Sample preparation in analysis of pharmaceuticals

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Sample preparation is a very important and essential step in environmental analysis. This article presents an overview of extraction methods for environmental samples, focusing especially on pharmaceuticals as there is great concern about them as pollutants.

Keywords: Environmental samples; Pharmaceuticals; Sample-preparation techniques

Abbreviations: AIBN, 2,2′-azo-bis-isobutyronitrile; ASE, Accelerated solvent extraction; CAR, Carboxen; DAD, Diode-array detector; DI-SPME, Direct-immersion solid-phase extraction; DSPE, Dispersive solid-phase extraction; EGDMA, Ethylene glycol dimethacrylate; ESE, Enhanced solvent extraction; ESI, Electrospray ionization; FD, Fluorescence detection; FLD, Fluorimetric detection; GC, Gas chromatography; HPLC, High-performance liquid chromatography; HS-SPME, Headspace solid-phase extraction; ISs, Immunosorbents; ITSPME, In-tube solid-phase microextraction; LC, Liquid chromatography; LC-MS^2, Liquid chromatography tandem mass spectrometry; LLE, Liquid–liquid extraction; LPME, Liquid-phase microextraction; MAA, Methacrylic acid; MASE, Microwave-assisted solvent extraction; MIP, Molecularly-imprinted polymers; MMLLE, Microporous membrane liquid-liquid extraction; MSPD, Matrix solid-phase dispersion; OTC, Oxytetracycline; PDMS, Polydimethylsiloxane; PFE, Pressurized fluid extraction; PLE, Pressurized liquid extraction; PSE, Pressurized solvent extraction; RAM, Restricted access materials; SBSE, Stir bar sorptive extraction; SCF, Supercritical fluid; SFE, Supercritical fluid extraction; SLM, Supported liquid-membrane extraction; SMETH, Sulfamethazine; SPE, Solid-phase extraction; SPME, Solid-phase microextraction; St-DVB, Styrene divinylbenzene; TC, Tetracycline; TFA, Trifluoroacetic acid; TMP, Trimethoprim; TOPO, Tri-*n*-octyl phosphine oxide; USE, Ultrasonic extraction; WWTP, Wastewater-treatment plant.

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1. Introduction

Pharmaceuticals are ''emerging contaminants'', which, in most cases, correspond to unregulated contaminants that may be candidates for future regulation [1]. Their characteristic is that they do not need to persist in the environment to cause negative effects because they are continually being released into the environment, mainly from manufacturing processes, disposal of unused products and excreta [2]. In addition, there are no ecotoxicological data and risk assessment available for them, so it is difficult to predict what health effects they may cause on living organisms.

Among all the emerging contaminants, pharmaceuticals (Table 1) are of the greatest and increasing concern.

Effluents from wastewater-treatment plants (WWTPs) comprise one of the most important sources of pharmaceuticals being released into the environment. The wide spread of pharmaceutical chemical structures (e.g., sulfonamides, tetracyclines, macrolides, and β-lactams) makes sample preparation complex, especially when pharmaceuticals of different groups are in the mixture.

Unlike priority pollutants, the behavior of pharmaceuticals in the environment has not been studied extensively. Some general reports and reviews on the occurrence, fate and risk assessment of pharmaceuticals in the environment have been published, with veterinary drugs being the targets of most studies because of their systematic use [2].

Biological environmental sample matrices, especially sewage and marine-water samples and pharmaceutical products are complex and often contain interfering elements that can mask or interfere with the compounds of interest, so that direct analysis may not be possible. Moreover, the concentrations in which the pharmaceuticals are generally found have made it necessary to perform an initial stage of concentration and purification of the analytes prior to their analysis.

The analytical procedure usually comprises five steps: sampling, sample preparation, separation, detection, and data analysis. Each step is involved in obtaining correct results, but sampling and sample preparation are the key components of the analytical process. Over 80% of the analysis time is spent on these two steps. It is also important to keep in mind that all five

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Table 1. Classes of pharmaceuticals

Therapeutic classes	Examples
Veterinary and human antibiotics	
– β-lactams	Amoxicillin, Ampicillin, Benzylpenicillin
– macrolides	Erythromycin, Azithromycin, Tylosin
– sulfonamides	Sulfamethazine, Sulfadiazine, Sulfaguanidine
– tetracyclines	Oxytetracycline, Tetracycline
Analgesics and anti-inflammatory drugs	Codeine, Ibuprofen, Acetoaminofen, Diclofenac, Fenoprofen
Lipid regulators	Bezafibrate, Clofibric acid, Fenofibric acid
Psychiatric drugs	Diazepam
β-blockers	Metoprolol, Propranolol, Timolol, Solatol
X-ray contrast media	Iopromide, Iopamidol, Diatrizoate
Anti-depressants	Fluoxetine
Hormones	Estradiol, Estrone, Estriol, Diethylstilbestrol

of these analytical steps are consecutive, and the next step cannot begin until the preceding one has been completed. If one of these steps is not followed properly, performance of the procedure would be poor overall, errors would be introduced, and the results would be inconsistent [3,4].

There is therefore no doubt that proper sample preparation is a prerequisite for most analytical procedures. Analysts have responded to this challenge, so this article reviews recent sample-preparation techniques for analyzing pharmaceuticals in various samples. We give an overview of current developments in sample preparation and cite several applications in detail.

2. Sample preparation

The basic concept of sample-preparation methods is to convert a real matrix into a sample suitable for analysis. This process almost inevitably changes the interactions of compounds with their concrete chemical environment. These interactions are determined by the physical and chemical properties of both analytes and matrices, and they affect the applicability of different sample-preparation techniques and analytical methods as well as their efficiency and reproducibility. Hence, characterization of the initial physicochemical state of a sample is a precondition of all further sample-preparation steps [5]. It is very important to have information on the physical and chemical properties (Table 2) of an analyte (e.g., log K_{ow}, pK_a) because that may help determine whether a compound is likely to concentrate in some specific conditions [2].

Log K_{ow} is an indicator of the lipophilicity of the compound. A high log K_{ow} is typical for hydrophobic compounds, whereas a low K_{ow} signifies a compound soluble in water.

Most pharmaceuticals have acidic and/or basic functionalities; their ionization rate depends on acidic dissociation constants (i.e. pK_a values) and is controlled by solution pH (e.g., p$K_{a,1}$ and p$K_{a,2}$ values for certain sulfonamides are in the ranges 2–3 and 5–8, respectively). In the pH ranges of 3–5, the compound is primarily in its neutral form, whereas, at higher pH, the compound is predominantly anionic. Most β-blockers and anti-ulcer agents are basic in nature, with pK_a values in the range 7.1–9.7, but non-steroidal anti-inflammatory drugs (NSAIDs) are acidic with pK_a of 4.0–4.5. These different chemical species (cationic, neutral, or anionic) often have vastly different properties. Unfortunately, the pK_a values of many relevant pharmaceuticals are either not known accurately or not available at all. But, with this knowledge, one can choose the best option for analyzing pharmaceuticals (pK_a value enables adjustment of the pH value of sample solution; log K_{ow} shows affinity of pharmaceuticals towards water (polar/non-polar compounds)).

