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Preconcentration of pharmaceuticals residues in sediment samples using microwave assisted micellar extraction coupled with solid phase extraction and their determination by HPLC–UV

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Abstract

An analytical method combining microwave assisted micellar extraction (MAME) and solid phase extraction (SPE) has been developed to extract and preconcentrate a selected group of eight pharmaceutical compounds in sediment samples prior to their determination using liquid chromatography with an UV–DAD detector. A non-ionic surfactant, Polyoxyethylene 10 lauryl ether (POLE) was used for the MAME extraction and the different parameters for the optimization process were studied. Then, SPE was used to clean-up and preconcentrate the target analytes in the extract, prior to their determination using HPLC–UV. The method was applied to the determination of the selected pharmaceuticals compounds in several kinds of sediment samples with different characteristics. Relative recoveries for spiked sediment samples were over 70% and relative standard deviations (RSDs) were under 11% for all recoveries tested. Detection limits between 4 and 167 ng g^{-1} were obtained. The method was validated using Soxhlet extraction procedure.

Keywords: Pharmaceuticals residues; Microwave assisted micellar extraction; Solid phase extraction; HPLC–UV–DAD

1. Introduction

Residues of pharmaceutical compounds end up in the environment due to the common practices to improve the state of health not only humans but also in animals [1].

Concentration of pharmaceuticals in the environment, their evolution with time and their possible effects depend not only on the quantity of drugs manufactured and the dosage frequency but also the amount discharged from wastewater treatment plants (WWTPs) [2,3]. Effluents from WWTPs can be considered one of the most important sources of pharmaceuticals residues in the environment. Conventional wastewater treatment processes are not specifically designed to remove pharmaceuticals, so they often do not eliminate them efficiently [4].

It is only recently that attention has been given to the potential contamination caused by these pharmaceuticals residues in sediments samples [5]. Published analytical methods for these compounds are typically specific to a simple contamination or pharmaceuticals class [6–10].

For the efficient and timely determination of the variety of pharmaceutical residues present in the environment, a multi-residue analytical method must be established. The main objective of this research was develop a time and cost-effective analytical method for the simultaneous determination of eight common pharmaceutical compounds including anti-inflammatory drugs (ketoprofen, naproxen, ibuprofen), lipid regulating agents (bezafibrate, clofibric acid), a β-blocker (propranolol), an antiepileptic (carbamazepine) and an analgesic (phenazone) in sediment samples.

Environmental concentrations of pharmaceuticals compounds are very low, depending on sample matrices. Therefore, analytes need to be extracted and preconcentrated prior to instrumental analysis.

For soil samples, various extraction methods have been selected, such as supercritical fluid extraction (SFE) [11–13], pressurized liquid extraction (PLE) [14], and microwave assisted extraction (MAE) [15–17], which have been used to enhance the extraction efficiency.

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Although the advantages of these procedures include reduced solvent volume and shorter analysis time, they use organic solvent. A new possibility for the application of microwave assisted extraction is the use of micellar media as extractants (MAME), which has been applied to the extraction of several compounds present in different environmental samples [18–20].

Sample extracts obtained from solid matrices are frequently interfered with other components which may affect to the signal of target analytes. For this reason, it may be necessary to introduce an additional clean-up step before HPLC–UV determination [21]. In this case, solid phase extraction (SPE) is the selected method for sample clean-up. Solid phase extraction is an extraction/preconcentration procedure commonly applied to liquid samples. However, another possibility is intensity target analytes signal by means of extract clean-up and preconcentration. In this field, the use of solid phase extraction was investigated as a secondary step of several extraction methods such as MAE [22]. SPE allows clean-up and preconcentration to be carried out at the same time.

This paper describes the performance of MAME methodology using a non-ionic surfactant, Polyoxyethylene 10 lauryl ether (POLE), as extractant and coupling this with to solid phase extraction to clean-up and preconcentrate eight pharmaceutical compounds in solid samples, following determination by liquid chromatography using UV–DAD detection. The performance and application of this method in sediments samples is important due to the difficulty in extracting the pharmaceuticals from such complex matrices. In fact, there are very few publications that cover these types of matrices.

