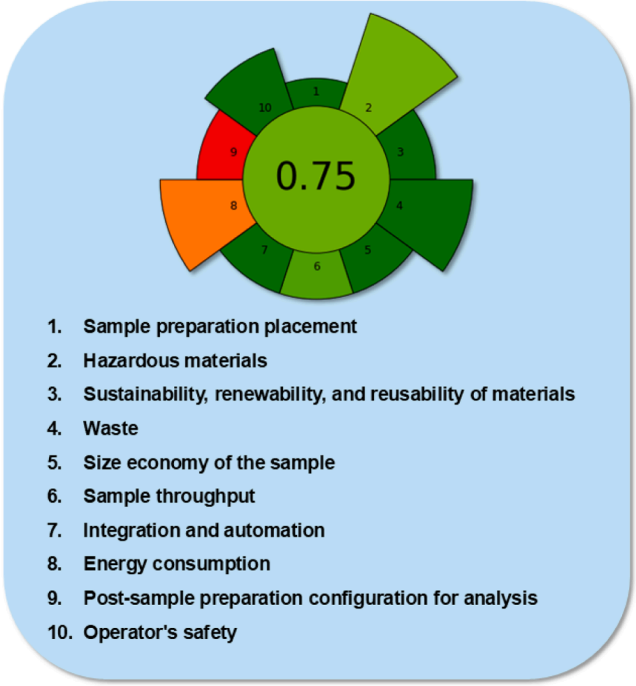


Fig. 7. Ten criteria and output image of AGREEprep.

output enables an equitable comparison of different sample preparation procedures, either based on the overall score or by evaluating individual criteria [140,141].

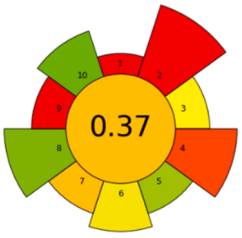
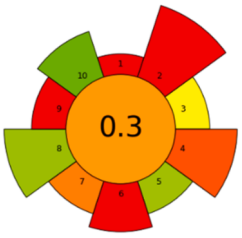
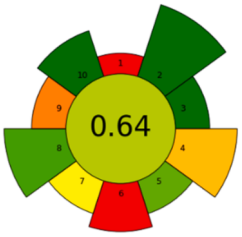
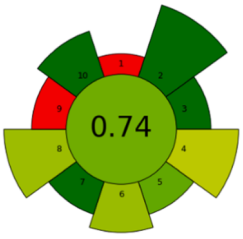
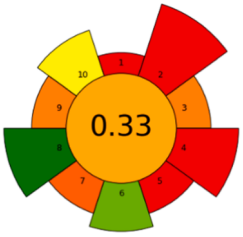
The AGREEprep metric offers a distinct advantage over other greenness assessment tools by specifically targeting the sample preparation stage, often underrepresented in broader metrics. Its ten well-defined criteria focus on the sustainability of each step from sample collection to instrumental analysis, thereby providing a more accurate and comparable evaluation of sample preparation methods. For better recognition of the greenness in miniaturized sample preparation techniques, the AGREEprep tool was applied to selected studies reviewed in this work compared to traditional SPE and LLE procedures, for NAs extraction. The comparisons are presented in Table 3.

Through the analysis using AGREEprep, it is notable that conventional SPE and LLE methods exhibit lower sustainability when compared to their miniaturized counterparts. The traditional methods yielded scores below 0.4, which, according to AGREEprep's criteria, classify them as unsustainable, primarily due to their reliance on large volumes of hazardous solvents and high waste generation [140]. In contrast, the miniaturized techniques, whether based on solid or liquid-phase extraction principles, achieved notably higher scores, reflecting their improved sustainability profile through reduced solvent consumption, decreased analyst exposure to hazardous materials, and minimized waste production. However, it is worth noting that many of these miniaturized techniques are still predominantly implemented using lab-made systems, which can hinder their commercial viability and, more critically, their integration into automated analytical workflows for higher throughput. Thus, the miniaturization of extraction techniques is a trend that will endure, restrictedly aligned with the green analytical principles, to achieve more sustainable and environmentally friendly methods.

