Review Article

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A review of current bioanalytical approaches in sample pretreatment techniques for the determination of antidepressants in biological specimens

Abstract: Antidepressants are a class of compounds widely used in clinical settings for the treatment of several diseases. In the last years there has been a considerable increase in their consumption, representing an important public health issue in several countries. Because they are substances with narrow therapeutic windows, and since they are capable of interacting with other classes of compounds, monitoring of these compounds is of relevance, minimizing the risk of medical interactions as well as side and toxic effects. In addition, understanding the extent of their use, their detection through routine toxicology tests and development of new methods for detection and monitoring is of extreme importance concerning public health, patient well-being, and implications in clinical and forensic situations. The main objective of this work is to perform a critical review on the biological samples used in the detection and quantification of antidepressants with special focus on the techniques for sample preparation.

Keywords: antidepressants, biological specimens, sample pretreatment techniques, analytical instrumentation

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1 Brief introduction: Antidepressants - Classification and relevance from the toxicological point of view

Depression is a common chronic or recurrent disease that affects individuals regardless of social or economic status. According to the World Health Organization, this mental disorder was predicted to be for all ages and both sexes, the second cause of global disease by the year 2020. Patients with these serious mental disorders usually die 10 to 20 years earlier than the rest of the population due to their physical health and difficulties in accessing comprehensive health services by most of them [1]. Antidepressants are used for the treatment of conditions such as depression, anxiety, and other mental health problems and depressive disorders have been diagnosed in more patients attending health centres. In addition, health specialists are using more and more antidepressant and antipsychotic medications, and the consumption of tranquilizers and drugs to control hyperactivity by children and adolescents is still too high, which has led to several expert alerts [2].

Several classes of antidepressants are available nowadays. These classes are: monoamine oxidase inhibitors (MAOI) like iproniazid, tricyclic antidepressants (TCA) like imipramine, selective serotonin re-uptake inhibitors (SSRI) like fluoxetine, serotonin-norepinephrine re-uptake inhibitors (SSNRI) like venlafaxine, noradrenergic, and specific serotonergic antidepressant medication (NaSSA) like mirtazapine. Bupropion is an "atypical" antidepressant and belongs to a single chemical class (aminoketone), being mainly a norepinephrine-dopamine re-uptake inhibitor (NDRI). Piperazines are a chemical class that has been marketed as "multi-modal" drugs with

high binding affinity and complementary mechanisms of action for several serotonin receptors and serotonin transporters like vortioxetine [3-6].

These drugs are capable of, on the one hand, reduce the symptoms of depression, but on the other hand several important drug interactions are likely to occur [2]. These drugs present large inter-individual differences and their therapeutic windows are relatively small which makes patient compliance monitoring extremely important. Although there are several antidepressant drug classes, there is still a population of patients that do not respond to these medications [3]. Multimodal mechanisms of action of new generation antidepressants (vortioxetine, desvenlafaxine, vilazodone, and levomilnacipran) show that depression may not be caused solely by a simple serotonin deficiency but rather be related to the distribution of $5HT_{1A}$ receptors in different areas and its relationship with serotonin action mediated or not by glutamate, norepinephrine, and histamine. Research on the use of these new antidepressants in human subjects is limited and more studies are needed to reveal substantial differences concerning the mechanism of action and other pharmacological characteristics of these new drugs [7]. An important aspect relevant in the treatment with these drugs is the inter-individual differences in genes. In the case of antidepressants, the relevant genetic variation is related to phase I drug metabolism, in particular polymorphisms of CYP2C19 and CYP2D6. This assumes particular importance in the effectiveness of each drug [8]. Future research is likely to focus on any of the strategies for combining different antidepressants, expanding the indications or increasing their efficacy [9]. Future developments should consider new agents for the most refractory cases or even the use of new drugs such as esketamine [10] or psilocybin [11].

Besides, as a consequence of their excessive use, and also because of the concomitant use of other medicines, alcohol or drugs of abuse, this class of compounds is often involved in both clinical and forensic situations, namely drug abuse or suicide attempts which can result in severe intoxications of voluntary or accidental nature [12]. Therefore, developing analytical methods for their determination and quantification is of interest not only to clinical toxicology, but also in the field of forensic sciences [13]. Monitoring these compounds allows minimizing the risk of side effects, reducing the possibility of drug-drug interactions, adjusting the dose as needed and evaluating patient compliance as well [14,15].

The high consumption rate of these compounds can be considered a public health problem and therefore, it is important to compile the existing literature on the matter (including analytical methods for their determination) to assist health professionals and improve patients' quality of life.

2 Biological specimens

Efforts have been made in order to identify and quantify antidepressant drugs given the increased use of these compounds in recent years. The fact that there is a wide spectrum of substances, the interaction of these compounds with other drugs and the possibility of concomitant effects with other substances, represents a constant challenge for a rigorous determination by clinic and forensic toxicology laboratories. This requires the use of highly specific and sensitive analytical techniques to determine these compounds and metabolites and the choice of the biological sample to be analyzed is important as well.

The most commonly used samples are plasma [16,17], serum [18], and urine [19]. Hair [20] and oral fluid [21] are also commonly used and whole blood [22,23] is sometimes preferred. Urine is the sample preferably used because of the ease of collection but with the disadvantage that it can be adulterated and/or tampered with and does not always allow the detection and/or quantification of the compounds due to their rapid biotransformation [19]. A good alternative would be oral fluid because of the ease of collection and the difficulty of adulteration; this sample has been gaining more and more importance in the field of drug monitoring not only in what concerns drugs of abuse, but concerning medicinal drugs as well [24,25]. The collection procedure of this sample is painless, non-invasive and the concentrations can be possibly correlated to those of plasma which is advantageous particularly with regard to mentally unstable patients and children [24,26,27]. Another unlikely and complex biological sample is vitreous humor (HV). Filonzi dos Santos et al. [28] developed a methodology for the determination of the TCA amitriptyline, nortriptyline, imipramine, and desipramine which were extracted from 0.5 mL of HV samples by means of hollow fiber-liquid phase microextraction and detection by gas chromatography-mass spectrometry (GC-MS) with electron ionization. This method proved to be appropriate for the analysis of real post-mortem cases and based on the articles that performed the measurements of these compounds in femoral whole blood (FWB) and HV. In real cases, the HV/FWB ratio was of about 0.1 [29].

Moreover, as the consumption of antidepressants has been increasing, it is important that methods are developed for the identification and determination of this class of compounds in a wide range of biological matrices.

3 Sample pretreatment

In the absence of a review of the newest techniques for the preparation of biological samples used for the determination of antidepressants and given that this is the most time-consuming process for laboratories, a comprehensive review focusing on the possible approaches and especially new trends in the treatment of specimens for the identification and determination of these compounds is relevant. The reviewed articles were independently selected by the three authors in order to determine their relevance in the context of the current review and only the articles selected by at least two of the authors were included in this paper.

As previously mentioned, antidepressants are normally detected in whole blood, serum, plasma, and urine samples, requiring a specific treatment of each of these specimens, according to the investigated compounds. This process can be initiated by a pretreatment step which is important to remove matrix compounds that may interfere with the analytes resulting in better results, reduced noise, and is more relevant when applied to more complex matrices. Therefore, the most common pretreatment procedures applied to antidepressants are: liquid-liquid extraction (LLE), solid-phase extraction (SPE), protein precipitation (PPT), and dilution. In general, sample treatment has an effect on precision, accuracy and robustness of analytical methods, and the preconcentration [30] of analytes increases sensitivity and allows lower limits of detection (LODs).

3.1 Liquid-liquid extraction

The classical LLE extraction is one of the most used techniques for sample pretreatment in the toxicology field.

However, this is a less used technique for the extraction of antidepressants from biological samples with only few papers available.

The most recent work is that of Degreef et al. [31] who developed a method for the determination of 40 antidepressants and metabolites in 0.2 mL plasma samples using methyl-tertiary-butyl-ether as extracting solvent. The quantification based on liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) should be highlighted. Quantification limits (LOQs) between 0.5 and 25 ng/mL were obtained. The authors concluded that the method was simple with a relatively short chromatographic run, wide calibration range and could be implemented in therapeutic drug monitoring and forensic or clinical research.

For the LLE technique applied to the extraction of antidepressants, the most used organic solvent is ethyl acetate which can be utilized either by itself or in a mixture. This solvent is volatile, poorly soluble in water, polar but has advantages such as low cost, low toxicity, and allows adequate extraction of several classes of compounds.

Although having benefits, this technique, features some limitations such as considerable volume of sample and organic solvents, low recovery and poor selectivity. In addition, it presents constraints when extracting compounds with distinct lipophilicities and produces high matrix effects when liquid chromatography-mass spectrometric methods are used [32]. Figure 1 represents the LLE technique.

3.2 Solid-phase extraction

For many years, SPE has been widely used in the field of toxicology for drugs determinations in several biological
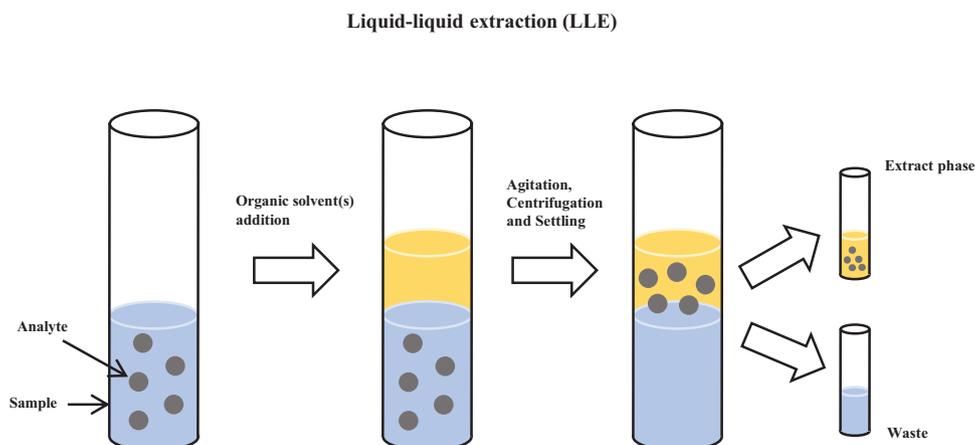
Liquid-liquid extraction (LLE)

Figure 1: Schematic representation of LLE.

specimens. This technique allows the use of different types of cartridges depending on the cost, availability, and nature of the analytes to be determined. This procedure has been applied several times to the sample pretreatment in the determination of antidepressants, mainly in water samples.

