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Review

Modern Approaches to Preparation of Body Fluids for Determination of Bioactive Compounds

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Abstract: The current clinical and forensic toxicological analysis of body fluids requires a modern approach to sample preparation characterized by high selectivity and enrichment capability, suitability for micro-samples, simplicity and speed, and the possibility of automation and miniaturization, as well as the use of small amounts of reagents, especially toxic solvents. Most of the abovementioned features may be realized using so-called microextraction techniques which cover liquid-phase techniques (e.g., single-drop microextraction, SDME; dispersive liquid–liquid microextraction, DLLME; hollow-fiber liquid-phase microextraction, HF-LPME) and solid-phase extraction techniques (solid-phase microextraction, SPME; microextraction in packed syringes, MEPS; disposable pipette tip extraction, DPX; stir bar sorption extraction, SBSE). Some other extraction methodologies like dispersive solid-phase extraction (d-SPE) or magnetic solid-phase extraction (MSPE) can also be easily miniaturized. This review briefly describes and characterizes the abovementioned extraction methods, and then presents their current applications to the preparation of body fluids analyzed for bioactive compounds in combination with appropriate analytical methods, mainly chromatographic and related techniques. The perspectives of the analytical area we are interested in are also indicated.

Keywords: microextraction techniques; body fluids; bioactive compounds; clinical and forensic analysis

1. Introduction

Body fluids belong to the most often analyzed samples in clinical investigations, and in related fields such as forensic toxicology. Plasma/serum, whole blood, urine, and saliva constitute biological materials of special interest.

Due to complexity of the matrix, body fluids are a challenge for those who are interested in bioanalysis. In most cases of such analyses, the selection of an appropriate sample preparation method is required because of main factors such as the chemical nature of the analyte and type of sample matrix, the analytical interferences of the target compounds with their metabolites and/or with constituents of the matrix, the low concentration levels of analytes, which are not detectable by analytical instruments, and the contamination and subsequent shortening of life of the used instrument or its accessories (e.g., due to interaction of matrix constituents with sorbents of chromatographic columns).

At present, conventional extractions techniques, i.e., liquid–liquid extraction (LLE) and solid-phase extraction (SPE), are still extensively applied to the analysis of body fluids for determination of bioactive compounds [1]. Although they demonstrate many advantageous features, they also possess some drawbacks, e.g., time consumption, large consumption of toxic organic solvents and possible formation of emulsion (LLE), or the requirement of relatively expensive extraction columns and multi-step processes (SPEs) [1]. Therefore, the development of alternative sample preparation methods is desired.

The development of novel sample preparation approaches is strongly stimulated by the following requirements of modern clinical or forensic analysis: (a) high selectivity and enrichment capability, (b) simplicity and rapidity, (c) suitability for micro-amount biological samples, (d) automation, and (e) miniaturization [2]. The other key factor affecting the dynamic development of extraction methods is the introduction of new materials, which may be used as potential sorbents in bioanalysis. There are MOFs (metal–organic frameworks), [3] ionic liquids [4], fabric phases [5], graphene oxide tablets [6], cork [7], bract [8], or coated papers [9].

This review focuses on modern approaches to the preparation of body fluid samples. The principles of microextraction techniques are briefly described and then exemplified using selected applications for the preparation of body fluids analyzed for medicines, drugs of abuse, and other bioactive compounds.

2. Modern and Novel Sample Preparation Techniques

There are several sample treatment methodologies that may be applied to the preparation of body fluids analyzed for bioactive compounds. The selection of the below sample preparation approaches was mainly based on the consideration of efficient and cost-effective miniaturized techniques, so-called microextraction methods. Generally, microextraction methods may be divided into two groups: Solid-phase extractions and Liquid-phase extractions.

2.1. Solid-Phase Extractions

2.1.1. Solid-Phase Microextraction and Derived Techniques

A solvent-free solid-phase microextraction (SPME) was proposed by Arthur and Pawliszyn in 1990 [10], and then, in 1993, Supelco introduced the first commercial version of an SPME device [11]. The SPME technique, like conventional SPE, involves the partitioning of the target compound between an organic phase coating a fiber (usually made of fused silica) and the sample matrix. This extraction methodology may be conducted into two ways: directly by placing a small-diameter fiber coated with a stationary phase film in an aqueous sample or in headspace mode, by placing the fiber on the headspace of the sample. The adsorbed/absorbed analytes are then thermally desorbed from the stationary phase in the injector of a gas chromatograph. However, a new trend in developing faster analytical procedures involving SPME, i.e., direct coupling of this extraction approach with mass spectrometry, was noted [12]. Although, the SPME technique is known to have some drawbacks (fragile coating layers, degradation of the fibers with multi-use, carryover problems, batch-to-batch variations of fiber coatings, and relatively high cost), currently, it is dynamically developing, mainly through the introduction of new SPME fiber materials and selective coatings [2].

The SPME approach may be also performed "in-tube", where an open tubular fused-silica capillary column is exploited as the SPME device instead of an SPME fiber [13]. A comparison of fiber and in-tube SPME techniques in combination with liquid chromatography was also provided [13]. In-tube SPME is superior to fiber SPME mainly due to the shorter equilibrium time and better suitability for automation, as well as a wider diversity of stationary phases coating commercial capillary columns.

Thin-film microextraction (TFME) constitutes another geometry for SPME, which was proposed by Jiang and Pawliszyn [14]. In TFME, a sheet of flat film, employed as the extraction phase, is reinforced by an extra support such as a stainless-steel rod, stainless-steel mesh, or blade-shaped substrate [15]. Due to the large surface area-to-volume ratio, TFME is superior to SPME in terms of adsorption capability and extraction rate. This technique, in combination with novel biocompatible matrix membranes, may be especially useful in the medicine field as a non-invasive diagnostic tool in the analysis of breath, skin, and saliva.

