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RESEARCH PAPER

Eco-friendly LC–MS/MS method for analysis of multi-class micropollutants in tap, fountain, and well water from northern Portugal

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Abstract Organic micropollutants present in drinking water (DW) may cause adverse effects for public health, and so reliable analytical methods are required to detect these pollutants at trace levels in DW. This work describes the first green analytical methodology for multi-class determination of 21 pollutants in DW: seven pesticides, an industrial compound, 12 pharmaceuticals, and a metabolite (some included in Directive 2013/39/EU or Decision 2015/495/EU). A solid-phase extraction procedure followed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (offline SPE–UHPLC–MS/MS) method was optimized using eco-friendly solvents, achieving detection limits below 0.20 ng L^{-1}. The validated analytical method was successfully applied to DW samples from different sources (tap, fountain, and well waters) from different locations in the north of Portugal, as well as before and after bench-scale UV and ozonation experiments in spiked tap water samples. Thirteen compounds were detected, many of them not regulated yet, in the following order of frequency: diclofenac > norfluoxetine > atrazine > simazine > warfarin > metoprolol > alachlor > chlorfenvinphos > trimethoprim > clarithromycin ≈ carbamazepine ≈ PFOS > citalopram. Hazard quotients were also estimated for the quantified substances and suggested no adverse effects to humans.

Keywords Drinking water · Priority substances · Contaminants of emerging concern · Solid-phase extraction · Ultra-high-performance liquid chromatography–tandem mass spectrometry

Introduction

Many micropollutants are not completely removed during conventional domestic wastewater treatment and are discharged into water bodies (such as rivers) that are then used to supply drinking water treatment plants (DWTPs) providing tap water. Amoxicillin, naproxen, metoprolol, phenacetin, indomethacin, sulfamethoxazole, and caffeine are some of these refractory micropollutants, and despite their low concentrations in DW, they are of increasing public health concern [1, 2]. Moreover, even if public health effects are not expected, chemical compounds may cause ecotoxicological adverse effects after long-term exposure, particularly when present as complex mixtures [3, 4].

Some regulations on water pollution have been published in recent years. In the particular case of the European Union (EU), the requirements for a good chemical status of groundwater have been set out in Directive 2006/118/EC [5] and the values for wholesome and clean water for human consumption in Directive 1998/83/EC [6]. Moreover, the EU identified surface water protection as one of the top work priorities due to the increasing demand for water protection and treatment by environmental organizations and the general public. Directive 2000/60/EC [7] was the first mark in the European water policy, which set up a strategy to define high-risk substances to be prioritized. A set of 33 priority substances/groups of substances (PSs) and the respective environmental quality standards (EQS) were ratified by Directive 2008/105/EC [8]. In 2013, Directive 39/2013/EU [9] recommended attention to the monitoring and the progress of

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innovative water/wastewater treatment technologies, identifying 45 PSs to meet requirements for the protection of the aquatic compartments and human health. More recently, a set of substances for EU monitoring in surface water bodies was defined in the Watch List of Decision 2015/495/EU [10]. The occurrence and removal of these substances was already reviewed [11]. However, reports focused on the determination of organic micropollutants in DW, and in particular regarding contaminants of emerging concern (CECs), are still scarce and most countries do not have monitoring programs to routinely determine these micropollutants. In fact, the analytical challenge of measuring pollutants at low concentrations in environmental matrices, such as sludge and wastewater [12, 13], has been a major research focus for scientists in recent decades, but much less attention has been given to DW [14]. In this context, it is crucial to develop sensitive and reproducible analytical methods that enable the determination of organic micropollutants belonging to different classes in DW.

The employment of an accurate and precise sample preparation as well as analytical techniques with high standards of sensitivity and reproducibility, such as ultra-high-performance liquid chromatography (UHPLC), is required to assess the occurrence and respective removal of micropollutants after water treatment. Hyphenated chromatography–mass spectrometry techniques are presently the methods of choice for DW analysis (Electronic Supplementary Material (ESM) Table S1), with only few works dealing with both pharmaceuticals and pesticides [15, 16], some with pesticides and/or their metabolites [17–19], and most referring only to pharmaceuticals and/or their metabolites [14, 20–27]. Considering the resources and time consumed in these tasks, new analytical methods should incorporate multi-residue and environmentally friendly approaches, being able to determine trace levels of a wide range of chemically heterogeneous compounds and simultaneously reduce the cleanup and extraction steps using green solvents [28, 29].

Green chemistry principles were introduced in the 1990s, aiming to reduce the environmental impact of diverse chemical activities, including those used in research [30, 31]. In this scenario, green analytical chemistry (GAC) plays an important role, e.g., by reducing hazardous wastes, using reusable materials, and/or employing "eco-friendly solvents" or "green solvents". The last two terms refer to solvents that have a lower environmental impact resulting from their production, use, and disposal (life cycle assessment), and/or that allow health and safety impacts to be minimized [32]. The main goals of GAC include the multi-analyte determination and the development of new (or modification of) analytical methodologies through the replacement of toxic reagents by smaller amounts of safer reagents, preferentially obtained from renewable sources [29, 32]. Several strategies have been used in LC–MS/MS, such as the reduction of the internal diameter and particle size (sub-2 μm) of chromatographic columns (to diminish eluent consumption), and the replacement of conventional mobile phases (consisting of acetonitrile and/or methanol) by environmental friendly alternatives like water, ethanol, and carbon dioxide in the particular case of supercritical fluid chromatography [30, 33].

The aim of this work was the optimization and validation of an eco-friendly analytical method based on offline SPE-UHPLC–MS/MS for the multi-class determination of organic micropollutants (12 pharmaceuticals, one metabolite, seven pesticides, and one industrial compound) in DW from northern Portugal. The targeted organic contaminants (ESM Table S2) were selected on the basis of their inclusion in EU regulations; some of the compounds are specified in Directive 2013/39/EU or in the Watch List of Decision 2015/495/EU. The selected micropollutants were previously reported as toxic and frequently found in the aquatic environment [14, 20, 34]. The occurrence of the multi-class contaminants was investigated for the first time in DW samples from different sources (tap, fountain, and well waters) and locations in northern Portugal, and the related hazard quotients (HQs) were determined. The HQs evaluation for these micropollutants could be a predictive way to assess the human health risk of exposure to CECs, but only a few reports focused on this approach for organic contaminants in DW [2, 14, 34–37]. The efficiency of two processes (UV and ozonation) typically employed for DW disinfection and/or degradation of organic pollutants in DWTPs was also verified using the analytical strategy proposed.

Experimental

Chemicals and materials

All reference standards (diclofenac sodium, tramadol hydrochloride, azithromycin dihydrate, clarithromycin, trimethoprim, warfarin, clopidogrel hydrogen sulfate, metoprolol tartrate, carbamazepine, citalopram hydrobromide, venlafaxine hydrochloride, fluoxetine hydrochloride, norfluoxetine oxalate, alachlor, atrazine, simazine, isoproturon, chlorfenvinphos, pentachlorophenol, clofibric acid, and perfluorooctanesulfonic acid; >98 % purity) were purchased from Sigma-Aldrich (Steinhein, Germany). Individual stock solutions of approximately 1000 mg L^{-1} were prepared in methanol, ethanol, or acetonitrile, depending on the solubility of each analyte. Two working standard solutions containing all the target analytes at 200 μg L^{-1} and 20 μg L^{-1} were prepared by diluting each stock solution in ethanol. Surrogate standards (ketoprofen-d_3, fluoxetine-d_5 solution, and atrazine-d_5) were purchased from Sigma-Aldrich (Steinhein, Germany). Individual stock solutions of 1000 mg L^{-1} of the isotopically labeled internal standards ketoprofen-d_3 and atrazine-d_5 were prepared in methanol, the same solvent as the fluoxetine-d_5 solution. An ethanolic working solution containing 1 mg L^{-1} of each isotopically labeled internal standard was prepared.

