MEMORIA DE LA ACREDITACIÓN A LA ETAPA DE INVESTIGACIÓN

DOCTORADO EN GESTIÓN COSTERA

Optimización y aplicación de un método basado en SPE-LC-MS/MS para la determinación de multiresiduos de compuestos farmacéuticos en aguas depuradas

Autor: Cristina Afonso Olivares

Directores:

Prof. Dra. Dña. Zoraida Sosa Ferrera

Prof. Dr. D. José Juan Santana Rodríguez

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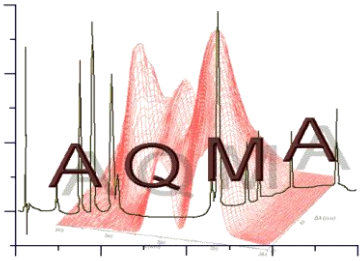
DE GRAN CANARIA



La siguiente memoria recoge el trabajo de investigación titulado “Optimización de un método basado en SPE-LC-MS/MS para la determinación de multiresiduos de compuestos farmacéuticos en aguas depuradas”. Se ha realizado en los laboratorios del Grupo de investigación de Análisis Químico Medioambiental (AQMA) de la Universidad de Las Palmas de Gran Canaria, bajo la dirección de los Profesores Doctores María Zoraida Sosa Ferrera y José Juan Santana Rodríguez. Esta memoria se presenta con el objetivo de obtener la Acreditación a la Etapa de Investigación que forma parte del programa de Doctorado en Gestión Costera.

OPTIMIZACIÓN DE UN MÉTODO BASADO EN SPE-LC-MS/MS PARA LA DETERMINACIÓN DE MULTIRESIDUOS DE COMPUESTOS FARMACÉUTICOS EN AGUAS DEPURADAS.

CRISTINA AFONSO-OLIVARES, ZORAIDA SOSA-FERRERA,
JOSÉ J. SANTANA-RODRÍGUEZ*

Departamento de Química, Universidad de Las Palmas de Gran Canaria. 35017 Las Palmas de Gran Canaria, Spain.

RESUMEN

Este trabajo presenta un método de preparación de muestra para la evaluación de veintitrés compuestos farmacéuticos que pertenecen a diferentes clases terapéuticas en aguas depuradas. Entre los compuestos de interés se incluyen cinco antiinflamatorios (diclofenaco, ketoprofeno, ibuprofeno, naproxeno y metamizol), tres estimulantes (nicotina, cafeína y paraxantina), dos antihipertensivos (propranolol y atenolol), un antiepiléptico (carbamazepina), un antidepresivo (fluoxetina), seis antibióticos (ofloxacina, ciprofloxacina, eritromicina, trimetoprim, sulfametoxazol y metronidazol) y dos antiulcerosos (omeprazol y ranitidina). La extracción en fase sólida (SPE) y la cromatografía líquida con detección por espectrometría de masas en tándem (LC-MS/MS) fueron las técnicas seleccionadas para la extracción y detección-cuantificación, respectivamente. El método desarrollado se podrá aplicar como método analítico para determinar la eficacia de los tratamientos de eliminación de contaminantes orgánicos de los efluentes procedentes de estaciones depuradoras de aguas residuales.

Se realizó un estudio detallado de las condiciones experimentales de extracción. En condiciones óptimas, las recuperaciones obtenidas varían en el intervalo de 29.4% a 140.6%, y las desviaciones estándar relativas (RSD) se encuentran por debajo de 19,9%. Los límites de detección (LOD) y cuantificación (LOQ) del método se encuentran en el rango de 0.16 a 67.9 y 0.53 a 226 ng L^{-1}, respectivamente. El método desarrollado se ha aplicado con éxito para evaluar la presencia de estos compuestos farmacéuticos en muestras de efluentes de plantas de tratamiento de aguas residuales ubicadas en la isla de Gran Canaria (España). Una gran mayoría de los compuestos se detectaron en las muestras analizadas y las concentraciones alcanzadas llegaron a un máximo de 645.9 ng L^{-1}.

OPTIMIZATION OF A SOLID PHASE EXTRACTION COMBINATED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY PROCEDURE TO SIMULTANEOUS DETERMINATION OF PHARMACEUTICALS COMPOUNDS IN TREATED WATER SAMPLES.

CRISTINA AFONSO-OLIVARES, ZORAIDA SOSA-FERRERA and

JOSÉ J. SANTANA-RODRÍGUEZ*

Departamento de Química, Universidad de Las Palmas de Gran Canaria. 35017 Las Palmas de Gran Canaria, Spain.

ABSTRACT

This work presents a sample preparation method for the evaluation of twenty-three pharmaceutical compounds belonging to different therapeutic classes in treated water samples. The target compounds include five anti-inflammatories (diclofenac, ketoprofen, ibuprofen, naproxen and metamizole), three stimulants (nicotine, caffeine and paraxanthine), two antihypertensive (propranolol and atenolol), an antiepileptic (carbamazepine), an antidepressant (fluoxetine), six antibiotics (ofloxacin, ciprofloxacin, erythromycin, trimethoprim, sulfamethoxazole and metronidazole) and two antiulcers (omeprazole and ranitidine). Solid-phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS/MS) were selected as extraction and detection techniques, respectively. The developed method will be applied like analytical method to determine the effectiveness of treatments for removing organic pollutants from treated wastewater effluents.

A detailed study of the experimental conditions of extraction was performed. Under optimal conditions, recoveries obtained were in the range of 29.4% to 140.6%, and the relative standard deviations (RSD) were below 19.9%. The detection (LOD) and quantification (LOQ) limits of the method were in the range 0.16-67.9 and 0.53-226 ng L^{-1}, respectively. The developed method was successfully applied to evaluate the presence of these pharmaceutical compounds in samples from wastewater treatment plants located on the Gran Canaria Island (Spain). Most of the compounds were detected at concentrations up to 645.9 ng L^{-1} in the WWTP effluents that were studied.