2.1.2. Microextraction in Packed Syringe

Microextraction in a packed syringe (MEPS) is a relatively new technique because it was first applied in a fully automated procedure by Abdel-Rehim in 2004 [16] to extract local anesthetics from

human plasma. The MEPS technique is a miniaturized version of conventional solid-phase extraction (SPE), which can be connected online to GC or LC, without modification [16]. In MEPS, 1 mg of the sorbent material is usually placed into a syringe (100–250 μL) as a plug. A sample is drawn through the syringe (e.g., by an autosampler) before passing through the solid material where analytes are adsorbed. The sorbent is washed to remove the biological matrix, and then the analytes are eluted with an appropriate organic solvent (e.g., directly into the instrument's injector). In comparison with conventional SPE, the main advantages of MEPS are congruence for full automation (which minimizes the sample preparation time to a minute), as well as the possibility to reuse a packed syringe (more than 100 times with body fluid samples), whereas an SPE column usually can only be used once. Compared with SPME, the MEPS technique is more powerful for the preparation of biological samples with complex matrices. Moreover, much higher extraction yields can be obtained (60–90%) compared to standard SPME (1–10%), and smaller sample volumes can be handled (10 μL) compared to SPME (>1000 μL) [16].

2.1.3. Disposable Pipette Extraction

Disposable pipette extraction (DPX, TIPS, PT-SPE) is a miniaturization of conventional SPE, which was developed by Brewer [17]. In DPX, the sorbent is placed in a pipette tip and it is mixed well with the sample. This reduces the sorption material needed to retain analytes, and leads to faster and more efficient extraction process [18]. Similarly to conventional SPE or MEPS, a four-step extraction process takes place: (1) conditioning for activation of the sorbent sites, (2) aspiration of the sample, (3) removing sample matrix interferences, and (4) elution of analytes. In the steps 1, 3, and 4, an appropriate solvent (or solvents) is aspirated with air and removed one or more times. After conditioning (step 1), the sample is suctioned with air (step 2) for mixing with the adsorbent. The mixing/contact time must be controlled to reach a dynamic equilibrium during the interaction of analytes with the sorbent, and then the sample is removed from the tip. DPX is a simple and fast sample preparation approach, which minimizes the consumption of the sample and organic solvents. Recently, some miniaturized concepts for chromatographic analysis involving a pipette tip and spin column were reviewed [19]. However, until now, their application to routine analysis is limited mainly due to the small number of commercially available extracting materials and the higher cost compared to traditional SPE columns [20].

2.1.4. Dispersive Micro-Solid-Phase Extraction and Magnetic Solid-Phase Extraction

Dispersive micro-solid-phase extraction (D-μ-SPE) is a miniaturized dispersive solid-phase extraction (d-SPE) consisting of the dispersion of micro- or nanosorbents in the sample solution, followed by separation of the solid sorbent from the extracted analytes by centrifugation and filtration [21]. Among the advantageous features in favor of the D-μ-SPE technique in relation to conventional SPE are rapidity (due to the dispersion phenomenon which increases the sorbent surface for interaction with analyte molecules), possibility to use a large spectrum of sorbents (which are able to disperse in the sample solution), simplicity, and reducing costs in terms of the used sorbent amounts and laboratory equipment. One of the d-SPE variants is magnetic solid-phase extraction (MSPE), where magnetic nanoparticles (MNPs) are used as sorbents in the sample preparation process. MNPs attract the consideration of many analytical chemists because they considerably simplify and accelerate the extraction process due to their capacity to be easily isolated from the sample solution through an external magnet [2]. Regarding the trend of MNPs to form agglomerates and the loss of magnetism due to their chemical activity, these materials are modified into core–shell composites by coating them with an organic (e.g., surfactant) or an inorganic (e.g., graphene or carbon nanotubes) layer. The magnetic composites created, which are used as sorbents, may also have a higher extraction ability than simple magnetic nanoparticles (e.g., Fe_3O_4) when samples with a complex matrix are analyzed. A recent general review of D-μ-SPE [22] focused mainly on the dispersion strategies and sorbents used, and also indicated several trends in the near future for the development of this extraction technique.

2.1.5. Stir Bar Sorption Extraction

Stir bar sorption extraction (SBSE) was introduced by Baltussen with his co-workers in 1999 [23]. In this approach, a stir bar (magnetic element) is coated with a sorbent and immersed in a sample solution. The sample is stirred with appropriate speed for a specified time to reach equilibrium. After adsorption of the analyte on the sorption material, the magnetic element is transferred to a small amount of a selected organic solvent to desorb the analyte into it. Although SBSE is theoretically similar to SPME, its capacity is greater than SPME. This results from the fact that more sorbent mass is usually present in SBSE than in SPME and more analyte is transferred to the sorbent in SBSE. Currently, polydimethylsiloxane (PDME) is mainly employed as the sorbent coating, and the SBSE technique is not used very often. However, the current trend is to employ this technique in combination with many novel materials, including restricted access materials, carbon adsorbents, molecularly imprinted polymers, ionic liquids, microporous monoliths, sol–gel prepared coatings, and dual-phase materials [24]. The main advances in various "extraction/stirring integrated techniques", including the source SBSE technique, emphasizing their analytical potential, were also previously reviewed and compared [25].