Matrix effects are major problem in extracting analytes (e.g., pharmaceuticals). A matrix effect can be defined as the influence of a property of the sample, independent of the presence of the analyte, on recovery efficiency and thereby on the quantity extracted (e.g., pharmaceuticals may sorb to organic matter in the samples, causing the concentrations of freely dissolved pharmaceuticals to be lower and therefore more difficult to detect).

Sample preparation can be achieved by employing a wide range of techniques, but all methods have the same goal [13]:

- to remove potential interferences;
- to increase the concentration of an analyte;
- if necessary, to convert an analyte into a more suitable form; and,
- to provide a robust, reproducible method that is independent of variations in the sample matrix.

Although many traditional sample-preparation methods are still in use, there have been trends in recent years towards [13]:

- use of smaller initial sample sizes, small volumes or no organic solvents;

Table 2. Physico-chemical properties (log K_{ow}, pK_a, K_d, log K_{oc}) of some pharmaceuticals found in the references (in square brackets, e.g., [7])

Compounds	CAS	log K_{ow}	pK_a	K_d	log K_{oc}
Amoxicillin	26787-78-0	0.87 [6]; 0.97 [7]	2.4 [6]; 2.8, 7.2 [8]	1.06 [6]	2937 [7]
Ampicillin	69-53-4	1.45 [6]; 1.45 [7]	2.53 [6]; 2.7, 7.3 [8]	na	2728 [7]
Benzylpenicillin	61-33-6	1.87 [6]; 1.85 [7]	2.79 [6]; 2.8 [8]	na	2625 [7]
Bezafibrate	41859-67-0	4.25 [6]; 4.25 [7]	3.6 [6]	na	3166 [7]
Carbamazepine	298-46-4	2.45 [6]; 2.25 [7]	13.9 [6]	25.52 [6]	3588 [7]
Chloramphenicol	56-75-7	1.14 [6]; 0.92 [7]	na	na	1000 [7]
Chlortetracycline	57-62-5	−0.62 [6]; −0.36 [9]; −0.68 [7]	6.5 [6]; 3.3, 7.4, 9.3 [9,10]	na	1979 [7]
Ciprofloxacin	85721-33-1	0.4 [6]; −0.00 [7]	6.38 [6]	416.9 [6]	1550 [7]
Clofibric acid	882-09-7	2.57 [6]; 2.84 [7]	na	na	1640 [7]
Diclofenac	15307-86-5	4.51 [6]; 4.02 [7]	4.15 [6]	0.72 [6]	2921 [7]
Enrofloxacin	93106-60-6	1.1 [6]; 0.70 [7]	6.27 [6]	na	1922 [7]
Erythromycin	114-07-8	3.06 [6,9]; 2.48 [7]	8.9 [6]; 8.88 [9]	164.76 [6]	1000 [7]
Estriol	50-27-1	2.81 [6]; 2.81 [7]	na	na	2904 [7]
Ibuprofen	15687-27-1	3.97 [6]; 3.79 [7]	4.4 [6]	453.79 [6]	2596 [7]
Norfloxacin	70458-96-7	−1.0 [6]; −0.31 [7]	6.4 [6]	na	1964 [7]
Oxytetracycline	79-57-2	−1.22 [6]; −2.87 [7]	3.27 [6]; 3.3, 7.3, 9.1 [9,10]	0.02 [6]	1988 [7]
Paracetamol	1580-83-2	1.70 [7]; 0.46 [10]	9.5 [6]	0.4139 [6]	3944 [7]
Propranolol	525-66-6	2.60 [7]	9.49 [6]	na	3086 [7]
Roxithromycin	80214-83-1	2.75 [6]; 2.75 [7]	8.8 [6]	na	1390 [7]
Sulfadiazine	68-35-9	−0.09 [6]; −0.34 [7]	6.15 [9]; 6.50 [11]; 2.0, 6.4 [12]	na	2276 [7]
Sulfaguanidine	57-67-0	−1.07 [7]	11.3 [11]	na	2109 [7]
Sulfamethazine	57-68-1	0.89 [6]; 0.76 [7]	2.65 [6,11]; 2.4, 7.4 [12]	na	2695 [7]
Sulfamethoxazole	723-46-6	0.89 [6]; 0.48 [7]	5.7 [6]; 5.9 [11]; 1.8, 6.0 [12]	na	3185 [7]
Tetracycline	60-54-8	−1.19 [6]; −1.33 [7]	3.3 [6,10], 7.7, 9.7 [10]	na	1760 [7]
Trimethoprim	738-70-5	0.73 [7];	6.6 [6]	na	2957 [7]
Tylosin	1401-69-0	3.5 [6]; 1.05 [7]	7.1 [6]; 7.73 [9]	na	1000 [7]

na = Data not available; log; K_{ow} = Logarithm of the octanol/water partition coefficient; pK_a = Acidic dissociation constant; K_d = Sludge/water partition coefficient; log K_{oc} = Logarithm of the organic carbon normalized sorption coefficient.

- greater specificity or greater selectivity in extraction; and,
- increased potential for automation.

Fig. 1 shows different sample preparation procedures in the analytical process.

Sample preparation must also be tailored to the final analysis, considering the instrumentation to be used and the degree of accuracy required, whether quantitative or qualitative [14].

2.1. Solid-phase extraction (SPE)

SPE has gradually replaced classical liquid–liquid extraction (LLE) and become the most common sample-preparation technique in environmental areas. SPE offers the following advantages over LLE:

1. higher recoveries;
2. improved selectivity, specificity and reproducibility;
3. elimination of emulsions;
4. less organic solvent usage;
5. shorter sample preparation time; and,
6. easier operation and the possibility of automation.

In SPE, the analytes to be extracted are partitioned between a solid phase and a liquid phase, and these analytes must have greater affinity for the solid phase than for the sample matrix. SPE is mostly used to prepare liquid samples and extracts of semi-volatile or non-volatile analytes, but it can be also used for solids pre-extracted into solvents.

SPE products are excellent for extraction, concentration, and clean-up. Clean-up procedures on SPE sorbents are not limited to extracts from solid samples but could also be used for all the extracts obtained from environmental samples, especially wastewater samples. Clean-up is an important step in determination of analytes at low levels and depends, of course, on the complexity of the sample matrix and detection mode, especially when the analysis is performed by liquid chromatography (LC). Fig. 2 shows common SPE procedures and Tables 3–5 give references for SPE procedures for extraction of pharmaceuticals from environmental samples.