Keywords: Multi-residue, Pharmaceutical compounds, SPE, LC-MS/MS, wastewater.

1. INTRODUCTION

Economic growth and globalization have led to obvious benefits but at the same time, have led to the emergence of new environmental risks. Because of this rapid development of human civilization, many environmental problems are affecting, directly or indirectly, to the water resources and this is causing increased water shortages in several regions [1]. Exposure to hazardous chemicals represents a threat that should be subject to assessment measures and the reduction and control of irrigation and this may be possible thanks to developing the sensitive methods of analysis [2].

The presence of pharmaceutical compounds in wastewater is one of the challenges in environmental monitoring. They are part of the so-called emerging contaminants [3] and they are very widespread in rivers [4], lakes [5] and sea [6], because it is dispersed through wastewater [7]. Discharge of emerging contaminants is a health and environmental problems that has not yet been sufficiently investigated and whose legal regulation is still unsatisfactory [8]. Control of these pollutants is particularly difficult due to the wide dispersion of emission sources ranging from household waste, hospital and industrial to discharges from farming and ranching [9]. These are subject to a constant discharge and also customary purification systems are not designed to remove, so that their levels in the medium can easily achieve high values [10, 11].

The control difficulty of these drugs in water samples is linked not only with the dispersion, but also the complexity of environmental matrices. Moreover, there is a significant dilution factor in the environment so that the concentrations of these compounds in the water samples are in the $ng \cdot L^{-1}$ range [12]. There are many common use pharmaceutical compounds with different physicochemical properties. The simultaneous detection of different classes of these pollutants, which require complex analytical processes, has become a major issue [13, 14]. Therefore, a multi-residue method permits analysis of a wide range of contaminants of different properties in a single run [15].

Multi-residue analytical methods use liquid chromatography with tandem-mass spectrometry (LC-MS/MS) with different ionization techniques [16]. Normally it is necessary to concentrate and purify the organic contaminants in aqueous matrices before analysis. The most frequently used concentration and purification method is solid-phase extraction (SPE) [17].

In this study, we develop a SPE procedure combined with LC-MS/MS to the determination of twenty-three pharmaceutical compounds including diclofenac, ketoprofen, ibuprofen, naproxen, nicotine, atenolol, propranolol, metamizole, caffeine, paraxanthine, gemfibrozil, bezafibrate, carbamazepine, fluoxetine, ofloxacin, ciprofloxacin, erythromycin, trimethoprim, sulfamethoxazole, metronidazole, omeprazole, ranitidine and clofibric acid, which are all of different therapeutic classes. These pharmaceutical compounds were selected because of their high consumption rates. Table 1 shows characteristics of the target compounds, which influence their behavior in the environment. The parameters involved in SPE process and LC-MS/MS are optimized. The developed method could be applied to evaluate the presence of these pharmaceutical compounds in wastewater samples from wastewater treatment plants (WWTPs) of the Gran Canaria Island (Spain) and like analytical method to determine the effectiveness of treatments for removing organic pollutants from treated wastewater effluent.

Table 1. List of pharmaceuticals compounds, chemical structure, pKa values, Log K_{ow} values and retention times (R_T)

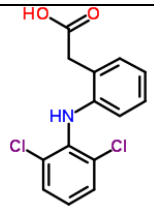
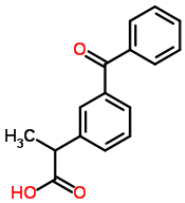
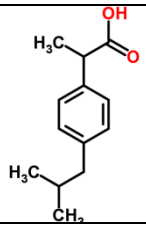
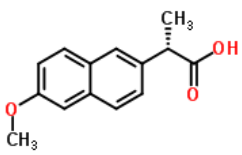
Application	Compound	Structure	pK_a[18]	Log K_{ow}*	R_T (min)
Anti-inflammatory	Diclofenac		4.15	4.51	41.93
	Ketoprofen		4.45		36.14
	Ibuprofen		4.51	3.97	40.70
	Naproxen		4.20	3.18	36.38

Table 1. Cont.

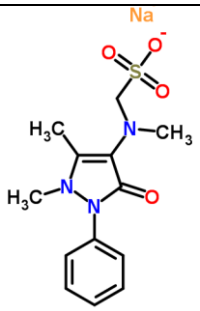
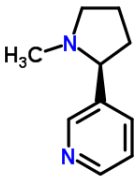
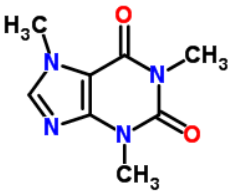
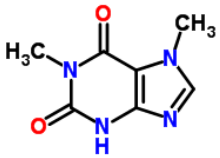
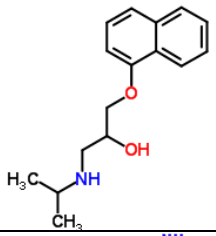
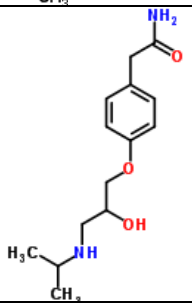
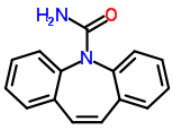
Application	Compound	Structure	pK_a[18]	Log K_{ow}*	R_T (min)
Anti-inflammatory	Metamizole		-1.20	1.07	9.07
Stimulant	Nicotine		8.00	1.17	2.02
	Caffeine		14.0	-0.07	17.69
	Paraxanthine		8.50		14.01
Antihypertensive	Propanolol		9.49	0.16	17.04
	Atenolol		9.16	2.45	3.90
Antiepileptic	Carbamazepine		13.90	2.45	31.78

Table 1. Cont.