Finally, the optimized methodology was successfully applied to the analysis of target compounds in sediment samples with different characteristics.

2. Experimental

2.1. Reagents

Pharmaceutical standards (phenazone, carbamazepine, clofibric acid, ketoprofen, naproxen, bezafibrate, ibuprofen and propranolol) were provided by Sigma–Aldrich (Steinheim, Germany). All pharmaceutical standards were 97–99% pure, and are listed in Table 1 (numbers in tables and figures).

Table 1
Analytes under study and chromatographic parameters

Compound	Identification number	t_r[a] (min)	λ[b] (mn)
Phenazone	1	2.00	255
Carbamazepine	2	9.40	220
Clofibric acid	3	14.50	220
Ketoprofen	4	15.90	255
Naproxen	5	18.50	230
Bezafibrate	6	21.20	230
Ibuprofen	7	27.80	220
Propranolol	8	32.50	220

[a] Retention time.
[b] Determination UV wavelength.

Individual standard solution of these compounds were prepared in methanol at 100 μg mL^{-1}, and stored in the dark at 4 °C prior to use.

The non-ionic surfactant Polyoxyethylene 10 lauryl ether (POLE) was obtained from Sigma (St. Louis, MO, USA) and prepared in ultra-high quality water (Milli-Q Water System).

HPLC-grade methanol was obtained from Panreac Quimica S.A. (Barcelona, Spain) and the acetic acid glacial was obtained from Scharlau Chemie S.A. (Barcelona, Spain). All the solvents were filtered through a 0.22 μm nylon membrane filter.

The SPE cartridges used were: 6-mL disposable OASIS HLB (200 mg, Waters Corp., Milford, MA), 6-mL disposable Sep-Pak C18 (500 mg, Waters Corp., Milford, MA), 6-mL disposable ENVIRELUT-PESTICIDE (500 mg Varian Corp., Madrid Spain), 6-mL BOND ELUT ENV (500 mg Varian Corp., Madrid Spain), 6-mL BOND ELUT FL (1000 mg Varian Corp., Madrid Spain).

2.2. Apparatus

The microwave system was a Multiwave with a 6 EVAP rotor and 6 MF100 vessels (Anton Paar, Gra 2 Austria).

A pH-meter (Crison, Spain) was used for the characterization of the sediment samples.

The HPLC system was equipped with Millenium chromatography manager software, two Waters 515 pump (Waters, Milford, MA, USA), fitted with an injector Rheodyne model 7725, and Waters 996 photodiode array detection (DAD) system (Waters, Milford, MA, USA) to detect the target compounds.

The stationary phase column was a Waters Nova-Pack C18, 150 mm × 3.9 mm, 4 μm particle diameter (Waters Associates).

2.3. Procedure

2.3.1. Characterization of the samples: organic matter and pH determination

Several soil samples were obtained from different areas of Gran Canaria Island: Maspalomas (in the South), the Cicer (Northeast), Sardina (North) and Taurito (Southwest). The Sauerlandt and Berwecke method [23] was used to determine the organic matter content in the samples, which consists in organic matter oxidation using potassium dichromate and sulphuric acid.

The Official Method 994.6 of the AOAC [24] was followed to determine the sediment pH. Five grams of each sediment samples were mixed with double distilled water, stirred and then the pH in the supernatant water was measured.

2.3.2. Spiking of samples

Two grams of sediment sample were spiked with the pharmaceutical mixture in methanol to obtain a final concentration of 2 μg g^{-1} of each analyte. Before analysis, the samples were shaken and stored overnight in the dark, in order to obtain a dry and homogeneous sample.