With regard to human biological samples, Kall et al. [33] developed a methodology for the determination of vortioxetine and its major human metabolite (Lu AA34443) in plasma samples using two different methods for quantification. An isocratic cation exchange (SCX) with analysis by high-performance liquid chromatography-tandem mass spectrometry (ESI) method utilizing SPE (C8 and 96-well plate) sample extracts and secondly, a reversed-phase ultra-performance liquid chromatography-tandem mass spectrometry method (UPLC-MS/MS) (positive ionization mode) with gradient elution following protein precipitation with acetonitrile was employed. For the first method described, they obtained extraction recoveries between 102% and 104% and LOQs of 0.4 ng/mL for vortioxetine and 2.0 ng/mL for Lu AA34443; for the second method, with better results, they obtained LOQ values of 0.2 ng/mL for vortioxetine and 0.5 ng/mL for Lu AA34443. Rosado et al. [19] developed a method for the determination of a relevant number of antidepressants (fluoxetine, venlafaxine, amitriptyline, mianserin, trimipramine, nortriptyline, mirtazapine, sertraline, dothiepin, citalopram, and paroxetine) and four of their metabolites (desmethyltrimpramine, *O*-desmethylvenlafaxine, norfluoxetine, and desmethylmirtazapine) in urine and plasma samples by GC-MS analysis. SPE was used and the extraction was performed using Strata™ X cartridges. LOQ values varied from 1 to 15 ng/mL, while recoveries in the ranges from 40% to 89% in urine and from 68% to 98% in plasma were obtained. This method has proven to be suitable for application in the monitoring of those drugs. The most recent work is that of Shin et al. [25] who developed and validated a method to quantify 18 antidepressants (amitriptyline, bupropion, citalopram, clomipramine, cyclobenzaprine, desipramine, desvenlafaxine, doxepin, duloxetine, fluoxetine, imipramine, mirtazapine, nortriptyline, paroxetine, sertraline, trazodone, trimipramine, and venlafaxine) in oral fluid samples with extraction by SPE and analysis by LC-MS/MS (ESI). For sample collection, Quantisal devices were used, with which 1 mL of oral fluid was collected and 3 mL of buffer was added. For the extraction of SPE, with Cerex® Trace-B cartridges, 500 μL of the Quantisal sample was applied. The authors obtained LOD and LOQ values of 10 ng/mL and reported recoveries between 91% and 129%. They considered this method perfectly applicable to routine laboratories with advantages such as the rapid run time (5 min) and low sample volume useful to determine the concentrations of antidepressants in this specimen.

For the extraction of antidepressants, there are several SPE sorbents available, but the most used are Oasis® HLB, Strata® X and mixed-mode reversed phase-strong cation exchange cartridges. Despite being a clean technique, this extraction procedure presents numerous disadvantages such as extensive production of residues, high cost per sample, time-consuming and laborious sample preparation (including also method development). Figure 2 represents the SPE technique.

3.3 Protein precipitation and specimen dilution approaches

For the pretreatment of complex matrices such as whole blood, plasma, or serum, it is common to apply at the beginning of the extraction process a protein precipitation step. This is very useful, simple, and rapid minimizing subsequent treatment approaches. For the class of antidepressants, the most commonly used solvent is acetonitrile but methanol [34] and trichloroacetic acid [35] have also been used. For instance, Farajzadeh and Abbaspour [36] applied acetonitrile as precipitant solvent to determine three antidepressants in plasma samples by gas chromatography-flame ionization detection (GC-FID), reporting recoveries from 79% to 98%. Nezhadali et al. [37] applied acetonitrile for plasma and serum samples pretreatment in the determination of fluoxetine by ultraviolet-visible spectrophotometer and reported recoveries around 100%. Another example is that of Hegstad et al. [38] that developed a method for the enantiomeric separation and quantification of R/S-citalopram using ultra-high performance supercritical fluid chromatography-tandem mass spectrometry (ESI) in 0.1 mL of serum samples. For the preparation of these samples, they used protein precipitation with acidic acetonitrile and filtration through a phospholipid removal plate. They obtained values of LOD and LOQ of 1.3 and 2.0 nM, respectively, and recoveries between 81% and 91%.

Another form of pretreatment of the sample is dilution and shoot approaches, namely, specimens of urine and plasma; this is performed most often with water, reducing interferences in the analysis. Nojavan et al. [39], Ríos-Gómez et al. [40], and Hamidi et al. [41] have diluted urine or plasma samples with deionized water, while Mohebbi et al. [42] have used ammoniacal buffer.
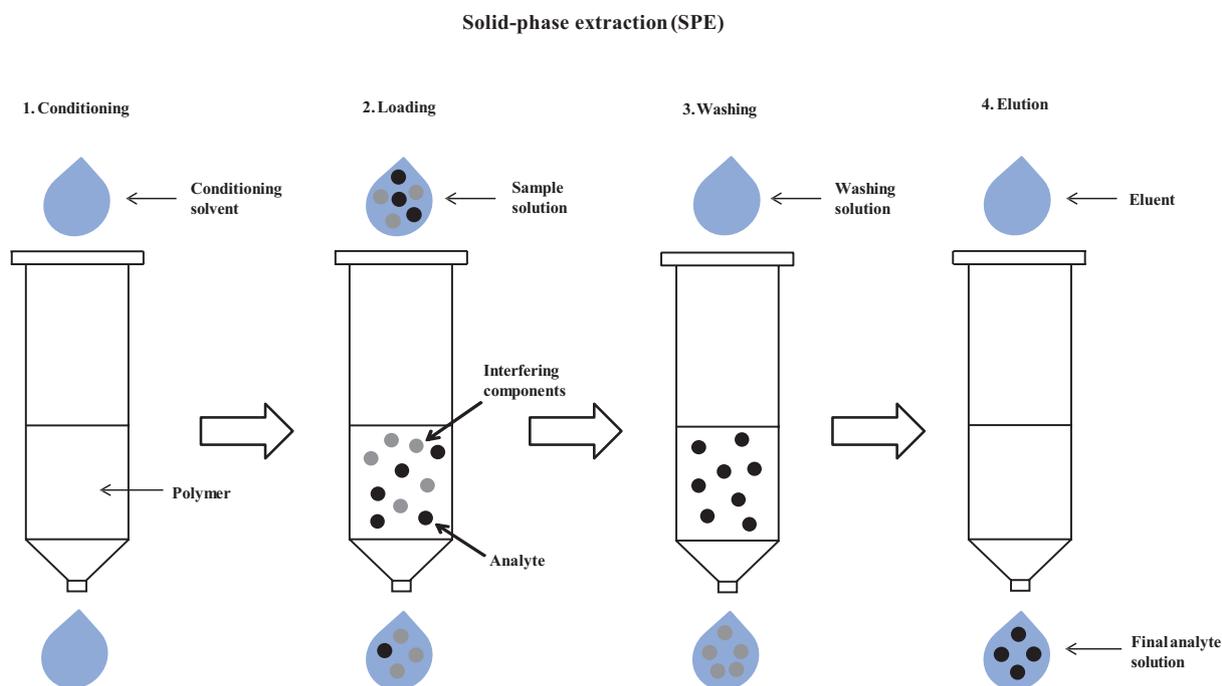
Solid-phase extraction (SPE)

Figure 2: Schematic representation of SPE.

3.4 New trends for sample preparation

In recent years, there has been an increasing interest in using miniaturized or microextraction techniques in several areas for the analysis of numerous compounds in order to avoid classical techniques such as SPE and LLE which demand the use of higher volumes of both organic solvents and biological samples. Therefore, the use of this type of technique for sample preparation should be highlighted taking into account not only the aforementioned advantages, but also the possibility of reuse the extraction devices and the fact that they are less expensive techniques. The first article described is from 1997, by Lee et al. [43]; the authors used 0.5 mL of whole blood for the extraction of four TCA by headspace solid-phase microextraction (HS-SPME) and analysis by GC-FID.

Ide and Nogueira [44] in 2018, developed a methodology with extraction by bar adsorptive microextraction and liquid desorption (BAµE-LD) with a SX phase and with analyte detection by high performance liquid chromatography with diode array detector and LC-MS/MS (ESI). Four of these compounds (amitriptyline, bupropion, citalopram, and trazodone) were studied using deionized water samples. This new generation device proved to be innovative and robust, further combined with the advantage of microextraction through the use of LD, proving to be particularly efficient in the analysis of antidepressants in trace amounts. With the implementation of the BAµE-LD technique, it becomes possible to select the best

sorbent phase, reducing the associated cost, facilitating the preparation, and handling of the device, allowing the use of residual amounts of organic solvents, making it an environmentally friendly technique and a good alternative to other sorption-based microextraction approaches. The difficulty in keeping the bar under constant stirring in the vortex and manipulation during back-extraction are the most important disadvantages of the procedure [44].

Another example is the article from 2018 by Moghadam et al. [45] who developed a methodology, also using deionized water for the detection of desipramine, escitalopram, and imipramine with a extraction technique of air agitated-emulsification microextraction based on a low density-deep eutectic solvent (AA-EME-LD-DES) and analysis by high performance liquid chromatography with ultraviolet detection (HPLC-UV). After validating this method, they applied it to human plasma samples where they were able to achieve recoveries between 88.75% and 95.12% for desipramine, 90.27% and 93.46% for escitalopram, and 94.88% and 95.74% for imipramine. This new version of low-density solved based emulsification, first used in 2018, was introduced for highly effective enrichment of three antidepressant drugs. Due to its specificities, choline chloride was easily synthesized using a safe and easy alternative that does not need extra purification phases. Given this, the authors concluded about the advantages of this version of the technique which proved to be simple, safer, fast, efficient, and low cost. In addition, it is viable for compounds analysis in the interval

between therapeutic and potentially toxic in plasma matrix [45].

Furthermore, LLE technique has gained interest in what concerns miniaturized techniques and in recent years it has been reported in several papers where the classical approach was not used but instead, a miniaturized version of the technique was utilized. Fernández et al. [46] in 2016 developed an ultrasound assisted-dispersive liquid-liquid microextraction (UA-DLLME) method for the simultaneous determination of six antidepressants (mirtazapine, venlafaxine, escitalopram, fluvoxamine, fluoxetine, and sertraline) by ultra-performance liquid chromatography with photodiode array detector using 0.5 mL of plasma samples. This extraction technique used acetonitrile as dispersant and chloroform as extracting solvents. They obtained LOD values of 4 ng/mL for mirtazapine, venlafaxine, and sertraline and 5 ng/mL for escitalopram, fluvoxamine, and fluoxetine. They also obtained LOQ values of 12 ng/mL for mirtazapine, 13 ng/mL for venlafaxine and sertraline and 17 ng/mL for escitalopram, fluvoxamine, and fluoxetine with recoveries in the range from 93% to 110%. UA-DLLME presents an advance in the field of eco-friendly analytical chemistry since it is sensitive, very fast, simple, of low cost, and reliable requiring a considerable small volume of sample and extraction solvent. On the other hand, this microextraction method is not adequate for unstable analytes, emulsions are easily formed, extraction time is long and the equilibrium is incomplete leading to poor repeatability in this method [46,47]. Hamedi and Hadjmohammadi [30] developed, in 2016, a methodology based on alcohol-assisted dispersive liquid-liquid microextraction (AA-DLLME) for preconcentration and determination of fluoxetine in human plasma and urine samples, followed by reverse-phase high performance liquid chromatography with ultraviolet detection. The conditions included 1-octanol as extraction solvent and methanol as disperser solvent. For plasma samples, LOD and LOQ of fluoxetine were 3 and 10 ng/mL, respectively with a recovery of 90.15%. In the case of urine samples, LOD and LOQ values were 4.2 and 10 ng/mL, respectively, with a recovery around 89%. This technique of extraction showed to be better for the environment having less toxicity over dispersive liquid-liquid microextraction. Moreover, it is also well known for its simpler extraction, for the small solvent volume needed, being fast, easily operated, reliable and precise [30,48]. The prefix "AA" in this technique represents the disperser agent (alcohol). Nevertheless, it shares the same advantages and disadvantages than the DLLME technique. The positive aspects are the short period of extraction and also the good cost-effective value, while the disadvantages are

the restriction in the selection of extraction solvents, low extraction efficiency, difficulty of automation and the large consumption of disperser solvent [30,47].

