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Ultra-high-performance liquid chromatography—Time-of-flight high resolution mass spectrometry to quantify acidic drugs in wastewater

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ABSTRACT

A novel analytical approach involving an improved rotating-disk sorptive extraction (RDSE) procedure and ultra-high-performance liquid chromatography (UHPLC) coupled to an ultraspray electrospray ionization source (UESI) and time-of-flight mass spectrometry (TOF/MS), in trap mode, was developed to identify and quantify four non-steroidal anti-inflammatory drugs (NSAIDs) (naproxen, ibuprofen, ketoprofen and diclofenac) and two anti-cholesterol drugs (ACDs) (clofibric acid and gemfibrozil) that are widely used and typically found in water samples. The method reduced the amount of both sample and reagents used and also the time required for the whole analysis, resulting in a reliable and green analytical strategy. The analytical eco-scale was calculated, showing that this methodology is an excellent green analysis, increasing its ecological worth. The detection limits (LOD) and precision (%RSD) were lower than 90 ng/L and 10%, respectively. Matrix effects and recoveries were studied using samples from the influent of a wastewater treatment plant (WWTP). All the compounds exhibited suppression of their signals due to matrix effects, and the recoveries were approximately 100%. The applicability and reliability of this methodology were confirmed through the analysis of influent and effluent samples from a WWTP in Santiago, Chile, obtaining concentrations ranging from 1.1 to 20.5 μg/L and from 0.5 to 8.6 μg/L, respectively.

1. Introduction

Pharmaceuticals are synthetic chemicals that belong to a wide range of different chemical families and may also react differently in the environment. Due to the risks to human health and the aquatic environment, the determination of pharmaceutical residues in environmental matrices has become a field of special interest [1–3]. Most pharmaceuticals are not completely removed from wastewater treatment plants (WWTPs) and can enter ground and drinking water at low concentrations, from ng/L to μg/L [4]. In particular, non-steroidal anti-inflammatory drugs (NSAIDs) and anti-cholesterol drugs (ACDs) are among the pharmaceuticals most consumed worldwide. Because of this, four NSAIDs (ketoprofen, ibuprofen, naproxen and diclofenac) and two ACDs (clofibric acid and gemfibrozil) were considered in this work as representative analytes. These compounds are derivatives of aromatic carboxylic acids, having dissociation constants (p*K*a) of 3.4 to 4.8 and log $K_{o/w}$ of 2.9 to 4.4. Consequently, despite their hydrophobicity, the pH normally observed in environmental waters favors their ionization, increasing their mobility and environmental risk.

Globally, concentrations between 0.7 and 3.4 μg/L have been observed for diclofenac, ibuprofen, naproxen and ketoprofen [5–7]. Similarly, values of 1.2 μg/L for gemfibrozil were determined in different WWTPs from Europe [5,6]. In Chile, concentrations between 1.3 and 2.0 μg/L were reported for NSAIDs [3] in WWTP effluents.

According to the literature, several analytical methods have been proposed to determine NSAIDs and ACDs while promoting efficient and green technologies [5–8]. On the one hand, extraction processes are being improved because this is considered one of the most crucial steps in analytical methods, having a significant impact on the quality of the results. On the other hand, with the same purpose in mind, new generations of ultra-high-performance liquid chromatography (UHPLC) are growing. Furthermore, the use of UHPLC coupled to an electrospray ionization source (ESI) and mass spectrometry (MS) also provides a significant improvement in methods sensitivity and feasibility, as well as a reduction in analysis time [8,9]. Among the different mass analyzers available, triple quadrupole (MS/MS) and time of flight (TOF) are the most frequently used [10,11]. It is important to note that TOF/MS is able to provide full-scan product ion spectra with accurate fragment ion masses, thus providing structural information for compounds [12].

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To the best of our knowledge, this is the first time that UHPLC with a novel dual-probe ultraspray-ESI (UESI) and TOF/MS detector has been used to this end, resulting in a significant challenge in the complexity of developing appropriate accompanying chromatographic and mass spectrometry methods.