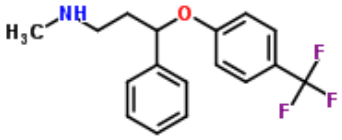
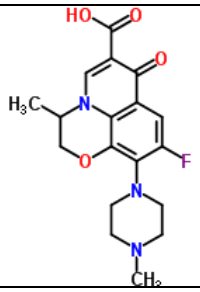
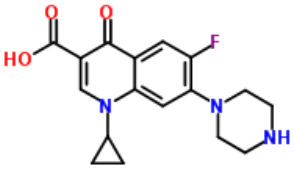
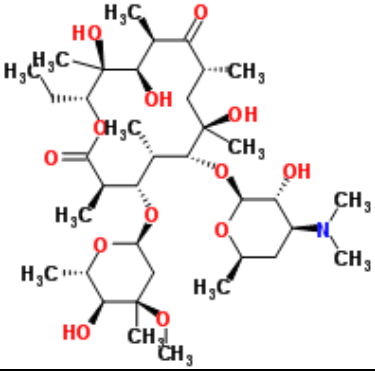
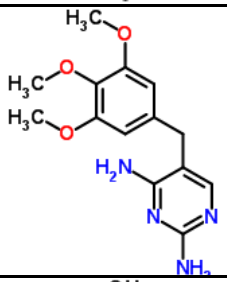
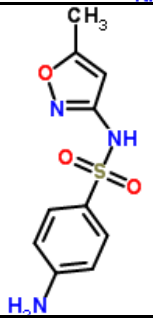
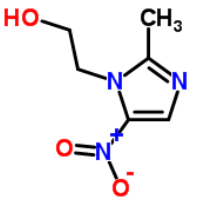
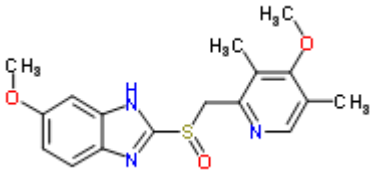
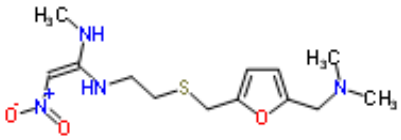
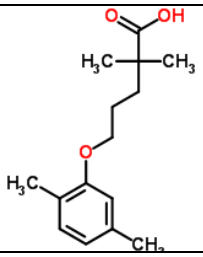
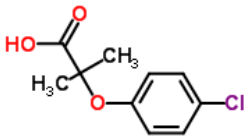
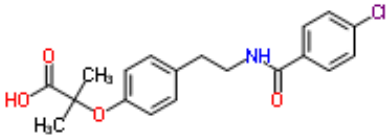
Application	Compound	Structure	pK_a[18]	Log K_{ow}*	R_T (min)
Antidepressant	Fluoxetine		8.70		25.10
Antibiotic	Ofloxacin		7.37	-0.39	11.13
	Ciprofloxacin		6.38	0.28	12.21
	Erythromycin		8.16	3.06	24.20
	Trimethoprim		6.60	0.91	8.59
	Sulfamethoxazole		5.70	0.89	20.14

Table 1. Cont.

Application	Compound	Structure	pK_a [18]	Log K_{ow} *	R_T (min)
Antibiotic	Metronidazole		14.44	-0.02	11.74
Antiulcer	Omeprazol		8.78		29.89
	Ranitidine		8.35		4.00
Lipid regulator	Gemfibrozil		4.75	4.77	42.00
	Clofibric acid		3.18		43.05
	Bezafibrate		3.60		41.05

* Extracted from Hazardous Substances Data Bank.

2. MATERIALS AND METHODS

2.1. Reagents

The pharmaceutical compounds used in this study (Table 1) were purchased from Sigma-Aldrich (Madrid, Spain). Their stock solutions (1 g L^{-1}) were prepared by dissolving appropriate amounts of pharmaceutical standards in methanol (HPLC gradient-grade PAI-ACS) from Panreac Química

(Barcelona, Spain) and the solutions were then stored in glass stoppered bottles at 4°C prior to use. Appropriate volumes of the stock solutions were diluted weekly to prepare work solutions containing the pharmaceutical compounds at 1 mg L^{-1}.

LC-MS quality methanol and water were used to prepare the mobile phase for LC-MS/MS. All of the dissolvent and formic acid that were used to adjust the pH of the mobile phase were obtained from Panreac Química (Barcelona, Spain).

Ultra high purity water was obtained from a Milli-Q (Millipore, Bedford, MA, USA) water purification system and was used for conditioning the process of solid-phase extraction and for preparing aqueous standard solutions.

2.2. Sample Preparation

Water samples were taken from the output of two wastewater treatment plants located on the island of Gran Canaria (Spain). The samples were collected in 1 L amber glass bottles that have been pre-rinsed with methanol and deionized water, filtered through 0.65 µm membrane filters (Millipore, Ireland) and stored in the dark in the refrigerator. The samples were extracted within 48 h.

2.3. Instrumentation and Chromatographic Conditions

LC-MS/MS

Analysis of the selected pharmaceuticals was performed by a Varian system (Varian Inc., Madrid, Spain) consisting of a 212-LC Binary Gradient LC/MS Chromatography Pump fitted with a Prostar 410 HPLC Autosampler and a 320-MS LC/MS/MS system (triple quadrupole) equipped with an electrospray ionisation (ESI) interface. The system and the data management were controlled by MS Varian LC/MS Workstation Version 6.9 SP1 software.