Choice of sorbent is the key point in SPE because it can control parameters such as selectivity, affinity and capacity. This choice depends strongly on the analytes of interest and the interactions of the chosen sorbent through the functional groups of the analytes. However, it also depends on the kind of sample matrix and its interactions with both the sorbent and the analytes [23]. Classical SPE sorbents range from chemically-bonded silica with the C8 or C18 organic group among others and carbon or ion-exchange materials to polymeric materials (St-DVB), immunosorbents (ISs), molecularly-imprinted

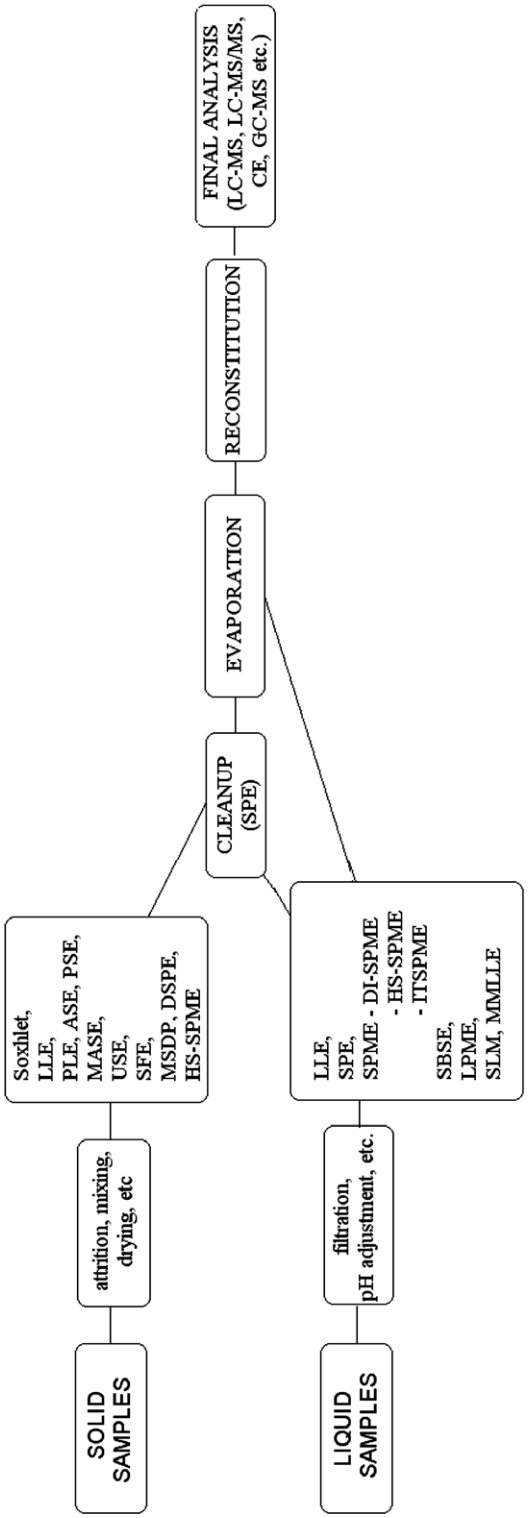

Figure 1. Sample-preparation procedures in the analytical process.

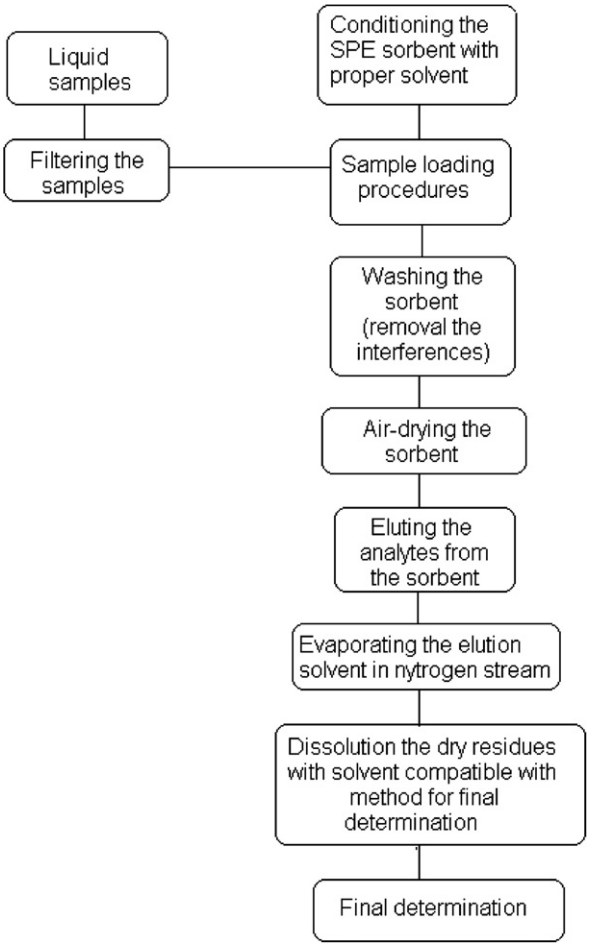

Figure 2. Common procedures for solid-phase extraction (SPE).

polymers (MIPs) and restricted access materials (RAMs) [23,24].

Silica sorbents have several disadvantages compared with polymeric sorbents. They are unstable in a broader pH range and contain the silanols, which are not a good choice for tetracyclines because they have been found to bind irreversibly [25], but, for estrogens, silica-gel clean-up is followed by C18 SPE enrichment [6].

Pharmaceuticals of adequate hydrophobicity (log K_{ow} in the range 1.5–4.0) can easily be preconcentrated using any reversed-phase material (e.g., C18, C8, St-DVB). Deprotonation of acidic compounds and protonation of basic compounds should be suppressed to ensure sufficient hydrophobicity of the analytes. Acidic pharmaceuticals should therefore be preconcentrated under acidic conditions opposite to basic analytes [26]. Whereas silica-based sorbents as well as St-DVB are not a good option for polar compounds, new materials have been developed in the past few years, so there are many commercially available polymeric sorbents with high specific surface areas [23].