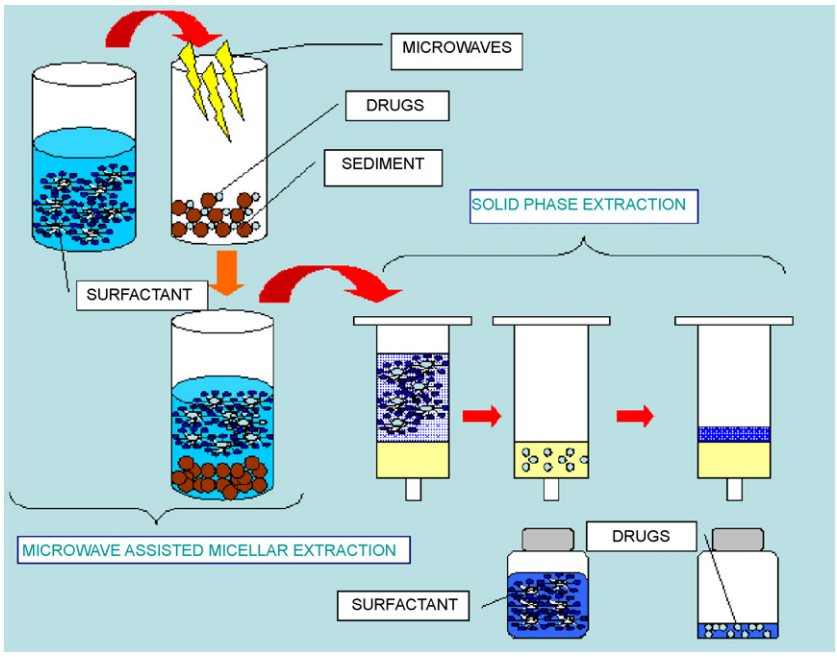

Fig. 1. Scheme of MAME–SPE procedure.

2.3.3. Microwave assisted micellar extraction coupled Solid phase extraction

The spiked samples were introduced into Teflon vessels. The optimum volume and concentration of non-ionic surfactant was added. The vessels were placed in the microwave oven, irradiated at the optimized conditions and then allowed to cool at room temperature. The surfactant extract was carefully removed, filtered and introduced into the cartridge. The cartridges were conditioned before use in the absorption process, by washing with 2 × 5 mL methanol and 2 × 5 mL Milli-Q water. The surfactant extracts (8 mL) with 14 mL of water acidulated to pH 3.0 were subsequently passed through the cartridges under vacuum at a flow rate of 10 mL min^{-1} and washed with 2 × 5 mL Milli-Q water. The cartridges were vacuum-dried for 5 min and eluted with 2 × 0.75 mL methanol at a flow rate of 1 mL min^{-1}. The eluted mixture was introduced into hermetically closed vials before analysis in the HPLC–UV system. Fig. 1 shows a scheme of the MAME–SPE procedure with all the different specific steps and stages represented.

2.3.4. Chromatographic analysis

Quantification was carried out using a HPLC–UV system by injecting 50 μL of extract into the liquid chromatograph. The chromatographic parameters are shown in Table 1.

Separation of the analytes was achieved by a methanol/water (pH 3.0 with acetic acid) gradient programme (Fig. 2) with a flow 1 mL min^{-1}.

The conditions used were the same for the analysis of both the MAME–SPE and the Soxhlet extracts.

The range of the calibration curve concentration was between 0.1 and 7.0 μg mL^{-1} for phenazone, clofibric acid, bezafibrate and propanolol and between 0.01 and 7.0 μg mL^{-1} for carbamazepine, ketoprofen, naproxen and ibuprofen.

2.3.5. Conventional Soxhlet extraction

Three grams of the spiked sediment were placed in a cellulose thimble (25 mm × 88 mm, Albet, Barcelona, Spain) and extracted with dichloromethane for 24 h at 4–6 cycles/h.

After extraction, the dichloromethane extracts were evaporated with nitrogen to dryness and redissolved with 1 mL of methanol prior to injection into in the HPLC–UV system.

2.3.6. Statistical analysis

All statistical tests (ANOVA experimental design) were performed using Stagraphics Plus software, version 5.0 (Manugistic, Rockville, MD, USA).