The multiple reaction monitoring (MRM) parameters were optimised for subsequent quantitative analysis. This procedure was conducted using a 1 mL syringe (Hamilton Company, Reno, NV, USA) and a continuous flow rate of 20 µL min^{-1}. Each standard or mixture was prepared at a concentration of 10 mg L^{-1} in methanol. Each solution was taken up by the Hamilton syringe at a volume of 0.1 mL, and the remaining 0.9 mL of syringe volume was filled with mobile phase.

Ionization in the ESI source was achieved using nitrogen as a nebuliser and drying gas. The housing and desolvation temperatures were set to 60°C and 250°C, respectively, for the optimisation of

the syringe pump injections for MS/MS. The drying and nebulising gas pressures were fixed at 30 psi and 65 psi, respectively. The capillary voltage was set to 5.0 kV in positive mode (ESI+) and −4.5 kV in negative mode (ESI−). The shield voltage was maintained at −600/600V (ESI+/ESI−) and the cone voltage was optimised for each individual compound. Collision-induced dissociation (CID) was conducted with argon as the collision gas at a fixed pressure of 1.94 psi.

The stationary-phase column was a 3.0 mm x 100 mm, 3.5 µm particle SunFireTM C18. The mobile phase consisted of water (containing 0.015% formic acid) /methanol (90:10) (v/v) for 1 min, during 20 min it changed to 60:40 (v/v) for 19 min up to 10:90 (v/v) and it maintains during 3 min. Finally, during 3 min it returns to the initial condition. A prudential time (4 min) was employed to equilibrate the system. The injection volume was 10 µL, and the flow rate was 200 µL min^{-1}.

Solid Phase Extraction

The SPE cartridges that were used were Oasis HLB (6 mL, 200 mg) and ExtraBond ECX (6 mL, 200 mg) from Scharlau. The cartridges were conditioned with 5 mL of methanol followed by 5 mL of Milli-Q water at pH 6 and a flow rate of 10 mL min^{-1} for each run. Water samples were then loaded onto the cartridges at a flow rate of 5 mL min^{-1} and thereafter, the cartridges were washed with 5 mL of Milli-Q water at a flow rate of 10 mL min^{-1} to remove possible interferences. Finally, the cartridges were dried under vacuum for approximately 5 min and further eluted with 2 mL of methanol at 1 mL min^{-1}. Blanks were run to evaluate any carryover during SPE.

3. RESULTS AND DISCUSSION

3.1. Optimisation of LC-MS/MS Detection

The optimisation of the mass spectrometer parameters, such as cone voltage and collision gas energy, was carried out by directly injecting standard solutions of each individual compound into the MS. The obtained fragment ions and the collision potential are displayed in Table 2. Figure 1 presents the fragment ions in the MRM mode produced by the collision of selected precursor ions into the collision cell of the triple quadrupole. Two transitions were acquired for the confirmation for most analytes.