Similarly to LLE, since 2014 it has become possible to observe evolution in the SPE technique concerning its application in miniaturized methods. For example, in 2015, Asgharinezhad et al. [49] developed a study using dispersive-micro solid-phase extraction (D-µ-SPE) for the isolation and preconcentration of two antidepressants (citalopram and sertraline) onto the surface of Fe_3O_4@ polypyrrole nanocomposite (Fe_3O_4@PPy NPs) with $NaClO_4$ sorbent in plasma and urine samples using HPLC-UV analysis. They obtained a LOD of 0.6 and 1.0 ng/mL for citalopram in plasma and urine samples, respectively, while for sertraline, the obtained LOD were 0.7 in plasma and 0.6 ng/mL in urine. For both compounds and biological samples, they obtained LOQ values of 2.0 ng/mL. The recoveries were in the range from 93% to 99%. Fe_3O_4@PPy NPs with core-shell structure featuring electrical and ferromagnetic characteristics was synthesized by an oxidative polymerization method. The authors concluded that with the enhancement of the stability of the NPs and their dispersibility in aqueous media and because of new interactions, namely hydrogen bonding, hydrophobic and π-π interactions amid sorbent and target analytes, the coating of NPs with PPy can enhance the sorption ability of the target analytes. They also concluded that this method demonstrated good results and higher extraction efficiency than Fe_3O_4 NPs which they had previously developed. The authors claimed advantages such as the short time of extraction, low sorbent and organic solvent consumption, high efficiency, low cost and ease of application when compared to SPE [49].

In 2014, Banitaba et al. [50] applied a fiber coating based on electrochemically reduced graphene oxide for the cold-fiber headspace solid-phase microextraction (HS-CF-SPME) of antidepressants (amitriptyline, trimipramine, and clomipramine) in diluted plasma samples and GC-FID analysis. They obtained a LOQ of 1.0 ng/mL for amitriptyline, 1.47 ng/mL for clomipramine and 1.77 ng/mL for trimipramine while recoveries of 96%, 73%, and 80%, respectively, were obtained. SPME presents advantages such as simplicity, speed, low cost of analysis, automation, selectivity, sensibility combined with the nonuse of organic solvents when gas chromatography is used. The reuse of fibers is also possible which is advantageous when compared to SPE. On the other hand, SPME usually presents low recovery values. When this extraction method is performed using the headspace approach, it presents good selectivity and longer fiber lifetime since the matrix is not in direct contact with the

coating, providing cleaner extracts. However, efficiency could be lower when compared to the direct immersion (DI) SPME method. CF-SPME was introduced with the aim of enhancing HS concentration as well as the distribution coefficient. In this technique, while the sample matrix is heated, enhancing the mass-transfer rate of analytes, the fiber coating is being cooled resulting in a distribution coefficient improvement. Further results showed the considerable advance in extraction efficiency with cooling even attained exhaustive extraction in some cases [51,52].

Over the years, there have been continuous developments in this area and a compilation of the studies carried out since 2017 to the present year was made for this review (Table 1). Only those papers that relied on the validation and determination of antidepressants in biological samples were selected. De Boeck et al. [53] developed, in 2018, a method capable of identifying a large number of antidepressants using an innovative extraction technique based on ionic liquid (IL) dispersive liquid-liquid microextraction (IL-DLLME) in which whole blood samples (1 mL) were extracted and analyzed by LC-MS/MS (ESI). They obtained LOD values between 0.78 and 35.15 ng/mL and recoveries from 53% to 133%. This technique takes advantage from the characteristics of ILs such as low vapour pressure at room temperature and lower toxicity when compared to conventional organic solvents. Nevertheless, the number of ILs and the number of possible variations of DLLME is high (e.g., involving the nature of the dispersive solvent, the absence of a dispersive solvent, the use or not of hydrophilic ILs, the use of surfactants, the way the droplet is removed, and the necessity or not of a cooling step, and the stirring mode), making method development and optimization very complicated [54].

Also for blood samples, in 2018, Ask et al. [35] developed a new extraction technique based on dried blood spots (DBS) with a previous step of clean-up by parallel artificial liquid membrane extraction (PALME) for the determination of amitriptyline. They used sample amounts as low as 5 µL of blood (up to 20 µL), detection by ultra-high performance liquid chromatography-tandem mass spectrometry (ESI) and obtained recoveries between 74% and 78% and a LOQ of 2.9 ng/mL. Using the same DBS extraction technique, followed by SPE, Moretti et al. [55] developed a methodology for the determination of 20 antidepressants in 85 µL post-mortem blood samples with chromatographic analysis by LC-MS/MS (ESI); the stability of the samples was evaluated for a period of three months. It should be noted that this new method allowed obtaining LOD values between 0.1 and 3.2 ng/mL for all compounds and permitted to quantify 9 of them. From the data provided by the authors, recoveries between 32.1% and 120.0%

were obtained and they concluded that DBS might represent a good complementary sample storage device in forensic investigations. Furthermore, the DBS technique presents other advantages; for instance, since it is a dried sample of blood, its transport and storage are particularly easy, it is stable at room temperature, as well as there is a reduced risk of being contagious for the professional that collects or manipulates this type of sample. Along with this, it requires minimum volume of biological sample. On the other hand, given its characteristics, if the sample is contaminated by the external environment, does not dry out sufficiently or if it is a sample with a reduced volume, the final concentration may be considerably affected [56]. Using PALME, DBS were processed allowing desorption, extraction, and high efficiency cleaning to occur simultaneously in 96-well plates [35].

In 2020, Behpour et al. [57] developed a methodology for the determination of desipramine and citalopram in serum and breast milk samples combining a gel electromembrane extraction (GEL-EME) method with the switchable hydrophilicity solvent-based homogeneous liquid-liquid microextraction (SHS-HLLME) method. With the analysis performed by GC-FID, they obtained LOD values of 0.7 and 0.3 ng/mL for desipramine and citalopram, respectively, and recoveries between 75.4% and 83.5%. GEL-EME is more advantageous when compared to EME, essentially because the membrane that composes it is prepared using an environmentally friendly process since it does not use toxic organic solvents. The combination of these two methods gives better results due to the low volume of organic solvent and the GEL-EME makes complex matrices cleaner. The authors concluded the work emphasizing the advantages of the GEL-EME/SHS-HLLME system since the injection of water in the GC is avoided with the use of organic solvent as extraction solvent in the SHS-HLLME.

Microfluidic systems were also used to determine antidepressants; in fact, Hedeshi et al. [58] have published recently their work concerning the use of modified paper extractive phases in a microfluidic device for the determination of some compounds in urine including a number of antidepressants (amitriptyline, trimipramine, and clomipramine). This approach presented excellent relative recoveries, ranging from 95% to 103%. Detailed information concerning the use of microfluidic systems, paper-based substrates, and gel electromembrane, as well as other microextraction techniques, for the determination of antidepressants are described in Table 1.

The most commonly used equipment for the analysis of antidepressants is HPLC-UV as applied both for urine and plasma samples. Fresco-Cala et al. (2018) [59]

Table 1: Bioanalytical procedures using microextraction techniques for the determination of antidepressants in biological samples published between 2017 and 2021

Compounds	Sample volume	Extraction procedure	Detection mode	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	Reference
Paroxetine Fluoxetine Sertraline Maprotiline	100 µL; plasma	SLE (96 well plates; dilution of the samples with 95 µL of water; addition of 1.5 mL of ethyl acetate)	LC-MS/MS (ESI)	-	0.10 (Paroxetine) 0.03 (Fluoxetine) 0.09 (Sertraline) 0.11 (Maprotiline)	0.34 (Paroxetine) 0.10 (Fluoxetine) 0.30 (Sertraline) 0.37 (Maprotiline)	[64]
Mirtazapine Doxepin Imipramine Amitriptyline	1 mL; serum	Dilution with water (1:6) and PT-µSPE (MF@PDA)	UHPLC-QTOF (ESI)	96.66-107.31 (Mirtazapine) 84.24-96.75 (Doxepin) 80.04-101.78 (Imipramine) 86.01-89.72 (Amitriptyline)	0.1 (Mirtazapine, Imipramine) 0.005 (Doxepin) 0.01 (Amitriptyline)	0.3 (Mirtazapine, Imipramine) 0.05 (Doxepin) 0.03 (Amitriptyline)	[65]
Fluoxetine Amitriptyline Nortriptyline Imipramine Desipramine Sertraline	250 µL; urine	Dilution with water (1:1) and µP-SHS-HLLME (N,N-dimethylcyclohexyla-mine: hydrochloric acid and sodium hydroxide)	GC-MS (EI)	96-98 (Fluoxetine) 88-101 (Amitriptyline) 88-94 (Nortriptyline) 68-96 (Imipramine) 85-102 (Desipramine) 86-97 (Sertraline)	0.88 (Fluoxetine) 0.07 (Amitriptyline) 0.05 (Nortriptyline) 0.40 (Imipramine) 0.03 (Desipramine) 0.02 (Sertraline)	2.92 (Fluoxetine) 0.23 (Amitriptyline) 0.18 (Nortriptyline) 1.32 (Imipramine) 0.09 (Desipramine) 0.05 (Sertraline)	[66]
Amitriptyline Doxepin Imipramine Nortriptyline	25 mL; urine	SUPRAS (1-decanol and tetrahydro-furan)	PS-MS	-	5.2 (Amitriptyline) 8.6 (Doxepin) 6.4 (Imipramine) 8.3 (Nortriptyline)	17.4 (Amitriptyline) 28.7 (Doxepin) 21.2 (Imipramine) 27.8 (Nortriptyline)	[67]
Amitriptyline Imipramine	100 µL; saliva, urine and plasma	Plasma was diluted with water (1:4) and On-disc EME-DLLME (methanol and tetrachloroethene)	GC-MS (EI)	62.5 (Amitriptyline) 56.4 (Imipramine) for saliva samples; 65.8 (Amitriptyline) 58.0 (Imipramine) for urine samples; 59.9 (Amitriptyline) 43.0 (Imipramine) for plasma samples	0.25 (Amitriptyline) 0.5 (Imipramine) for saliva samples; 0.5 (Amitriptyline, Imipramine) for urine samples; 0.5 (Amitriptyline, Imipramine) for plasma samples	0.5 (Amitriptyline) 1.0 (Imipramine) for saliva samples; 1.0 (Amitriptyline, Imipramine) for urine samples; 1.0 (Amitriptyline, Imi-pramine) for plasma samples	[68]
Desipramine Citalopram	7 mL; serum and breast milk	Serum and breast milk diluted with water (1:10) and (1:7), respectively; and GEL-EME with SHS-HLLME (dipro-pylamine)	GC-FID	75.4-80.5 (Desipramine) 77.1-83.5 (Citalopram)	0.7 (Desipramine) 0.3 (Citalopram)	5.0 (Desipramine) 2.5 (Citalopram)	[57]

(continued)