Weigel et al. [27] have compared several sorbents for the extraction efficiency of a group of acidic, neutral and basic pharmaceuticals from water samples. Among these sorbents, most presented similar recoveries for neutral analytes whereas the largest differences have been

Table 3. Survey of SPE methods for extraction of pharmaceuticals from aquatic samples

Pharmaceuticals	Sample	Sorbent type	Conditioning solvent	Elution solvent	Final analysis	Refs.
β-lactam antibiotics (e.g., Penicillin G, Ampicillin, Amoxicillin)	Wastewater, 250 mL pH 7.5	Oasis MAX 500 mg/6 mL, Waters	1. Methanol, 6 mL 2. Milli Q-water, 6 mL 3. 0.05M phosphate buffer (pH 7.5), 6 mL	2 × 1 mL of 0.05M tetra-*n*-butylammonium hydrogen sulphate in methanol	HPLC-DAD	[8]
	pH 8.0	Bond Elut C18 500 mg/6 mL, Varian	1. Methanol, 10 mL 2. Milli Q-water, 10 mL 3. 2% NaCl, 5 mL 4. 0.1M phosphate buffer solution (pH 8.0), 5 mL	2 × 1 mL methanol/water (0.1M phosphate buffer), 60/40 (v/v)		
Tetracyclines (e.g., Oxytetracycline, Chlortetracycline, Tetracycline), Sulfonamides (e.g., Sulfamethazine, Sulfamethoxazole), Macrolides (Erythromycin, Roxithromicin, Tylosin)	River water 120 mL	Oasis HLB 60 mg/3 mL, Waters	1. Methanol, 3 mL 2. Deionized water, 3 mL	Methanol, 5 mL	LC-MS^2	[10]
Sulfonamides (e.g., Sulfaguanidine, Sulfadiazine, Sulfamethazine, Sulfamethoxazole)	Wastewater 500 mL, pH 3.0	Oasis MCX, Waters	1. Water, 5 mL 2. Methanol, 5 mL 3. 5% NaOH in methanol, 5 mL 4. Water (pH 3), 5 mL	5% Ammonium hydroxide in methanol, 2 mL	LC-ESI-MS^2	[11]
Macrolides (e.g., Roxithromycin, Erythromycin, Azithromycin)	River water 250 mL, pH 6.0	Oasis HLB 30 mg, Waters	1. Acetonitrile, 5 mL 2. Milli Q-water, 5 mL	Mixture of 10 mM ammonium acetate (pH 6)/acetonitrile (50:50, v/v), 1 mL	LC-MS^2	[15]
Tetracyclines (e.g., Oxytetracycline, Tetracycline, Chlortetracycline)	Seawater, pH 3.4	Oasis HLB 30 mg, 200 mg, Waters; Isolute ENV+ 25 mg, IST	1. Methanol, 5 mL 2. Water, 5 mL 3. Formic acid buffer (pH 3.4)	Methanol containing 1% TFA, 1 mL	LC-FLD	[16]
Macrolides (e.g., Clarithromycin, Erythromycin, Tylosin), Fluoroquinolones (e.g., Ciprofloxacin), β-lactam antibiotic (Amoxicillin), Sulfonamide (Sulfomethoxazole), Tetracycline (Oxytetracycline), Anti-inflammatory drug (Ibuprofen), Psychiatric drug (Diazepam), Antiepileptic drug (Carbamazepin)	Municipal wastewater 500 mL, pH 2.0	Oasis MCX 60 mg/3 mL, Waters	1. Methanol, 6 mL 2. Milli-Q water, 3 mL 3. Water pH 2.0	1. Methanol, 2 mL 2. 2% Ammonium in methanol, 2 mL 3. 0.2% NaOH in methanol, 2 mL	HPLC-MS^2	[17]

Table 3 (*continued*)

Pharmaceuticals	Sample	Sorbent type	Conditioning solvent	Elution solvent	Final analysis	Refs.
	pH 7.0	LiChrolut EN 200 mg/3 mL, Merck	1. Methanol, 6 mL 2. Milli-Q water, 6 mL	1. Methanol, 3 mL 2. Ethyl acetate, 3 mL		
Benzimidazole anthelmintics (Albendazol, Fenbendazol, Mebendazol, Oksibendazol, Tiabendazol)	Water 1.5 mL	Isolute HCX 130 mg/6 mL, International Sorbent Technology,	Mixture of acetonitrile : acetic acid = 95 : 5, 1.5 mL	Mixture of acetonitrile : 29.3% Ammonium = 95 : 5, 3 mL	LC-ESI-MS	[18]
		Oasis MCX 60 mg/3 mL, Waters	0.75 mL	1.5 mL		
Anti-inflammatory drugs (Ibuprofen, Diclofenac), Antiepileptic drug (Carbamazepin), Lipid regulator (Clofibric acid)	Water samples (tap water, river water, ground water), 500 mL pH 5.0	Superclean ENVI-18, 500 mg/3 mL, Supelco	1. Elution solvent, 3 mL 2. Methanol, 3 mL 3. Deionized water, 3 mL	I. aceton-ethyl acetate (1:1, v/v), 8 mL or II. aceton-ethyl acetate (2:1, v/v), 8 mL	GC-MC	[19]
		Lichrolut EN 200 mg/3 mL, Merck	1. Elution solvent, 3 mL 2. Deionized water, 3 mL	aceton-methanol (3:2, v/v), 5 mL		
		Oasis HLB 60 mg/3 mL, Waters	1. Methanol, 3 mL 2. Deionized water, 3 mL	methanol, 2 mL		
Lipid regulators (Clofibric acid, Bezafibrate), Anti-inflammatory drug (Ibuprofen)	Wastewater 50 mL pH 2.0–2.5	Oasis HLB, 60 mg/3 mL, Waters	1. Methanol, 5 mL 2. Ultrapure water pH 2.0-2.5, 5 mL	Methanol, 2 mL	LC-ESI-MS^2	[20]

observed for acidic analytes (bezafibrate, ibuprofen, diclofenac and clofibric acid). For these acidic as well as all other pharmaceuticals mentioned in this article (except paracetamol), the highest retentions (>80%) were realized with Oasis HLB. Lindsey and co-workers [25] reached the same conclusion on Oasis HLB in the extraction of tetracyclines and sulfonamides from groundwater and surface water.

Another sorbent, Strata-X, was compared [28,29] with other commercially available sorbents for the

Table 4. Survey of SPE methods for the extraction of pharmaceuticals from liquid samples

Pharmaceuticals	Sample	Sorbent type	Conditioning solvent	Elution solvent	Final analysis	Refs.
Benzimidazole anthelmintics (Albendazol, Fenbendazol, Mebendazol, Oksibendazol, Tiabendazol)	Milk urine 1.5 mL	Isolute HCX 130 mg/6 mL, International Sorbent Technology,	Mixture of acetonitrile : acetic acid = 95 : 5, 1.5 mL	Mixture of acetonitrile : 29.3% Ammonium = 95 : 5; 3 mL	LC-ESI-MS	[18]
		Oasis MCX 60 mg/3 mL, Waters	0.75 ml	1.5 mL		
Sulfonamides (e.g., Sulfadiazin, Sulfamethazin), Tetracyclines (Oxytetracycline, Chlorotetracycline, Tetracycline)	Milk 5.0 g	Oasis HLB, 500 mg/6 mL, Waters	1. Methanol, 5 mL 2. 0.5M HCl, 5 mL 3. Deionized water, 5 mL	1. Methanol, 5 mL 2. 5% Methanol in 2% Ammonium hydroxide, 5 mL	LC-ESI-MS	[21]