Methanol and acetonitrile (MS grade) were obtained from VWR International (Fontenay-sous-Bois, France). Ethanol (HPLC grade) and ethylenediaminetetraacetic acid (EDTA) (99 %) were acquired from Fisher Scientific UK Ltd. (Leicestershire, UK). Sodium thiosulfate and L-ascorbic acid (99 %) were purchased from Sigma-Aldrich (Steinhein, Germany). Ammonium acetate, ammonium hydroxide 25 %, sulfuric acid, and formic acid were obtained from Merck (Darmstadt, Germany). Ultrapure water was supplied by a Milli-Q water system (resistivity of 18.2 MΩ cm, at 25 °C). HPLC-grade solvents were filtered with 0.22-μm nylon membrane filters (Membrane Solutions, TX, USA). Oasis® HLB (Hydrophilic-Lipophilic-Balanced), Oasis® MCX (Mixed-mode Cation eXchange), and Oasis® MAX (Mixed-mode Anion-eXchange) cartridges (150 mg, 6 mL), obtained from Waters (Milford, MA, USA), were tested for SPE optimization. A pHenomenal® pH 1100L pH meter (VWR, Germany) was used for the pH adjustments.

Sample preparation

Tap water samples were collected from the water supply network for use as matrix for the SPE optimization and method validation. The vacuum extraction and drying devices LiChrolut® used for SPE procedure were acquired from VWR (Merck Millipore, Billerica, MA, USA). In order to assess the best performance of SPE cartridges to extract the overall compounds, SPE optimization was performed by comparing Oasis® HLB, MCX, and MAX cartridges. Oasis® MAX and MCX cartridges were conditioned sequentially with 4 mL of methanol and 4 mL of ultrapure water at a flow rate of 1 mL min^{-1}. For HLB cartridges, the conditioning was performed at the same flow with 4 mL of methanol or ethanol and 4 mL of ultrapure water. The sample pH was optimized for HLB cartridges using methanol as conditioning solvent by comparing the recoveries achieved with initial sample pH adjusted to 3, 7, and 9. For MAX and MCX SPE procedures, samples were respectively alkalinized to pH 9 or acidified to pH 3, before loading. The pH adjustments were done with ammonium hydroxide or sulfuric acid. Sample loading was carried out with 250 mL of blank and spiked (35 ng L^{-1}) tap water samples at a constant flow rate of 10 mL min^{-1}, using the vacuum manifold unit connected to a vacuum pump. The washing step was performed with 4 mL of ultrapure water, 5 % ammonium hydroxide aqueous solution, or 2 % formic acid aqueous solution for HLB, MAX, and MCX, respectively. After the washing steps, the cartridges were dried under vacuum for 45 min. The elution step was performed at a flow rate of 1 mL min^{-1} with 4 mL of methanol or ethanol for Oasis® HLB cartridges, 4 mL of methanol to extract the neutral compounds and weak bases in the case of Oasis® MAX, and neutrals and weak acids in the case of Oasis® MCX. A second elution was performed for mixed-mode cartridges Oasis® MAX and MCX with a 2 % formic acid methanolic solution (elution of acids) or 5 % ammonium hydroxide methanolic solution (elution of basic compounds), respectively. The LiChrolut® drying device was coupled to the vacuum extraction unit to evaporate the extracts to dryness with a gentle nitrogen stream. The dry residues were reconstituted in 300 μL of ethanol and the ethanolic extracts were filtered using 0.22-μm polytetrafluoroethylene syringe filters (Membrane Solutions, TX, USA). To assess the breakthrough volume, sample loading was tested with three volumes of non-spiked (blanks) and 35 ng L^{-1} spiked tap water samples, namely 250, 500, and 1000 mL, using the optimized SPE procedure. In order to improve the recovery rates, the chelating agent EDTA (100 mg L^{-1}) was tested as well as two dechlorination agents, ascorbic acid (10 mg L^{-1}) and sodium thiosulfate (30 mg L^{-1}). Analysis of reuse efficiency for the optimized SPE protocol was performed in three consecutive days.

UHPLC–MS/MS

A Kinetex™ 1.7 μm XB-C18 100 Å column (100 × 2.1 mm, i.d.) (Phenomenex, CA, USA) was used and different mobile phases were tested (acetonitrile, ethanol, or methanol as organic phase and ammonium acetate, formic acid aqueous solutions, or water as aqueous phase). The optimized mobile phase was ethanol/water (70:30, v/v), pH 7.0, performed in isocratic mode using a flow rate of 0.20 mL min^{-1}. Column oven and autosampler temperatures were set respectively at 35 and 4 °C, and the volume of injection was 5 μL. An electrospray ionization source was used operating in both positive and negative ionization modes. The precursor ion and the two most abundant fragments were used for quantification by selected reaction monitoring (SRM) and identification (ESM Table S3). The mass spectrometer parameters declustering potential, collision energy, and collision cell exit potential of each analyte are described elsewhere [38]. The optimized conditions for MS parameters, using argon at 230 kPa as CID gas, were 2.5 dm^3 min^{-1} for nebulizing gas flow, 10 dm^3 min^{-1} for drying gas flow, 0.5 kV for capillary voltage, 450 °C for source temperature, and 200 °C for desolvation temperature.

Quality assurance/quality control

The offline SPE–UHPLC–MS/MS method validation was performed according to the international guidelines [39] and previous works [38, 40, 41], through the evaluation of the following parameters: selectivity, linearity and range, limits of detection and quantification, accuracy, precision, and recovery. Chromatograms of non-spiked tap waters (blank extracts), standards extracted from the spiked tap waters at 35 ng L^{-1}, and an ethanolic solution containing all the standards at a concentration corresponding to the theoretical concentration after SPE were compared to assess the selectivity. For

recovery experiments, three quality control (QC) standard solutions were prepared in triplicate in three consecutive days by extracting tap water samples spiked with three different concentrations (3.5, 15, and 35 ng L^{-1}). The peak areas of the standards extracted from the spiked tap waters were compared with those of ethanolic solutions containing all the standards at the theoretical concentration of recovered extracts to assess the recovery of each SPE procedure. For target compounds detected in the blank matrix, the peak areas were subtracted from those obtained with the spiked matrix.

The internal standard calibration method was used to define the linearity and range for each target analyte. Triplicates of 250 mL tap water samples spiked with seven different standard concentrations (0.75, 1.5, 2.0, 4.0, 8.0, 20, and 40 ng L^{-1}) were prepared, the pH was adjusted to 3, and sodium thiosulfate solution was added to obtain a concentration of 30 mg L^{-1}. Then 10 μL of a working internal standards solution of 1 mg L^{-1} was added to each sample. These standard solutions were extracted by the optimized SPE procedure and reconstituted in 300 μL of ethanol to create the calibration curves by injecting 5 μL into the UHPLC apparatus. Method detection (MDL) and quantification (MQL) limits were determined as described elsewhere [38, 41], spiking water samples prior to the SPE procedure with ethanolic standard solution to achieve successively diluted samples. The minimum detectable amount of each compound giving a signal-to-noise (S/N) ratio of 3.3 and 10 gave MDL and MQL, respectively. The three triplicate QC solutions, described above, were also used to evaluate the accuracy of the method as well as the precision (intra- and interbatch). The concentrations of the analytes in the SPE extracts calculated using the calibration curves were compared with the nominal concentration, in percentage, to determine the accuracy. The relative standard deviation (RSD) of the intra- and interbatch replicate analyses expressed the precision of the method [42, 43]. In order to evaluate the possible carry-over effect, ethanol was injected after each set of triplicates. The stability of the compounds was assessed by calculating the RSD of the three QC extracts stored at 4 °C in the autosampler 24 and 48 h after reconstitution.