In this context, this study considers the extraction of NSAIDs and ACDs from aqueous samples using rotating-disk sorptive extraction (RDSE) in which the extraction device is a rotating disk with a central cavity containing Oasis™ HLB as the sorbent phase. This sample preparation technique was coupled to UHPLC–UESI–TOF/MS, achieving high selectivity and sensitivity. RDSE has been previously described by Manzo et al. [3] for NSAIDs using GC–MS for quantitation, which required previous derivatization of the analytes. Analytical features were considerably improved in this study in terms of a reduction in sample volume, reagents, wastes, and extraction time, together with the increase in another analyte family. Ion suppression/enhancement was evaluated, and the different response of each compound to this matrix-dependent effect is discussed. The eco-efficiency of this methodology was also considered and compared with several relevant references. To identify and quantify NSAIDs (naproxen, ibuprofen, ketoprofen and diclofenac) and ACDs (clofibric acid and gemfibrozil) in real samples, the method was finally applied to the analysis of real samples from WWTPs in two locations in Santiago, Chile.

2. Materials and methods

2.1. Reagents

Water from a Millipore Milli-Q Plus water system (Billerica, MA) was used throughout the experiment. All NSAIDs (ketoprofen, ibuprofen, naproxen, and diclofenac), ACDs (clofibric acid and gemfibrozil) and surrogate standard (meclofenamic acid) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The standard stock solution of the analytes and the surrogate standards (200 mg/L) were prepared separately in methanol (Fisher Scientific, Fair Lawn, NJ, USA). The pH was adjusted with 37% p.a. hydrochloric acid and p.a. sodium hydroxide from Merck (Darmstadt, Germany).

Nitrogen with a purity of ≥99.999% was used for final extract evaporation; it was supplied from Linde (Santiago, Chile). Acetonitrile (ACN) (LC–MS grade), methanol (MeOH) (LC–MS grade), ethyl acetate (HPLC grade), water (LC–MS grade), and glacial acetic acid (HPLC grade) were purchased from Merck. Oasis™ HLB extraction cartridges were obtained from Waters Corporation (Milford, MA, USA).

2.2. Sample preparation

An aliquot of 25 mL of a standard or a water sample was extracted by RDSE. A 250 μL aliquot of 1 mg/L meclofenamic acid was added as surrogate standard to the real samples. Hydrochloric acid (0.1 mol/L) and sodium hydroxide (0.1 mol/L) were used to adjust the pH of the water samples (pH 2). The rotating disk was loaded with 50 mg of Oasis™ HLB phase, which had previously been removed from the conventional cartridges; thereafter, the disk was placed inside the beaker containing the sample and was then rotated at 3000 rpm for 60 min at room temperature. After extraction, the disk was placed into a 15 mL beaker containing 5 mL of methanol as a desorbing solvent and was stirred for 10 min at 2000 rpm twice. Then, the methanol extract containing the concentrated analyte was evaporated under an N_2 stream to dryness. The extract was redissolved in 500 μL of methanol and filtered through a 0.22 μm pore size and 13 mm diameter PVDF filter. Ten microliters of this solution were injected in the UHPLC–UESI–TOF/MS.

2.3. UHPLC–UESI–TOF/MS analysis

Chromatographic analyses were performed on a PerkinElmer Flexar FX-15 ultra-high-performance liquid chromatography system (PerkinElmer, USA) with a binary pump system, a vacuum degasser, a cooling autosampler and a thermostated column compartment.

Separation was carried out using a Brownlee SPP-C18 (2.1 mm × 75 mm, 2.7 μm) analytical column (PerkinElmer, USA). Optimum separation was achieved with a binary mobile phase gradient at a flow rate of 0.3 mL/min. The column temperature was maintained at 20 °C, and the injection volume was 10 μL. Solvents were (A) water/acetic acid (0.1%) at pH 3.3 and (B) acetonitrile. The program used 0.5 min to equilibrate the system prior to sample injection (0% B). Afterwards, the gradient elution program was as follows: 0–1 min, 0–80% B; 1–2 min, 80–90% B; 2–5 min, 90–100% B; and finally returning to the initial conditions in 2 min.

Identification and quantification of NSAIDs and ACDs were performed using an AxION 2 TOF MS system equipped with a dual-probe ultraspray electrospray ionization source (UESI) and controlled by Chromera Software and TOF MS Driver Software (PerkinElmer, USA).

The working conditions of the source were as follows: capillary voltage of 6000 V, capillary exit of −125 V, gas flow rate of 10 L/min, gas temperature of 300 °C and nebulizer pressure in both probes of 80 psi. LC–MS accurate mass spectra were recorded across a range of 100–1200 *m*/*z*. The delta mass was 20 ppm for all compounds. To improve the sensitivity of the analysis, the acquisition function was in trap mode, setting the parameters “IG Exit Low” and “Trap/Pulse Delay” at 38 and 46 μs, respectively.