Table 5. Survey of sample-preparation methods for the extraction of pharmaceuticals from solid samples. SPE is used for clean-up

Pharmaceuticals	Sample	Sorbent type	Conditioning solvent	Elution solvent	Final analysis	Refs.
Tetracyclines (Chlorotetracycline, Oxytetracycline), Macrolides (Erythromycin, Tylosin), Sulfonamide (Sulfadiazin)	Soil 10 g Liquid sample after PLE (methanol : 0.2M citric acid buffer pH 4.7 = 1:1)	Clean-up: tandem SAX, 500 mg/6 mL, Isolute – Oasis HLB, 200 mg/6 mL, Waters	1. Methanol, 2 mL 2. 0.04 M citric acid buffer pH 4.7, 2 mL	Methanol, 2 mL	LC-ESI-MS^2	[9]
Tetracyclines (e.g., Oxytetracycline, Chlortetracycline, Tetracycline), Sulfonamides (e.g., Sulfamethoxazole, Sulfamethazine), Macrolides (Erythromycin, Roxithromycin, Tylosin)	Sediment 1 g Liquid sample after LLE (McIlvaine buffer solution or ammonium hydroxide buffer solution with 200 µL of 5% Na_2EDTA (1 mmol in solution), 20 mL)	Clean-up: Oasis HLB, 60mg/3mL, Waters	1. Methanol, 3 mL 2. Deionized water, 3 mL	Methanol, 5 mL	LC-MS^2	[10]
Oxytetracycline, Sulfachloropyridazine, Tylosin	Soil 4g, pig slurry 2 mL Liquid sample after USE (methanol : 0.1M EDTA : McIlvaine buffer, pH 7 = 50:25:25)	Clean-up: tandem SAX, IST - Oasis HLB, Waters	1. Methanol 2. Conditioning buffer (mixture of methanol, 0.1M EDTA and McIlvaine buffer; pH 2.9)	Methanol, 2 × 1 mL (SAX was remove)	HPLC-UV HPLC-FD	[22]

retention of pharmaceutical compounds from water samples. In these studies, Strata-X was selected as the best phase for extracting sulfonamides, tetracyclines, [28,29], fluoroquinolones, penicillin G procaine and trimethoprim in mixture [29] by off-line SPE. For example, with this type of sorbent material, high recoveries were obtained for all the pharmaceuticals investigated (i.e. >80%). A big challenge was solving the extraction problem of sulfaguanidine (e.g., C18, C8, St-DVB, CN, and ion-exchange sorbents, except Strata-X, gave poor recoveries for sulfaguanidine). The reason for the problem was probably because sulfaguanidine is a polar molecule with extremely high pK_a value (see Table 2) and is the smallest molecule.

In many of the analytical methods described in the literature, the target compounds are analyzed simultaneously with other pharmaceuticals (often with quite different physico-chemical characteristics) in a multi-residue method. This simultaneous analysis of several groups of compounds generally requires a compromise

Table 6. Survey of MIP solid-phase extraction methods for extraction of pharmaceuticals from environmental samples

Pharmaceuticals	Sample	Sorbent type	Conditioning solvent	Elution solvent	Final analysis	Refs.
Sulfonamides (Sulfamethazin, Sulfamethoxazole)	Standard solution in water	MAA = functional monomer; EGDMA = cross-linker; SMETH = print molecule; AIBN = initiator	–	–	HPLC	[32]
Trimethoprim	Human urine, 25 mL	MAA = functional monomer; EGDMA = cross-linker; TMP = print molecule; AIBN = initiator; chloroform = proton solvent	Ethanol, 3 mL	7% TFA in methanol	HPLC	[33]
Tetracyclines (e.g., Oxytetracycline, Tetracycline)	Tissue 5 g Liquid sample after LLE with 3 × 20 mL of EDTA-McIlvain buffer	Clean-up: MAA = functional monomer; EGDMA = cross-linker; TC and OTC = print molecules; AIBN = initiator	Deionized water (pH 11.0 with 0.1M NaOH)	10% KOH in methanol, 20 mL	HPLC-UV	[34]

in the selection of experimental conditions, which, in some cases, means not obtaining the best performance for each compound [30].

RAMs and MIPs are special types of very specific and selective sorbents. RAMs are SPE sorbents that are often used for the analysis of small drugs, their impurities and metabolites. MIPs are highly stable polymers that possess recognition sites adapted to the three-dimensional shape and functionalities of an analyte of interest [31]. Several papers have outlined the development of MIP sorbents for sulfonamides [32], trimethoprim [33] and tetracyclines [34]. Table 6 shows the basic conditions for MIPs.

MIPs can solve every extraction problem especially with polar compounds (e.g., sulfaguanidine, as mentioned above). MIPs comprise a very promising type of sorbent, but work with them is time consuming, and requires patience and some skill. In future, MIPs will probably be adjusted to many other classes of pharmaceuticals.

2.2. Solid-phase microextraction (SPME)

SPME is a modern sampling or sample-preparation method used for isolating and pre-concentrating organic molecules from gaseous, liquid and solid samples. It is highly sensitive and can be used for polar and non-polar analytes with different types of matrix. The mechanism of SPME is similar to that of SPE because SPME is a miniature version of SPE, the only difference being the volume of sorbent. SPME uses a short piece of a fused-silica fiber coated with a polymeric stationary phase placed on a syringe. During transport, storage and manipulation, the fiber is retracted into the needle of the device. The process continues until equilibrium is reached between coating and sample. When gas chromatography (GC) is used, analytes are thermally desorbed from the fiber in a GC injector. Coupling of SPME with high-performance liquid chromatography (HPLC) requires a special interface with liquid desorption [35].

There are currently three SPME modes that require fused-silica fibers or GC capillary columns:

- headspace (HS) and direct-immersion (DI)-SPME are the two fiber-extraction modes; and,
- the GC capillary column mode is in-tube SPME (ITSPME).

DI-SPME is the most common mode and is conducted by direct insertion of the fiber into the sample matrix [36]. ITSPME is an effective sample-preparation technique based on the use of a fused-silica capillary column as an extraction device [37].

The main disadvantage of SPME in fieldwork is its lack of robustness. The needle can be easily bent and the fiber has limited time of usage [3,37].

SPME has become prominent as a sample-preparation technique for analyzing pharmaceuticals from environmental samples.

Balakrishnan et al. [11] compared DI-SPME with SPE procedures for extracting sulfonamides from wastewater. SPE was not effective for the determination of sulfasalazine (not detectable after SPE) as opposed to SPME, which extracted all the sulfonamide compounds with an efficiency of >75% (except sulfamethazine (39.8%) and sulfamethoxazole (59.2%)). The same paper described optimization of the SPME method, and the results are shown in Table 7. Table 8 displays a few ITSPME applications for the determination of pharmaceuticals.