2.2. Liquid-Phase Extractions

2.2.1. Single-Drop Microextraction

The term "liquid-phase microextraction (LPME)" means that the extraction technique consists of a microsyringe playing two roles: a funnel for extraction and a syringe for injection into a GC port. Single-drop microextraction (SDME), the simplest mode of LPME, was introduced by Liu and Dasgupta [26], as well as by Jeannot and Cantwell in 1996 [27]. In the SDME technique, analytes are isolated from an aqueous sample (being stirred) into a small (ca. one μL) drop of a water-immiscible organic solvent hanging on the needle of a microsyringe [28]. After the extraction process, the drop is withdrawn into the syringe and usually injected directly into a GC instrument. The SDME technique may be realized via three ways: through direct immersion, in the headspace mode, and as three-phase SDME. In contrast to the direct immersion mode, where the extracting solvent drop is submerged into the aqueous sample, in the headspace mode, the drop hangs in the headspace of the sample. In three-phase SDME, the droplet contains two immiscible solvents, i.e., the polar acceptor solution and the polar donor solution. These two solvents are separated by a non-polar solvent. The major problem of the SDME technique is drop instability, but attempts are being made to solve this issue via the creation of new devices for SDME. Current trends in developments in SDME, such as the use of non-conventional solvents, novel materials (for combining sorbent and liquid-phase extractions), and the creation of more suitable SDME devices, as well as their automation and implementation in microfluidic chip technologies, were presented in a recent review paper [29].

2.2.2. Dispersive Liquid–Liquid Microextraction

Dispersive liquid–liquid microextraction (DLLME) was proposed by Rezaee and co-workers in 2006 [30]. This technique is based on a ternary-component solvent system consisting of an extraction solvent, a dispenser, and an aqueous sample. In DLLME, a mixture of extraction and dispenser solvents is rapidly injected into an aqueous sample via a syringe. The extraction solvent is dispersed into the aqueous phase and forms a cloudy solution of fine droplets, which interact with an analyte. The centrifugation allows the separation of two phases, whereby the sediment phase is enriched with the analyte. The extraction solvent must be a high-density water-immiscible solvent, whereas the dispenser solvent must be a polar water-miscible one. Carbon tetrachloride, tetrachloroethylene, chlorobenzene, and ionic liquids may be used as the extraction solvent, while acetone, methanol, ethanol, acetonitrile, or tetrahydrofuran may play the role of dispensers. One of the more interesting varieties of the DLLME technique involves the solidification of a floating organic drop (DLLME-SFO) [31]. In DLLME-SFO, dodecanol or 2-dodecanol are usually used as extraction solvents. After centrifugation, the floating

organic phase is solidified quickly by cooling (e.g., in an ice bath). The solidified extraction solvent with the isolated analytes is separated, melted at room temperature, and then subjected to analysis.

2.2.3. Hollow-Fiber Liquid-Phase Microextraction

The idea of a supported liquid membrane (SLM) was for first time integrated with single-use extraction units for liquid–liquid–liquid microextraction (LLLME) by Pedersen-Bjergaard and his co-workers in 1999 [32]. The authors employed a polypropylene hollow fiber as the membrane for extraction of the model compound (methamphetamine) from body fluids. In SLM, an organic solvent (e.g., 1-octanol) is usually impregnated in the small pores of a hollow fiber, which protects the extracting solvent, thus permitting extraction only on the surface of the solvent immobilized in the membrane pores. Hollow-fiber liquid-phase microextraction (HF-LPME), as a mode of liquid-phase microextraction, was firstly published by Shen and Lee in 2002 [33]. To impregnate the pores of the fiber wall with an appropriate solvent, the needle tip inserted into the hollow fiber is immersed in an organic solvent for several minutes. In order to remove the excess organic solvent from the inside of the fiber, water is injected to flush it before being removed from the solvent. For the extraction, the prepared fiber is immersed in an aqueous sample while the organic solvent in the syringe is injected completely into the hollow fiber. During the extraction, the solution is agitated using a magnetic stirrer. After the extraction process, the solvent with the isolated analyte is withdrawn into the syringe and subsequently injected into a GC instrument. The disadvantages of HF-LPME include the relatively low repeatability of the extraction process due to the air bubbles forming on the hollow-fiber surface (reduction of transport speed), and the formation of a membrane barrier between the sample and the acceptor solvent (usually an organic solvent), reducing the rate of extraction [30]. Electromembrane extraction (EME) is an extended concept of HF-LPME, which was introduced in 2006 [34]. In the EME approach, a charged analyte is extracted from a sample solution through the SLM into an acceptor solution, where its transfer is facilitated by an external electrical field applied across the SLM [35]. A review article concerning the EME technique, covering its principles, new SLMs, support materials for the SLM, new sample additives, and novel technical configurations, as well as the applications of EME in pharmaceutical analysis, was published [35].

3. Applications

Data concerning exemplary applications of solid-phase extractions and liquid-phase extractions for clinical and forensic analysis are gathered in Tables 1 and 2, respectively.

Table 1. Methods of sample preparation by solid-phase extraction.