3. Results and discussion

3.1. Method optimization to determine NSAIDs and ACDs

The RDSE procedure used in this work was based on the previous procedure reported by Manzo et al., which was specifically designed for NSAID determination in water samples [3]. Some variations were tested to improve the analytical features of the original RDSE method. First, 25 mL of a water sample was extracted by RDSE for various periods of time from 15 to 90 min. The results obtained suggest that extraction with 25 mL for 60 min was the most efficient process, as shown in Fig. A.1 for ketoprofen and clofibric acid as representative analytes. This improvement in the extraction method reduces the sample consumption to half, avoids the need for derivatization, and reduces the extraction time by 30 min.

It is important to note that the TOF analyzer was combined with a dual-probe ultraspray-ESI, which represents a large improvement over conventional ionization sources by allowing simultaneous calibration and sample analysis. Thus, the mass precision is guaranteed; improving in this way the feasibility of the results. To correctly use this probe, the ionization of the analytes must be enabled using an appropriate mobile phase. In the present study, all analytes are acidic drugs with p*K*a values less than 5, and, consequently, a mobile phase of approximately pH 7 should be adequate; however, several studies have indicated that weak acids facilitate the formation of negative ESI ions when the pH is lower than the p*K*a [13].

Therefore, to achieve efficient deprotonation and separation of the analyzed compounds, different mobile phases (methanol, acetonitrile and aqueous solutions of formic acid, acetic acid and ammonia) were tested in gradient programs. Fig. 1 shows the significant differences observed in the abundances of each compound when different mobile phases were used. A mobile phase of formic

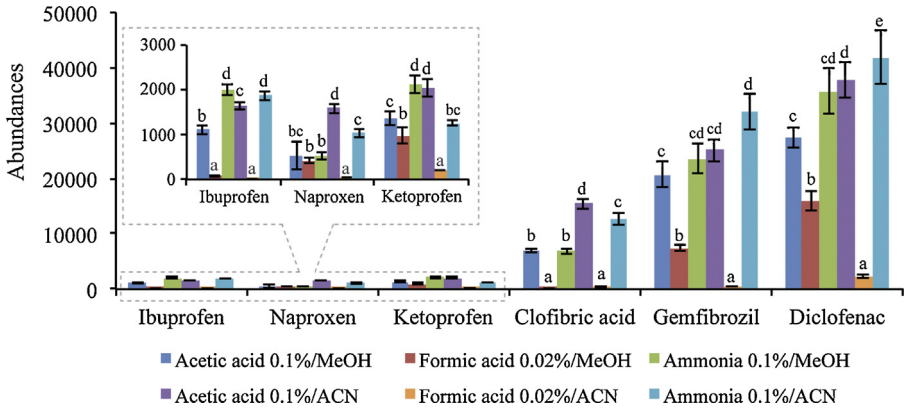

Fig. 1. Abundances of NSAIDs and ACDs obtained using the following different mobile phases: acetic acid 0.1%/MeOH, formic acid 0.02%/MeOH, ammonia 0.1%/MeOH, acetic acid 0.1%/ACN, formic acid 0.02%/ACN and ammonia 0.1%/ACN. Values followed by the same letter show no significant differences for $p < 0.05$, according to the Fisher's protected LSD test.

acid was discarded because a low sensitivity of the signal (abundance) was observed, as can be seen in Fig. 1.

Based on these results and contrary to the expected outcome, the mobile phases consisting of ammonia 0.1%/ACN and acetic acid 0.1%/ACN showed comparable results; using both in a gradient elution program. Although the mobile phase consisting of ammonia 0.1%/ACN showed good results, a lower peak resolution was observed. This can result from the pH of ammonia 0.1%/ACN (pH 9) being inappropriate for the column's stability and lifetime. The addition of acetic acid to water increased the overall sensitivity and improved the peak shape of the analyzed compounds. Because of this, mobile phase consisted of acetic acid 0.1%/ACN was selected. Acetonitrile was chosen as the organic modifier because it decreases the background noise and peaks and resulted in the best resolution [14].