2.3. Stir-bar sorptive extraction (SBSE) [40]

This sorptive and solventless extraction technique is based on the same principles as SPME, but, instead of a polymer-coated fiber, a large amount of the extracting phase is coated on a stir-bar. The most widely used sorptive extraction phase is polydimethylsiloxane (PDMS) (as in SPME).

Extraction of an analyte from the aqueous phase into an extraction medium is controlled by the partitioning coefficient of the analyte between the silicone phase and the aqueous phase ($K_{PDMS/w}$). Recent studies have correlated this partitioning coefficient with octanol–water

Table 7. Example of SPME method for the extraction of pharmaceuticals from aquatic samples

Pharmaceuticals	Sample	SPME optimization parameters	Desorption	Final analysis	Ref.
Sulfonamides (e.g., Sulfaguanidine, Sulfadiazine, Sulfamethazine, Sulfamethoxazole)	Wastewater 25 mL	PDMS 100 μm; CW/DVB 65 μm; CW/TPR 50 μm*; PA 85 μm; PDMS/DVB 60 μm ionic strength: 5%, 10%*, 15%, 20% KCl; extraction time: 5, 10, 15, 20*, 25, 30, 35, 40 min; pH: 3.0, 4.5*, 5.3, 6.0	Methanol, 100 μL 30* min 60 min	LC-ESI-MS^2	[11]

*Optimal conditions.

Table 8. Survey of ITSPME methods for the extraction of the pharmaceuticals from liquid samples

Pharmaceuticals	Sample	Fiber	Desorption	Final analysis	Refs.
Sulfonamides (e.g., Sulfadiazine, Sulfamethazine, Sulfamethoxazole)	Milk	Poli(MAA-EGDMA)	Methanol : 0,02M Na_2HPO_4 = 3:7, v/v; pH 3.0	HPLC-UV	[38]
Fluoroquinolones (e.g., Ciprofloxacine, Norfloxacine, Enrofloxacine)	Surface water, Wastewater samples 1 mL	CAR 1010 PLOT 17 μm, Supelco*; CAR 1006 PLOT, Supelco; Supel-Q-PLOT, Supelco; CP-sil 5CB, Varian; CP-sil 19CB, Varian; CP-wax 52CB, Varian	5mM ammonium formate pH 3.0 : acetonitrile = 85:15, v/v	LC-MS^2	[39]

*Optimal conditions.

distribution coefficients (K_{ow}). Due to the similarity of $K_{PDMS/w}$ to K_{ow}, chemists can predict extraction efficiencies (SBSE can be used only for hydrophobic compounds with log $K_{ow} \geqslant 2$; and, a high enrichment factor could be obtained for analytes even with log $K_{ow} > 5$). However, in SPME, the amount of extraction medium (e.g., the amount of PDMS coated on the fiber) is very limited. For a typical 100-μm PDMS fiber, the volume of the extraction phase is approximately 0.5 μL. However, the amounts of the extraction phase in SBSE are 50–250 times greater.

After extraction and thermal desorption, the analyte can be introduced quantitatively into the analytical system. This process provides high sensitivity, since the complete extract can be analyzed. In contrast to SPME, the desorption process is slower because the extraction phase is extended, so desorption needs to be combined with cold trapping and reconcentration. Alternatively, analysts can use liquid desorption.

In the past few years, SBSE has been developed rapidly and successfully applied to the trace analysis of various target analytes in environmental and biological samples with extremely low limits of detection (LODs) of 0.1 ng/L.

Tienpont et al. [41] successfully applied SBSE to the analysis of drugs (e.g., barbiturates and benzodiazepams) and metabolites in urine and blood. For that purpose, they used a glass stir bar coated with a thick layer (24 μL) of PDMS.

2.4. Membrane extraction

Membrane extraction is one of the attempts to automate LLE. A membrane can act as a selective filter, either just limiting diffusion between two solutions or as an active membrane in which the chemical structure of the membrane determines the selectivity of sample transfer [13].

The most important technique of membrane extraction is supported liquid membrane (SLM) extraction, which is based on a three-phase system (aqueous/organic/aqueous) wherein a thin film of an organic phase is immobilized in a hydrophobic porous polymer membrane, which is placed between two aqueous liquids (the donor and the acceptor) in a flow system. Different transport mechanisms can be utilized, but all the analytes have to pass through the organic membrane liquid as uncharged species by a diffusion process. Distribution coefficients determine the driving force of the analytes into the organic solvent [42]. Moreover, SLM can easily be combined with various analytical instruments online.

The second membrane-extraction technique is microporous membrane LLE (MMLLE) based on a two-phase system (aqueous/organic).

These techniques offer a number of advantages compared to classical LLE, such as higher selectivity, higher volume ratios and enrichment factors, very clean extracts, less or no consumption of organic solvents, and considerably easier automation [42]. They are mostly suitable for analytes with high or moderate polarity (e.g., sulfonamides) and they are particularly useful when size or charge can be used to achieve selection.

In food analysis, membrane extraction is also used to separate high molecular weight species. For trace contaminants, direct application of membrane separations is limited and automation is difficult [14].

Table 9 shows methods for pharmaceutical determination that use SLM extraction for sample preparation. Msagati et al. [18] compared SLM with SPE on Waters Oasis MCX sorbents (Table 3) for extraction of benzimidazole compounds. When water was spiked with analyte concentrations in the range 0.1–1 ng/L and enriched, recovery obtained by SLM was in the range 63–99% (i.e. 63–95% for fenbendazole), while recovery obtained by SPE was 67–99% (i.e. 70–92.88% for fenbendazole). This shows the validity and the applicability of SLM.

Table 9. Survey of membrane-extraction procedures for the extraction of pharmaceuticals from environmental samples

Pharmaceuticals	Sample	Membrane	Membrane liquids	Final analysis	Refs.
Benzimidazole anthelmintics (Albendazol, Fenbendazol, Mebendazol, Oksibendazol, Tiabendazol)	Water, milk, urine 1.5 mL	Porous PTFE, type FG Millipore (impregnated by 5% TOPO in *n*-undecane: di-*n*-hexylether = 1:1*; hexylamine; *n*-undecane, di-*n*-hexylether; *n*-undecane/di-*n*-hexylether (1:1))	Donor phase: buffer $NaOH/NaHCO_3$ pH 9.6; 1.2 mm Acceptor phase: 0.6 mm	LC-ESI-MS	[18]
Sulfonamides (e.g., Sulfaguanidine, Sulfamethoxazole, Sulfamethazine, Sulfadiazin)	Water 10 mL, milk, urine pH 6.0, liver tissue 5.0 g, kidney tissue 5.0 g	Porous PTFE, type FG Millipore (impregnated by 5% TOPO in hexylamine; di-*n*-hexylether; *n*-undecane; di-*n*-hexylether : *n*-undecane = 1:1; 5% TOPO u di-*n*-hexylether : *n*-undecane = 1:1; hexylamine)	Donor phase: buffer pH 6.0 Acceptor phase: buffer pH 10.0	HPLC-MS	[43]

*Optimal conditions.

