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Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gche20

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Available online: 07 Nov 2011

To cite this article: S. Giandomenico, N. Cardellicchio, C. Annicchiarico, L. Lopez, M. Maggi, L. Spada & A. Di Leo (2011): Simultaneous determination of pharmaceutical compounds in environmental samples by solid-phase extraction and gas chromatography-mass spectrometry, Chemistry and Ecology, 27:sup2, 127-136

To link to this article: http://dx.doi.org/10.1080/02757540.2011.625936

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Chemistry and Ecology
Vol. 27, No. S2, December 2011, 127–136

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Simultaneous determination of pharmaceutical compounds in environmental samples by solid-phase extraction and gas chromatography–mass spectrometry

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(*Received 28 January 2011; final version received 16 September 2011*)

Pharmaceutical substances are synthetic compounds with very widespread usage due to their therapeutic biological effects. These compounds and their bioactive metabolites are continually introduced into the aquatic environment as complex mixtures via sewage treatment plants (incomplete destruction), animal farms or leaching from landfills. In this study, an analytical procedure involving solid-phase extraction and gas chromatography–mass spectrometry was developed to determine pharmaceutical compounds (caffeine, diclofenac, ketoprofen and ibuprofen) in aqueous samples (wastewater and surface water). The results demonstrated the suitability of the method at trace levels ($ng \cdot L^{-1}$) for multi-residue analysis of different types of water matrices.

Keywords: pharmaceuticals; SPE; GC-MS

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1. Introduction

During the last three decades, studies on the impact of chemical pollution have focused almost exclusively on the conventional 'priority pollutants', especially those acutely toxic/carcinogenic and industrial intermediates displaying persistence in the environment. This spectrum of chemicals, however, is only one piece of the larger puzzle of risk assessment. Another group of bioactive chemicals, receiving comparatively little attention as potential environmental pollutants, includes the pharmaceuticals. These compounds and their bioactive metabolites are continually introduced into the aquatic environment as complex mixtures via sewage treatment plants (STEP) (incomplete destruction), animal farms or leaching from landfills [1]. They represent a significant environmental risk if one considers on the one hand quantities potentially introduced into the aquatic environment and that they have been produced to be biologically active. STEPs have proved to be the main entry points of this contamination into the aquatic environment. Recent studies have documented the presence of a wide variety of pharmaceuticals in the environment worldwide, including antibiotics, anaesthetics, anti-inflammatories, antitumour compounds, oestrogens, lipid-reducing agents, diuretics, antidepressantsand illicit drugs [2–6].

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ISSN 0275-7540 print/ISSN 1029-0370 online
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http://dx.doi.org/10.1080/02757540.2011.625936
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Relatively few studies are available on the occurrence and fate of by-products [7]. By-products include both metabolites excreted via urine or faeces and transformation products, which can be formed in the environment from a pharmaceutical substance and/or metabolites, released under physicochemical and biological condtions, for example, in a wastewater treatment plant (WWTP). The difference between metabolites and transformation products is not always clear because reactions may be influenced by human metabolism and biodegradation in the environment or in treatment plants. Carbamazepine and non-steroidal anti-inflammatory drug derivates (i.e. hydroxyl- and carboxy-ibuprofen and , 4′- and 5′-hydroxy diclofenac) are the more important metabolites determined in the environment [8–10].