This relationship between mobile phase pH and analyte response in ESI has also been observed by other research groups, termed the "wrong-way-around" [15–17]. Wu et al. [17] explained several reasons for this phenomenon. The electrochemical reaction in the ESI capillary tip could be facilitated in a weakly acidic environment (versus a neutral pH solution). Near the ESI tip, weak acids could provide protons that facilitate the production of excess negative charge by reducing the number of protons to hydrogen gas. These excess charges likely accumulate to a greater extent on the surface of ESI droplets, increasing the local pH and promoting the deprotonation of analytes.

To optimize the identification and quantification of the analytes in standard solutions, and influent and effluent WWTP samples, all possible combinations among two different mobile phase flows (from 0.2 to 0.5 mL/min) and two injection volumes (5 and 10 μL) were tested. The combination of a 0.3 mL/min flow rate and 10 μL injection volume produced optimal compound separation within 5 min. ESI in positive- and negative-ion mode was also evaluated, with all compounds being ionized in negative mode, as expected.

Detection was monitored in trap mode. The improvement of this mode with respect to conventional pulse mode is the use of specific times (μs) for both parameters, "IG Exit Low" and "Trap/Pulse Delay", which were evaluated between 2 and 333 μs. A good combination of both parameters significantly improves the sensitivity of the signals in the mass range of interest. After several assays, the optimum values of these parameters were 38 and 46 μs, respectively, increasing the sensitivity of the signals by two orders of magnitude with respect to the pulse mode and concomitantly reducing the detection and quantifications limits. Fig. 2 shows the extracted ion chromatograms (EICs) and scan spectra $[M-H]^-$ corresponding to the quantified NSAIDs and ACDs in a multi-standard solution containing 500 μg/L of each analyte. In this figure, the experimental mass is observed for each compound as follows: ketoprofen (m/z 253.0869), naproxen (m/z 229.0859), clofibric acid (m/z 213.0321), ibuprofen (m/z 205.1229), diclofenac, meclofenamic acid (m/z 294.0093) and gemfibrozil (m/z 249.1487). The calibrant (m/z 112.9784) was observed in all experiments, demonstrating the mass accuracy of the method.

Diclofenac and meclofenamic acid have the same exact mass; therefore, these compounds were identified by their retention times (Fig. 2). Table 1 shows the empirical formula, theoretical and experimental m/z, error (ppm) and retention time (min) for each compound. The ppm error was less than 2.2 for all the studied compounds, demonstrating the high resolution of TOF/MS detection; in addition, the excellent stability of the retention times increased the reliability of the analysis.

3.2. Analytical features of the method

After separation and optimization of the UPLC–UESI–TOF/MS conditions, the quality parameters of the extraction and chromatographic methods were studied to establish their performance characteristics, assuring suitable identification, confirmation and quantification of the studied compounds. The calibration curves, determination coefficients, detection (LODs) and quantification limits (LOQs) were obtained. Linearity was evaluated through the direct injection of standard solutions at seven concentration levels (from 10 μg/L to 1000 μg/L). Coefficients of determination (r^2), regression equations, method precision, LODs and LOQs are detailed in Table 2.

Method precision expressed as repeatability (within-day precision) and reproducibility (between-day precision) was evaluated for each compound using different extraction disks with NSAIDs and ACDs at 10 μg/L in drinking water and influent. The relative standard deviations (%RSDs) for the repeatability were consistently lower than 7% (with the only exception being clofibric acid at 9.5%) in drinking water; whereas the %RSD values in influent were typically 10–16%. On the other hand, the reproducibility exhibited %RSD values typically in the range of 5–13% in water and 15–19% in influent (Table 2).

The LOD and LOQ of the method were determined by following the $3-\sigma$ criteria using 10 blank samples for the determination of these compounds in water. These values ranged from 1 to 90 ng/L and 4 to 160 ng/L, respectively.