Table 1: *(continued)*

Compounds	Sample volume	Extraction procedure	Detection mode	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	Reference
Amitriptyline Desipramine Imipramine Nortriptyline Venlafaxine Citalopram Fluoxetine Paroxetine	200 µL; post-mortem blood and bone marrow	DI-SPME (LC Probe 45 µm C18-Silica fiber)	LC-TOF-MS (ESI)	-	2.98 (Amitriptyline) 9.49 (Desipramine) 3.08 (Imipramine) 4.93 (Nortriptyline) 5.46 (Venlafaxine) 9.98 (Citalopram) 5.80 (Fluoxetine) 5.34 (Paroxetine)	8.95 (Amitriptyline) 28.48 (Desipramine) 9.23 (Imipramine) 14.80 (Nortriptyline) 16.39 (Venlafaxine) 29.95 (Citalopram) 17.40 (Fluoxetine) 16.02 (Paroxetine)	[62]
Sertraline Norsertraline Fluoxetine Norfluoxetine Citalopram N-Desmethylcitalopram N,N-Desmethylcitalopram Vortioxetine	100 µL; blood and oral fluid	VAMS and MEPS (C$_2$)	HPLC-UV-FL	89-93 (Sertraline) 87-90 (Norsertraline) 90-94 (Fluoxetine) 87-91 (Norfluoxetine) 89-95 (Citalopram) 87-89 (N-Desmethylcitalopram) 86-90 (N,N-Desmethylcitalopram) 87-91 (Vortioxetine) for blood samples; 90-95 (Sertraline) 85-92 (Norsertraline) 91-96 (Fluoxetine) 88-91 (Norfluoxetine) 91-95 (Citalopram) 85-90 (N-Desmethylcitalopram) 85-91 (N,N-Desmethylcitalopram) 89-93 (Vortioxetine) for oral fluid samples	2.5 (Sertraline, Norsertraline) 3.0 (Fluoxetine, Norfluoxetine) 0.3 (Citalopram, N-Desmethylcitalopram, N,N-Desmethylcitalopram) 1.5 (Vortioxetine) for blood samples; 1.5 (Sertraline, Norsertraline) 2.5 (Fluoxetine, Norfluoxetine) 0.3 (Citalopram, N-Desmethylcitalopram, N,N-Desmethylcitalopram) 1.0 (Vortioxetine) for oral fluid samples	7.0 (Sertraline, Norsertraline) 10.0 (Fluoxetine, Norfluoxetine) 1.0 (Citalopram, N-Desmethylcitalopram, N,N-Desmethylcitalopram) 5.0 (Vortioxetine) for blood samples; 5.0 (Sertraline, Norsertraline) 7.0 (Fluoxetine, Norfluoxetine) 1.0 (Citalopram, N-Desmethylcitalopram, N,N-Desmethylcitalopram) 3.0 (Vortioxetine) for oral fluid samples	[69]
Amitriptyline Nortriptyline Doxepin	NA; plasma and urine	SPME (Polyoxomolybdate$_{368}$/polyaniline nanocomposite fiber)	HPLC-UV	92-96 (Amitriptyline) 91-94 (Nortriptyline) 95-97 (Doxepin) for plasma samples; 94-97 (Amitriptyline, Nortriptyline) 91-92 (Doxepin) for urine samples	0.1	0.5	[70]

(continued)

Table 1: (*continued*)

Compounds	Sample volume	Extraction procedure	Detection mode	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	Reference
Nortriptyline Fluoxetine	20 µL; plasma	Internal surface reversed-phase RAM synthesized onto a paper surface	PSI-MS	92.5-101.4 (Nortriptyline) 92.9-101.8 (Fluoxetine)	0.6 (Nortriptyline) 0.7 (Fluoxetine)	1.8 (Nortriptyline) 2.0 (Fluoxetine)	[71]
Amitriptyline Trimipramine Clomipramine	4 mL; urine	Microfluidic device with two pieces of PTES (phenyltrimethoxysilane)-modified papers	GC-MS (EI)	95-103.2 (Amitriptyline) 93-103.9 (Trimipramine) 95.3-98 (Clomipramine)	0.01 (Amitriptyline, Trimipramine) 0.005 (Clomipramine)	0.04 (Amitriptyline, Trimipramine) 0.02 (Clomipramine)	[58]
Amitriptyline Citalopram Desipramine N-Desmethyl-mirtazapine Desvenlafaxine Dibenzepin Dothiepin Fluoxetine Fluvoxamine Maprotiline Mianserin Mirtazapine Nortriptyline Paroxetine Protriptyline Reboxetine Sertraline Trazodone Trimipramine Venlafaxine	85 µL; post-mortem blood	DBS (Whatman 903TM cards) followed by SPE (Bond Elut Certify I cartridges - C8 and strong cation exchanger)	LC-MS/MS (ESI)	66.9-99.2 (Citalopram) 87.1-117.6 (N-Desmethyl-mirtazapine) 85.4-95.2 (Desvenlafaxine) 76.5-117.8 (Dibenzepin) 32.1-120.0 (Fluoxetine) 69.2-118.8 (Fluvoxamine) 77.0-116.7 (Mirtazapine) 59.8-112.4 (Trazodone) 87.3-118.7 (Venlafaxine) No data presented for the remaining compounds	0.6 (Amitriptyline, Maprotiline, Paroxetine) 2.3 (Citalopram) 0.9 (Desipramine) 1.4 (N-Desmethyl-mirtazapine) 3.2 (Desvenlafaxine) 0.3 (Dibenzepin) 1.8 (Dothiepin) 1.6 (Fluoxetine) 1.9 (Fluvoxamine) 1.0 (Mianserin) 2.2 (Mirtazapine) 0.5 (Nortriptyline, Sertraline, Trazodone) 2.0 (Protriptyline) 1.1 (Reboxetine) 0.1 (Trimipramine, Venlafaxine)	5.0 (Citalopram, N-Desmethyl-mirtazapine, Desvenlafaxine, Dibenzepin, Fluoxetine, Fluvoxamine, Trazodone, Trimipramine, Venlafaxine) 10.0 (Mirtazapine) The remaining compounds were not quantified	[55]
Amitriptyline Nortriptyline	2.5 mL; urine	Dilution with water (1:2) and EA-DM-µSPE ($Fe_3O_4@SiO_2@N_3$)	HPLC-UV	85-86 (Amitriptyline) 80-81.2 (Nortriptyline)	0.05 (Amitriptyline) 0.03 (Nortriptyline)	0.10 (Amitriptyline) 0.07 (Nortriptyline)	[72]

(*continued*)

Table 1: *(continued)*

Compounds	Sample volume	Extraction procedure	Detection mode	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	Reference
Venlafaxine Paroxetine Fluoxetine Amitriptyline Clomipramine	50 µL; serum	FPSE (sol-gel PCL-PDMS-PCL coated polyester membrane)	HPLC-DAD	5.6 (Venlafaxine) 23.8 (Paroxetine) 27.6 (Fluoxetine) 35.8 (Amitriptyline) 45.6 (Clomipramine)	150	500	[73]
Venlafaxine Paroxetine Fluoxetine Amitriptyline Clomipramine	500 µL; urine	FPSE (sol-gel Graphene coated)	HPLC-DAD	25.5 (Venlafaxine) 33.9 (Paroxetine) 67.0 (Fluoxetine) 43.0 (Amitriptyline) 29.0 (Clomipramine)	150	500	[74]
Amitriptyline Imipramine Desipramine Maprotiline Sertraline Doxepin	13 mL of urine and 10 mL of plasma	Dilution with water (1:2) and SUPRAS-ME (octanol and tetrahydrofuran)	GC-MS (EI)	58.7 (Amitriptyline) 57.5 (Imipramine) 48.1 (Desipramine) 46.2 (Maprotiline) 53.7 (Sertraline) 61.2 (Doxepin)	0.005 (Amitriptyline) 0.020 (Imipramine, Desipramine, Maprotiline) 0.030 (Sertraline) 0.003 (Doxepin)	0.01 (Amitriptyline and Doxepin) 0.05 (Imipramine, Desipramine, Maprotiline and Sertraline)	[75]
Mirtazapine Venlafaxine Escitalopram Fluoxetine Fluvoxamine Sertraline	300 µL; urine	MEPS (C8-SCX)	UPLC-PDA	92-107 (Mirtazapine) 80-102 (Venlafaxine) 92-112 (Escitalopram) 98-126 (Fluoxetine) 95-115 (Fluvoxamine) 93-122 (Sertraline)	0.7 (Mirtazapine) 4.6 (Venlafaxine) 1.8 (Escitalopram) 0.5 (Fluoxetine) 3.8 (Fluvoxamine) 7.0 (Sertraline)	2.1 (Mirtazapine) 13.8 (Venlafaxine) 5.4 (Escitalopram) 1.5 (Fluoxetine) 11.4 (Fluvoxamine) 21.0 (Sertraline)	[76]
Amitriptyline Nortriptyline Desipramine Clomipramine	NA; urine	SFO-DLPME (sodium sulfate)	GC-MS	89 (Amitriptyline) 74 (Nortriptyline) 76 (Desipramine) 81 (Clomipramine)	0.016 (Amitriptyline) 0.025 (Nortriptyline) 0.013 (Desipramine) 0.017 (Clomipramine)	0.025 (Amitriptyline) 0.041 (Nortriptyline) 0.024 (Desipramine) 0.027 (Clomipramine)	[77]
Amitriptyline Nortriptyline Quetiapine Venlafaxine O-desmethyl-venlafaxine Fluoxetine	125 µL; plasma	PALME (96-well plates)	LC-MS/MS (ESI)	71-73 (Amitriptyline) 72-78 (Nortriptyline) 64-65 (Quetiapine) 82-89 (Venlafaxine) 47-49 (O-desmethyl-venlafaxine) 64-73 (Fluoxetine)	-	-	[78]

(continued)

Table 1: *(continued)*

Compounds	Sample volume	Extraction procedure	Detection mode	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	Reference
Duloxetine Venlafaxine Atomoxetine	10 mL; urine	UAMDSPME (MrGOQDs-PD@ Ni)	HPLC-PDA	94.7-96.2 (Duloxetine) 95.3-95.7 (Venlafaxine) 93.3-94.0 (Atomoxetine)	1.0 (Duloxetine) 0.7 (Venlafaxine) 1.1 (Atomoxetine)	2.9 (Duloxetine) 2.3 (Venlafaxine) 3.4 (Atomoxetine)	[79]
Amitriptyline Desipramine Mianserine Trimipramine	600 µL; urine	microSPE (MWCNTs incorporated polymer monolith)	HPLC-UV	83-85 (Amitriptyline) 72-108 (Desipramine) 74-86 (Mianserine) 77-93 (Trimipramine)	15 (Amitriptyline, Trimipramine) 9 (Desipramine) 13 (Mianserine)	30 (Amitriptyline) 14 (Desipramine) 25 (Mianserine) 29 (Trimipramine)	[59]
Clomipramine Imipramine	6 mL of sample solution; urine	Dilution with water (1:5) and EME-EA-LLME	GC-FID	45.3-51.2 (Clomipramine) 61.9-67.8 (Imipramine)	0.15	0.5	[39]
Agomelatine Amitriptyline Bupropion Clomipramine Dosulepine Doxepin Duloxetine Escitalopram Fluoxetine Fluvoxamine Imipramine Maprotiline Mianserin Mirtazapine Nortriptyline Paroxetine Reboxetine Trazodone Venlafaxine	1 mL; blood	IL-DLLME (1-butyl-3-methylimidazolium hexafluorophosphate)	LC-MS/MS (ESI)	96.52-98.33 (Agomelatine) 113.60-120.92 (Amitriptyline) 73.08-77.60 (Bupropion) 90.35-105.24 (Clomipramine) 110.26-115.50 (Dosulepine) 131.95-132.98 (Doxepin) 82.40-104.93 (Duloxetine) 128.98-125.41 (Escitalopram) 94.16-96.95 (Fluoxetine) 53.11-61.66 (Fluvoxamine) 116.47-119.12 (Imipramine) 95.54-107.34 (Maprotiline) 115.59-121.67 (Mianserin) 119.85-123.80 (Mirtazapine) 93.12-106.74 (Nortriptyline) 90.99-103.54 (Paroxetine) 89.77-96.11 (Reboxetine) 108.68-111.98 (Trazodone) 56.21-66.69 (Venlafaxine)	1.10 (Agomelatine) 1.54 (Amitriptyline) 1.63 (Bupropion) 1.41 (Clomipramine) 0.99 (Dosulepine) 1.26 (Doxepin) 1.04 (Duloxetine, Maprotiline) 0.78 (Escitalopram) 1.55 (Fluoxetine) 2.14 (Fluvoxamine) 1.08 (Imipramine) 1.38 (Mianserin) 141 (Mirtazapine) 1.68 (Nortriptyline) 1.74 (Paroxetine) 1.26 (Reboxetine) 35.15 (Trazodone) 0.80 (Venlafaxine)	250 (Trazodone) 10 (for all the other compounds)	[53]