Statistical tests were carried out using SPSS 11.0 (Chicago, IL, USA).

3. Results and discussion

3.1. Optimization of the microwave assisted micellar extraction (MAME)

Several parameters can alter the extraction efficiency of the MAME process such as: surfactant volume and concentration, extraction time and power. The effects of these parameters were studied using marine sediment samples from Cicer beach (in the Northeast of Gran Canaria Island) with the characteristics as specified in Table 7.

3.1.1. Effect of the extractant volume

A preliminary study was carried out in order to check if the volume of extractant to be added would affect the analytes extraction due to possible evaporation, losses or incomplete interaction with the sample. In this way, measurements of the analyte recoveries were carried out using 5, 8 and 20 mL of

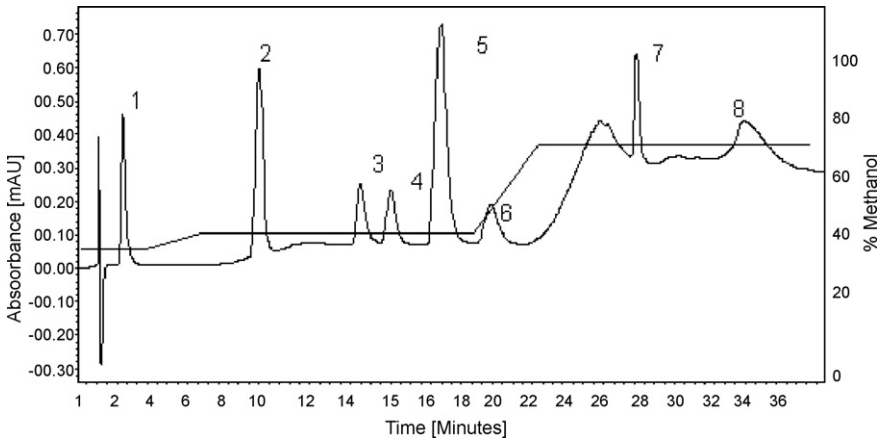

Fig. 2. HPLC–UV chromatogram of pharmaceuticals mixture (5 μg mL^{-1} for each analytes) in methanol. The numbering refers to Table 1.

POLE at a concentration of 5% (v/v) and 2 g of sample. At extraction volumes under than 5 mL, irreproducible data were obtained due to the insufficient covering of the extractant. On the other hand, a volume of 20 mL leading to evaporation loss due to the high temperatures reached. This latter effect may be due to the high capacity of the aqueous surfactant solutions to absorb the microwave radiation and transform it in heat [25]. Thus, a volume of 8 mL was chosen for subsequent studies in order to ensure that the sample was totally covered by the surfactant.

3.1.2. *Effect of the microwave conditions and the surfactant concentration*

The irradiation time and power are parameters that are interrelated, and the surfactant concentration may be of great influence in the extraction efficiency. Multivariable factorial design or central composite design has been used to find the optimal extraction conditions [26,27]. To that end, a 2^3 factorial design was made. The experimental design parameters for the screening are shown in Table 2. In this study, the variable correlations showed that microwave radiation power and surfactant concentration were the parameters that bear greatest influence on the extraction efficiency.

The surfactant concentration and microwave power were optimized together using a response surface with 3^2 factorial design with duplicated central points. This allowed for the direct evaluation of the variable under consideration [28]. Ranges of microwave power between 50 and 800 W and a surfactant concentration between 0.5 and 5% (v/v) were studied. Fig. 3 shows the response surface profile for two of the pharmaceutical compounds: clofibric acid (a) and bezafibrate (b). In both cases, it can be observed that the optimum extraction conditions were obtained for intermediate powers and intermediate surfactant concentration. The behaviour was similar over the rest of