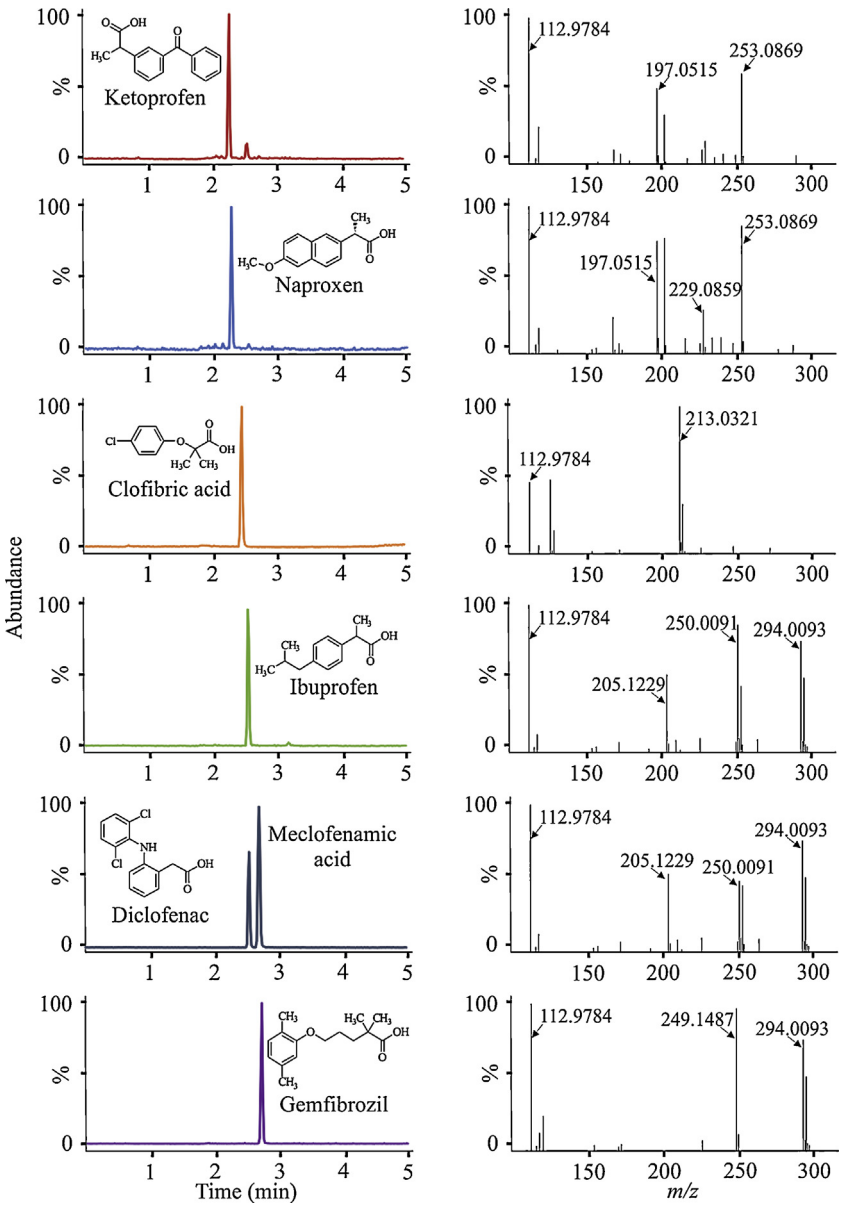

Fig. 2. Analytical signals of NSAIDs and ACDs in a multi-standard solution containing 500 μg/L of each analyte: (left panel) extracted ion chromatograms of $[M-H]^-$ obtained in TOF mode of *m/z* 253.0869 (ketoprofen), *m/z* 229.0859 (naproxen), *m/z* 213.0321 (clofibric acid), *m/z* 205.1229 (ibuprofen), *m/z* 294.0093 (diclofenac) and *m/z* 249.1487 (gemfibrozil); (right panel) scan spectra obtained in TOF mode.

Table 1
Formula, theoretical and experimental mass/charge (*m/z*), error (ppm) and retention time (min) of the compounds.

Compounds	Formula	Theoretical (*m/z*)	Experimental (*m/z*)	Error (ppm)	Retention time (min)
Ketoprofen	$C_{16}H_{14}O_3$	253.0864	253.0869	−2.0	2.32
Naproxen	$C_{14}H_{14}O_3$	229.0864	229.0859	2.2	2.33
Clofibric acid	$C_{10}H_{11}ClO_3$	213.0317	213.0321	−1.9	2.35
Ibuprofen	$C_{13}H_{18}O_2$	205.1227	205.1229	−1.0	2.67
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	294.0087	294.0093	−2.0	2.67
Gemfibrozil	$C_{15}H_{22}O_3$	249.1489	249.1487	0.8	2.70
Meclofenamic acid[a]	$C_{14}H_{11}Cl_2NO_2$	294.0087	294.0093	−2.0	2.87

[a] Surrogate standard.