(continued)

Table 1: *(continued)*

Compounds	Sample volume	Extraction procedure	Detection mode	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	Reference
Amitriptyline	20 µL; blood	DBS (Whatman® FTA® DMPK-C cards), Protein precipitation with trichloroacetic acid and PALME (96-well plates)	UHPLC-MS/MS (ESI)	74.4-77.9	0.9	2.9	[35]
Amitriptyline Doxepin	500 µL; plasma	Protein precipitation with methanol and ADLLM-LDS (toluene)	GC-FID	55 (Amitriptyline) 74 (Doxepin)	2	20	[34]
Amitriptyline Clomipramine Desipramine Imipramine Nortriptyline	2.5 mL of urine and 1 mL of plasma	Dilution of the plasma at a ratio of 1:4 and dilution of the urine at a ratio of 1:1 with an ammoniacal buffer and DSPE-DES-AALLME (C_{18})	GC-MS (EI)	72 (Amitriptyline, Nortriptyline) 74 (Clomipramine) 62 (Desipramine) 67 (Imipramine) for urine samples; 70 (Amitriptyline) 72 (Clomipramine) 64 (Desipramine) 68 (Imipramine) 65 (Nortriptyline) for plasma samples	0.008 (Amitriptyline) 0.014 (Clomipramine) 0.009 (Desipramine) 0.015 (Imipramine) 0.011 (Nortriptyline) for urine samples; 0.032 (Amitriptyline) 0.056 (Clomipramine) 0.036 (Desipramine) 0.060 (Imipramine) 0.044 (Nortriptyline) for plasma samples	0.027 (Amitriptyline) 0.046 (Clomipramine) 0.030 (Desipramine) 0.049 (Imipramine) 0.036 (Nortriptyline) for urine samples; 0.108 (Amitriptyline) 0.184 (Clomipramine) 0.120 (Desipramine) 0.191 (Imipramine) 0.144 (Nortriptyline) for plasma samples	[42]
Amitriptyline Clomipramine Desipramine Imipramine Nortriptyline	2 mL; urine	SI-HLLE (ammoniacal buffer and acetonitrile), DSPE (PSA, GCB, C_{18}), and DLLME-SFO (menthol and acetonitrile)	HPLC-UV	84 (Amitriptyline) 81 (Clomipramine) 75 (Desipramine) 69 (Imipramine) 76 (Nortriptyline)	0.22 (Amitriptyline) 0.29 (Clomipramine) 0.26 (Desipramine) 0.31 (Imipramine) 0.24 (Nortriptyline)	0.71 (Amitriptyline) 0.93 (Clomipramine) 0.84 (Desipramine) 1.1 (Imipramine) 0.79 (Nortriptyline)	[61]
Amitriptyline Desipramine Mianserin Trimipramine	1.2 mL; urine	Dilution with water (1:1) and SWCNHs (Thin film microextraction)	UPLC-DAD and direct infusion MS (ESI)	70-130	0.1 (Amitriptyline, Desipramine, Mianserin) 0.2 (Trimipramine)	10	[40]
Nortriptyline	6-8 µL; blood and urine	HF-DDSME	GC-MS (EI)	97.33-98.00 for blood samples 97.66-99.00 for urine samples	21 for blood samples 9 for urine samples	-	[80]
Amitriptyline Clomipramine Imipramine	1 mL; plasma	Protein precipitation with acetonitrile and LLLE in combination with DLLME (n-hexane and acetonitrile)	GC-FID	98 (Amitriptyline) 79 (Clomipramine) 93 (Imipramine)	1 (Amitriptyline) 3 (Clomipramine) 2 (Imipramine)	3 (Amitriptyline) 11 (Clomipramine) 6 (Imipramine)	[36]

(continued)

Table 1: (continued)

Compounds	Sample volume	Extraction procedure	Detection mode	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	Reference
Fluoxetine	0.6 mL; serum and plasma	Protein precipitation with acetonitrile and SPE with MIP sorbent	UV-Vis spectrophotometer	100-108 for serum samples 100-103 for plasma samples	6.56×10^{-9} M	1×10^{-8} M	[37]
Agomelatine Bupropion Citalopram Fluoxetine Mirtazapine Paroxetine Trazodone	One drop; blood	PS-DVB-coated glass blood spot	CLC-MS (API-ES)	90.9-94.1 (Agomelatine) 88.8-91.2 (Bupropion) 92.3-95 (Citalopram) 95.1-103 (Fluoxetine) 106-110 (Mirtazapine) 104-107 (Paroxetine) 89.7-105 (Trazodone)	33 (Agomelatine) 32 (Bupropion) 19 (Citalopram) 34 (Fluoxetine) 38 (Mirtazapine) 18 (Paroxetine, Trazodone)	96 (Agomelatine) 95 (Bupropion) 61 (Citalopram) 99 (Fluoxetine) 100 (Mirtazapine) 52 (Paroxetine) 53 (Trazodone)	[81]
Amitriptyline Imipramine	1 mL; plasma	Protein precipitation of the plasma with methanol, dilution with water at a ratio of 1:3 and MSPE (Magnetic framework composites (Fe3O4@TMU-10))	HPLC-UV	57.33 (Amitriptyline) 60.00 (Imipramine)	4	8	[82]
Agomelatine Citalopram Fluoxetine Fluvoxamine Maprotiline Melitracen Mirtazapine Moclobemide N-Desmethyl-mirtazapine Norfluoxetine Paroxetine Sertraline	500 µL; blood	UA-LDS-DLLME (toluene)	GC-MS (EI)	54.9-65.4 (Agomelatine) 69.9-79.6 (Citalopram) 67.1-84.2 (Fluoxetine) 88.8-94.2 (Fluvoxamine) 76.1-95.4 (Maprotiline) 43.0-48.4 (Melitracen) 73.3-99.4 (Mirtazapine) 30.3-34.6 (Moclobemide) 62.9-85.6 (N-Desmethylmirtazapine) 70.7-79.6 (Norfluoxetine) 59.9-80.7 (Paroxetine) 51.4-56.6 (Sertraline)	-	5 (Agomelatine, Mirtazapine, Moclobemide, N-Desmethylmirtazapine, Paroxetine, Sertraline) 15 (Citalopram, Fluoxetine, Fluvoxamine, Maprotiline, Melitracen, Norfluoxetine)	[83]
Citalopram Fluoxetine Sertraline	500 µL; urine	PMME (POM incorporated)	HPLC-UV	98.3-104.7 (Citalopram) 82.8-95.7 (Fluoxetine) 91.7-103.4 (Sertraline)	0.7-1.4	2.7 (Citalopram) 4.7 (Fluoxetine) 2.2 (Sertraline)	[60]
Fluoxetine Norfluoxetine Paroxetine	500 µL; plasma	Protein precipitation with acetonitrile and MEPS (C18)	LC-FLD	58.9-65.2 (Fluoxetine) 58.7-66.9 (Norfluoxetine) 70.4-77.3 (Paroxetine)	5 (Fluoxetine, Norfluoxetine) 1 (Paroxetine)	20 (Fluoxetine, Norfluoxetine) 5 (Paroxetine)	[84]

(continued)

Table 1: (continued)

Compounds	Sample volume	Extraction procedure	Detection mode	Recovery (%)	LOD (ng/mL)	LOQ (ng/mL)	Reference
Clomipramine	10 mL; plasma	Protein precipitation with acetonitrile, diluted with deionized water (1:4) and UADM-SPE (Fe3O4@SiO2-NH2 magnetic nanoparticles)	HPLC-UV	90.6	5	16.7	[41]
Mirtazapine Paroxetine Citalopram Sertraline Imipramine Clomipramine Fluoxetine	200 µL; plasma	Dilution with phosphate buffer solution (200 µL, 5 mmol/mL, pH8.0) and DPX (RAM phase (C18-BSA))	LC-MS/MS (ESI)	-	-	0.5 (Mirtazapine, Citalopram) 1.0 (Paroxetine, Imipramine) 5.0 (Sertraline) 10.0 (Clomipramine, Fluoxetine)	[85]

µP-SHS-HLLME: "µ-pipette" combined with switchable hydrophilicity solvent homogeneous liquid–liquid microextraction; **ADLM-LDS:** Air-dispersed liquid–liquid microextraction using low-density solvent; **API-ES:** atmospheric pressure ionization source electrospray; **CLC-MS:** Capillary liquid chromatography-mass spectrometry; **DBS:** Dried blood spots; **DI-SPME:** Direct immersion solid-phase microextraction; **DLLME-SFO:** Dispersive liquid–liquid microextraction based on the solidification of floating organic droplet; **DPX:** Disposable pipette extraction; **DSPE:** Dispersive solid-phase extraction; **DSPE-DES-AALLME:** Dispersive solid-phase extraction–deep eutectic solvent-based air-assisted liquid-liquid microextraction; **EA-DM-µSPE:** Effervescent salt-assisted dispersive magnetic micro solid-phase extraction; **EI:** Electron ionization; **EME-EA-LME:** Electromembrane extraction combined with electro-assisted liquid-liquid microextraction; **ESI:** Electrospray ionization; **FPSE:** Fabric phase sorptive extraction; **GC-FID:** Gas chromatography-flame ionization detector; **GC-MS:** Gas chromatography-mass spectrometry; **GEL-EME:** Gel electromembrane extraction; **HF-DDSME:** Hollow-fiber drop-to-drop solvent microextraction; **HPLC-DAD:** High performance liquid chromatography-diode array detector; **HPLC-PDA:** High performance liquid chromatography-photodiode array detector; **HPLC-UV:** High performance liquid chromatography-ultraviolet detection; **HPLC-UV-FL:** Liquid chromatography with sequential spectrophotometric and spectrofluorimetric detection; **IL-DLLME:** Ionic liquid dispersive liquid-liquid microextraction; **LC-FLD:** Liquid chromatography-fluorescence detector; **LC-MS/MS:** Liquid chromatography-tandem mass spectrometry; **LC-TOF-MS:** Liquid chromatography time-of-flight mass spectrometry; **LLE-DLLME:** Liquid-liquid-liquid extraction-dispersive liquid-liquid microextraction; **LOD:** Limit of detection; **LOQ:** Limit of quantitation; **MEPS:** Microextraction by packed sorbent; **microSPE:** micro Solid-phase extraction; **MIP-SPE:** Molecularly imprinted polymer-solid-phase extraction; **MSPE:** Magnetic solid-phase extraction; **MWCNTs:** Single- and multi-walled carbon nanotubes; **NA:** Not available; **On-disc EME-DLLME:** On-disc electromembrane extraction-dispersive liquid-liquid microextraction; **PALME:** Parallel artificial liquid membrane extraction; **PMME:** Polymer monolith microextraction; **PS-DVB-coated blood spot:** poly(styrene-co-divinylbenzene)-coated glass blood spot; **PSI-MS:** Paper spray ionization mass spectrometry; **POM:** Polyoxometalate; **PS-MS:** Paper spray ionization mass spectrometry; **PT-µSPE:** Pipette-tip micro-solid phase extraction; **RAM phase:** Restricted access material phase; **SFO-DLPME:** Solidification of floating organic droplets-dispersive liquid phase microextraction; **SHS-HLLME:** Switchable hydrophilicity solvent-based homogeneous liquid-liquid microextraction; **SI-HLLE:** Salt induced-homogeneous liquid–liquid extraction; **SLE:** Solid-phase supported liquid–liquid extraction; **SPE:** Solid-phase extraction; **SPME:** Solid-phase microextraction; **SUPRAS:** Supramolecular solvent-based microextraction; **SUPRAS-ME:** Supramolecular microextraction; **SWCNHs:** Single-walled carbon nanohorns; **UADM-SPE:** Ultrasound-assisted dispersive magnetic-solid-phase extraction; **UA-LDS-DLLME:** Ultrasound-assisted low-density solvent dispersive liquid–liquid microextraction; **UAMDSPME:** Ultrasonic assisted magnetic dispersive solid phase microextraction; **UHPLC-MS/MS:** Ultra-high performance liquid chromatography-tandem mass spectrometry; **UHPLC-QTOF:** Ultra-high-performance liquid chromatography–quadrupole–time-of-flight mass spectrometry; **UPLC-DAD:** Ultra-high performance liquid chromatography-diode array detector; **UPLC-PDA:** Ultra-high performance liquid chromatography-photodiode array detector; **UV-Vis spectrophotometer:** Ultraviolet-visible spectrophotometer; **VAMS:** Volumetric absorptive microsampling.