Table 2
Design matrix in the screening design 2^3

Run number	Power (W)	Time (min)	Surfactant concentration (%, v/v)
1	100	2	0.5
2	800	2	5.0
3	800	10	5.0
4	800	10	0.5
5	100	10	0.5
6	800	2	0.5
7	100	10	5.0
8	100	2	5.0
9	50	2	5.0

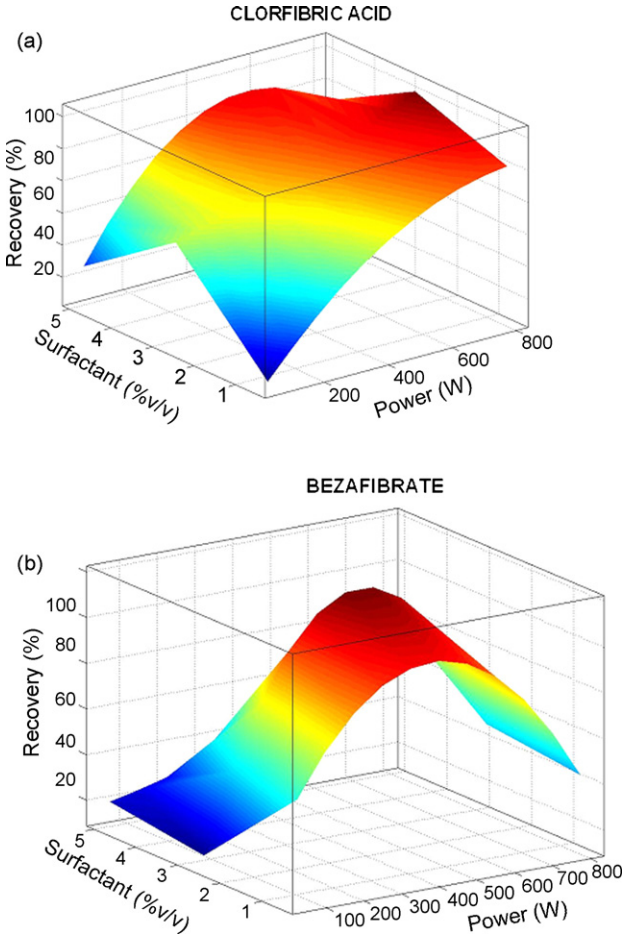

Fig. 3. Response of surface profile of the extraction of clofibric acid (a) and bezafibrate (b) using POLE as extractant.

Table 3
Recovery percentages obtained to different kinds of cartridges in the SPE process

Compound	Type of sorbent				
	OASIS HLB	BOND ELUT FL	SEP-PAK C18	ENVIRELUT-PESTICIDE	BOND ELUT ENV
Phenazone	6	49	0.3	0.3	102
Carbamazepine	116	34	56	44	91
Clofibric acid	99	n.d.	27	20	92
Ketoprofen	100	1	61	68	61
Naproxen	86	2	59	66	46
Bezafibrate	104	8	46	40	67
Ibuprofen	114	n.d.	84	88	69
Propanolol	106	11	65	79	78

the pharmaceuticals that were extracted with this surfactant. In order to determine more precisely the surfactant concentration and microwave power, the equation adjusted to the behaviour of each pharmaceutical was used to calculate the percentage of maximum recovery for each analyte. Indeed the averages obtained demonstrated that a surfactant concentration of 2.75% (v/v) POLE and a radiation power of 500 W were the optimum conditions.

3.2. Optimization of the solid phase extraction

Before analysing the MAME extract using HPLC–UV, a clean-up and preconcentration stage was required to remove the interferences that could be extracted together with the target analytes. Solid phase extraction is widely used in sample extraction and analyte enrichment [29].

There are various different parameters that may influence in the preconcentration process such as the cartridge type and the solvent volume.