Matrix effect

The post-extraction addition method was used to assess the matrix effect [38, 41, 43]. The method was carried out on tap water samples, by comparison of three post-spiked extracts of blank samples and three extracts of non-spiked blank samples, using the optimized SPE procedure. The matrix effect (ME) was calculated as the ratio of the peak areas obtained for blank extracts spiked after SPE, subtracting those of the non-spiked blanks (A) and the peak areas of the standards solution with a similar concentration as the post-spiked extracts (B) through the following equation: ME (%) = $A/B \times 100$ [41, 43]. The absence of matrix effect, the ionization enhancement, and the ionization suppression are given respectively by values of 100 %, >100 %, or <100 %.

Application to drinking water samples and chemical treatment

Grab DW samples from different sources, namely tap water ($n = 13$), fountain water ($n = 5$), and well water ($n = 5$), were collected at the end of May 2015 from various locations in northwest Portugal and analyzed by the proposed method. Samples were immediately stored at 4 °C until extraction, which was performed within 24 h. Before SPE, samples were acidified with sulfuric acid (pH 3), and sodium thiosulfate was added to each sample (30 mg L^{-1}) to reduce any residual chlorine that might be added as a disinfectant.

Tap water samples collected from the water supply network were spiked with the target analytes at 30 ng L^{-1} to assess the applicability of the present UHPLC–MS/MS method to assess the removal of the target micropollutants by chemical processes. UV and ozonation experiments were performed as described elsewhere [44], and the removal of the target micropollutants was evaluated after 30 min using a 1 L reactor loaded with 750 mL of the spiked samples under magnetic stirring at 350 rpm.

Human health risk assessment

For those substances found in DW, a preliminary human health risk assessment was performed through the estimation of the HQ according to previous works [35, 45]. HQ is given by the quotient of the estimated daily intake (EDI) and the acceptable daily intake (ADI):

$$\mathrm{HQ} = \frac{\mathrm{EDI}}{\mathrm{ADI}} \tag{1}$$

where EDI values were calculated for the higher concentration of each substance quantified in tap, fountain, or well water as follows:

$$\mathrm{EDI} = \frac{\text{Concentration} \times \text{Ingestion rate}}{\text{Body weight}} \tag{2}$$

by considering an average body weight of 70 kg for adults based on the average life expectancy at birth of the global population in 2013 of the World Health Organization and a water intake of 2 L day^{-1} [35]. ADI for each pesticide was based on the Australian ADI list [46], whereas the values for pharmaceuticals were calculated from Eq. 3:

$$\mathrm{ADI} = \frac{\mathrm{ADD}}{\mathrm{AF}} \tag{3}$$

where ADD is the average daily dose and AF is an assessment factor of 1000, which accounts for 10 from intraspecies variability, 10 for sensitivity in susceptible population groups, and 10 for the differences between the ADD and the no observed effect concentration [35, 37].

Results and discussion

UHPLC–MS/MS optimization

Chromatographic separation was optimized using a sub-2-μm-particle Kinetex™ column, allowing short and high resolution chromatographic runs. Since the present work deals with different groups of compounds with a vast range of physicochemical characteristics (ESM Table S2), the ideal mobile phase for certain target compounds might lead to low sensitivity for many other analytes. The mobile phase consisting of ethanol and ultrapure water gave the best signal intensity and symmetric peaks as previously found for a wastewater matrix [38]. The variation of organic/aqueous phase proportion and flow rate was optimized, and a mixture of ethanol and ultrapure water (70:30, v/v) was used with a flow rate of 0.20 mL min^{-1} in isocratic mode. The column oven temperature was set at 35 °C, thereby improving the resolution and peak shape of the analytes and reducing the analysis time to 15 min because raising the temperature reduces the viscosity of the mobile phase.

MS/MS optimization

The tandem MS detection using a triple quadrupole enabled the simultaneous quantification of the 21 analytes at trace levels, as well as confirming their identity. The precursor ions of each compound were selected through the flow injection analysis of each target analyte in full scan mode, under both positive and negative modes. From all the compounds studied in this work, 18 compounds and two internal standards had a higher intensity under positive mode of ionization, with the protonated molecular ion of each compound $[M+H]^+$ chosen as precursor ion, whereas four substances (three compounds and one internal standard) were more intense in the negative ionization mode using the deprotonated molecular ion of each compound $[M-H]^-$ as precursor ion. Most compounds presented two or more SRM; the most abundant product ion from each precursor ion (SRM1) was selected for quantification and the second most abundant (SRM2) was monitored for identity confirmation (ESM Table S3), with a scan time of 100 ms per transition. In order to confirm the identity of the compounds, both the retention time (Table 1) and the ion ratio (SRM1/SRM2) of each analyte were used, according to European Commission Decision 2002/657/EC. Two pharmaceuticals and one pesticide (tramadol, fluoxetine, and pentachlorophenol) had a poor fragmentation and only one SRM was monitored, a drawback overcome by the internal standard calibration using the respective surrogate standard.

SPE optimization

A detailed optimization study was carried out on the most relevant parameters that affect recovery rates and matrix effects, namely the sample pH, the extraction solvents, the type of cartridges, the sample volume, and the addition of chelating and dechlorination additives. Preliminary studies were performed to evaluate the performance of different sample pH, by extracting 250 mL of tap water samples through the versatile Oasis® HLB cartridges. The water samples were adjusted to different pH (3, 7, and 9) and extracted using a conventional solvent, i.e., methanol, as conditioning and eluting solvent. Acidic pH provided higher recoveries for acidic compounds, and in particular for pesticides and some pharmaceuticals (ESM Fig. S1); whereas, basic analytes were recovered better at higher pH, but a lower influence of pH on the extraction efficiencies was found for these compounds. Thus, the best compromise was to adjust the sample pH to 3 in order to get the best recovery for as many analytes as possible.

Recoveries of Oasis® MCX cartridges useful for extraction of basic compounds and Oasis® MAX adequate for extraction of acidic compounds were then compared to Oasis® HLB cartridges. A recovery higher than 70 % was achieved using Oasis® MCX for the antidepressants (citalopram, venlafaxine, fluoxetine) and for trimethoprim (Fig. 1). These results were expected owing to the high pK_a of these compounds (near 9). Clofibric acid and diclofenac were better recovered when extracted by Oasis® MAX cartridges (Fig. 1), owing to their acidic nature (pK_a values of approximately 4). However, the versatile Oasis® HLB cartridges suitable for most compounds (acidic, basic, and neutrals) provided higher recoveries for most analytes (Fig. 1), as observed in other works [15, 20]. Thus, Oasis® HLB was the adsorbent selected for the next recovery experiments, using sample pH adjusted to 3.

Different sample volumes were tested (250, 500, and 1000 mL) using Oasis® HLB cartridges and sample pH adjusted to 3 to determine the breakthrough volume, the volume that allows the maximum extraction efficiency and from which extraction efficiency declines [41]. A sample volume of 250 mL provided the highest recoveries for the majority of the compounds, except for fluoxetine and norfluoxetine, and was therefore selected as the optimized sample volume (data not shown). Although a higher volume would give a theoretical higher enrichment factor, the results showed that recovery rates for most compounds decreased using higher sample volumes because of the aforementioned phenomenon of decrease of extraction efficiency above the so-called breakthrough volume, as previously described [47]. Although a clean matrix was studied in the present work, it is reported in other studies

Table 1 Retention time, range, linearity, method detection (MDL) and quantification (MQL) limits, accuracy, precision (intra- and interbatch), and matrix effect for each target analyte