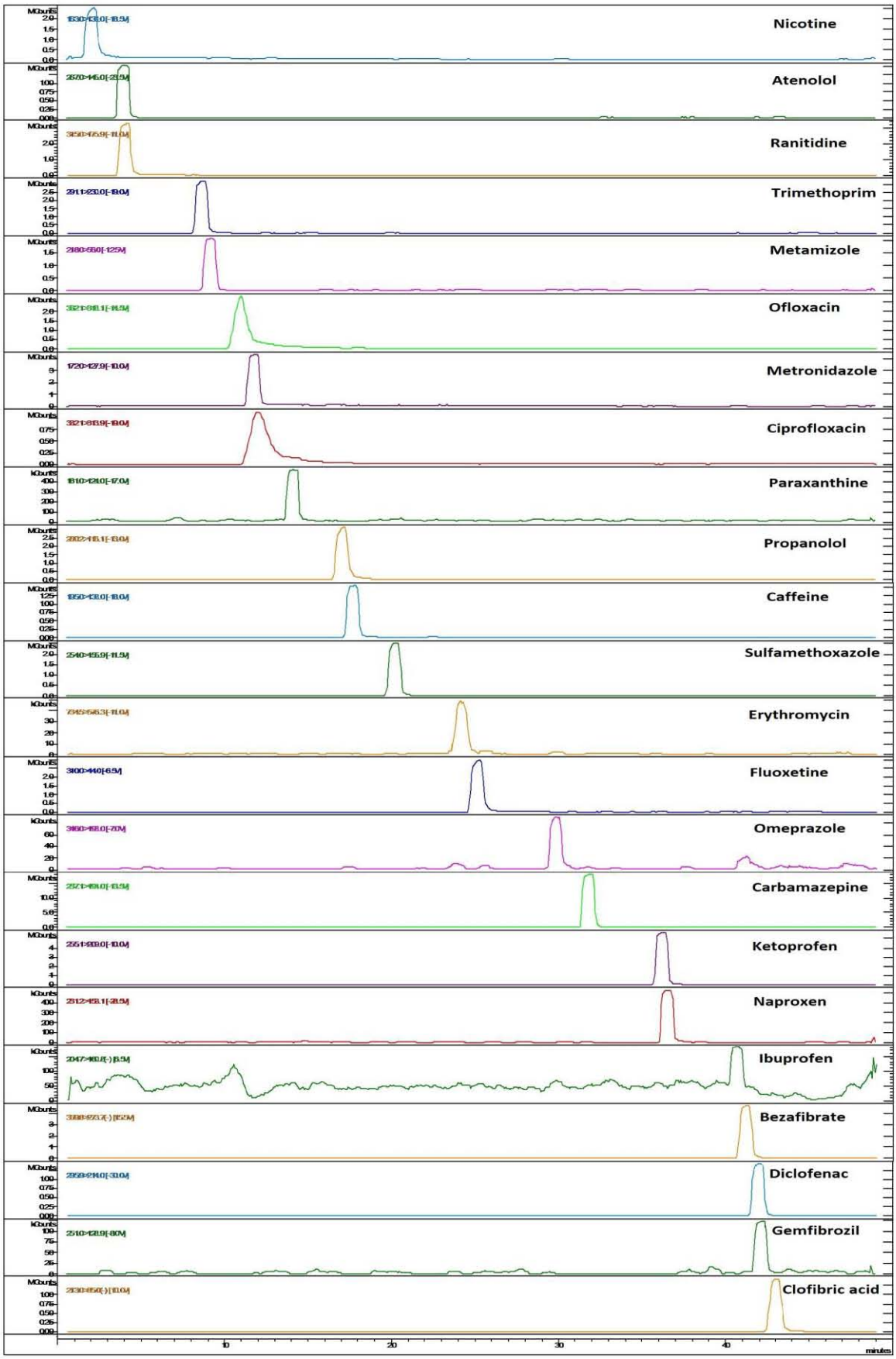


Figure 1: The fragment ions in the multiple reaction monitoring (MRM) mode.

Table 2. Mass spectrometer parameter for the determination of target analytes.

Nº	Compound	Precursor ion (m/z)	Cone V	Fragment ions (collision potential)	Ion mode
1	Nicotine	163	30	130(18.5)[a], 84 (17)	ESI +
2	Atenolol	267	52	145 (23.5)[a], 190 (16.5)	ESI +
3	Ranitidine	315.0	44	175.9 (11)[a], 129.8 (20)	ESI +
4	Trimethoprim	291.1	64	230 (19)[a], 122.9 (21)	ESI +
5	Metamizole	218	30	56 (12.5)[a], 97 (11.5)	ESI +
6	Ofloxacin	362.1	52	318.1 (14.5)[a], 261.0 (22.5)	ESI +
7	Metronidazole	172	40	127.9 (10.0)a, 81.9 (21.0)	ESI +
8	Ciprofloxacin	332.1	52	313.9 (19.0)[a], 230.8 (36.0)	ESI +
9	Paraxanthine	181	40	124 (17)[a]	ESI +
10	Propanolol	260.2	48	116.1 (13)[a], 183.1 (12)	ESI +
11	Caffeine	195	56	138 (18)[a]	ESI +
12	Sulfamethoxazole	254	44	155.9 (11.5)[a], 91.9 (23)	ESI +
13	Erythromycin	734.5	48	576.3 (11)[a], 157.8 (22.5)	ESI +
14	Fluoxetine	310	30	44 (6.5)[a], 148 (5.5)	ESI +
15	Omeprazole	346	32	198.0 (7)[a], 135.8 (27.5)	ESI +
16	Carbamazepine	237.1	40	194 (13.5)[a], 192 (17)	ESI +
17	Ketoprofen	255.1	52	209 (10)[a], 104.9 (18.5)	ESI +
18	Naproxen	231.2	36	153.1 (28.5)[a], 170 (22)	ESI +
19	Ibuprofen	204.7	40	160.8 (6.5)[a], 158.5 (6.0)	ESI -
20	Bezafibrate	359.8	64	273.7 (15.5)[a], 153.5 (28.5)	ESI -
21	Diclofenac	295.9	32	214.0 (30)[a], 250.0 (11.0)	ESI +
22	Gemfibrozil	251	30	128.9 (8.0)[a], 233 (5.0)	ESI +
23	Clofibric acid	213	32	85 (10)[a], 127 (13.5)	ESI -

a) Fragment ion used for quantitation (MRM).

3.2. Optimisation of the Extraction Process in SPE

Our aim is to find suitable conditions for the extraction of twenty-three pharmaceutical compounds from purified water. Extraction and preconcentration are important steps in the development of the method. The optimisation of SPE included the evaluation of the following experimental variables: cartridge type, pH, ionic strength, sample volume, wash step and desorption volume. Initially, the sample volume of Milli-Q water were spiked with 200 µL of work solution, and a desorption volume of 2 mL of methanol were used. The samples were passed through cartridges under the conditions described in Section 2.3.

3.3. Optimisation of SPE cartridge and relation between variables

To optimize the SPE process, we tested two different cartridges, including a reversed phase (Oasis HLB) and ion-exchange adsorbent (ExtraBond ECX). The pH value, ionic strength and sample

volume of the water play important roles in SPE efficiency. For this reason, we used an initial experimental design 2^3 (two levels, three parameters) to study the influence of pH, ionic strength and sample volume for each cartridge. The experimental design was obtained using Statgraphics Plus software 5.1 and the statistics study was done with IBM SPSS Statistics 19.We have chosen acid and basic pH values of 3 and 9, ionic strength values of 0% and 30% (w/v) NaCl and sample volumes of 100 mL and 1000 mL. It was found that, under the different studied conditions, the Oasis HLB cartridge is most suitable than ExtraBond ECX cartridge. The influence between parameters is varied for each analyte, but using the Pearson correlation (partial and bivariate), we have observed that the most influential variables are the pH and ionic strength. For the greatest number of analytes, the sample volume does not affect the extraction process. Due to that method will be used to analyze samples of treated water, we have chosen a sample volume that can be passed easily through the cartridges without become clogged and have a good preconcentration factor. In summary, we have chosen the Oasis HLB cartridge and a sample volume of 250 mL.