First, we studied various different SPE cartridges to obtain the best extraction efficiencies. In this study, five cartridges were selected for the evaluation of the extraction efficiency of pharmaceutical residues: 6-mL disposable OASIS HLB, 6-mL disposable Sep-Pak C18, 6-mL disposable ENVIRELUT-PESTICIDE, 6-mL BOND ELUT ENV and 6-mL BOND ELUT FL. As methanol is recommended as the most efficient for eluting polar contaminants from the SPE cartridge, it was chosen to evaluate the SPE performance. The selected conditions were 8 mL MAME extract as a sample volume, and 2 mL of methanol for desorption. Samples were spiked with 0.5 $\mu g\,mL^{-1}$ of each pharmaceutical residue.

The results obtained (as % recovery) over the different SPE cartridges are shown in Table 3, where it is observed that phenazone is seen to give the worst result over all the cartridges except for Bond Elut Env. However, the Oasis HLB cartridge generated the best recovery for the most compounds. Therefore, the Oasis HLB cartridge was chosen as the optimum SPE absorbent for further experiments.

3.2.1. Effect of the elution volume

The elution volume may be an important factor that affecting the recovery of target compounds. Volumes of 1, 1.5 and 2 mL of methanol were investigated. As it can be observed in Table 4, in this case, the solvent volume was not a significant parameter over all the recovery values. However, an extraction volume of 1 mL was not sufficient to obtain reproducible results. Although it was obtained similar results with 1.5 and 2.0 mL, 1.5 mL was chosen because a better preconcentration factor (5.3) was leaded.

The elution conditions were carried out in two consecutive steps using 0.75 mL of methanol in each one. The extracts were introduced into hermetically closed vials before analysis in the HPLC–UV system.

3.2.2. pH effect

pH effect in the extraction efficiency of pharmaceuticals was studied by adjusting the pH value of the MAME extract.

Generally, the sample is acidified in order to suppress dissociation [30–32]. In order to study this influence, we compared the results obtained using a MAME extract at neutral pH and another acidified at pH 4 with acetic acid.

For most of compounds, this study indicates that the extractions were better or similar, under acidic conditions than under neutral conditions, except in the case of phenazone where very low recoveries were obtained at both pHs. The overall results led us to the conclusion that the extractions should be performed under acid conditions to ensure the neutral form of the target analytes and a better absorption in the selected sorbent.

In summary, the optimum conditions in the microwave assisted extraction coupled with solid phase extraction (MAME–SPE) were: a radiation time of 6 min; radiation power 500 W, 8 mL of POLE solution 2.75% (v/v) and 1.5 mL of desorption volume. A typical chromatogram containing the

Table 4
Effect of desorption volume in percentage recovery in the SPE process

Compound	Desorption volume		
	1 mL	1.5 mL	2 mL
Phenazone	4	5	4
Carbamazepine	84	96	98
Clofibric acid	53	96	66
Ketoprofen	93	102	100
Naproxen	70	88	80
Bezafibrate	103	89	86
Ibuprofen	87	112	106
Propanolol	88	92	80

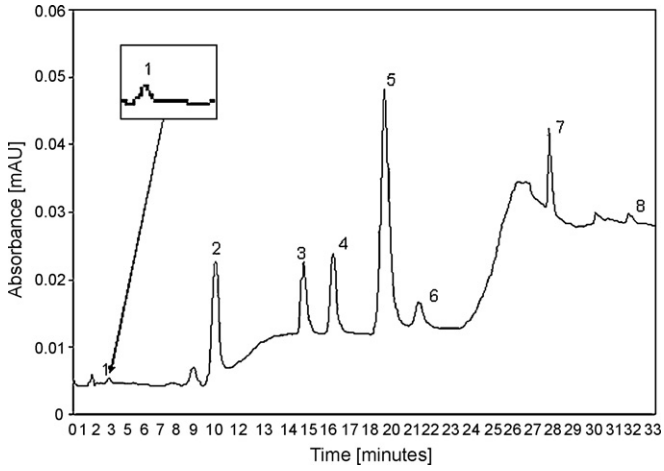

Fig. 4. Chromatogram of the pharmaceuticals mixture (2 μg g^{-1} for each analyte) in Milli-Q water under MAME–SPE optimum conditions. The numbering refers to Table 1. Chromatographic conditions specified in the text.

target compounds under optimal separation conditions, after MAME–SPE procedure was applied, is shown in Fig. 4.