Class and subclass	Analyte	Retention time (min)	Range (n L^{-1})	r^2	MDL (ng L^{-1})	MQL (ng L^{-1})	Accuracy (%)	Intrabatch precision RSD (%)	Interbatch precision RSD (%)	Matrix effect (%)
Pharmaceuticals										
Anti-inflammatories	Diclofenac	1.27	0.75–40	0.9982	0.17	0.52	106.3 ± 10.5	1.67–8.48	10.1	22.2 ± 2.3
	Tramadol	5.65	0.75–40	0.9976	0.07	0.22	103.7 ± 9.3	2.28–3.55	12.9	117.1 ± 0.1
Antibiotics	Azithromycin	8.08	0.75–40	0.9969	0.20	0.61	93.4 ± 13.3	7.93–9.75	9.38	23.7 ± 8.4
	Clarithromycin	8.47	0.75–40	0.9957	0.11	0.32	104.1 ± 6.1	7.75–10.0	11.2	26.4 ± 11.5
	Trimethoprim	4.00	0.75–40	0.9993	0.07	0.21	97.1 ± 15.7	2.99–5.80	7.21	64.9 ± 13.3
Anticoagulant	Warfarin	1.28	0.75–40	0.9965	0.17	0.52	97.6 ± 15.1	7.67–15.2	10.6	193.4 ± 1.7
Antiplatelet agent	Clopidogrel	2.11	0.75–40	0.9982	0.01	0.04	112.1 ± 6.6	2.75–8.24	6.89	77.4 ± 10.3
Beta-blockers	Metoprolol	6.29	0.75–40	0.9984	0.05	0.15	109.3 ± 0.7	3.26–14.0	13.2	113.1 ± 5.6
Psychiatric drugs	Carbamazepine	1.32	0.75–40	0.9966	0.19	0.59	100.6 ± 3.5	9.76–15.0	8.38	30.4 ± 8.4
	Citalopram	6.06	0.75–40	0.9961	0.09	0.26	86.6 ± 6.4	5.09–11.4	14.5	113.2 ± 11.7
	Venlafaxine	6.84	0.75–40	0.9978	0.10	0.32	105.2 ± 5.4	1.11–4.60	14.5	108.9 ± 2.1
	Fluoxetine	8.86	0.75–40	0.9963	0.04	0.13	118.1 ± 0.3	0.77–3.96	5.19	95.2 ± 6.4
Metabolite	Norfluoxetine	8.93	0.75–40	0.9975	0.05	0.16	119.0 ± 0.2	3.04–6.79	6.99	95.2 ± 4.4
Pesticides										
Chloroacetanilide	Alachlor	1.65	0.75–40	0.9975	0.09	0.28	98.8 ± 0.3	6.39–14.9	8.97	99.2 ± 10.3
Triazine	Atrazine	1.33	0.75–40	0.9945	0.12	0.37	92.3 ± 2.8	2.60–6.47	7.86	52.5 ± 15.4
	Simazine	1.21	0.75–40	0.9983	0.15	0.46	84.9 ± 4.6	3.86–9.23	8.35	49.8 ± 2.4
Organophosphorus	Chlorfenvinphos	1.62	0.75–40	0.9971	0.18	0.54	98.6 ± 6.2	5.01–14.7	14.8	96.9 ± 2.0
Phenylurea	Isoproturon	1.34	0.75–40	0.9968	0.04	0.12	99.2 ± 3.4	2.00–4.10	5.02	34.4 ± 9.4
Organochlorine	Pentachlorophenol	1.55	0.75–40	0.9986	0.20	0.60	94.1 ± 6.8	7.75–13.2	8.65	57.5 ± 9.0
Herbicide	Clofibric acid	1.23	0.75–40	0.9995	0.14	0.42	92.7 ± 5.5	6.20–11.0	6.57	19.1 ± 6.5
Industrial compound	PFOS	1.07	0.75–40	0.9957	0.06	0.19	80.6 ± 6.2	5.30–13.5	4.51	48.7 ± 1.4

MDL method detection limit, *MQL* method quantification limit

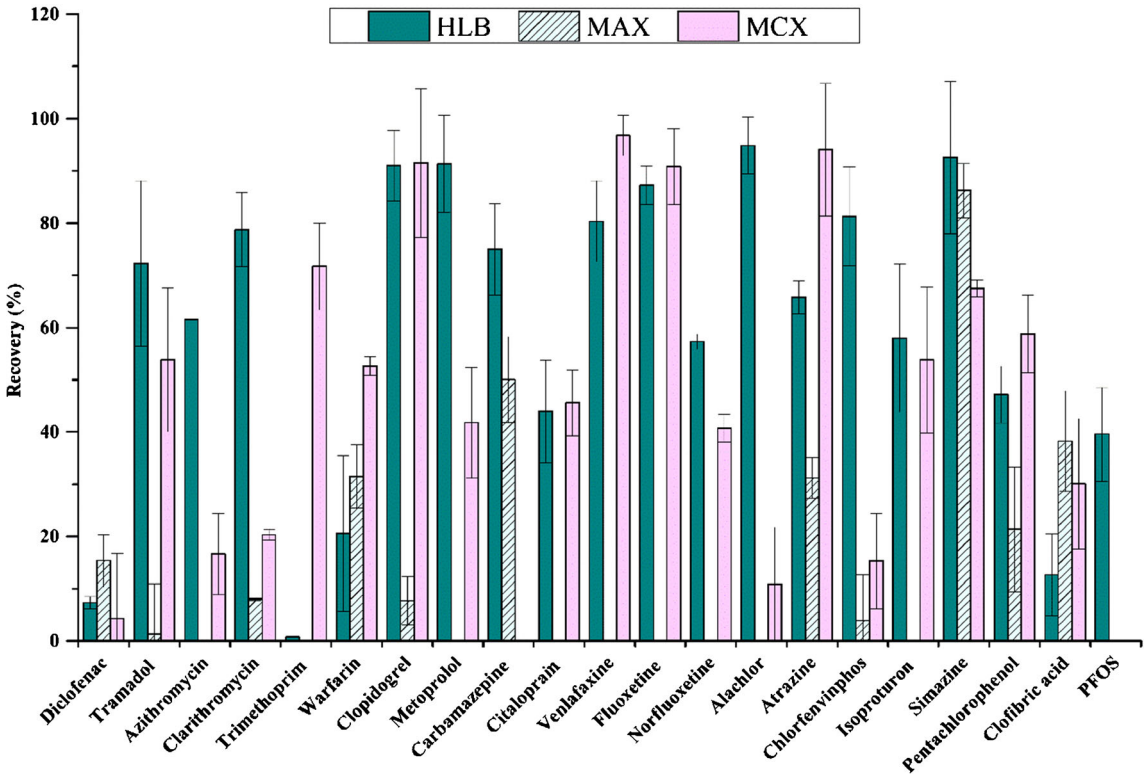

Fig. 1 Recoveries obtained for the target analytes with the following SPE conditions: HLB, MAX, and MCX using methanol and extracting 250 mL of tap water samples, adjusted to pH 3 for HLB and MCX and pH 9 for MAX cartridges

dealing with different matrices that even when using the same method, the recovery is not always better for matrices that are supposed to be cleaner [20, 48].

Afterwards, Oasis® HLB cartridges were employed to extract 250 mL of tap water samples at pH 3 (optimized for methanol) using ethanol as conditioning and elution solvent because of the known toxicity of methanol, usually used for SPE. Ethanol (Fig. 2) gave recoveries slightly higher than methanol (Fig. 1) for the majority of compounds. Moreover, ethanol is considered a "green" solvent, i.e., minimizes the environmental impact resulting from the use of solvents, and follows the guidelines of GAC [28, 29]. In fact, several methods reported in the literature employ solvents such as methanol or acetonitrile, presenting high toxicity [14, 15, 20, 22, 23, 27]. Thus, ethanol was selected as solvent for the next experiments. This is the first SPE procedure proposed for extraction and cleanup of DW samples that employs ethanol as extracting and eluting solvent.