3.3. Matrix effects and recoveries

The performance of the UESI–MS interface is considerably influenced by the composition of the liquid reaching the detector. The type and amount of organic mobile phase and the sample matrix components play key roles. Co-extracted substances present in the sample can suppress/enhance the analyte signal [18,19]. Because of this effect, the UESI–TOF/MS signal response obtained from standard and matrix samples are expected to differ significantly. However, these variations may also result from inefficient extraction of the compounds of interest [20].

Table 2
Linearity, regression equation, %RSDs, LOD and LOQ, ME and Re.

Compounds	Linearity (r^2)	Regression equation	Repeatability[a] (%RSD)		Reproducibility[b] (%RSD)		LOD (μg/L)	LOQ (μg/L)	ME (%)	Re (%)
			Water	Influent	Water	Influent				
Ketoprofen	0.998	$y = 400.3954x - 3378.4049$	7.0	11.8	12.3	15.5	0.002	0.007	31.5	107.8
Naproxen	0.999	$y = 154.7174x + 674.0056$	4.8	14.9	7.1	18.2	0.003	0.008	81.1	98.6
Clofibric acid	0.999	$y = 479.4310x - 8921.5497$	9.5	14.3	13.5	17.9	0.091	0.103	55.4	74.3
Ibuprofen	0.997	$y = 171.1217x + 4687.6833$	4.8	16.4	6.5	19.8	0.002	0.009	36.3	99.4
Diclofenac	0.998	$y = 379.6915x - 11473.6584$	4.7	13.4	5.9	16.0	0.089	0.163	54.3	95.1
Gemfibrozil	0.998	$y = 49.0005x + 2028.5373$	6.2	10.2	10.0	15.1	0.001	0.004	44.4	110.6
Meclofenamic acid[c]	0.996	$y = 735.9433x + 19272.3506$	5.0	16.9	7.1	19.7	0.053	0.061	45.7	100.0

[a] $n = 5$.
[b] $n = 5$.
[c] Surrogate standard.

In an effort to evaluate the matrix effect (ME) and recovery (Re) independently, two specific tests were carried out on 4 samples (S1, S2, S3 and S4). S1 was a multi-standard solution of 500 μg/L of each analyte; S2 was an influent extract obtained using RDSE that had been enriched with 500 μg/L of the analytes before injection; S3 was an extract obtained directly from an influent water sample; S4 corresponded to the extract obtained from an influent sample enriched with 10 μg/L of each NSAID and ACD (notice that, after the application of the concentration factor resulting from the extraction process, the actual concentration of each analyte was 500 μg/L).

The ME and Re were calculated using the following equations:

$$\text{ME}\ \ (\%) = \frac{Ax_{S2} - Ax_{S3}}{Ax_{S1}} \times 100 \quad (1)$$

$$Re\ \ (\%) = \frac{Ax_{S4} - Ax_{S3}}{Ax_{S2}} \times 100 \quad (2)$$

where Ax_{S1}, Ax_{S2}, Ax_{S3} and Ax_{S4} correspond to the abundances obtained for the NSAIDs and ACDs x from samples S1, S2, S3 and S4, respectively. Using this configuration, the recovery value obtained is free of any MEs.

Table 2 shows the ME and Re for each of the analytes. On the one hand, the ME results show a suppression effect in the analytical responses of all compounds. Most analytes presented a high ME of approximately 50%, except naproxen (81%). This is remarkable in the present study for two reasons. To the best of our knowledge, this is the first time that a detailed study of the MEs in these matrixes has been developed, with surprising results. The other reason is that it represents a turning point in NSAID and ACD research in water samples because it highlights the importance of developing an ME study when analyzing these compounds by UHPLC–UESI–TOF/MS to obtain representative and reliable results.

On the other hand, in analyzing the Re values obtained, several considerations must be taken into account. In SPE procedures, the cartridge is of primary importance in extracting the target compounds from water samples. Previous studies have shown that Oasis™ HLB cartridges generate the best absolute recoveries for most pharmaceuticals, achieving values of 60–85% [21–23]. Therefore, good recoveries were expected for RDSE using Oasis™ HLB. The results presented in Table 2 shows that the method achieved excellent quantitative recoveries of most of the analyzed compounds (95–110%) with RSDs <16% when analyzed by RDSE and UHPLC–UESI–TOF/MS.

3.4. Eco-efficiency

To achieve the highest eco-efficiency possible, analytical trends are working to reduce or eliminate reagents, minimize wastes, consume as little energy as possible and perform miniaturized, in-situ and non-invasive measurements. Based on a recent publication [24], the analytical eco-efficiency of this methodology was calculated.