5. Concluding remarks and future trends

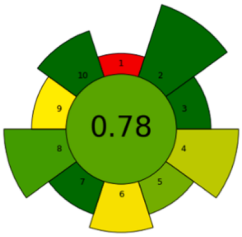
The occurrence of NAs in pharmaceutical products presents a significant challenge to traditional analytical protocols due to the low concentrations of these impurities in complex pharmaceutical matrices. Consequently, the sample preparation step becomes crucial.

Table 3
AGREEprep tool applied to evaluate the sample preparation methods for NAs determination.

Extraction principle	Matrix	Extraction technique	Score	Ref.
Solid Phase Extraction Based	Medicine Tablets	SPE		[13]
	Medicine Tablets	SPE		[5]
	Ranitidine tablets	HS-SPME		. [88]
	Sartan tablets	DPX		[10]
	Losartan tablets	MEPS		[11]
Liquid Phase Extraction Based	Industrial effluent	LLE		[142]

(*continued on next page*)

Table 3 (*continued*)

Extraction principle	Matrix	Extraction technique	Score	Ref.
	Losartan tablets	HS-SDME		[128]
	Ranitidine tablets	DLLME		[54]

SPE: Solid phase extraction; **LLE:** Liquid-liquid extraction; **HS-SPME:** Headspace solid phase microextraction; **MEPS:** Microextraction by packed sorbent; **DPX:** Disposable pipette extraction; **DLLME:** Dispersive liquid liquid microextraction; **HS-SDME:** Headspace single-drop microextraction.

Miniaturization of this step contributes to increased sustainability and applicability of analytical methods, enhancing throughput while maintaining reliable performance. This review highlights that miniaturized techniques based on SPE and LLE have proven effective in various applications, particularly in protocols relevant to the pharmaceutical industry. In recent years, techniques such as SPME, MEPS, DPX, and SDME have been successfully applied for the determination of NAs in medicines, including sartan drugs, ranitidine, metformin, and even packaging materials. These approaches reduce the consumption of samples, solvents, and sorbents, shorten analysis time, lower energy demands, and offer potential for automation. Moreover, these techniques meet the criteria established by regulations worldwide for the determination of NAs in pharmaceutical products.

The continuous advancement of these protocols should be encouraged to overcome the inherent limitations of miniaturized sample preparation techniques. The incorporation of novel tailored materials, such as MIPs and MOFs for NAs determination, represents a prominent trend in this field. These materials offer high chemical stability and selectivity toward specific classes of analytes, thereby enhancing key analytical performance parameters such as LOD and accuracy, while also reducing matrix effects. Furthermore, such materials may provide cost-effective alternatives, contributing to the overall reduction of sample preparation costs. Moreover, the miniaturization of various sample preparation protocols has paved the way for the development of microfluidic and point-of-care devices. With the continuous advancement of analytical platforms such as LC-MS and GC-MS, commonly employed in NAs analyses, the reduction of sample and/or solvent volumes used in purification and preconcentration steps has become increasingly important. Microfluidic systems enable faster and more cost-effective analyses, while also offering a more sustainable approach, as they operate with reagent and sample volumes typically in the microliter range.

Given the significant improvements brought by miniaturized techniques to analytical protocols, it is essential to promote their integration into standardized procedures established by global regulatory agencies. Current analytical methods for the determination of NAs in pharmaceuticals, as outlined by agencies such as the FDA and EMA, are still largely based on conventional techniques that carry the limitations discussed in this review. The incorporation of miniaturized sample preparation approaches into these official protocols would represent a significant advancement in pharmaceutical analysis, contributing to the development of more sustainable and high-throughput strategies for the quality control of medicines and been in accordance with green analytical principles.

CRediT authorship contribution statement

Almir Custodio Batista Junior: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Yuri Arrates Rocha:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Andrea Rodrigues Chaves:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of Competing Interest

The authors have declared no conflicts of interest.

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Data Availability

Data will be made available on request.

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