Name of Analytes	Material	Extraction Efficiency (%)	Analytical Method	Precision (RSD %)	LOD/LOQ	Reference
Solid-Phase Microextraction (SPME) and Derived Techniques						
Methadone	Plasma Urine	95–97	GC–FID	5 5.3	0.035/0.10 µg/L 0.035/0.10 µg/L	[35]
Beta-blockers (acebutolol, atenolol, fenoterol, nadolol, pindolol, procaterol, sotalol, timolol)	Plasma Urine	98–115 85–119	LC–MS/MS	1.0–17.4 0.8–20.7	0.018–0.29/0.09–1.15 ng/mL 0.018–0.31/0.06–1.04 ng/mL	[36]
Chemotherapeutics (amoxicillin, cefatoxime, ciprofloxacin, daptomycin, fluconazole, gentamicin, clindamycin, linezolid, metronidazole, moxifloxacin)	Whole blood	65–83	LC–MS/MS	0.11–3.19	0.126–0.198/0.392–0.652 ng/mL	[37]
Testosterone cortisol dehydroepiandrosterone	Saliva	99.5–101.9 102.4–105.5 94.0–99.9	LC–MS/MS	4.9–9.5	- [a]/0.01 ng/mL - [a]/0.03 ng/mL - [a]/0.28 ng/mL	[38]
Estron 17 β-estradiol 17 µ-ethinylestradiol Estrol	Urine	75–103 87–101 71–92 80–91	HPLC–FLD [b]	3–19 1–9 2–186 2–9	3.03/10 µg/L 0.03/0.1 µg/L 0.15/0.5 µg/L 0.1/1 µg/L	[39]
Microextraction in Packed Syringe (MEPS)						
Immunosuppressive drugs (cyclosporine, everolimus, sirolimus, tacrolimus)	Whole blood	102–103 103–109 102–108 103–106	LC–MS/MS	4.2–5.1 3.5–13.7 3.6–8.9 2.0–9.1	0.9/3.0 ng/mL 0.15/0.5 ng/mL 0.15/0.5 ng/mL 0.15/0.5 ng/mL	[40]
Ecgonine methyl ester, Benzoylecgonine, Cocaine, Cocaethylene	Urine	113–116 93–104 100–109 100–105	TOF–MS/DART [c]	- [a]	22.9/75.0 ng/mL 23.7/65.0 ng/mL 4.0/95.0 ng/mL 9.8/75.0 ng/mL	[41]
Linezolid Amoxicillin	Plasma	99.1–108.3 96.2–10.9	LC–MS/MS	1.66–6.83 0.24–3.26	0.1407/0.3814 ng/mL 0.1341–0.4249 ng/mL	[42]
Disposable Pipette Extraction (DPX, Other Acronyms: TIPS, PT-SPE)						
Carbamazepine	Urine	98.2–99.4	HPLC–UV	2.5	0.04/- [a] µg/L	[43]
Metoprolol Pindolol	Plasma	101–103 94–114	LC–MS/MS	8–15 9–11	- [a]/5 nM - [a]/5 nM	[44]
Busulfan Cyclophosamid	Whole blood	99–113 103–110	LC–MS/MS	13–16 7–12	- [a]/5 nM - [a]/10 nM	[45]

Table 1. *Cont.*

Name of Analytes	Material	Extraction Efficiency (%)	Analytical Method	Precision (RSD %)	LOD/LOQ	Reference
Dispersive Micro-Solid Phase Extraction (D-μ-SPE) and Magnetic Dispersive Extraction (MSPE)						
7-Aminoflunitrazepam	Whole blood (post-mortem)	72.7–106.8	GC–MS (PTV–LVI [d]–GC–MS)	5.1–17.0	- [a]/0.2 ng/L	[46]
Amitriptyline		108.1–139.7		1.92–15.7	- [a]/0.2 ng/L	
Carbamazepine		74.6–111.3		3.4–5.7	- [a]/0.2 ng/L	
Carbaryl		100.6–118.4		4.2–15.7	- [a]/0.3 ng/L	
Carbofuran		93.3–143.5		5.4–9.0	- [a]/0.2 ng/L	
Cocaine		103.2–116.3		0.94–4.45	- [a]/0.2 ng/L	
Diazepam		85.1–91.0		2.4–3.7	- [a]/0.2 ng/L	
Haloperidol		68.4–115.8		6.1–9.1	- [a]/0.3 ng/L	
MDMA		86.7–104.6		5.3–7.2	- [a]/0.3 ng/L	
Methiocarb		99.2–116.4		4.0–12.35	- [a]/0.3 ng/L	
Pirimicarb		107.3–119.8		2.6–17.1	- [a]/0.3 ng/L	
Terbufos		87.5–108.1		8.4–11.6	- [a]/0.2 ng/L	
Citalopram	Plasma urine	93.4–99	HPLC–UV	4.8–8.4	0.2–1.0 μg/L	[47]
Sertraline		94–98.4		4.3–9.2	0.3–0.7 μg/L	
Levofloxacin	Serum	78.7–83.4	HPLC–UV	4.1–4.8	- [a]	[48]
Stir Bar Sorptive Extraction (SBSE)						
Fluoxetine	Plasma	101.1–115.9	HPLC–FLD [b]	4.1–14.8	9.8/32.7 ng/mL	[49]
Losartan	Plasma	98–107	HPLC–UV	3–5	7 ng/mL/- [a]	[50]
Valsartan		98–117		3–8	27 ng/mL/- [a]	
Amphetamine	Urine	99.3–99.6	HPLC–UV	4.7–6.5	11/39.7 ng/mL	[51]
Methamphetamine		99.5–99.8		4.3–5.9	10/35.2 ng/ mL	

[a] Lack of data; [b] fluorescence detector; [c] time-of-flight mass spectrometer with direct analysis in real time; [d] large volume injection programmed temperature vaporization.

Table 2. Methods of sample preparation by liquid-phase extraction.