In the absence of the LOQ value, the lowest point of the calibration curve was considered.

developed a new micro solid-phase extraction technique (microSPE) with incorporation of carbon nanotubes for the determination of four antidepressants in urine samples reporting LOQ values between 14 and 30 ng/mL and recoveries between 72% and 108%. The authors concluded that due to the ease of retention resulting from the additional π interactions, carbon nanotubes in the monolith improve the sensitivity to the antidepressants making this method simple and economical. Cai et al. [60] developed, in 2017, a polymer monolith microextraction technique (PMME) (with polyoxometalate) for the determination of three compounds in urine samples achieving lower LOQ values, between 2.2 and 4.7 ng/mL, and recoveries ranging from 83% to 105%. The authors reinforce economic, solvent-saving, ease of operation and convenience, as well as ease of preparation characteristics of this technique of microextraction. In addition, Mohebbi et al. (2018) [61] for the determination of five antidepressants in urine samples used a dispersive solid-phase extraction technique (DSPE) and classify this technique as a clean-up method based on SPE. However, the sorbent is added directly to the extract without any conditioning or pretreatment when compared to SPE. Hamidi et al. (2017) [41] also developed a new technique of dispersive solid-phase extraction (ultrasound assisted dispersive magnetic solid-phase extraction (UADM-SPE)). They obtained recoveries between 69 and 84%, and 90%, respectively, with some of the same analyzed compounds. UADM-SPE advantages go from its cost-effective relation, ease of operation and short extraction period to the low consumption of toxic organic solvent and short time of analysis.

But more recently, analytical techniques such as mass spectrometry and tandem mass spectrometry coupled to gas and liquid chromatography have been increasingly implemented in the determination of antidepressants in biological samples, due to the specificity and sensitivity due to unambiguous molecular weight separation. In addition, powerful high-resolution analysis techniques associated with mass spectrometry can be used which can improve described advantages mentioned above by improving analyte resolution. An example of this is the work developed by Majda et al. in 2020 [62] who developed a methodology for the determination of 8 antidepressants (amitriptyline, desipramine, imipramine, nortriptyline, venlafaxine, citalopram, fluoxetine, and paroxetine) in 200 μL samples of post-mortem blood and bone marrow with extraction by direct immersion solid-phase microextraction (DI-SPME) and chromatographic analysis by liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS). The authors achieved LOD values between

2.98 and 9.98 ng/mL and LOQ values between 8.95 and 29.95 ng/mL for complex biological matrices. DI extraction commands higher efficiency of the analytes in study, even with low volatility, since the fiber is in direct contact with the sample. This results in a reduction of fiber lifetime due to increased detrition. Thus, contamination probability rises along with carry-over effect during extractions. For these reasons, DI should be avoided in the analysis of complex matrices but rather be used in cleaner samples [51,63].

Figure 3 summarizes microextraction approaches for sample preparation for the extraction of antidepressants.

4 Detection of antidepressants

The increase in the prescription and the consumption of antidepressants has led to the need of developing analytical methods for the detection and quantification of this class of medicines in a wide range of matrices. Among the methods published in scientific journals, the most described are gas chromatography (GC) coupled to mass spectrometry (GC-MS) [19,42,66,68,75,77,80,83] and flame ionization detector (GC-FID) [34,36,39,57], high performance liquid chromatography (HPLC) coupled to ultraviolet detector (HPLC-UV) [41,59-61,70,72] and liquid chromatography (LC) coupled to tandem mass spectrometry (LC-MS/MS) [25,31,53,55], or ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) [33,35]. Mass spectrometric detection provides better sensitivity and specificity allowing separating co-eluting compounds and using deuterated analogues as internal standards. In the case of GC-MS, particularly for the detection of metabolites, a derivatization step is often deemed necessary prior to chromatography, which usually makes the procedures more time consuming and laborious [19]. Taking into account the chemical structure of these compounds (namely secondary amines), the main derivatizing agents used are N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and trimethyl chlorosilane (TMCS). Agents such as trifluoroacetyl and heptafluorobutyryl can also be used; however, these agents are relatively unstable and damage capillary GC columns. Another problem usually associated with GC analysis for antidepressants is their poor fragmentation pattern since usually the main ions have a low m/z (an example of this is fragment 58, which is common to many of these drugs). This poor fragmentation means that the other ions have to be carefully chosen for qualification
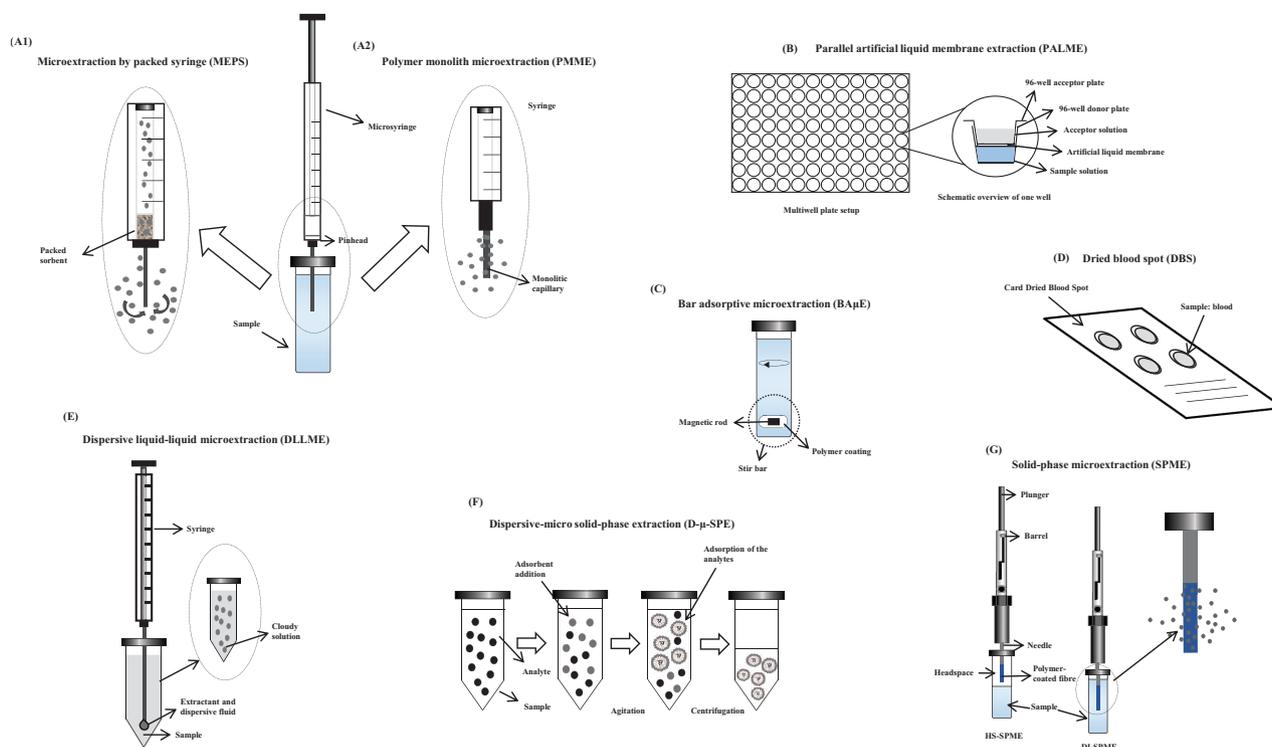
Figure 3: Microextraction approaches for sample preparation: (a1) microextraction by packed syringe (MEPS) and (a2) polymer monolith microextraction (PMME); (b) parallel artificial liquid membrane extraction (PALME); (c) bar adsorptive microextraction (BAµE); (d) dried blood spot (DBS); (e) dispersive liquid-liquid microextraction (DLLME); (f) dispersive-micro solid-phase extraction (D-µ-SPE); (g) solid-phase microextraction (SPME).

(positivity criteria). Rosado et al. [19] evaluated, in 2017, the contribution between different ions in a multi-method that allowed the determination of 15 antidepressants and metabolites by GC/MS. In order to solve this problem, different mixtures of antidepressant drugs were used.

Most of these drawbacks were overcome with the implementation of liquid chromatographic-mass spectrometric procedures allowing the detection of metabolites at very low concentrations. In addition, no derivatization is needed using this type of instrumentation decreasing analysis time in general [31,35]. In most applications, the compounds are ionized in the positive electrospray ionization (ESI+) mode of the mass-spectrometer despite of being less prone to ion suppression phenomena than the way less used atmospheric pressure chemical ionization (APCI). The influence of matrix effects must be carefully evaluated during method validation since it is capable of impairing sensitivity, precision, and accuracy. Compounds usually associated to ion suppression include carbohydrates, salts, lipids, highly polar compounds, and even metabolites of the analytes being tested.

The mobile phases were always a binary or ternary combination of some of the following reagents: methanol, acetonitrile, acetate or phosphate buffer, and water.

Meanwhile, ~ 1% of various additives (formic acid, trifluoroacetic acid, triethylamine, phosphoric acid, ammonium acetate, and ammonium formate, etc.) were often added to solvent systems to improve peak shape. However, as it also occurs in GC/MS, different classes of antidepressants have very similar masses and as such comparable transitions may be expected. Therefore, with an open window of alternatives both to targeted and non-targeted analysis, it is advantageous to use highly sensitive and resolution techniques, such as LC-TOF-MS as published by Majda et al. [62] in 2020 and UHPLC-QTOF as published by He et al. [65] or an analytical technique using HRMS (ORBITRAP analysers). Indeed, these instruments are considered a better choice as they present high sensitivity, selectivity and provide also accurate mass measurements. Due to its high efficiency in generating and registering a relevant quantity of information, this type of instrumental techniques offers a new perspective in chemical analysis. Furthermore, these techniques have the flexibility to expand the panel of analytes, decrease the volume of sample used, detect trace concentrations, and allow also a better understanding of metabolism patterns [86,87] due to the possibility of accurate mass measurements.

5 Conclusion and future perspectives

Antidepressant drugs are widely used and increasingly prescribed by health professionals as a common practice for the treatment of several pathologies. The overuse of these medications increases the importance of developing methods to monitor their concentrations in patients. It is essential that laboratories provide responses concerning the determination of these compounds in biological samples. Several extraction procedures have been applied to antidepressants, such as LLE and SPE, but also their miniaturized versions. The trend towards the development of new methods of identification and quantification follows an idea of using smaller amounts of biological samples, lower volumes of organic solvents, with reusable materials and less waste, resulting in fast, simple, and efficient techniques. It should be noted that this must be combined with robust analyzes where high resolution techniques have been gaining more and more interest allowing maximum specificity and sensitivity, making possible the identification of the analytes even if they are present at low concentrations. It is also of great interest to automate the extraction and/or analysis procedures. The main objective will be the improvement of patients' lives and improving the medical condition of each individual by a better management of their situation or by improving the treatment of other patients by compiling numerous data and support public health authorities.