3.3. Analytical performance characteristics

The performance method was evaluated under optimal detection conditions by determination of the linearity, detection limits and reproducibility. Calibration curves were obtained by using different concentration levels ranging from 0.1 to 7.0 μg mL^{-1} for phenazone, clorfibric acid, bezafibrate and propanolol and between 0.01 and 7.0 μg mL^{-1} for carbamazepine, ketoprofen, naproxen and ibuprofen. Curves were linear over these concentration ranges. In all cases, the regression coefficients were over 0.99. Relative recoveries of the method proposed, MAME–SPE–HPLC, were determined by processing spiked six sediment samples with 2 μg g^{-1} of mixture of pharmaceutical compounds through out the entire procedure (Table 5).

As it can be observed, the recoveries are higher than 70% exception made of the case of phenazone (5.6%). This low response may be due to the low adsorption extraction yields for the SPE cartridge. Although the recovery obtained for phenazone was not sufficient for reliable quantitative analysis, other validation parameters, such as sensitivity (LOQ 556 ng g^{-1}) or reproducibility (RSD < 11%) were fairly satisfactory. Therefore, an acceptable quantitative estimation of this compound in the sample could be obtained.

The reproducibility of the method was evaluated through analysis of six replicates of sediment samples containing 2 μg g^{-1} of each pharmaceuticals residues. The relative standard deviations (RSDs) were under 11% for all the target compounds (Table 5). Therefore, the accuracy obtained using the proposed method is satisfactory.

The limits of detection (LODs) and limits of quantification (LOQs) of individual compounds were determined by calculating signal/noise ratio (S/N = 3) [33] and (S/N = 10), respectively, for each compound. With the exception of bezafibrate, the LODs were under 46 ng g^{-1} and the LOQ varied between 12 and 151 ng g^{-1}. The results are in line with those obtained by other authors for this type of solid samples [34].

The results obtained using the proposed method was compared with those obtained using the traditional Soxhlet extraction procedure as proposed by EPA [35]. It was observed that, for most compounds studied, the results obtained are comparable over both methods (Table 6).

However, the proposed method is faster and allows the analysis of the target compounds in a shorter time.

Table 5
Analytical parameters of the proposed method

Compound	Recovery[a] (%)	RSD[b] (%)	LOD[c] (ng g^{-1})	LOQ[d] (ng g^{-1})
Phenazone	6	6	12	39
Carbamazepine	78	10	15	48
Clofibric acid	84	10	45	151
Ketoprofen	78	9	26	87
Naproxen	70	10	5	15
Bezafibrate	114	8	167	556
Ibuprofen	89	8	4	12
Propranolol	78	11	19	62

[a] Mean of six determinations.
[b] Relative standard deviation ($n = 6$).
[c] Limit of detection.
[d] Limit of quantification.

Table 6
Application of MAME–SPE procedure and Soxhlet extraction to a sediment sample containing a mixture of pharmaceuticals

Compound	MAME –SPE Procedure		Soxhlet	
	Added (μg g^{-1})	Found (μg g^{-1})	Added (μg g^{-1})	Found (μg g^{-1})
Phenazone	2	0.1 ± 0.02	2	1.1 ± 0.52
Carbamazepine	2	1.5 ± 0.24	2	1.3 ± 0.17
Clofibric acid	2	1.7 ± 0.36	2	1.8 ± 0.24
Ketoprofen	2	1.6 ± 0.29	2	2.0 ± 0.56
Naproxen	2	1.4 ± 0.27	2	2.2 ± 0.22
Bezafibrate	2	2.3 ± 0.36	2	1.6 ± 0.54
Ibuprofen	2	1.8 ± 0.28	2	2.1 ± 0.45
Propranolol	2	0.8 ± 0.17	2	1.2 ± 0.41