Name of Analytes	Material	Extraction Efficiency (%)	Analytical Method	Precision (RSD %)	LOD/LOQ	Reference
Single-Drop Microextraction (SDME)						
Atorovastatin	Serum	87.6–105.6	LC–MS	4.2–6.9	0.02 ng/L/- [a]	[52]
Fluvastatin		88.3–92.8		3.4–5.9	2.12 ng/L/- [a]	
Lovastatin		84.5–104.2		4.2–6.9	0.03 ng/L/- [a]	
Mevostatin		87.4–96.3		4.6–6.4	2.0 ng/L/- [a]	
Simvastatin		82.2–97.8		4.8–6.7	0.03 ng/L/- [a]	
Caffeine	Horse urine	83.5 ± 1.22	OT–CEC [b]	- [a]	9.07/10.73 ng/mL	[53]
Cocaine		103.1 ± 2.14			8.27/9.783 ng/mL	
Ephedrine		80.3 ± 1.25			7.71/9.123 ng/mL	
Morphine		108.3 ± 2.03			5.12/5.983 ng/mL	
Piroxicam		94.8 ± 1.46			17.6/19.03 ng/mL	
Strychnine		98.8 ± 1.54			0.94/1.193 ng/mL	
Theophylline		87.5 ± 1.3			1.32/2.023 ng/mL	
Berberine	Urine	105.5–107.7	MEKC [c]–UV	4.6–8.8	0.2/1.5 ng/mL	[54]
Palmatine		88.5–95.0		7.3–12.4	0.5/0.7 ng/mL	
Tetrahydropalmatine		93.1–115.5		6.4–9.0	1.5/4.8 ng/mL	
Dispersive Liquid–Liquid Microextraction (DLLME)						
Aloe-emodin	Urine	87.1–105.0 (OS-DLLME [d])	HPLC–UV	7.2–8.7	0.07 ng/mL/- [a]	[55]
Chryophoanol					0.4 ng/mL/- [a]	
Danthon					0.08 ng/mL/- [a]	
Emodin					0.08 ng/mL/- [a]	
Physicon					0.1 ng/mL/- [a]	
Rhenib		94.8–103.0 (IL-DLLME [e])		5.7–6.4	0.3 ng/mL/- [a]	
					0.01 ng/mL/- [a]	
					0.08 ng/mL/- [a]	
					0.12 ng/mL/- [a]	
					1.0 ng/mL/- [a]	
					0.5 ng/mL/- [a]	
					0.5 ng/mL/- [a]	
Flurbiprofen	Urine	84.4 ± 1.5	HPLC–UV	8.1	16.3 ng/mL/- [a]	[56]
Indomethacin		84.61 ± 1.4		8.6	8.3 ng/mL/- [a]	
Ketoprofen		73.7 ± 1.4		2.5	32.0 ng/mL/- [a]	
Naproxen		76.6 ± 1.3		3.6	9.2 ng/mL/- [a]	

Table 2. *Cont.*

Name of Analytes	Material	Extraction Efficiency (%)	Analytical Method	Precision (RSD %)	LOD/LOQ	Reference
Carvedilol	Plasma	93–99	HPLC–UV	3.1–10.0	6/19 ng/mL	[57]
Diltiazem		93–100		4.9–13.4	3/9 ng/mL	
Metoprolol		94–104		5.1–11.1	2/7 ng/mL	
Propranolol		94–104		7.6–10.4	5/18 ng/mL	
Verapamil		90–100		2.0–5.4	3/10 ng/mL	
Hollow-Fiber Liquid-Phase Microextraction (HF-LPME) and Electromembrane Extraction						
Hydrochlorothiazide	Urine	91.5–92.5	HPLC–UV	4.4–7.9	- [a]/0.5 µg/L	[58]
Triamterene		89.5–89.0		5.8–9.3	- [a]/0.5 µg/L	
Ketamine	Urine	85.2–101.0	GC–MS	2.9–10.1	0.25/0.50 ng/mL	[59]
Norketamine		86.9–94.3		3.6–9.2	0.10/0.50 ng/mL	
Dehydronorketamine		64.6–69.7		9.3–16.9	0.10/0.50 ng/mL	
Cyproterone acetate	Urine	47.1	HPLC–UV	5.8	1.0/ µg/L	[60]
	Plasma	46.3		6	0.5/ µg/L	
Dydrogesterone		20.6		6.1	2.0/ µg/L	
		21.4		6.3	1.2/ µg/L	

[a] Lack of data; [b] open tubular capillary electrochromatography; [c] micellar electrokinetic chromatography; [d] organic solvent dispersive liquid–liquid microextraction; [e] ionic liquid dispersive liquid–liquid microextraction.

3.1. Solid-Phase Extractions

The SPME process may be fully automated and effectively accelerated using the 96-well plate format of thin-film solid-phase microextraction (TFME). A PPy–CH_2–COOH (nanostructured α-carboxy polypyrrol) coating for SPME fiber, used in headspace mode (HS-SPME), was synthetized and applied to the extraction of methadone at its trace level from plasma and urine samples [36]. The carboxy end-capped polypyrrole film was electrochemically deposited on a platinum wire, and a nano-fibrous structure with a diameter of 120 nm was obtained. The nanostructure of the film provided a high surface area that allowed for the high extraction efficiency of methadone. The extraction efficiency was 95–97%. Advantageous features of the used nanostructured fiber include high mechanical stability, strong adhesion of the coating to the substrate, fast extraction equilibrium and desorption, and relatively low cost.

Eight beta-blockers and bronchodilators were isolated from body fluids (plasma and urine) employing the 96-well plate format of the TFME system and an extraction phase made of hydrophilic–lipophilic balance particles (HLB) [37]. Methanol–acetonitrile (80:20 *v*/*v*) with 0.1% formic acid was used as a desorption solvent, which was compatible with the used mobile phase of LC–MS/MS method. The developed extraction method described in the form of a protocol is fast (time of full preparation of one sample less than 2 min), automated, and efficient. The authors also emphasized that the protocol could be modified through compromising sensitivity; finally, the proposed extraction approach could be shortened to 10–15 min for all 96 samples.

The combination of LC–MS/MS with SPME, involving polymeric sorption coatings with molecular imprints (MIP), was used for the simultaneous determination of 10 antibiotic drugs in-whole blood samples [38]. Three conducting polymers, polypyrrole, polythiophene, and poly(3-methylthiophene), used as coatings in SPME, were selected for the extraction of the target compounds. Optimization of the extraction conditions included parameters such as the extraction time, kind of desorption solution, and pH of organic extraction solvent. The obtained results showed that the tested antibiotics could be divided into three groups due to sorption capacity and selectivity to SPME coatings used and, thus, the appropriate sorbent was chosen for each group of analytes. According to the authors, the proposed MIP–SPME–LC–MS/MS method is suitable for therapeutic drug monitoring (TDM) of antibiotics in clinical laboratories, as well as in forensic laboratories for assays at higher concentration levels in body fluids.