In this study, the compounds analysed were diclofenac, paracetamol, ketoprofen, ibuprofen (non-steroidal anti-inflammatory drugs), a group of the most commonly prescribed drugs [11,12] and caffeine (a stimulant). Because pharmaceuticals are usually present in environmental water samples at trace levels, a pre-concentration technique such as solid-phase extraction (SPE) is necessary. Materials for SPE typically include the use of an octadecyl (C_{18})-bonded silica cartridge [13], graphitised carbon black [14], ethinylbenzene–divinylbenzene co-polymer [15] and polystyrene–divinylbenzene [16] and co-polymers composed of both lipophilic and hydrophilic monomers [17]. The selection of an appropriate solid phase is a difficult, because the recoveries obtained for some compounds can be low. This problem is more evident in the simultaneous determination of several classes of pharmaceuticals. Following sample pre-concentration, the analytical technique for the quantification of pharmaceuticals can be carried out with gas chromatography coupled to mass spectrometry (GC-MS), although for acid compounds, it requires an additional step of derivatisation [18]. The most commonly used derivatisation reagents are: pentafluorobenzyl bromide [19] and diazomethane [20] for carboxylic groupa and *N*,*O*bis(trimethylsilyl)trifluoroacetamide [21], *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)trifluoroacetamide [18] and *N*−methyl-*N*-(trimethylsylil) trifluoroacetamide (MSTFA) [13,22] for hydroxyl and carboxyl functional groups. The purpose of our study was to present a simple procedure for the simultaneous determination of ibuprofen, paracetamol, caffeine, diclofenac and ketoprofen at trace levels ($ng \cdot L^{-1}$) in environmental samples using SPE WCX (weak cation exchange) pre-concentration, followed by derivatisation with MSTFA and GC-MS analysis. In order to evaluate the reliability of the method, it was applied to the analysis of STEP wastewater, surface water and drinking water.

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2. Materials and methods

2.1. *Chemicals and reagents*

Acetone, ethyl acetate, methanol (HPLC reagent grade) and 37% hydrochloric acid (reagent grade) were purchased from J.T. Baker. Ultrapure water was obtained using a Milli-Q system (Purelab Option Q-ELGA). The silylation reagent dichlorodimethylsilane, MSTFA (purity >98%) and pharmaceutical products (ibuprofen, paracetamol, caffeine, diclofenac and ketoprofen), as well as pyrene and 1-hydroxypyrene used as internal standards, were purchased from Sigma-Aldrich (purity >98%). A Whatman GF/F filter was obtained from VWR International. Mecoprop [2-(4-chloro-2-methylphenoxy)propanoic acid] used as surrogate standard was obtained from Dr Ehrenstorfer. For SPE, WCX and Oasis HLB cartridges were purchased from Agilent Technology and Waters, respectively.

2.2. *Sample collection and solid-phase extraction*

Water samples were collected in July 2010 (Figure 1). The samples were: surface water from Galeso River (station 1), Battendieri River (station 2) and D'Aiedda Channel, which carried