2.5. Liquid-phase microextraction (LPME)

LPME, or miniaturized LLE, is a relatively recent technique. Normally, it is carried out using a membrane as an interface between the sample (donor) and the organic solvent (acceptor), as that avoids mixing the two phases and other problems encountered in classical LLE. The main advantages of LPME are very low consumption of organic solvent, low cost, high selectivity and clean extracts [20,44].

Hollow-fiber LPME is an alternative to LPME based on a porous polypropylene hollow fiber, which is placed in an aqueous sample (0.1–4 mL). Prior to extraction, the hollow fiber has been soaked in an organic solvent to immobilize the solvent (15–20 μL) in the pores of the hollow fiber. This solvent is immiscible with water and forms a thin layer within the wall of this hollow fiber (thin layer thickness = 200 μm). Analytes are therefore extracted from the aqueous sample, through the organic phase in the pores of the hollow fiber, and further into an acceptor solution inside the lumen of the hollow fiber. In that way, the final micro-extract is not in direct contact with the sample solution. If the acceptor solution is the same organic solvent as that inside the hollow-fiber pore, then we have two-phase LPME. If it is aqueous, we talk about three-phase LPME. Thus, hollow-fiber LPME is a more robust and reliable alternative of LPME. In addition, the equipment needed is very simple and inexpensive [20,44].

Most published works on LPME focus on fundamental aspects, but its applicability in drug analysis (human plasma, whole blood, urine, saliva, and breast milk) and environmental monitoring has been also discussed [44].

Several different classes of drugs have been extracted by LPME. Special attention has been paid to anti-inflammatory agents, analgesics, psychoanaleptics, antihistamines and some drugs of abuse. Most of these compounds are relatively hydrophobic bases and are generally extracted in three-phase LPME with recoveries in the range 40–90%.

Quintana et al. [20] have evaluated the applicability of hollow-fiber LPME for the extraction or enrichment of acidic pharmaceuticals (e.g., ibuprofen, clofibric acid, bezafibrate, and diclofenac) from water samples prior to the determination by LC-ESI-MS^2. The mean recovery of these acidic drugs stays within the range $93 \pm 35\%$ for treated wastewater and $123 \pm 45\%$ for raw wastewater. A large relative standard deviation is a consequence of relatively low precision of LPME as a result of a small extract volume.

2.6. Supercritical fluid extraction (SFE)

Supercritical fluids (SCFs) include properties of both liquids and gases while their density correlates with temperature and pressure. They offer a considerable promise as a media for selective isolation of target compounds for complex matrices. The main advantages of using SCFs for extraction are because they are inexpensive, extract analytes in a faster manner and are more environmentally friendly than organic solvents. For these reasons, CO_2 is a reagent widely used as a supercritical solvent in SFE [13]. Apart from CO_2, other potential SCF solvents are N_2O, xenon, C_2H_6, C_3H_8, n-C_5H_{12}, NH_3, CHF_3, SF_6 and water [45]. However, some of them are dangerous (e.g., N_2O, due to its oxidizing power) whereas some are more exotic solvents (e.g., xenon) and have been ruled

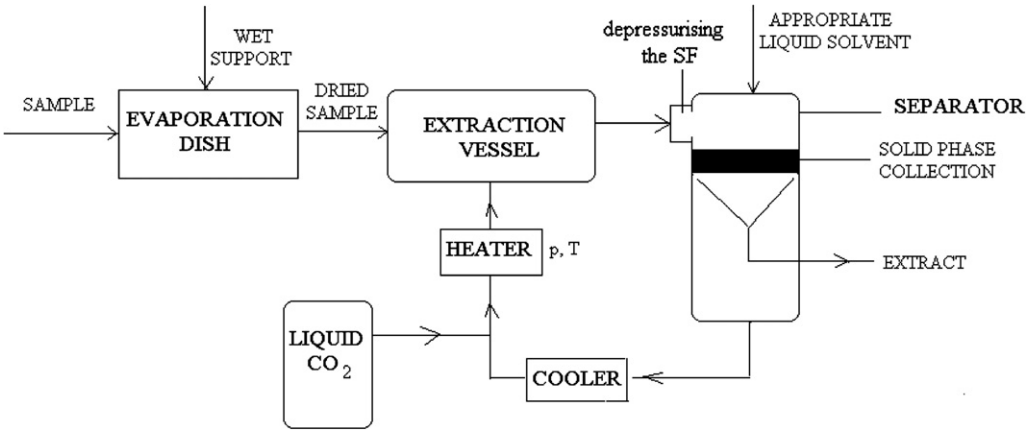

Figure 3. Supercritical fluid extraction (SFE).

Table 10. References involving the superfluid extraction of pharmaceuticals from environmental samples

Pharmaceuticals	Sample	Solvent	Conditions	Clean-up	Final analysis	Refs.
Sulfonamides (Sulfamethazine, Sulfadimethoxine, Sulfaquinoxaline)	Chicken liver 1.0 g	CO_2	680 bars, 40°C, 5 min, flow rate 2.5–2.7 L/min	Al_2O_3 2.0 g/6 mL Elution: 4 mL HPLC mobile phase (0.05 M phosphate buffer (pH 7.0) containing 0.1% tetrabutylammonium hydroxide : methanol = 68:32)	HPLC-DAD	[46]
Benzodiazepines (Diazepam, Oxazepam, Nordiazepam, Prazepam, Temazepam), Anabolic agents (e.g., Fluoxymestrone, Nortestosterone, Methyltestosterone), Non-steroidal anti-inflammatory drugs (Tolmetin, Ketoprofen, Fenbufen, Indometacin, Naproxen)	Water serum	CO_2	45°C, 329 MPa	–	HPLC	[47]

out because of their cost. Sometimes, the relatively low polarity of CO_2 may be a major problem, especially for most pharmaceuticals and drug samples. By adding modifiers to SCF (like methanol to CO_2), its polarity can be changed to make separation more selective. Fig. 3 shows a scheme for SFE.

Two of the main problems with SFE are the robustness of the method compared to other techniques and that conditions must be consistent for reproducible extractions. The automated systems available are aimed mainly at the environmental area, rather than trace analysis in food. The presence of water and fat in food samples can require extensive sample preparation and development of more on-line clean-up procedures [14].

SFE is used a sample-preparation technique for the extraction of pharmaceuticals from liquid and solid samples. Few authors have reported the SFE of environmental samples that contain different therapeutic classes of pharmaceuticals (Table 10).