Two modes of solid-phase microextraction (in-tube and thin-film SPME) were applied to the estimation of hormone (or similar substances) levels in saliva [38] and urine [39]. Testosterone, cortisol, and dehydroepiandrosterone were assayed by online in-tube SPME in combination with LC–MS/MS [39], and estrogens(estrone, 17β-estradiol, 17α-ethinylestradiol, and estriol) were determined employing a combination of thin-film SPME (TF-SPME) with a 96-well plate system and HPLC–FLD [8]. In the second case [39], a biosorbent—bract—as a novel extraction phase for TF-SPME, was used. In both cases, the methods were fast, with a high throughput and low sample consumption (100 µL of saliva and 37 µL of urine), and only ultrafiltration of samples was required prior to analysis.

Microextraction in packed sorbent (MEPS) is a new sample preparation technology, used in combination with liquid chromatography–mass spectrometry [40] or mass spectrometry alone [41]. An automated sample work-up and quantification of four immunosuppressive drugs in whole blood, using combined MEPS with LC–MS/MS, was presented [40]. The proposed method required 50 µL of whole-blood sample. The obtained quantitative results were in good agreement with a reference LC–MS/MS method involving protein precipitation. The analytical parameters of the developed method revealed that it can be useful for TDM of the studied immunosuppressive drugs.

The MEPS technology was also used in a fast screening of cocaine and its metabolites in human urine samples examined by direct analysis in a real-time source coupled to time-of-flight mass spectrometry (DART–TOF) [41]. As the type of sorbet is one of the most important parameters in solid-phase extraction, four various adsorbent materials (C8, ENV^+, Oasis MCX, Clean Screen DAU) were studied. The last material (Clean Screen DAU) worked best, showing satisfactory extraction efficiency for all studied analytes. In the described sample preparation method, a few microliters of the sample were used, and the extraction time was less than 2 min. Finally, the authors stated that coupling

MEPS technology to an autosampler and connecting it with DART/TOF mass spectrometer would allow for its full automation, making it a very useful tool for screening drugs of abuse in biological matrices.

Developing a new approach for antibiotic drug (linezolid and amoxicillin) determination in human plasma by LC/UV–MS/MS, three sample preparation methods were compared [42]. The pretreatments of samples were performed using protein precipitation (PP), solid-phase extraction (SPE), and microextraction in a packed syringe (MEPS). Among three studied extraction methods, MEPS appeared to be superior to PP and SPE, in terms of accuracy and precision.

A bio-inspired sponge, an amino-functionalized metal–organic framework (Zr–MOF–NH_2), was successfully applied as a sorbent in PT-SPE to extract carbamazepine from urine samples [43]. This extraction method was combined with the HPLC–UV method. The best extraction conditions were achieved when the sample volume was 100 µL, with sample pH adjusted to 7.5, and 5 mg of the sorbent and 10 µL of methanol as eluent solvent were used. The total time of analysis, including the sample preparation step, was less than 12 min. The sorbent was used for at least eight extractions without significant loss of its capacity and extraction repeatability.

Two automated clean-up methodologies, based on monolithic packed 96-tip sets and combined with LC–MS/MS, were employed for determination of the beta-blockers (pindolol and metoprolol) in plasma [44] and anti-cancer drugs (cyclophosphamide and busulfan) in whole-blood samples [45]. In the case of the beta-blocker analysis, a 100-µL sample volume was handled, and, for the determination of the anti-cancer drugs, 1 mL of a sample was needed. In both cases, sample preparation was performed in only about 2 min for 96 samples. However, in the case of whole-blood analysis, the monolithic packed 96 tips can be re-used up to only five times, inferior to the 100 times achieved with MEPS methodology [45]. Comparing the determination of the beta-blockers in plasma, using monolithic packed 96 tips, with the protein precipitation method (using 0.1% formic acid in acetonitrile), the tips method resulted in a higher (2–3 times) S/N ratio. Generally, the tips methodology provided better results in terms of selectivity, accuracy, and precision.

A d-SPE protocol based on the modified QuEChERS procedure (using 50 mg of PSA and 150 mg of anhydrous $MgSO_4$), followed by large volume injection programmed temperature vaporization (LVI–PTV)–gas chromatography–mass spectrometry (GC-MS) analysis, was developed for the simultaneous determination of 16 drugs and pesticides in postmortem blood samples without derivatization [46]. The validated d-SPE/LVI–PTV/GC–MS method is suitable for routine analysis in a forensic laboratory. The usability of the proposed method was confirmed by the analysis of 10 postmortem blood samples. Six samples contained cocaine, two contained MDMA, and two contained carbamazepine. Other found analytes were carbofuran, the metabolite 7-aminoflunitrazepam, amitriptyline, and diazepam.

Magnetically modified nanomaterials based on conductive polymers [47] and molecularly imprinted polymers (MIPs) [48] can be used as examples of new sorbents widely employed in MSPE for preconcentration and determination of pharmaceuticals in a complex biological matrix. Polypyrrole (PPy) with magnetic nanoparticles (MNPs)—Fe_3O_4—doped by sodium perchlorate ($NaClO_4$), exhibited high extraction efficiency: 93.4–99% and 94–98.4% for citalopram (CIT) and sertraline (STR), respectively. The isolation of the medicines was performed in the optimized extraction conditions: sample pH—9.0, sorbent amount—10 mg, sorption time—7 min, elution solvent (0.06 mol/L HCl in methanol) volume—120 µL, elution time—2 min. The performance of the developed extraction method was estimated by spiking CIT and STR at trace levels in urine and plasma samples [47].