3.4. Optimisation of pH and ionic strength

The pH is an important parameter in the extraction process because, depending on the acidity or alkalinity of the compounds under study, the interactions with the phase of the cartridge will vary. The addition of salt can improve the extraction process and is an important issue because two processes can occur simultaneously. It can produce the phenomenon known as "salting out" in which the increase in salt concentration results in higher recoveries of the analyte, because the water molecules form hydration spheres around the ionic salt molecules. They reduce the concentration of water that is available to dissolve analyte molecules, so the recovery is expected to increase [19]. In contrast, there may be another process in which polar molecules may participate in electrostatic interactions with salt ions in solution, thereby reducing their ability to move into the extraction phase and decreasing the recovery [20].

Holding other variables constant (250 mL sample volume, 5 mL wash step and 2 mL elution volume of methanol), we have used an experimental design 3^2 (two variables, three parameters) to study the influence of pH and ionic strength. The effect of ionic strength in the recovery of the compounds under study was determined by addition sodium chloride to the aqueous medium in the range of 0% to 30% (w/v) and the pH values studied were 3, 6 and 9.

If analysed the response surfaces, obtained from the Matlab software, we can see there are many contrasts in the behavior of different compounds for the studied variables. Figure 2 shows the response surface obtained for diclofenac and carbamazepine. Due to the differences, we performed a balance sheet and have chosen as optimal values pH 9 and 15% ionic strength, which coincide with one of the peaks of the response surface plot of diclofenac is the patron of many of the compounds studied.

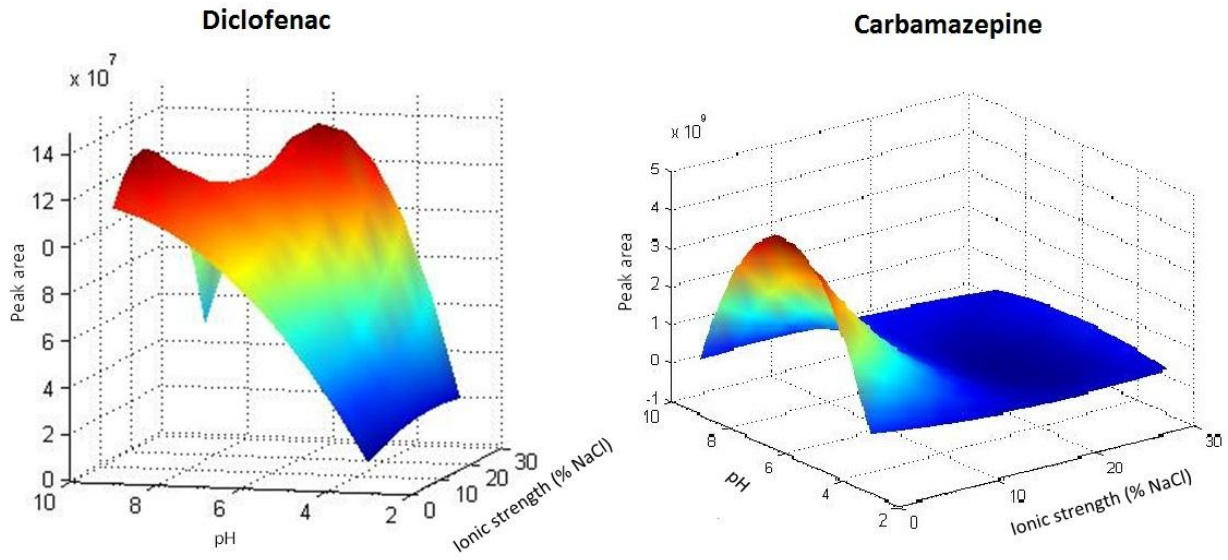


Figure 2: Effect of ionic strength and pH on the SPE extraction for diclofenac and carbamazepine

3.5. Optimisation of desorption volume

Desorption volume employed must be sufficient to ensure the total extraction of the analytes. The solvent that was used for desorption was methanol. For the evaluation of the required volume, the desorption volumes chosen were 1 and 2 mL (in one or two steps). Figure 3 shows the results for desorption volume optimisation for some analytes. Eluation at 1 mL is not feasible because all analytes do not achieve high recovery, as in the case of paraxanthine, with achieves only half the recovery of that at 2 mL. Finally, desorption volume of 2 mL, done in one step, was chosen, although the results are very similar if it is done in two steps. These conditions results in a preconcentration factor of 125.

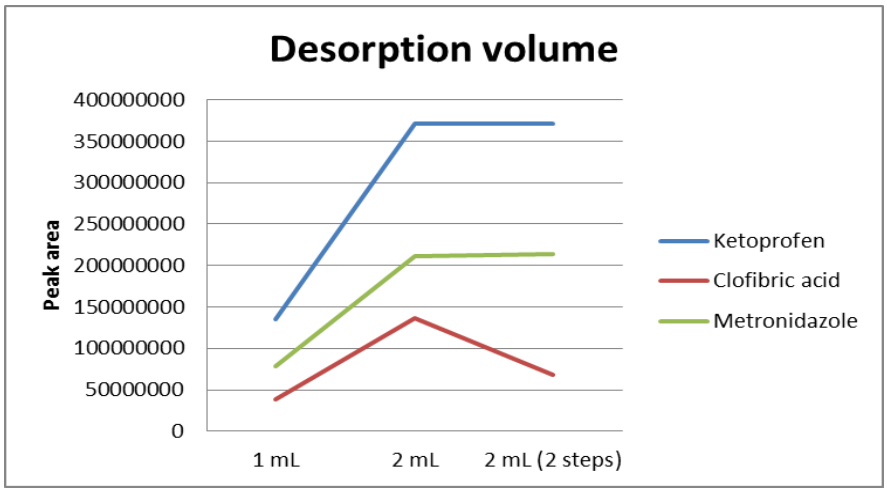


Figure 3: Results of desorption volume optimisation for ketoprofen, clofibric acid, and metronidazole.