Author contributions: Sofia Soares: conceptualization, investigation, writing – original draft; Mário Barroso: conceptualization, methodology, writing – review and editing; Eugenia Gallardo: conceptualization, methodology, project administration, supervision, writing – review and editing.

Conflicts of interest: Authors state no conflict of interest.

References

[1] World Health Organization. New WHO guidelines to improve the physical health of people with severe mental disorders [Internet]. World Health Organization; 2018. Available from: https://www.who.int/mental_health/en/

[2] Spielmans GI, Berman MI, Linardatos E, Rosenlicht NZ, Perry A, Tsai AC. Adjunctive atypical antipsychotic treatment for major depressive disorder: a meta-analysis of depression, quality of life, and safety outcomes. PLoS Med. 2013;10(3):e1001403.

[3] Berm EJJ, Paardekooper J, Brummel-Mulder E, Hak E, Wilffert B, Maring JG. A simple dried blood spot method for therapeutic drug monitoring of the tricyclic antidepressants amitriptyline, nortriptyline, imipramine, clomipramine, and their active metabolites using LC-MS/MS. Talanta. 2015;134:165-72.

[4] Hillhouse TM, Porter JH. A brief history of the development of antidepressant drugs: from monoamines to glutamate. Exp Clin Psychopharmacol. 2015;23(1):1-21.

[5] Preskorn SH. Drug Development in Psychiatry: The Long and Winding Road from Chance Discovery to Rational Development. In: Springer, editor. Handbook of experimental pharmacology. Heidelberg, Berlin; 2018. 1-18.

[6] Heck E, MacQueen G. Noradrenergic and specific serotonergic antidepressants. In: Future Medicine, editor. Antidepressants and Major Depressive Disorder. London, UK; 2012. p. 68-80.

[7] Faquih AE, Memon RI, Hafeez H, Zeshan M, Naveed S. A Review of Novel Antidepressants: A Guide for Clinicians. Cureus. 2019;11(3):e4185.

[8] van Westrhenen R, Aitchison KJ, Ingelman-Sundberg M, Jukić MM. Pharmacogenomics of Antidepressant and Antipsychotic Treatment: How Far Have We Got and Where Are We Going? Front Psychiatry. 2020;11:1-11.

[9] Bleakley S. Review of the choice and use of antidepressant drugs. Prog Neurol Psychiatry. 2013;17(6):18-26.

[10] Food and Drug Administration. FDA approves new nasal spray medication for treatment-resistant depression; available only at a certified doctor's office or clinic [Internet]. 2019. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-new-nasal-spray-medication-treatment-resistant-depression-available-only-certified

[11] Vargas AS, Luís Â, Barroso M, Gallardo E, Pereira L. Psilocybin as a New Approach to Treat Depression and Anxiety in the Context of Life-Threatening Diseases—A Systematic Review and Meta-Analysis of Clinical Trials. Biomedicines. 2020;8(9):331.

[12] Moreno AMJ, Navas MJ, Asuero AG. HPLC-DAD determination of CNS-acting drugs in human blood, plasma, and serum. Crit Rev Anal Chem. 2014;44(1):68-106.

[13] Juan H, Zhiling Z, Huande L. Simultaneous determination of fluoxetine, citalopram, paroxetine, venlafaxine in plasma by high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-MS/ESI). J Chromatogr B. 2005;820(1):33-9.

[14] Haji EO, Hiemke C, Pfuhlmann B. Therapeutic Drug Monitoring for Antidepressant Drug Treatment. Curr Pharm Des. 2012;18(36):5818-27.

[15] Wille SMR, Cooreman SG, Neels HM, Lambert WEE. Relevant issues in the monitoring and the toxicology of antidepressants. Crit Rev Clin Lab Sci. 2008;45(1):25-89.

[16] Shinozuka T, Terada M, Tanaka E. Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. Forensic Sci Int. 2006;162(1-3):108-12.

[17] Ansermot N, Brawand-Amey M, Kottelat A, Eap CB. Fast quantification of ten psychotropic drugs and metabolites in human plasma by ultra-high performance liquid chromatography tandem mass spectrometry for therapeutic drug monitoring. J Chromatogr A. 2013;1292:160-72.

[18] Uřinovská R, Brozmanová H, Sištík P, Silhán P, Kacířová I, Lemr K, et al. Liquid chromatography-tandem mass spectrometry method for determination of five antidepressants and four atypical antipsychotics and their main metabolites in human serum. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;907:101-7.

[19] Rosado T, Gonçalves A, Martinho A, Alves G, Duarte AP, Domingues F, et al. Simultaneous Quantification of Antidepressants and Metabolites in Urine and Plasma Samples by GC-MS for Therapeutic Drug Monitoring. Chromatographia. 2017;80:301-28.

[20] Fisichella M, Morini L, Sempio C, Groppi A. Validation of a multi-analyte LC-MS/MS method for screening and quantification of 87 psychoactive drugs and their metabolites in hair. Anal Bioanal Chem. 2014;406(14):3497-506.

[21] Desrosiers NA, Huestis MA. Oral Fluid Drug Testing: Analytical Approaches, Issues and Interpretation of Results. J Anal Toxicol. 2019;43(6):415-43.

[22] Papoutsis I, Khraiwesh A, Nikolaou P, Pistos C, Spiliopoulou C, Athanaselis S. A fully validated method for the simultaneous determination of 11 antidepressant drugs in whole blood by gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2012;70:557-62.

[23] Sempio C, Morini L, Vignali C, Groppi A. Simple and sensitive screening and quantitative determination of 88 psychoactive drugs and their metabolites in blood through LC-MS/MS: Application on postmortem samples. J Chromatogr B. 2014;970:1-7.

[24] de Castro A, Concheiro M, Quintela O, Cruz A, López-Rivadulla M. LC-MS/MS method for the determination of nine antidepressants and some of their main metabolites in oral fluid and plasma. Study of correlation between venlafaxine concentrations in both matrices. J Pharm Biomed Anal. 2008;48(1):183-93.

[25] Shin SS, Borg D, Stripp R. Developing and Validating a Fast and Accurate Method to Quantify 18 Antidepressants in Oral Fluid Samples Using SPE and LC-MS-MS. J Anal Toxicol. 2020;00:1-8.

[26] Clement H, Preiskorn J, Studer S, Ebert K, Maurice E, Böckmann J, et al. Oral fluid as an alternative matrix for Therapeutic Drug Monitoring. In: Pharmacopsychiatry. New York: Georg Thieme Verlag KG; 2017. p. 213-27.

[27] Coulter C, Taruc M, Tuyay J, Moore C. Antidepressant drugs in oral fluid using liquid chromatography-tandem mass spectrometry. J Anal Toxicol. 2010;34(2):64-72.

[28] dos Santos MF, Yamada A, Seulin SC, Leyton V, Pasqualucci CAG, Muñoz DR, et al. Liquid-phase microextraction and gas chromatographic-mass spectrometric analysis of antidepressants in vitreous humor: Study of matrix effect of human and bovine vitreous and saline solution. J Anal Toxicol. 2016;40(3):187-93.

[29] Stiakakis I, Belivanis SD, Tzatzarakis MN, Fragoulis M, Tsatsakis AM. Disputed case of homicide by smothering due to severe amitriptyline intoxication of the victim. J Forensic Leg Med. 2009;16(5):280-3.

[30] Hamedi R, Hadjmohammadi MR. Optimization of alcohol-assisted dispersive liquid-liquid microextraction by experimental design for the rapid determination of fluoxetine in biological samples. J Sep Sci. 2016;39(24):4784-93.

[31] Degreef M, van Nuijs ALN, Maudens KE. Validation of a simple, fast liquid chromatography-tandem mass

spectrometry method for the simultaneous quantification of 40 antidepressant drugs or their metabolites in plasma. Clin Chim Acta. 2018;485:243-57.

[32] Kole PL, Venkatesh G, Kotecha J, Sheshala R. Recent advances in sample preparation techniques for effective bioanalytical methods. Biomed Chromatogr. 2011;25(1):199-217.

[33] Kall MA, Rohde M, Jørgensen M. Quantitative determination of the antidepressant vortioxetine and its major human metabolite in plasma. Bioanalysis. 2015;7(22):2881-94.

[34] Mofazzeli F, Asaadi Shirvan H, Mohammadi F. Extraction and determination of tricyclic antidepressants in real samples using air-dispersed liquid-liquid microextraction prior to gas chromatography and flame ionization detection. J Sep Sci. 2018;41(23):4340-7.

[35] Ask KS, Øiestad EL, Pedersen-Bjergaard S, Gjelstad A. Dried blood spots and parallel artificial liquid membrane extraction-A simple combination of microsampling and microextraction. Anal Chim Acta. 2018;1009:56-64.

[36] Farajzadeh MA, Abbaspour M. Development of new extraction method based on liquid-liquid-liquid extraction followed by dispersive liquid-liquid microextraction for extraction of three tricyclic antidepressants in plasma samples. Biomed Chromatogr. 2018;32(8):e4251.

[37] Nezhadali A, Motlagh MO, Sadeghzadeh S. Spectrophotometric determination of fluoxetine by molecularly imprinted polypyrrole and optimization by experimental design, artificial neural network and genetic algorithm. Spectrochim Acta A Mol Biomol Spectrosc. 2018;190:181-7.

[38] Hegstad S, Havnen H, Helland A, Falch BMH, Spigset O. Enantiomeric separation and quantification of citalopram in serum by ultra-high performance supercritical fluid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2017;1061-1062:103-9.

[39] Nojavan S, Shaghaghi H, Rahmani T, Shokri A, Nasiri-Aghdam M. Combination of electromembrane extraction and electro-assisted liquid-liquid microextraction: A tandem sample preparation method. J Chromatogr A. 2018;1563:20-7.

[40] Ríos-Gómez J, Fresco-Cala B, García-Valverde MT, Lucena R, Cárdenas S. Carbon Nanohorn Suprastructures on a Paper Support as a Sorptive Phase. Molecules. 2018;23(6):e1252.

[41] Hamidi F, Hadjmohammadi MR, Aghaie ABG. Ultrasound-assisted dispersive magnetic solid phase extraction based on amino-functionalized Fe3O4 adsorbent for recovery of clomipramine from human plasma and its determination by high performance liquid chromatography: Optimization by experimental design. J Chromatogr B Analyt Technol Biomed Life Sci. 2017;1063:18-24.

[42] Mohebbi A, Yaripour S, Farajzadeh MA, Afshar Mogaddam MR. Combination of dispersive solid phase extraction and deep eutectic solvent-based air-assisted liquid-liquid microextraction followed by gas chromatography-mass spectrometry as an efficient analytical method for the quantification of some tricyclic antidepressants drugs in biological fluids. J Chromatogr A. 2018;1571:84-93.

[43] Lee XP, Kumazawa T, Sato K, Suzuki O. Detection of Tricyclic Antidepressants in Whole Blood by Headspace Solid-Phase Microextraction and Capillary Gas Chromatography. J Chromatogr Sci. 1997;35(7):302-8.

[44] Ide AH, Nogueira JMF. New-generation bar adsorptive microextraction (BAμE) devices for a better eco-user-friendly

analytical approach-Application for the determination of antidepressant pharmaceuticals in biological fluids. J Pharm Biomed Anal. 2018;153:126-34.

[45] Moghadam AG, Rajabi M, Asghari A. Efficient and relatively safe emulsification microextraction using a deep eutectic solvent for influential enrichment of trace main antidepressant drugs from complicated samples. J Chromatogr B Analyt Technol Biomed Life Sci. 2018;1072:50-9.