Molecularly imprinted magnetic carbon nanotubes (MCNTs@MIPs) were applied to the MSPE of levofloxacin in serum samples [48]. The prepared sorbent was characterized by high specific surface area and high selectivity to template molecules of levofloxacin. Investigations indicated that the sorbent was very selective (exhibited excellent recognition) toward levofloaxacin. Under optimal extraction conditions (sorbent amount—15–20 mg, adsorption and desorption time—60 min, eluent—methanol/acetic (6:4, *v*/*v*), eluent volume—2 mL), extraction recoveries ranged from 78.7 ± 4.8% to 83 ± 4.1%. The sorbent stability was assessed as a reduction in average recovery after five cycles and was estimated at less than 7.6%.

A validated high-performance liquid chromatography with fluorescence detection (HPLC–FLD) method combined with stir bar sorption extraction (SBSE) was developed for determination of fluoxetine in human plasma [49]. The extractions of this antidepressant drug from plasma samples were performed using laboratory-made polydimethylsiloxane (PDMS) stir bars. Several factors such as temperature and time of sorption, stirring speed, and two modes of desorption (ultrasonic and magnetic stirring) were considered in the optimization of extraction conditions. The method was successfully applied to the analysis of real plasma samples originating from depressed patients treated with fluoxetine. Taking into account the time of sample preparation (3 h), low cost of each coated bar, and possibility to reuse it 50 times, the authors recommended the proposed method as a reliable tool for routine analysis. Novel coatings for bars in the SBSE approach were introduced for body fluid preparation for the determination of antihypertensive drugs [50] and illicit drugs [51]. A monolithic vinylpyrroli–doneethylene glycol dimethacrylate (VPD-EDMA) polymer was applied to the extraction of losartan and valsartan from plasma samples [50]. Compared to commercially available PDMS (polydimethylsiloxane) and PA (polyacrylamide) stir bars, the proposed coated stir bar exhibited higher physical stability and, therefore, it was suitable for the use of an ultrasonic stirring mode, which was profitable in terms of requiring less time and less solvent for the performed extraction. A nano graphene oxide sol–gel composite (NGO/sol–gel), used as the coating of a capillary glass tube stir bar, was employed for the isolation of amphetamine and methamphetamine from urine samples [51]. In both cases, the coated bars were coupled with HPLC in combination with relatively low-sensitivity UV detection, but the obtained results indicated the usefulness of both mentioned methods for the analysis of real samples in clinical and forensic laboratories.

3.2. Liquid-Phase Extractions

Jahan and co-workers [52] proposed a coupling microextraction device combining a microsyringe with a very short capillary. The designed device enabled manually controlling the shape and size of the aqueous organic droplet during the extraction process, as well as ensuring droplet stability even under vigorous stirring conditions. The performance of the developed method was checked using human serum samples spiked with five statins (lovastatin, simvastatin, mevastatin, Fluvastatin, and atorvastatin). Using 1.2 µL of a toluene–aqueous (0.2:1) droplet, a 350–1712-fold enrichment of the statins was achieved within four minutes.

A combination of SDME and open tubular capillary electrochromatography (OT–CEC) for the simultaneous determination of seven illicit drugs (caffeine, cocaine, ephedrine, morphine, piroxicam, strychnine, and theophylline) in horse urine was presented [53]. The extraction procedure enabled good compound recovery with good precision and appropriate reduction of interferences. The obtained enrichment factors were between 38 and 102 depending on the studied compound. The proposed SDME–OT–CEC method showed potential for application in toxicology investigations, as well as being a cheaper and more environmentally friendly method compared to LC–MS methods.

SDME was also used on-line coupled with sweeping micellar electrokinetic chromatography (MECK) for the isolation and preconcentration of three alkaloids: berberine, palmatine, and tetrahydropalmatine, present in human urine at trace level [54]. In this method, analytes were firstly extracted from a basic aqueous sample solution (donor phase) into *n*-octanol, and then back-extracted into the acidified aqueous solution (acceptor phase), which formed a droplet at the tip of a capillary. The acceptor phase was introduced into the capillary using the hydrodynamic injection mode and analyzed by sweeping MEKC. The extraction method was optimized considering a number of experimental factors: extraction solvent type, time of drop formation, stirring rate, duration of pre- and back-extraction, sample temperature, addition of NaCl, and composition of donor and acceptor phase. Under the optimized conditions, the developed method gave a 1583–3556-fold improvement in sensitivity for the studied analytes within 20 min.

Organic solvent dispersive liquid–liquid microextraction (OS–DLLME) and ionic liquid dispersive liquid–liquid microextraction (IL–DLLME) were applied to determine emodin and its six metabolites in urine samples. These two extraction approaches were evaluated and compared [55]. The analytes were

assayed using HPLC–UV and HPLC–MS methods. In order to optimize DLLME, several parameters were studied and optimized, including kind of extraction solvent, volumes of the extraction solvent and the disperser solvent, extraction and centrifugation time, sample pH, and concentration of added NaCl. At optimal extraction conditions, the enrichment factors for emodin and its metabolites ranged from 90–295 for the OS–DLLME method and from 63–192 for the IL–DLLME method. Comparing the abovementioned extraction methods, it was concluded that IL–DLLME was more rapid and simple, as well as more repeatable, than OS–DLLME. However, OS–DLLME exhibited higher enrichment factors, a wider linear range, and lower limits of detection for the target compounds.

A one-step in-syringe set-up for DLLME with the use of an ionic liquid was proposed, and pros and cons of the novel approach were discussed [56]. Among the advantages may be listed the use of a simple extraction unit (only a conventional plastic syringe), avoiding the centrifugation step, reducing the extraction time, and being more suitable for the automation of the whole extraction procedure than in the conventional approach. The novel approach broadens the used solvent range (not only solvents denser than water can be exploited) by simply changing the orientation of the syringe during the phase separation step [56]. Among the limitations (at the present state), low extraction recoveries and low precision of the procedure may be mentioned. The suitability of the proposed approach was evaluated via the determination of four non-steroidal anti-inflammatory drugs (ketoprofen, naproxen, flurbiprofen, and indomethacin) spiked with urine samples. The obtained wide concentration range (0.02–10 μg/mL) of the target drugs allowed assaying them at therapeutic and toxic concentration levels.