3.6. Optimisation of wash step

The last variable to be optimized was the wash step. This is an important step for removing impurities from the samples and in our study is primordial to eliminate sodium chloride. Keeping other variables constant, the principal solvent to be used is Milli-Q water (5 mL), and Milli-Q water with 5% of methanol. Figure 4 shows the results for optimisation of wash step for some analytes. The addition of a small percentage of organic solvent may not affect the elution of some analytes, however, has chosen to use as a wash step 5 mL of Milli-Q water to prevent any loss of analyte.

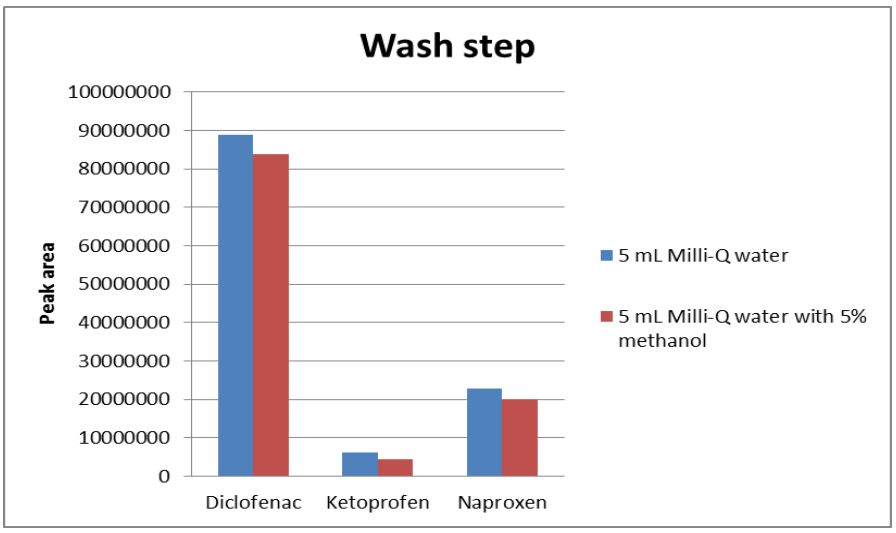


Figure 4: Results of wash step optimisation for diclofenac, ibuprofen and naproxen.

3.7. Analytical Parameters

The analytical parameters of the method are shown in Table 3. Calibration curves were established for almost all compounds in the range of 1-300 μg L^{-1}, and the correlation coefficients were equal to or higher than 0.9901 in all cases. The recovery of analytes through the optimised method (SPE extraction and LC—MS/MS detection) was evaluated at a final concentration of 50 μg L^{-1} of each compound. The recoveries obtained were higher than 29.4%.

Six standard mixes of pharmaceuticals (final concentration 50 μg L^{-1} of each pharmaceutical) were extracted and then injected to calculate the reproducibility (RSD, %) of each compound under study. Normal results were achieved for all compounds with RSDs lower than 19.9%.

Table 3. Analytical parameters for SPE procedure combined with LC-MS/MS.

Nº	Compound	LDR[a] (μg L^{-1})	r^2	50 μg L^{-1}		LOD[b] (ng L^{-1})	LOQ[c] (ng L^{-1})
				RSD (%)	Recovery (%)		
1	Nicotine	1-300	0.9971	10.6	29.4	30.8	103
2	Atenolol	1-300	0.9994	19.7	85.7	12.3	41.0
3	Ranitidine	1-300	0.9913	19.3	58.7	6.26	20.9
4	Trimethoprim	5-300	0.9959	6.82	66.6	4.29	14.3
5	Metamizole	50-300	0.9919	19.6	147	23.5	78.3
6	Ofloxacin	5-300	0.9980	17.6	32.3	28.4	94.8
7	Metronidazole	5-300	0.9988	14.7	119	5.50	18.3
8	Ciprofloxacin	5-300	0.9909	6.70	68.6	19.1	63.8
9	Paraxanthine	50-300	0.9948	2.44	93.1	35.3	117
10	Propanolol	5-300	0.9958	15.5	56.3	10.4	34.7
11	Caffeine	5-300	0.9986	10.5	37.6	5.38	17.9
12	Sulfamethoxazole	5-300	0.9979	12.7	106	0.58	1.93
13	Erythromycin	1-300	0.9982	19.1	57.7	0.21	0.69
14	Fluoxetine	1-300	0.9982	19.9	30.6	0.16	0.53
15	Omeprazol	1-300	0.9971	10.8	48.7	0.72	2.40
16	Carbamazepine	1-300	0.9905	14.7	31.3	1.17	3.90
17	Ketoprofen	1-300	0.9953	9.37	53.4	2.94	9.79
18	Naproxen	1-300	0.9983	17.6	95.6	0.72	2.40
19	Ibuprofen	50-300	0.9901	8.46	117	67.9	226
20	Bezafibrate	1-300	0.9988	14.2	68.8	1.99	6.63
21	Diclofenac	1-300	0.9950	18.6	73.6	0.19	0.63
22	Gemfibrozil	5-300	0.9942	9.12	83.0	2.34	7.79
23	Clofibric acid	5-300	0.9969	17.7	76.4	0.39	1.30

a) Lineal Dynamic Range
b) Limit of Detection
c) Limit of Quantification

The limit of detection (LOD) was defined as the lowest concentration that gave a signal-to-noise ratio (S/N) ≥ 3, and the limit of quantification (LOQ) was defined as the lowest concentration that gave a S/N ≥ 10 [21]. LODs were in the range of 0.16-.67.9 ng L^{-1}, and LOQs were in the range of 0.53-226 ng L^{-1}. When compared with the results from other authors, [22] we observe that recoveries and detection limits obtained with our proposed method were appropriate for the detection and determination of the pharmaceutical compounds in wastewater samples.