Subsequently, the chelating and dechlorination effects were studied. Whilst a solution of EDTA was added to the water samples to test the chelating effect, ascorbic acid or sodium thiosulfate was added to assess the dechlorination effect. Regarding to the addition of EDTA, it was possible to verify a slight improvement in the extraction efficiency of a few compounds (Fig. 2), compared with the results obtained for samples without additive, namely for chlorfenvinphos, clofibric acid, trimethoprim, and diclofenac. This could be explained by the fact that these compounds might bind to residual metals present in the sample matrix, resulting in lower extraction recoveries [20]. By adding EDTA, soluble metals bind to the chelating agent, increasing the extraction efficiency of some compounds that are available to be extracted and detected [20]. This phenomenon was previously observed in DW by several authors [14, 20, 23]. Concerning the dechlorination agents, the addition of sodium thiosulfate increased the overall extraction recoveries (Fig. 2), probably because it reduced the residual chlorine that had been added as a disinfectant in the DW supply [22]. The effects of filtering and/or aeration of the water samples and the simultaneous addition of EDTA and sodium thiosulfate were also studied; however, the recovery efficiency was not improved. Therefore, sodium thiosulfate was used before SPE to enhance the recovery rates.

The main objective of the optimization of the sample preparation methodology was the development of a single SPE procedure, allowing the extraction of a large group of compounds with different physicochemical characteristics. As a result, and according to the higher recoveries obtained for most of the target compounds, the selected conditions were Oasis® HLB cartridges, ethanol as conditioning and eluting solvent, and 250 mL of water samples (pH 3) with sodium thiosulfate at 30 mg L^{-1} as dechlorination agent.

The recoveries obtained for reuse performance assessment of the cartridges showed that each reuse led to a loss of retention capacity of the cartridges, reflected by the decrease of the

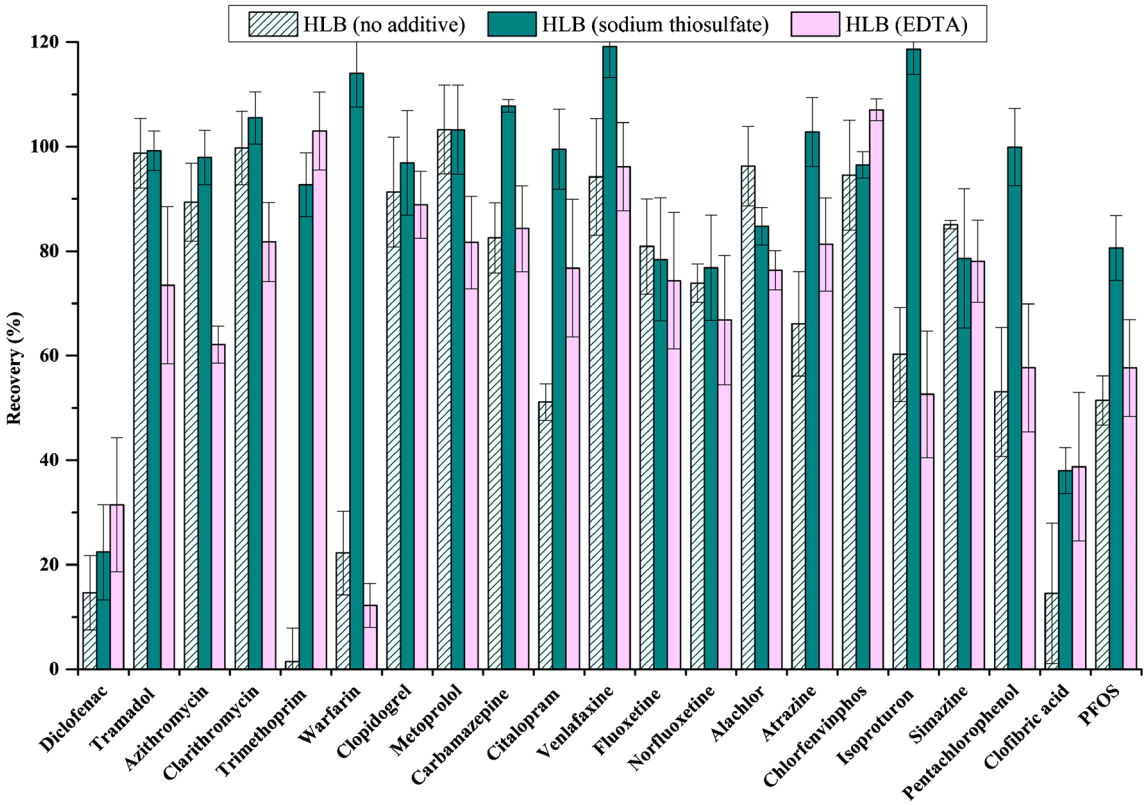

Fig. 2 Recoveries obtained for the target analytes with the following SPE conditions: HLB cartridges using ethanol, extracting 250 mL of tap water samples, adjusted to pH 3, without additives, with sodium thiosulfate, or EDTA as additives

recovery of the compounds. The first reuse of the cartridges led to an average decrease of 14 % in the recovery efficiency. The loss was higher for the second reuse, with a decrease of approximately 50 % in the recovery rates. Here, it was verified that although claimed by the supplier, reuse of cartridges is not a good practice for analytical purposes that require a high reproducibility.

Matrix effect

The matrix effect was determined by the post-extraction addition method to assess the influence of the matrix in the ionization process occurring in the ionization source of the mass spectrometer [38]. The percentage ratio between the post-spiked blank extracts and ethanolic standard solutions was between 19.1 % and 193 %. Although DW is considered a clean and simple matrix, a wide range of values was found for the matrix effect. Cotton et al. [49] also reported high matrix interferences for many compounds; only less than half of the analytes had matrix effect values within 80–120 %. When LC–MS/MS methods are developed to determine various micropollutants in different matrices, e.g., DW, surface water, and wastewater, matrix effects are usually calculated for only one of these matrices. Most compounds presented signal suppression, i.e., matrix effect < 100 %, namely diclofenac, azithromycin, clarithromycin, trimethoprim, clopidogrel, carbamazepine, atrazine, simazine, isoproturon, pentachlorophenol, clofibric acid, and PFOS (Table 1). Tramadol, metoprolol, citalopram, and venlafaxine had a slight ionization enhancement (matrix effect > 100 %) while the signal of warfarin was highly increased. Compounds with almost no matrix effect, under the conditions of the current work, were fluoxetine, norfluoxetine, alachlor, and chlorfenvinphos.

Quality assurance/quality control

The trends of GAC were applied in the chromatographic optimization, namely the use of low volumes of non-toxic solvents [28, 29]. Enhanced productivity and reduced cost are the main objectives for routine analysis, both being possible using stationary phases with reduced column length and diameter [30, 33]. Also the new instruments operating at higher pressure allow the use of more viscous solvents such as ethanol, which is less volatile than acetonitrile and has less toxicity and lower disposal costs than both acetonitrile and methanol, complying with the trends of GAC. The short run time and the low volume of a non-toxic organic phase such as ethanol are a great achievement in the method development, in comparison to chromatographic methods for DW analysis using methanol [15, 20, 21] or acetonitrile [14, 22, 23, 27] as organic mobile phases, as well as methanol as solvent for conditioning and eluting the SPE cartridges

[14, 15, 20, 22, 23]. In the present work, 21 compounds with diverse chemical nature (seven pesticides, one industrial compound, 12 pharmaceuticals, and one metabolite) were determined in a single run (ESM Fig. S2a, b). In the limited literature for DW analysis, the number of compounds analyzed by LC–MS/MS varies up to ca. 80, most reports deal with pharmaceuticals only [14, 20–23, 27], and a couple of them deal with both pharmaceuticals and pesticides [15, 16].