2.7. Pressurized liquid extraction (PLE)

PLE (Fig. 4) employs a closed flow-through system that uses conventional organic solvents at elevated temperatures above their atmospheric boiling points. A restriction or backpressure valve ensures that a solvent remains liquid but with enhanced solvation power and lower viscosities and hence higher diffusion rates. Both changes increase the extraction rate, and both static and flow-through designs can be used. In the latter, a fresh solvent is introduced to a sample, improving the extraction but diluting the extract [13].

PLE has advantages over other methods (e.g., better reproducibility, reduced use of extraction solvent and reduced time for sample preparation). Extracts are

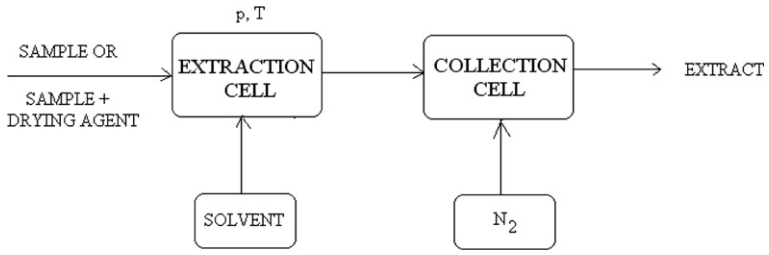

Figure 4. Pressurized liquid extraction (PLE).

generally much more concentrated than with conventional extraction methods. Depending on author or instrument manufacturer, the technique has been also referred to as pressurized fluid extraction (PFE), pressurized solvent extraction (PSE), enhanced solvent extraction (ESE) and accelerated solvent extraction (ASE).

PLE has been applied to a number of matrices. Many applications for soil and environmental samples have been reviewed [13]. Stoob et al. [12] have developed a method for the PLE of sulfonamide antibiotics from aged agricultural soils. The optimal extraction conditions are as follows: temperature of extraction, 200°C; pressure, 100 bar; extraction time, 9 min; pH of soil samples, 8.8; and, extraction solvent, 15% acetonitrile in water.

For antimicrobials, PLE has been a very effective technique for isolating analytes from fat-containing matrices. It can use water at high pressure and high temperature to extract polar drugs [48].

In PLE and other sample-preparation methods for solid samples, it is very important to take account of the temperatures of degradation of selected compounds and their specific behavior. For example, for efficient extraction of tetracyclines, the sample matrix is acidified with citrate buffer or EDTA solution at pH 4.7 to avoid complexation of these substances with cations (Ca^{2+}, Mg^{2+} or Fe^{3+}). Moreover, extraction at room temperatures is preferable for tetracyclines that may convert into their epi or anhydro forms when they are heated. The degradation of macrolides has also been observed at temperatures above 100°C [6].

2.8. Matrix solid-phase dispersion (MSPD)

MSPD involves blending a viscous, solid or semi-solid sample (approximately 0.5 g) with a solid support (a four-to-one ratio of support to sample) (e.g., silica) that has been derivatized to produce a bound organic phase (e.g., octadecylsilyl (C18)) on its surface. The materials for solid support are the same as those used for packing SPE columns. Once the MSPD blending process is complete, the material is transferred to a column similar to the SPE column [49].

This technique has found favor in many applications because it eliminates most of the complications of LLE and/or SPE of solid and semi-solid samples. MSPD columns permit isolation of analytes of different polarities or entire chemical classes of compounds [50]. The selectivity of MSPD depends on the sorbent/sample combination used. MSPD has been most frequently applied to the isolation of drugs and other pollutants from animal tissues, fruits and vegetables. Nevertheless, the use of MSPD for pharmaceutical extraction has been reported in only a limited number of publications, some of which are shown in Table 11.

2.9. Dispersive solid-phase extraction (DSPE)

DSPE is similar to MSPD, only a sorbent is added to an aliquot of the extract rather than to the original sample, as in MSPD. High cost of the sorbent limits the sample size that can be used in MSPD. This leads to a concern about sample representation and homogeneity. Nevertheless, DSPE relies on the extraction process to provide a homogenous aliquot from an original sample of any size and only a small amount of sorbent is used [52].

DSPE has found its way to environmental analysis. Posyniak et al. [52] developed DSPE for the determination of sulfonamide levels in chicken-muscle tissue.

2.10. Ultrasonic extraction (USE)

USE is often used for extraction of pharmaceuticals from solid samples [22]. This method puts in mechanical energy in the form of a shearing action, which is

Table 11. Example of MSPD method for the extraction of pharmaceuticals from solid samples

Pharmaceuticals	Sample	Sorbent type	Solvent	Final analysis	Ref.
Sulfonamides (e.g., Sulfadiazine, Sulfamethazine, Sulfamethoxazole)	Meat (beef, pork, chicken) 0.5 g	Al_2O_3-N-S, ICN Biochemicals	70% ethanol, 10 mL	LC-MS	[51]

produced by a low-frequency sound wave. The sample with added solvent is immersed in an ultrasonic bath and subjected to ultrasonic radiation for few minutes. Extracted analytes are separated from the matrix by vacuum filtration or centrifugation. The process is repeated two or three times to achieve higher extraction efficiency, and the extracts are combined for analysis.

USE has the benefit of shortened extraction times compared to classical liquid extraction methods. The main disadvantage of USE is poor reproducibility due to lack of uniformity in the distribution of ultrasound energy. However, as both selectivity and sample-enrichment capabilities are limited, further clean-up and/or concentration steps are usually required for determination of trace analytes [14].

2.11. Microwave-assisted solvent extraction (MASE)

MASE [53] involves heating solid sample-solvent mixtures in a closed vessel with microwave energy under temperature-controlled and pressure-controlled conditions. This closed extraction system enables analyte extraction with elevated temperatures and pressure accelerating the extraction process and yielding a performance comparable to the standard Soxhlet method. As extraction solvents, polar liquids or mixtures of polar and non-polar liquids are used because only polar compounds absorb microwave energy.

After the heating cycle is completed, samples are cooled and filtered in order to separate the extract for analysis. It is only applicable to thermally stable compounds [14].

This technique has always been used for extraction of different compounds from plant materials, soils and sediments. Akhtar and Croateau [54] developed MASE for extraction of salinomycin from finished feed with ethanol – 2-propanol (15+2) extraction solvent.

3. Conclusion

It is well known that sample preparation is one of the most critical steps in the determination of trace pollutants in different environmental matrices. Recently, sample-preparation methods have been significantly improved.

As the use of pharmaceuticals is increasing, more sample-preparation procedures are being developed. Among them, SPE is the most popular for drug analysis and has become an essential tool in laboratories all over the world. It has also largely replaced older techniques. The development of SPE has been fast and accompanied with many improvements. One of these improvements is MIPs. Because of their specific and selective properties, their use will probably be broader in the future, especially in forensic, clinical, pharmaceutical and biochemical analyses.

SPME and membrane extraction are becoming attractive alternatives to SPE in terms of liquid samples, while PLE, MSPD and MASE are good alternatives for pharmaceuticals involving solid samples.

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