[46] Fernández P, Taboada V, Regenjo M, Morales L, Alvarez I, Carro AM, et al. Optimization of ultrasound assisted dispersive liquid-liquid microextraction of six antidepressants in human plasma using experimental design. J Pharm Biomed Anal. 2016;124:189-97.

[47] Sereshti H, Khorram P, Nouri N. Recent trends in replacement of disperser solvent in dispersive liquid-liquid microextraction methods. Sep Purif Rev. 2019;48(2):159-78.

[48] Rahmani M, Ghasemi E, Sasani M. Application of response surface methodology for air assisted-dispersive liquid- liquid microextraction of deoxynivalenol in rice samples prior to HPLC-DAD analysis and comparison with solid phase extraction cleanup. Talanta. 2017;165:27-32.

[49] Asgharinezhad AA, Karami S, Ebrahimzadeh H, Shekari N, Jalilian N. Polypyrrole/magnetic nanoparticles composite as an efficient sorbent for dispersive micro-solid-phase extraction of antidepressant drugs from biological fluids. Int J Pharm. 2015;494(1):102-12.

[50] Banitaba MH, Davarani SSH, Ahmar H, Movahed SK. Application of a new fiber coating based on electrochemically reduced graphene oxide for the cold-fiber headspace solid-phase microextraction of tricyclic antidepressants. J Sep Sci. 2014;37(9-10):1162-9.

[51] Jalili V, Barkhordari A, Ghiasvand A. A comprehensive look at solid-phase microextraction technique: A review of reviews. Microchem J. 2020;152:104319.

[52] Souza-Silva ÉA, Jiang R, Rodríguez-Lafuente A, Gionfriddo E, Pawliszyn J. A critical review of the state of the art of solid-phase microextraction of complex matrices I. Environmental analysis. TrAC - Trends Anal Chem. 2015;71:224-35.

[53] De Boeck M, Dubrulle L, Dehaen W, Tytgat J, Cuypers E. Fast and easy extraction of antidepressants from whole blood using ionic liquids as extraction solvent. Talanta. 2018;180:292-9.

[54] Trujillo-Rodríguez MJ, Rocío-Bautista P, Pino V, Afonso AM. Ionic liquids in dispersive liquid-liquid microextraction. TrAC - Trends Anal Chem. 2013;51:87-106.

[55] Moretti M, Freni F, Valentini B, Vignali C, Groppi A, Visonà SD, et al. Determination of antidepressants and antipsychotics in dried blood spots (DBSs) collected from post-mortem samples and evaluation of the stability over a three-month period. Molecules. 2019;24(20):pii: E3636.

[56] Min KL, Ryu JY, Chang MJ. Development and clinical applications of the dried blood spot method for therapeutic drug monitoring of anti-epileptic drugs. Basic Clin Pharmacol Toxicol. 2019;125(3):215-36.

[57] Behpour M, Nojavan S, Asadi S, Shokri A. Combination of gel-electromembrane extraction with switchable hydrophilicity solvent-based homogeneous liquid-liquid microextraction followed by gas chromatography for the extraction and determination of antidepressants in human serum, breast milk and wastewater. J Chromatogr A. 2020;1621:461041.

[58] Hashemi Hedeshi M, Rezvani O, Bagheri H. Silane-based modified papers and their extractive phase roles in a microfluidic platform. Anal Chim Acta. 2020;1128:31-41.

[59] Fresco-Cala B, Mompó-Roselló Ó, Simó-Alfonso EF, Cárdenas S, Herrero-Martínez JM. Carbon nanotube-modified monolithic polymethacrylate pipette tips for (micro)solid-phase extraction of antidepressants from urine samples. Mikrochim Acta. 2018;185(2):127.

[60] Cai J, Zhu G-T, He X-M, Zhang Z, Wang R-Q, Feng Y-Q. Polyoxometalate incorporated polymer monolith microextraction for highly selective extraction of antidepressants in undiluted urine. Talanta. 2017;170:252-9.

[61] Mohebbi A, Farajzadeh MA, Yaripour S, Afshar Mogaddam MR. Determination of tricyclic antidepressants in human urine samples by the three-step sample pretreatment followed by HPLC-UV analysis: an efficient analytical method for further pharmacokinetic and forensic studies. EXCLI J. 2018;17:952-63.

[62] Majda A, Mrochem K, Wietecha-Posłuszny R, Zapotoczny S, Zawadzki M. Fast and efficient analyses of the post-mortem human blood and bone marrow using DI-SPME/ LC-TOFMS method for forensic medicine purposes. Talanta. 2020;209:120533.

[63] Zhang L, Gionfriddo E, Acquaro V, Pawliszyn J. Direct immersion solid-phase microextraction analysis of multi-class contaminants in edible seaweeds by gas chromatography-mass spectrometry. Anal Chim Acta. 2018;1031:83-97.

[64] Zheng M, Zhang C, Wang L, Wang K, Kang W, Lian K, et al. Determination of nine mental drugs in human plasma using solid-phase supported liquid-liquid extraction and HPLC-MS/ MS. Microchem J. 2021;160:105647.

[65] He X, Sun T, Wang L, Jiang X. Pipette-tip micro-solid phase extraction based on melamine-foam@polydopamine followed by ultra-high-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry for detection of psychotropic drugs in human serum. J Chromatogr B. 2021;1163:122499.

[66] Luiz Oenning A, Birk L, Eller S, Franco de Oliveira T, Merib J, Carasek E. A green and low-cost method employing switchable hydrophilicity solvent for the simultaneous determination of antidepressants in human urine by gas chromatography - mass spectrometry detection. J Chromatogr B Anal Technol Biomed Life Sci. 2020;1143:122069.

[67] Oliveira FM de, Scheel GL, Augusti R, Tarley CRT, Nascentes CC. Supramolecular microextraction combined with paper spray ionization mass spectrometry for sensitive determination of tricyclic antidepressants in urine. Anal Chim Acta. 2020;1106:52-60.

[68] Karami M, Yamini Y. On-disc electromembrane extraction-dispersive liquid-liquid microextraction: A fast and effective method for extraction and determination of ionic target analytes from complex biofluids by GC/MS. Anal Chim Acta. 2020;1105:95-104.

[69] Marasca C, Protti M, Mandrioli R, Atti AR, Armirotti A, Cavalli A, et al. Whole blood and oral fluid microsampling for the monitoring of patients under treatment with antidepressant drugs. J Pharm Biomed Anal. 2020;188:113384.

[70] Amoli HS, Yamini Y, Darmani H. Polyoxomolybdate 368 / polyaniline nanocomposite as a novel fiber for solid-phase microextraction of antidepressant drugs in biological samples. J Sep Sci. 2020;43(13):2636-45.

[71] Fernandes AR, Bernardo RA, Sousa JCP, Georg R de C, Vaz BG, Chaves AR. Restrict access material for paper spray ionization mass spectrometry: A versatile tool for catecholamines and antidepressants determination in plasma samples. Microchem J. 2020;158:105245.

[72] Fahimirad B, Rajabi M, Elhampour A. A rapid and simple extraction of anti-depressant drugs by effervescent salt-assisted dispersive magnetic micro solid-phase extraction method using new adsorbent Fe3O4@SiO2@N3. Anal Chim Acta. 2019;1047:275-84.

[73] Zilfidou E, Kabir A, Furton KG, Samanidou V. An improved fabric phase sorptive extraction method for the determination of five selected antidepressant drug residues in human blood serum prior to high performance liquid chromatography with diode array detection. J Chromatogr B Anal Technol Biomed Life Sci. 2019;1125:121720.

[74] Lioupi A, Kabir A, Furton KG, Samanidou V. Fabric phase sorptive extraction for the isolation of five common antidepressants from human urine prior to HPLC-DAD analysis. J Chromatogr B Anal Technol Biomed Life Sci. 2019;1118-1119:171-9.

[75] Salamat Q, Yamini Y, Moradi M, Farahani A, Feizi N. Extraction of antidepressant drugs in biological samples using alkanol-based nano structured supramolecular solvent microextraction followed by gas chromatography with mass spectrometric analysis. J Sep Sci. 2019;42(8):1620-8.

[76] Fuentes AMA, Fernández P, Fernández AM, Carro AM, Lorenzo RA. Microextraction by packed sorbent followed by ultra high performance liquid chromatography for the fast extraction and determination of six antidepressants in urine. J Sep Sci. 2019;42(11):2053-61.

[77] Mohebbi A, Farajzadeh MA, Nemati M, Sarhangi N, Afshar Mogaddam MR. Development of green sodium sulfate-induced solidification of floating organic droplets-dispersive liquid phase microextraction method: Application to extraction of four antidepressants. Biomed Chromatogr. 2019;33(11):e4642.

[78] Ask KS, Lid M, Øiestad EL, Pedersen-Bjergaard S, Gjelstad A. Liquid-phase microextraction in 96-well plates - calibration and accurate quantification of pharmaceuticals in human plasma samples. J Chromatogr A. 2019;1602:117-23.

[79] Ghorbani M, Chamsaz M, Aghamohammadhasan M, Shams A. Ultrasonic assisted magnetic dispersive solid phase microextraction for pre concentration of serotonin-norepineph-rine reuptake inhibitor drugs. Anal Biochem. 2018;551:7-18.

[80] Jagtap PK, Tapadia K. Pharmacokinetic determination and analysis of nortriptyline based on GC-MS coupled with hollow-fiber drop-to-drop solvent microextraction technique. Bioanalysis. 2018;10(3):143-52.

[81] Murtada K, de Andrés F, Ríos Á, Zougagh M. A simple poly(styrene-co-divinylbenzene)-coated glass blood spot method for monitoring of seven antidepressants using capillary liquid chromatography-mass spectrometry. Talanta. 2018;188:772-8.

[82] Safari M, Shahlaei M, Yamini Y, Shakorian M, Arkan E. Magnetic framework composite as sorbent for magnetic solid phase extraction coupled with high performance liquid chromatography for simultaneous extraction and determination of tricyclic antidepressants. Anal Chim Acta. 2018;1034:204-13.

[83] Chen X, Zheng S, Le J, Qian Z, Zhang R, Hong Z, et al. Ultrasound-assisted low-density solvent dispersive liquid-liquid microextraction for the simultaneous determination of 12 new antidepressants and 2 antipsychotics in whole blood by gas chromatography-mass spectrometry. J Pharm Biomed Anal J Pharm Biomed. 2017;142:19-27.

[84] Magalhães P, Alves G, Llerena A, Falcão A. Therapeutic Drug Monitoring of Fluoxetine, Norfluoxetine and Paroxetine: A New Tool Based on Microextraction by Packed Sorbent Coupled to Liquid Chromatography. J Anal Toxicol. 2017;41(7):631-8.

[85] Pinto MAL, de Souza ID, Queiroz MEC. Determination of drugs in plasma samples by disposable pipette extraction with C18-BSA phase and liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal. 2017;139:116-24.

[86] Reichl B, Himmelsbach M, Emhofer L, Klampfl CW, Buchberger W. Uptake and metabolism of the antidepressants sertraline, clomipramine, and trazodone in a garden cress (Lepidium sativum) model. Electrophoresis. 2018;39(9-10):1301-8.

[87] Peterman SM, Duczak N, Kalgutkar AS, Lame ME, Soglia JR. Application of a linear ion trap/orbitrap mass spectrometer in metabolite characterization studies: Examination of the human liver microsomal metabolism of the non-tricyclic anti-depressant nefazodone using data-dependent accurate mass measurements. J Am Soc Mass Spectrom. 2006;17(3):363-75.