The offline SPE–UHPLC–MS/MS method was validated according to the international guidelines [39] and works published elsewhere [38, 41, 50], regarding recovery, accuracy, intra and inter-batch precision (Table 1). The recovery of the target analytes using the optimized SPE procedure was assessed after preconcentration of blank samples and 35 ng L^{-1} spiked samples. The recoveries evaluated for the DW matrix were reproducible and between 22.4 % and 139 % (Fig. 2). Peak areas of the target analytes found in the DW blank matrix were deducted for recovery rate evaluation. The dissimilar recoveries are due to the wide chemical nature of the target compounds and were taken into account, using the matrix-matched calibration curves and addition of internal standards before SPE. For instance, Gros et al. [20] developed a multi-residue analytical method, with similar recoveries values for DW, namely for cimetidine (24 ± 16.5 %). In that work, recovery values for the same compounds were higher in other matrices such as surface and wastewaters. López-Serna et al. [48] also reported some low values of recovery (<10 %) for groundwater, and higher recoveries for matrices presumably more affected by interferents. Accuracy and intra- and interbatch precision were evaluated by analysis of the QC extracts. The accuracy ranged from 80.6 % to 119 % (Table 1), which is within the range of 80–120 %, according to the international criteria [39]. RSD of the triplicate measurements of the three QC was used to guarantee the precision of the method (Table 1), with intrabatch precision less than 15.2 % and interbatch precision less than 14.8 %, meeting the international guidelines (RSD lower than 15 % or 20 % for the lower concentration QC) [39]. RSD of the triplicate analysis of the three QC samples after 24 and 48 h of reconstitution was lower than 5 %. The calibration curves were generated using the internal calibration method through spiking samples with isotopically labeled internal standards before SPE extraction. Three internal standards were used for three sets of compounds that were defined depending on the acid/basic nature (see ESM Table S3), as in other published works dealing with multi-class determination [14, 20, 27], which use an internal standard for each set of compounds owing to the high cost for routine environmental monitoring and difficulty in finding suitable internal standards for each compound in a series of compounds with distinct properties. The coefficients of determination of the calibration curve extracts were higher than 0.99 in the range of 0.75–40 ng L^{-1} for all compounds (Table 1). The MDL and MQL were 0.01–0.20 ng L^{-1} and 0.04–0.61 ng L^{-1}, respectively, allowing one to detect the target contaminants at residual concentrations (few nanograms per liter levels).

Quantification of micropollutants in DW

The developed offline SPE–UHPLC–MS/MS method was applied to DW samples collected at the end of May 2015, from various locations of northwest Portugal and from different sources (Table 2), namely tap water ($n = 13$) (ESM Fig. S2c), fountain water ($n = 5$), and well water ($n = 5$). Of the 21 investigated chemicals, 13 were detected in DW samples at nanogram per liter levels, which is consistent with concentrations reported in other studies [14, 15, 20–23, 27, 51]. The most common chemicals observed were diclofenac, trimethoprim, warfarin, metoprolol, norfluoxetine, atrazine, and simazine.

Regarding tap water, diclofenac, warfarin, norfluoxetine, atrazine, and simazine were the compounds most frequently detected. The micropollutants found at the highest concentrations were diclofenac and the pesticide chlorfenvinphos considered a PS, although well below the 0.1 μg L^{-1} required for single pesticides in Directive 1998/83/EC [6]. Concerning fountain water samples, diclofenac and atrazine were the most common micropollutants, being also found at the highest concentrations. The results obtained for well water samples showed that diclofenac was quantified in all the samples. Diclofenac, carbamazepine, and the PS simazine were those found at the highest concentrations.

The comparison of the results obtained in this work with similar studies conducted by other authors (ESM Table S4) is difficult, since the consumption of pharmaceutical compounds as well as the intensity of agricultural and industrial activities vary among different regions. Carbamazepine, caffeine, ibuprofen, and sulfamethoxazole were often reported in DW, with carbamazepine being the most frequently found up to 40 ng L^{-1} [14, 15, 20–23, 27, 51]. Other compounds such as atenolol, clofibric acid, azithromycin, erythromycin, fluoxetine, and diclofenac were also detected but at very low levels [15, 20, 21, 23, 27]. It is important to emphasize the need for revision of the European policy regarding tap water, considering that Directive 1998/83/EC is outdated in view of the studies reported in the last decade. The more recent Directive 2013/39/EU regulates surface waters, demanding more rigorous acceptable values than Directive 1998/83/EC [6] regulating water for human consumption. The same issue should be considered for groundwater regulated by Directive 2006/118/EC [5], considering that fountain and well waters used for human consumption can be sourced from this type of water.

Human health risk assessment

The maximum values of each micropollutant in DW were used to estimate the respective HQ. This prediction gives insights about the human health risk assessment by evaluating the

Table 2 Concentrations of micropollutants (ng L^{-1}) detected in tap, fountain, and well water samples analyzed

Class and sub-class	Analyte	Tap water ($n = 13$)		Fountain water ($n = 5$)		Well water ($n = 5$)	
Pharmaceuticals		Concentration (ng L^{-1})	Frequency	Concentration (ng L^{-1})	Frequency	Concentration (ng L^{-1})	Frequency
Anti-inflammatories	Diclofenac	<MQL–7.87	7/13	3.95–7.66	4/5	1.60–36.20	5/5
	Tramadol	ND	ND	ND	ND	ND	ND
Antibiotics	Azithromycin	ND	ND	ND	ND	ND	ND
	Clarithromycin	<MQL	1/13	ND	ND	1.14	1/5
	Trimethoprim	<MQL	1/13	<MQL	1/5	0.86	1/5
Anticoagulant	Warfarin	0.39–3.89	5/13	4.07	1/5	11.2	1/5
Antiplatelet agent	Clopidogrel	ND	ND	ND	ND	ND	ND
Beta-blockers	Metoprolol	<MQL	5/13	ND	ND	<MQL	1/5
Psychiatric drugs	Carbamazepine	3.34	1/13	ND	ND	58.8	1/5
	Citalopram	<MQL	1/13	ND	ND	ND	ND
	Venlafaxine	ND	ND	ND	ND	ND	ND
	Fluoxetine	ND	ND	ND	ND	ND	ND
Metabolite	Norfluoxetine	<MQL	13/13	<MQL	1/5	<MQL	1/5
Pesticides							
Chloroacetanilide	Alachlor	<MQL	4/13	ND	ND	3.07	1/5
Triazine	Atrazine	1.14–2.24	6/13	1.59–103	3/5	1.66	1/5
	Simazine	<MQL–1.45	4/13	<MQL–2.20	2/5	2.84–28.40	2/5
Organophosphorus	Chlorfenvinphos	2.46–6.50	2/13	0.49–3.89	2/5	ND	ND
Phenylurea	Isoproturon	ND	ND	ND	ND	ND	ND
Organochlorine	Pentachlorophenol	ND	ND	ND	ND	ND	ND
Herbicide	Clofibric acid	ND	ND	ND	ND	ND	ND
Industrial compound	PFOS	<MQL	1/13	ND	ND	11.7	1/5

MQL method quantification limit, *ND* not detected

probability of adverse effects: HQ values below 0.1 indicate no expected adverse effects; values between 0.1 and 1.0 suggest potential for adverse effects that should be considered, despite the low risk; HQ values ranging from 1.0 to 10 indicate adverse effects or mild risk; a high risk is assumed only for HQ values above 10 [35]. The maximum measured concentrations observed for the targeted chemicals found in DW (Table 2) were used to calculate EDI, predicting the worst case scenario. Even so, the HQs for all micropollutants found in DW samples were between 4.56×10^{-6} and 4.49×10^{-3}, i.e., well below 0.1, so adverse effects are not likely to be expected at such concentrations. Risks assessment of simultaneous exposure to multiple contaminants was not considered, although some of these compounds are already recognized to trigger several additive, synergistic, or antagonist effects [34, 36].

Removal of micropollutants in DW using UV radiation or ozonation

Tap water samples collected from the water supply network were post-spiked with the target micropollutants at nanogram per liter level and exposed to UV radiation or ozonation to assess the removal of the target micropollutants using the eco-friendly analytical method (Fig. 3), since these processes are often applied in DWTPs.