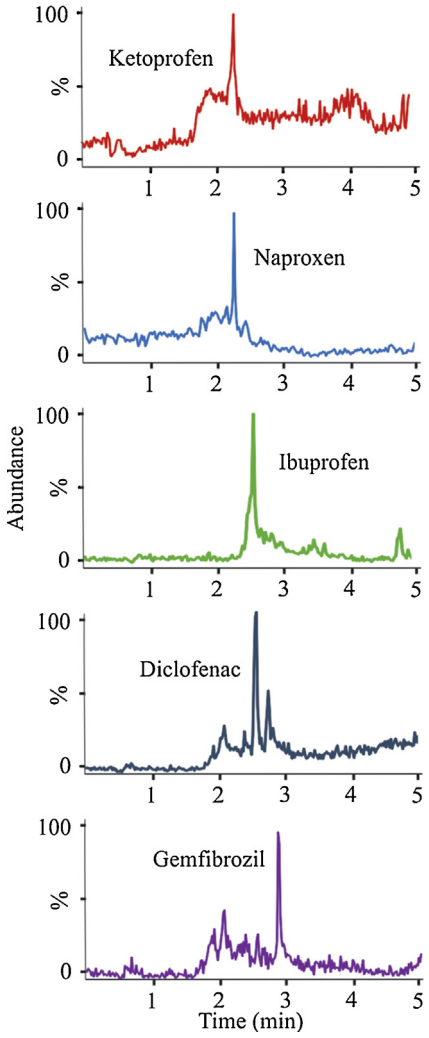

Fig. 3. Extracted ion chromatograms of $[M-H]^-$ obtained in TOF mode of NSAIDs and ACDs found in a real wastewater sample.

The methodology developed using RDSE implies a minimum consumption of reagents and samples with a concomitant lower generation of waste. Moreover, some wastes were recycled. For the determination of NSAIDs and ACDs, an UHPLC–UESI–TOF/MS was used, avoiding derivatization and reducing the consumption of energy due to the speed of the analysis (5 min per sample).

Table 3
NSAID and ACD concentrations (μg/L) in influent and effluent WWTP samples.

Compounds	WWTP1-I	WWTP1-E	WWTP2-I	WWTP2-E
Ketoprofen	1.6 ± 0.8	n.d.	1.2 ± 0.1	0.98 ± 0.07
Naproxen	1.13 ± 0.08	n.d.	1.2 ± 0.5	0.5 ± 0.1
Clofibric acid	n.d.	n.d.	n.d.	n.d.
Ibuprofen	2.6 ± 0.6	n.d.	1.9 ± 0.7	0.6 ± 0.1
Diclofenac	1.4 ± 0.6	1.39 ± 0.6	1.37 ± 0.08	1.3 ± 0.6
Gemfibrozil	20 ± 3	8.61 ± 2.7	13 ± 1	3.6 ± 0.1

n.d.: not detected.

Table 4
Comparison of different analytical methods for the extraction and determination of NSAIDs and ACDs in water samples.