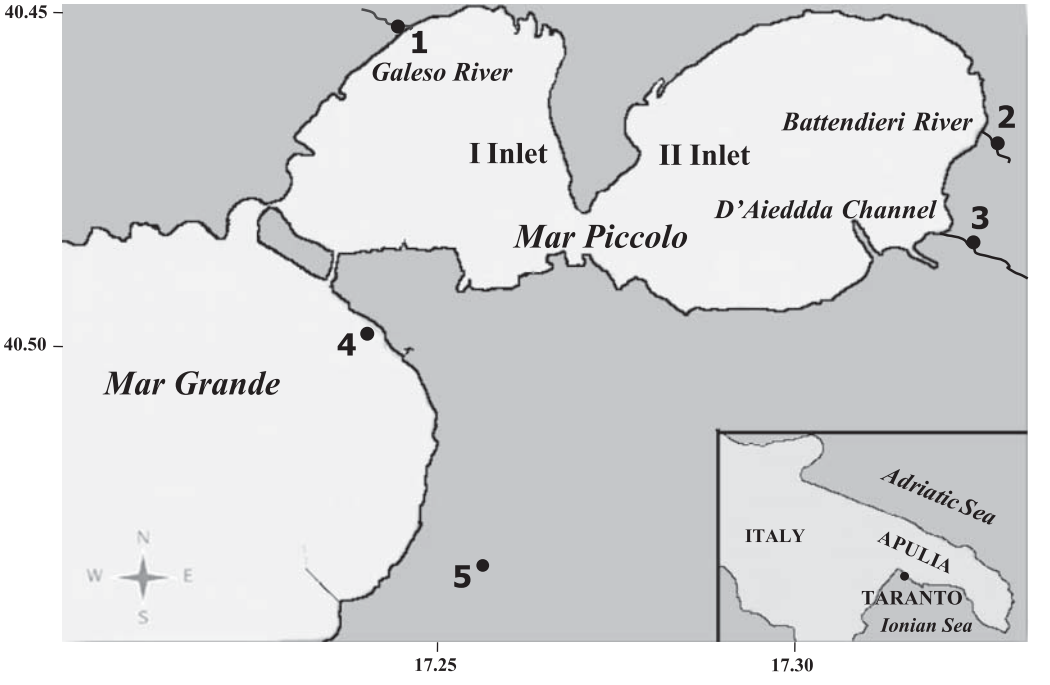

Figure 1. Sampling stations.

wastewater from sewage plants (station 3); surface seawater from Mar Grande basin (station 4) in an area influenced by urban wastewater; wastewater samples from WWTP effluent (∼100,000 PE (population equivalent)) (station 5) and tap water. Samples were taken using a Teflon Niskin bottle. After collection, samples were stored in an amber glass bottle and acidified to pH 2.0 with 3.5 M HCl. In the laboratory, samples were filtered on a GF/F filter (0.7 μm) and analysed as soon as possible, i.e. within 24 h. In addition, for the wastewater, sample was filtered with 0.45 μm cellulose filters, to eliminate possible clogging of the cartridges.

All glassware was silanised with dichlorodimethylsilane (10% v/v) in toluene to minimise the adsorption of target compounds on the glass walls. First, the glassware was rinsed with the silylation reagent, cleaned three times with toluene and three times with acetone and then heated to 150 °C for at least 12 h.

For SPE extraction, 3-mL cartridges packed with 60 mg of WCX sorbent, a co-polymer of poly(divinylbenzene)-co-*N*-vinylpyrrolidone containing carboxylic acid groups, was used. SPE was performed under vacuum using a 12-fold vacuum extraction box (Supelco) at a flow rate of 12–15 mL · min^{-1}. Before extraction loading, the SPE cartridge was conditioned with 3 mL of ethyl acetate and 3 mL of Milli-Q water at pH 2. The sample extraction volume was generally 1000 mL. After the enrichment phase, the cartridge was dried for 1 h under a vacuum. Analytes were eluted with 4.5 mL of ethyl acetate and 4.5 mL of ethyl acetate/acetone (1:1), respectively. The elutes were collected in a silanised glass vial and the volume was reduced under a gently stream of nitrogen to 100 μL of ethyl acetate. For the derivatisation step, 30 μL of MSTFA was added to the sample and the reaction was carried out at 65 °C for 35 min.

2.3. *GC-MS analysis*

Analyses were carried out using a gas chromatograph (model 7890, Agilent Technology) coupled to a mass detector (5975C Agilent Technology). The mass spectrometer was used in the electronic

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impact mode (70 eV electron energy) with ion source, quadrupole and transfer line temperatures of 230, 150 and 280 °C, respectively. Injection of 5 μL of sample was performed using the PTV injector in solvent mode and at the following temperature programme: 50 °C (0.5 min) to 250 °C at 600 °C · min^{-1} (10 min), while vent flow was adjusted to 100 mL · min^{-1}. The carrier gas was ultrapure helium, set at a constant flow mode (1.5 mL · min^{-1}). The chromatographic column was a PTE-5 (Supelco Inc. Bellefonte), 30 m × 0.32 mm ID × 0.25 μm film thickness. The GC oven was programmed as follows: 50 °C (2 min), set at 10 °C · min^{-1} to 250 °C (5 min) and 20 °C · min^{-1} to 280 °C (2 min). Mecoprop was added to the sample at the beginning of the extraction procedure, for recovery calculation, whereas pyrene and 1-hydroxypyrene were used as internal standards for neutral and acid compounds, respectively, and were added to the sample prior to the derivatisation step. For the calibration curve, 10 mg of each compound was dissolved in 10 mL of methanol to give a 1000 mg · L^{-1} stock solution. The diluted solutions were successively prepared diluting stock solution with ethyl acetate. A series of mixed working standard solutions were prepared daily in the range 1–500 μg · L^{-1}.