3.8. Matrix effect

The matrix effect is a decrease or increase of the instrumental response of the analyte due to the presence of other components. In other words, for the same analyte concentration, analysis of a real sample or a pure analyte standard solution does not provide the same instrumental response. The matrix effect causes a proportional systematic error. We evaluated the relative signal suppression caused by the matrix effects by using the algorithm (Ec.1) published by Vieno et al. [23].

$$100 - \left(\frac{\left(A_{sp} - A_{usp} \right) \cdot 100}{A_s} \right) \quad \text{Ec 1.}$$

where A_s corresponds to the peak area of the analyte in pure standard solution, A_{sp} corresponds to the peak area in the spiked matrix extract, and A_{usp} corresponds to the matrix extract of a real sample. The spiked concentration was 50 µg L^{-1}.

Table 4. Matrix effect evaluation. Percentage of signal reduction (ionization suppression) for pharmaceutical compounds effluent wastewater.

Nº	Compound	Matrix effect (%)	Nº	Compound	Matrix effect (%)
1	Nicotine	15.40	**13**	Erythromycin	54.86
2	Atenolol	39.74	**14**	Fluoxetine	80.91
3	Ranitidine	74.99	**15**	Omeprazol	27.78
4	Trimethoprim	-154.3	**16**	Carbamazepine	33.76
5	Metamizole	-85.30	**17**	Ketoprofen	-1.624
6	Ofloxacin	-23.71	**18**	Naproxen	72.82
7	Metronidazole	66.99	**19**	Ibuprofen	60.10
8	Ciprofloxacin	56.13	**20**	Bezafibrate	21.11
9	Paraxanthine	-119.8	**21**	Diclofenac	25.46
10	Propanolol	96.24	**22**	Gemfibrozil	60.37
11	Caffeine	47.97	**23**	Clofibric acid	34.93
12	Sulfamethoxazole	48.79			

Table 4 shows the relative signal suppression from target compounds. The results are varied (signal suppressions from -154.27% to 96.24%), and we can observe that matrix effect occurs for nearly all compounds studied, including in some cases, an increase in the signal (negative values). Studies by other authors [17] show suppression signal from 10% to 90%. Therefore, we must use the standard additions method which takes into account the effect of the matrix.

3.9. Evaluation of Selected Pharmaceutical Compounds in Wastewater

To validate the method, SPE extraction combined with LC-MS/MS was applied to the analysis of wastewaters from two different wastewater treatment plants (WWTPs) located on the island of Gran Canaria. Both WWTPs use the membrane bioreactor technique (WWTP1 and WWTP2).

Table 5. Concentrations in ng L^{-1} found in treated water from three different wastewater treatment plants in Gran Canaria island.[a]

Nº	Compound	WWTP1 (ng L^{-1})	WWTP2 (ng L^{-1})
1	Nicotine	nd[b]	nd
2	Atenolol	246.9 ± 11.5	147.1 ± 0.7
3	Ranitidine	nd	234.3 ± 38.1
4	Trimethoprim	202.9 ± 25.3	69.54 ± 1.07
5	Metamizole	nd	nd
6	Ofloxacin	253.7 ± 21.3	202.6 ± 8.6
7	Metronidazole	nd	nd
8	Ciprofloxacin	453.9 ± 30.9	416.8 ± 13.0
9	Paraxanthine	nd	nd
10	Propanolol	nd	nd
11	Caffeine	nd	nd
12	Sulfamethoxazole	106.8 ± 5.7	205.1 ± 5.3
13	Erythromycin	nd	nd
14	Fluoxetine	287.3 ± 38.5	312.6 ± 35.7
15	Omeprazol	nd	nd
16	Carbamazepine	444.0 ± 43.4	185.1 ± 31.9
17	Ketoprofen	189.9 ± 6.5	nd
18	Naproxen	615.9 ± 49.0	318.5 ± 51.6
19	Ibuprofen	645.9 ± 102.7	443.7 ± 82.0
20	Bezafibrate	nd	nd
21	Diclofenac	nd	35.37 ± 4.48
22	Gemfibrozil	nd	nd
23	Clofibric acid	nd	nd

a) n = 3
b) nd = no detected

Two samples were extracted and analysed in triplicate using the optimized conditions described above and the concentrations were quantified from the internal calibration curve. The results of these measurements are shown in Table 5. The majority of the compounds under study were found in different concentrations ranging from 35.37 to 645.9 ng L^{-1}. We can conclude that studied method is applicable for the evaluation of pharmaceutical compounds in treated wastewater.

4. CONCLUSIONS

In the present work, we have developed an analytical method for the evaluation of twenty-three drugs (nicotine, atenolol, ranitidine, thrimethoprim, metamizole, ofloxacin, metronidazole, ciprofloxacin, paraxanthine, propranolol, caffeine, sulfamethoxazole, erythromycin, fluoxetine, omeprazole, carbamazepine, ketoprofen, naproxen, ibuprofen, bezafibrate, diclofenac, gemfibrozil and clofibric acid) of different therapeutic classes in treated water samples. SPE with an Oasis HLB cartridge was used for the extraction step. Subsequently, the detection and quantification was made by high performance liquid chromatography with mass spectrometry detection (LC—MS/MS). The method developed is sensitive, reproducible, and applicable to wastewater samples. The detection limits that were achieved with the proposed method were appropriate for the detection of pharmaceutical compounds in real samples.

Application of the developed method to the analysis of treated wastewaters from two different wastewater treatment plants of the island of Gran Canaria indicates the presence of most of the compounds under study. The concentrations were in the range of 35.37 - 645.9 ng L^{-1}.

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