DLLME in combination with HPLC–UV was applied to the determination of five antiarrhythmic drugs, metoprolol, propranolol, diltiazem, carvedilol, and verapamil, present in human plasma [57]. The method required 660 μL of plasma sample, and the complete separation of all the analytes was achieved within 7 min. In the extraction process, acetonitrile, used as a disperser solvent (resulting from the protein precipitation), was mixed with the extracting medium (dichloromethane) and then quickly injected into an aqueous basic solution. After centrifugation, the sedimented phase with the concentrated drugs was taken and evaporated to dryness. The dried residue was dissolved in 50 μL of deionized acidified water and subjected to HPLC analysis. The developed method was successfully applied to the analysis of the selected antiarrhythmic drugs in real plasma samples at ng/mL. According to the authors and considering the determined analytical parameters, the proposed method seems to be appropriate for routine analysis in drug analysis laboratories for pharmacokinetic and pharmacodynamics studies, as well as for therapeutic drug monitoring.

In hollow-fiber liquid-phase microextraction (HF-LPME), a porous membrane of the polypropylene hollow fiber with the pore size of 0.2 μm prevents the entry of macromolecular compounds in the sample solution into the acceptor phase present inside the fiber [58]. The HF-LPME technique may be realized using two modes: two-phase and three-phase. In two-phase HF-LPME, the target compound is enriched into the organic solvent as the acceptor phase and then determined by an appropriate chromatographic method. In three-phase HF-LPME, the analyte is extracted from the aqueous donor phase to organic phase, followed by back-extraction into the aqueous donor phase which is directly subjected to analysis. The applications of two-phase and three-phase HF-LPME for the isolation and preconcentration of hydrochlorothiazide (HYD) and triamterene (TRM) in urine samples were presented, respectively [58]. Under optimal conditions, enrichment factors of 128 and 239 were achieved for HYD and TRM, respectively. Finally, the proposed method was successfully applied to the analysis of TRM and HYD in a urine sample obtained from a volunteer who received 500 mg of triameterene-H. The performed analysis showed that the concentrations of HYD and TRM were 18.2 and 7.3 μg/L, respectively.

Using HF-LPME in the three-phase mode combined with GC–MS, ketamine (KT) and its main metabolites, norketamine (NK) and dehydronorketamine (DHNK), were determined in urine samples [59]. A "green chemistry" approach to the sample extraction involved using eucalyptus essential oil as a supported liquid membrane in HF-LPME. After drying, the acceptor phase with the isolated analytes was derivatized with trifluoroacetic anhydride before analysis by GC–MS. The usefulness of the developed method was confirmed by the analysis of real urine samples originating from two

patients who were suspected of taking KT. KT, NK, and DHNK were determined in the concentrations 0.0873, 5.805, and 8.760 μg per 1 mL of urine, respectively (in one case) and 7.3, 5.3, and 6.8 ng per 1 mL of urine, respectively (in the second case).

A novel generation of deep eutectic solvent (DES) as an acceptor phase in three-phase HF-LPME was introduced for the extraction of two steroidal hormones, dydrogesterone (DYD) and cyproterone acetate (CPA), from urine and plasma samples [60]. The following factors influencing extraction efficiency were studied: nature and composition of DES, composition of supported liquid membrane, salt addition, length of hollow fiber, stirring rate, and duration of extraction. Under the optimized conditions, preconcentration factors ranged from 187 to 428. The method was validated via the analysis of real urine samples collected from two patients. In the case where patient 1 consumed one tablet (containing 2 mg of cyproterone acetate +0.035 mg of ethylene estradiol), the analysis showed that the medicine concentration was below the detection limit of the method. However, positive results of the analysis were achieved in the case of patient 2, who was subjected to hormonal therapy for a long time. The usefulness of the developed method was also confirmed via analysis of plasma samples spiked with 5 and 100 ppb of DYD and 10 and 200 ppb of CPA.

4. Summary and Conclusions

Microextraction methods constitute modern approaches for sample preparation in terms of efficiency, selectivity, simplicity, speed, enrichment capability, requirement of small sample volume, and congruence for automation and miniaturization. Due to low or very low (usually on the order of several hundred microliters) or no consumption (SPME combined with thermal desorption of analytes in GC injection port) of organic solvents, these techniques may be also classified as "green" approaches. The eight microextraction techniques belonging to solid-phase (SPME, MEPS, DPX, D-μ-SPE/MSPE, SBSE) and liquid-phase (SDME, DLLME, HF-LPME) extractions were briefly described and characterized. The usefulness of these techniques in the preparation of biological samples was supported by selected examples of their applications for the determination of medicines, drugs of abuse, and other bioactive compounds in body fluids. In the most reported cases, extraction recoveries were over 80% with an acceptable RSD lower than 10–15%. To quantify the studied bioactive compounds, the proposed microextraction techniques were usually combined with an appropriate chromatographic or capillary electrophoretic method. Using such combinations, limits of detection and quantification were at concentration levels equal to ppb or ppm. Considering sample consumption, solid-phase extractions generally require lower volumes, especially MEPS and DPX techniques, where sample volume consumption range from 5 to 600 μL. It is also worth noting that some proposed microextraction methods, at the optimized conditions, may be effective enough (they have a high enough enrichment capacity) to achieve LODs at low ppm levels of the studied compounds, even in combination with a relatively low-sensitivity UV detection method.

Microextraction techniques began being introduced during the last decade of the last century, and, based on the growing number of publications associated with them, it seems that they represent the current direction of development of sample preparation methods. Considering their numerous advantages, including "green" characteristics, without loss of analytical performance, it seems that these approaches will be extensively developed and employed in many important analytical areas, such as clinical and forensic investigations, as well as in environmental and food analysis.

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