Table 7
Physico-chemical characteristics of the different soil samples of Gran Canaria island (Spain)

No.	Samples	pH	O.M.[a] (%)	Granulometry (%)			
				300 μm	200 μm	150 μm	≤100 μm
1	Maspalomas (South)	9.3	0.4	58.30	31.79	9.12	0.79
2	Cicer (Northeast)	8.8	0.6	2.15	22.66	68.87	6.32
3	Sardina (North)	9.1	0.6	70.54	26.45	2.97	0.04
4	Taurito (Southwest)	9.3	0.7	64.68	21.75	7.61	5.95

[a] O.M., organic matter content.

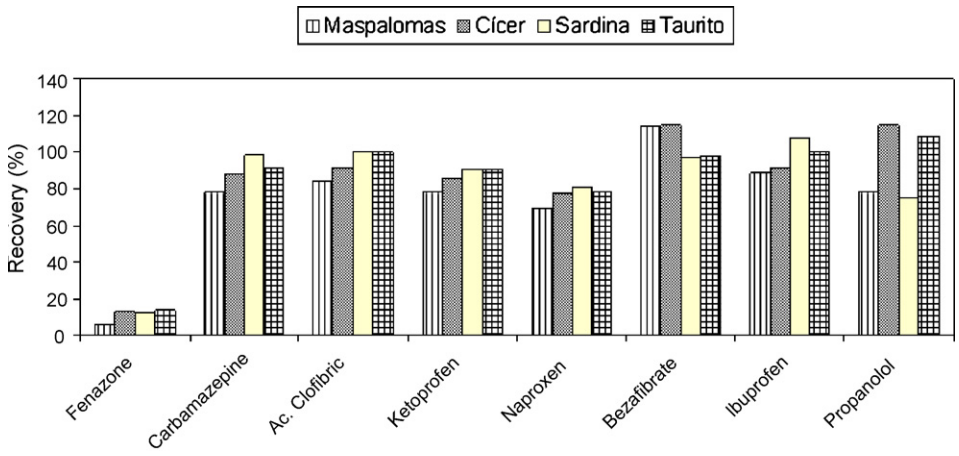

Fig. 5. Application of the optimized MAME–SPE procedure to different sediment samples of Gran Canaria island.

3.4. Analytical applications

The described method was applied to the determination of target pharmaceuticals in several spiked natural sediment samples collected over the island of Gran Canaria (Canary Islands, Spain) with different characteristics (Table 7). Unspiked samples (blanks) were previously analysed using the proposed method and no amounts of the target compounds were detectable.

Spiked sediment samples containing a concentration of $2\,\mu g\,g^{-1}$ of each pharmaceutical were then analysed by MAME–SPE–HPLC. As can be seen, in Fig. 5, all the pharmaceuticals were determined, in all the tested samples, with recoveries over 80% except in the case of phenazone. RSDs of all the recovery experiments were under 11%. Therefore, the results demonstrate that the proposed method can be applied to the determination of the pharmaceuticals studied in different kinds of sediments with satisfactory levels of accuracy and precision.

4. Conclusions

A group of pharmaceutical residues, with different structures and physico-chemical properties, were simultaneously determined using HPLC–UV after MAME–SPE extraction in sediment samples. The optimal conditions for the MAME and SPE procedure were determined. The developed method had a satisfactory recovery range of over 80% for most of the target compounds.

The combination of the MAME–SPE procedure provides a rapid, precise and accurate pretreatment procedure and an effective approach to improve the sensitivity of the HPLC–UV for the determination of pharmaceutical residues in environmental sediment samples.

MAME–SPE–HPLC–UV is an inexpensive analytical technique as compared HPLC–MS for routine analysis of pharmaceuticals in sediment samples. This routine analysis of pharmaceuticals may be a useful tool to ascertain the presence of target compounds in the environment and to evaluate the effect of their contamination.

The pharmaceuticals can be extracted more selectively and more quickly with similar or better recoveries as compared with conventional extraction processes.

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