Only seven pharmaceuticals were completely removed by these water treatments: (i) tramadol, venlafaxine, and azithromycin by both processes; note that azithromycin was recently included in the first Watch List by the EU Decision 2015/495; (ii) clopidogrel, carbamazepine, and isoproturon by ozonation; and (iii) the metabolite norfluoxetine by UV. Regarding the other micropollutants, the efficiency of the processes varied according to the substance. The results showed that, in general, UV radiation was more effective than ozonation for the removal of pesticides and for the industrial compound, whereas ozonation performed slightly better for pharmaceuticals. The feasibility of this UHPLC–MS/MS analytical method for monitoring chemical processes used to improve the quality of DW was shown.

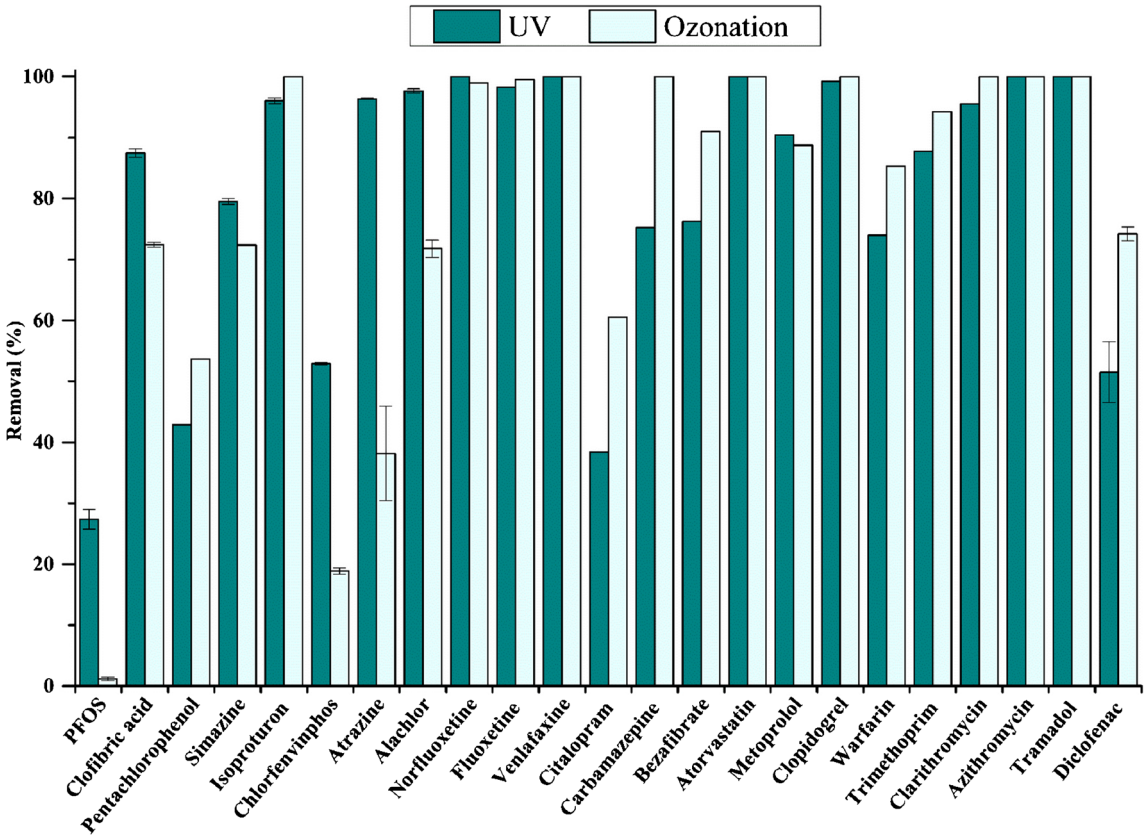

Fig. 3 Removal percentage of the micropollutants in spiked DW after the bench-scale UV or ozonation treatments

Conclusions

The offline SPE–UHPLC–MS/MS method that was developed and validated in this work for the assessment of the occurrence and removal of 21 multi-class micropollutants in DW has the great advantage of using an eco-friendly solvent (ethanol) for both SPE procedure and UHPLC analysis, according to the recent concerns about GAC applied to environmental analyses. Additional advantages of the method are (i) low detection limits (below 1 ng L^{-1}); (ii) short run time; (iii) low volume of eluent employed for each analysis; (iv) the use of a single cartridge/SPE procedure to extract all the target analytes; (v) and the low volume of sample used. The potential of the offline SPE–UHPLC–MS/MS method for monitoring programs and evaluation of advanced treatment options (UV and ozonation) was demonstrated in the selected case studies. For instance, analysis of tap, fountain, and well water samples from different locations of northwest Portugal showed the widespread occurrence of micropollutants in such matrices at nanogram per liter levels. Among the 13 micropollutants detected in DW samples, the most common were diclofenac, trimethoprim, warfarin, norfluoxetine, atrazine, and simazine; the feasibility of the method for monitoring DW treatment processes was also validated.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Benitez FJ, García J, Acero JL, Real FJ, Roldan G. Non-catalytic and catalytic wet air oxidation of pharmaceuticals in ultra-pure and natural waters. Process Saf Environ Prot. 2011;89(5):334–41.
2. Lin T, Yu S, Chen W. Occurrence, removal and risk assessment of pharmaceutical and personal care products (PPCPs) in an advanced drinking water treatment plant (ADWTP) around Taihu Lake in China. Chemosphere. 2016;152:1–9.
3. Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, et al. Collapse of a fish population after exposure to a synthetic estrogen. Proc Natl Acad Sci U S A. 2007;104(21): 8897–901.
4. Santos LH, Araujo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MC. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J Hazard Mater. 2010;175(1-3):45–95.
5. Directive_98/83/EC. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Off J Eur Commun. 1998;330:32–54.
6. Directive_2006/118/EC. Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. Off J Eur Union. 2006;372:1–31.
7. Directive. 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off J Eur Commun. 2000;L327:1–72.
8. Directive. 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Off J Eur Union. 2008;L348:84–97.
9. Directive_39. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Off J Eur Union. 2013;L226:1–17.
10. Decision_495. Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off J Eur Union. 2015;L78:40–2.
11. Barbosa MO, Moreira NFF, Ribeiro AR, Pereira MFR, Silva AMT. Occurrence and removal of organic micropollutants: an overview of the watch list of EU Decision 2015/495. Water Res. 2016;94:257–79.
12. Dasenaki ME, Thomaidis NS. Multianalyte method for the determination of pharmaceuticals in wastewater samples using solid-phase extraction and liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2015;407(15):4229–45.
13. Gago-Ferrero P, Borova V, Dasenaki ME, Thomaidis NS. Simultaneous determination of 148 pharmaceuticals and illicit drugs in sewage sludge based on ultrasound-assisted extraction and liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2015;407(15):4287–97.
14. Ferrer I, Zweigenbaum JA, Thurman EM. Analysis of 70 Environmental Protection Agency priority pharmaceuticals in water by EPA Method 1694. J Chromatogr A. 2010;1217(36):5674–86.
15. Maldaner L, Jardim IC. Determination of some organic contaminants in water samples by solid-phase extraction and liquid chromatography-tandem mass spectrometry. Talanta. 2012;100:38–44.
16. Rodil R, Quintana JB, Lopez-Mahia P, Muniategui-Lorenzo S, Prada-Rodriguez D. Multi-residue analytical method for the determination of emerging pollutants in water by solid-phase extraction and liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2009;1216(14):2958–69.
17. Kowal S, Balsaa P, Werres F, Schmidt TC. Fully automated standard addition method for the quantification of 29 polar pesticide metabolites in different water bodies using LC-MS/MS. Anal Bioanal Chem. 2013;405(19):6337–51.
18. Mann O, Pock E, Wruss K, Wruss W, Krska R. Development and validation of a fully automated online-SPE–ESI–LC–MS/MS multi-residue method for the determination of different classes of pesticides in drinking, ground and surface water. Int J Environ Anal Chem. 2016;96(4):353–72.
19. Sancho JV, Pozo OJ, Hernandez F. Liquid chromatography and tandem mass spectrometry: a powerful approach for the sensitive and rapid multiclass determination of pesticides and transformation products in water. Analyst. 2004;129(1):38–44.
20. Gros M, Rodriguez-Mozaz S, Barcelo D. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J Chromatogr A. 2012;1248:104–21.
21. Stolker AA, Niesing W, Hogendoorn EA, Versteegh JF, Fuchs R, Brinkman UA. Liquid chromatography with triple-quadrupole or quadrupole-time of flight mass spectrometry for screening and confirmation of residues of pharmaceuticals in water. Anal Bioanal Chem. 2004;378(4):955–63.
22. Wang C, Shi H, Adams CD, Gamagedara S, Stayton I, Timmons T, et al. Investigation of pharmaceuticals in Missouri natural and drinking water using high performance liquid chromatography-tandem mass spectrometry. Water Res. 2011;45(4):1818–28.
23. de Jesus Gaffney V, Almeida CM, Rodrigues A, Ferreira E, Benoliel MJ, Cardoso VV. Occurrence of pharmaceuticals in a water supply system and related human health risk assessment. Water Res. 2015;72:199–208.
24. Cimetiere N, Soutrel I, Lemasle M, Laplanche A, Crocq A. Standard addition method for the determination of pharmaceutical residues in drinking water by SPE–LC–MS/MS. Environ Technol. 2013;34(22):3031–41.
25. Idder S, Ley L, Mazellier P, Budzinski H. Quantitative on-line preconcentration-liquid chromatography coupled with tandem mass spectrometry method for the determination of pharmaceutical compounds in water. Anal Chim Acta. 2013;805:107–15.
26. Boleda MR, Galceran MT, Ventura F. Validation and uncertainty estimation of a multiresidue method for pharmaceuticals in surface and treated waters by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2013;1286:146–58.
27. Pinhancos R, Maass S, Ramanathan DM. High-resolution mass spectrometry method for the detection, characterization and quantitation of pharmaceuticals in water. J Mass Spectrom. 2011;46(11):1175–81.
28. de la Guardia M, Garrigues S. The social responsibility of environmental analysis. TrEAC Trends Environ Anal Chem. 2014;3–4:7–13.
29. Gałuszka A, Migaszewski Z, Namieśnik J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. TrAC Trends Anal Chem. 2013;50:78–84.
30. Shaaban H, Gorecki T. Current trends in green liquid chromatography for the analysis of pharmaceutically active compounds in the environmental water compartments. Talanta. 2015;132:739–52.
31. Farré M, Pérez S, Gonçalves C, Alpendurada MF, Barceló D. Green analytical chemistry in the determination of organic pollutants in the aquatic environment. TrAC Trends Anal Chem. 2010;29(11): 1347–62.
32. Pena-Pereira F, Kloskowski A, Namieśnik J. Perspectives on the replacement of harmful organic solvents in analytical methodologies: a framework toward the implementation of a generation of eco-friendly alternatives. Green Chem. 2015;17(7):3687–705.