Technique	Compounds	Sample volume (L)	Extraction time – chromatographic time (min)	LOD (ng/L)	Water sample	Recovery (%)	RSD (%)	Analytical eco-scale total score	Ref.
RDSE (Oasis HLB)–GC–MS	Diclofenac, ibuprofen, naproxen, ketoprofen	0.05	90, 60[a] – 39	1–33	WWTP-I WWTP-E	71–104[c]	3.5–25	78	[3]
SPE (Oasis MAX)–UHPLC–MS/MS	Gemfibrozil, diclofenac, ibuprofen	0.05	10 – 13	0.4–60	WWTP-I	65–73	7–14	62	[5]
		0.1		0.5–61	WWTP-E				
SPE (Oasis HLB)-UHPLC-MS/MS	Diclofenac, gemfibrozil, ibuprofen, ketoprofen, naproxen	0.1	30 – 9	48–642[b]	WWTP-I	55–124	4–6	53	[6]
				18–247[b]	WWTP-E	84–120	8–12		
SPE (STRATA X)–HPLC–UV–FL	Clofibric acid, diclofenac, gemfibrozil, ibuprofen, naproxen, ketoprofen	1	100 – 20	100–700[b]	River water	93–104	4–9	63	[7]
		0.5	50 – 20	30–970[b] 10–800[b]	WWTP-I WWTP-E	62–90 65–100	7–15 5–16		
SPE (Oasis HLB)–UPLC–QTOF/MS	Ibuprofen, ketoprofen	0.1	20 – 15	N.R.	WWTP-I	N.R.	<2%	74	[8]
		0.2	40 – 15		WWTP-E		N.R.		
		0.5	100 – 15		River water		N.R.		
SPE–GC–MS	Clofibric acid, diclofenac, gemfibrozil, ibuprofen, ketoprofen, naproxen	0.1	20, 60[a] – 23	40–84[b]	WWTP-I	56–82	3.9–8.3[c]	71	[26]
					WWTP-E	55–85			
SPE–UHPLC–MS/MS	Ketoprofen, naproxen, diclofenac, ibuprofen	0.5	60 – 6 .5	0.02–0.3	Seawater	86.5–101	1.1–2.6	73	[27]
SPE (Oasis HLB)–UHPLC–ESI-MS/MS	Diclofenac, gemfibrozil, ibuprofen, naproxen	1	100 – 5	0.4–1.0	Seawater	85–110	5.6–12.5	66	[28]
RDSE (Oasis HLB)–UHPLC–TOF/MS	Clofibric acid, diclofenac, gemfibrozil, ibuprofen, naproxen, ketoprofen	0.025	60 – 5	1–91	WWTP-I WWTP-E	74–110	10–16	80	This work

[a] Derivatization time.
[b] LOQ.
[c] Tap water.

Considering all of these advantages and calculating the penalty points of this work based on Gałuszka et al. [24], our method has an analytical eco-scale value of 80. These authors utilized a scale on which values >75 represent excellent green analyses. Therefore, it is reasonable to assume that our novel analytical procedure can be considered green analytical chemistry, increasing its environmental worth.

3.5. Real sample analysis

To evaluate the efficiency of the proposed method, it was applied to the analysis of real water samples. These samples were obtained from the influents (WWTP1-I and WWTP2-I) and effluents (WWTP1-E and WWTP2-E) of two WWTPs in Santiago, Chile. To ensure the reliability and feasibility of the developed methodology, samples were prepared in triplicate and each injected three times into the UHPLC–UESI–TOF/MS. An example of the chromatograms obtained is shown in Fig. 3. Mean values are shown in Table 3. Gemfibrozil had the highest concentration in both WWTPs, but, fortunately, its concentration was reduced to 60–80%. For the other compounds, the results agree with the levels determined in previous studies [25]. As shown in Table 3, the analyte concentrations decrease due to the WWTP treatment, sometimes even falling below the LOD, indicating either complete disappearance or degradation of the compounds [8].

After validation of this methodology with real samples to evaluate our work within the analytical chemistry field, a comparison between this work and other previous studies was performed. Table 4 compares different studies considering several parameters, as follows: technique, sample volume, extraction time and chromatographic time, LOD, water sample, recovery, RSD% and analytical eco-scale total score. According to the literature, this is the first study that combines RDSE and UHPLC–UESI–TOF/MS with concomitant analytical improvements. This methodology also has good LOD, recovery, RSD%, and analytical eco-scale values that are better than or similar to those of other studies.

4. Conclusions

A rapid and efficient method for the determination of four non-steroidal anti-inflammatory drugs (NSAIDs) and two anti-cholesterol drugs (ACDs) in water samples has been proposed. The rotating disk sorptive extraction (RDSE) method used in this study is able to combine high compound recovery (typically higher than 95%) with a reduction in the extraction time, the amount of sample and reagents used and the waste generated. The combination of this extraction method with a powerful analytical technique (ultra-high-performance liquid chromatography coupled to an ultraspray electrospray ionization source and time-of-flight mass spectrometry (UHPLC–UESI–TOF/MS) produced successful results. To the best of our knowledge, this is the first time that UHPLC coupled to an AxION 2 TOF MS was used for this purpose, opening up new fields and opportunities in research. A careful evaluation of matrix effects was necessary to ensure a reliable analytical method. The method designed consists of sample preparation in 60 min, together with reliable identification and quantification of NSAIDs and ACDs in 5 min. These compounds were determined in influent and effluent samples from wastewater treatment plants (WWTPs), leading to new possibilities in different matrixes, after sample preparation. This platform is a good candidate for an economical and green routine analytical method in industrial and scientific laboratories.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2015.10.071.

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