33. Shaaban H. New insights into liquid chromatography for more eco-friendly analysis of pharmaceuticals. Anal Bioanal Chem. 2016. doi:10.1007/s00216-016-9726-2.
34. Schriks M, Heringa MB, van der Kooi MME, de Voogt P, van Wezel AP. Toxicological relevance of emerging contaminants for drinking water quality. Water Res. 2010;44(2):461–76.
35. Mendoza A, Rodríguez-Gil JL, González-Alonso S, Mastroianni N, López de Alda M, Barceló D, et al. Drugs of abuse and benzodiazepines in the Madrid Region (Central Spain): seasonal variation in river waters, occurrence in tap water and potential environmental and human risk. Environ Int. 2014;70:76–87.
36. Bruce GM, Pleus RC, Snyder SA. Toxicological relevance of pharmaceuticals in drinking water. Environ Sci Technol. 2010;44(14):5619–26.
37. Houtman CJ, Kroesbergen J, Lekkerkerker-Teunissen K, van der Hoek JP. Human health risk assessment of the mixture of pharmaceuticals in Dutch drinking water and its sources based on frequent monitoring data. Sci Total Environ. 2014;496:54–62.
38. Ribeiro AR, Pedrosa M, Moreira NFF, Pereira MFR, Silva AMT. Environmental friendly method for urban wastewater monitoring of micropollutants defined in the Directive 2013/39/EU and Decision 2015/495/EU. J Chromatogr A. 2015;1418:140–9.
39. ICH. Validation of analytical procedures: text and methodology Q2(R1). International Conference on Harmonization. 1996;1–13.
40. Ribeiro AR, Maia AS, Moreira IS, Afonso CM, Castro PML, Tiritan ME. Enantioselective quantification of fluoxetine and norfluoxetine by HPLC in wastewater effluents. Chemosphere. 2014;95:589–96.
41. Ribeiro AR, Santos LHMLM, Maia AS, Delerue-Matos C, Castro PML, Tiritan ME. Enantiomeric fraction evaluation of pharmaceuticals in environmental matrices by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2014;1363:226–35.
42. US Food and Drug Administration. Bioanalytical method validation: guidance for industry. 2001. http://www.fda.gov/downloads/Drugs/Guidance\ComplianceRegulatoryInformation/Guidances/ucm070107.pdf. Accessed Mar 2013.
43. Madureira TV, Barreiro JC, Rocha MJ, Cass QB, Tiritan ME. Pharmaceutical trace analysis in aqueous environmental matrices by liquid chromatography-ion trap tandem mass spectrometry. J Chromatogr A. 2009;1216(42):7033–42.
44. Moreira NF, Orge CA, Ribeiro AR, Faria JL, Nunes OC, Pereira MF, et al. Fast mineralization and detoxification of amoxicillin and diclofenac by photocatalytic ozonation and application to an urban wastewater. Water Res. 2015;87:87–96.
45. Schwab BW, Hayes EP, Fiori JM, Mastrocco FJ, Roden NM, Cragin D, et al. Human pharmaceuticals in US surface waters: a human health risk assessment. Regul Toxicol Pharm. 2005;42(3):296–312.
46. Australian Governement. ADI LIST - acceptable daily intakes for agricultural and veterinary chemicals. 2015. Commonwealth of Australia, Canberra, Australia.
47. Bielicka-Daszkiewicz K, Voelkel A. Theoretical and experimental methods of determination of the breakthrough volume of SPE sorbents. Talanta. 2009;80(2):614–21.
48. López-Serna R, Petrović M, Barceló D. Development of a fast instrumental method for the analysis of pharmaceuticals in environmental and wastewaters based on ultra high performance liquid chromatography (UHPLC)–tandem mass spectrometry (MS/MS). Chemosphere. 2011;85(8):1390–9.
49. Cotton J, Leroux F, Broudin S, Poirel M, Corman B, Junot C, et al. Development and validation of a multiresidue method for the analysis of more than 500 pesticides and drugs in water based on on-line and liquid chromatography coupled to high resolution mass spectrometry. Water Res. 2016;104:20–7.
50. Maia AS, Ribeiro AR, Amorim CL, Barreiro JC, Cass QB, Castro PML, et al. Degradation of fluoroquinolone antibiotics and identification of metabolites/transformation products by liquid chromatography–tandem mass spectrometry. J Chromatogr A. 2014;1333:87–98.
51. K'Oreje KO, Vergeynst L, Ombaka D, De Wispelaere P, Okoth M, Van Langenhove H, et al. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. Chemosphere. 2016;149:238–44.