3. Results and discussion

3.1. *Optimisation of the derivatisation conditions*

Target compounds as diclofenac, paracetamol, ketoprofen and ibuprofen contain hydroxyl and/or carboxyl groups and have therefore low polarity. Gas chromatographic separation of these compounds can be performed only after derivatisation that converts functional groups into thermally stable, non-polar groups. Silylation is the most widely used technique and MSTFA represents a typical reagent for derivatisation of these pharmaceuticals [13,23,24]. Derivatisation involves the replacement of an acid hydrogen with $SiCH_3$ to form trimethylsilyl (TMS) derivates. For paracetamol, the derivatisation reaction is quite complex and leads mainly (>60%) to the formation of a ditrimethylsilyl-derived compounds.

The reaction for TMS derivates occurs cleanly without artefacts; moreover, because no underivatised compounds were found when analysed by GC-MS, derivatisation was considered complete. Derivatisation reactions are affected by many possible factors such as time, temperature, solvent and the concentration of the derivatisation reagent. With respect to reaction temperature and the amount of derivatisation reagent, 65 °C and 30 μL of MSTFA were commonly used [13]. A different reaction time with MSTFA has been reported [24,25]. This variation might be caused by structural differences in the target compounds. In this study, pharmaceutical mixed solutions were derivatised for different lengths of time (Figure 2).

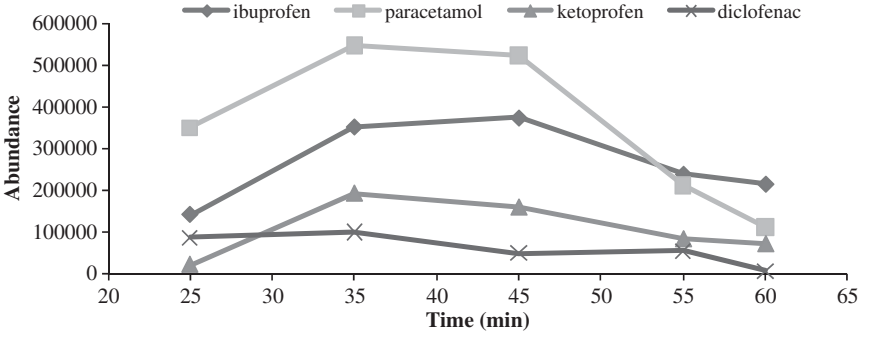

Figure 2. Influence of time on derivatisation reaction of pharmaceutical compounds (mixed standards solution of 100 μg · L^{-1} of each compound).

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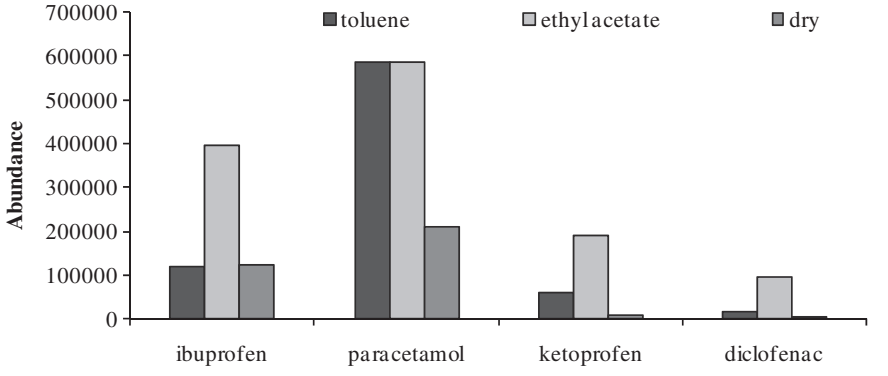

Figure 3. Influence of solvent on derivatisation reaction of pharmaceutical compounds (mixed standards solution of $100\,\mu g \cdot L^{-1}$ of each compound).

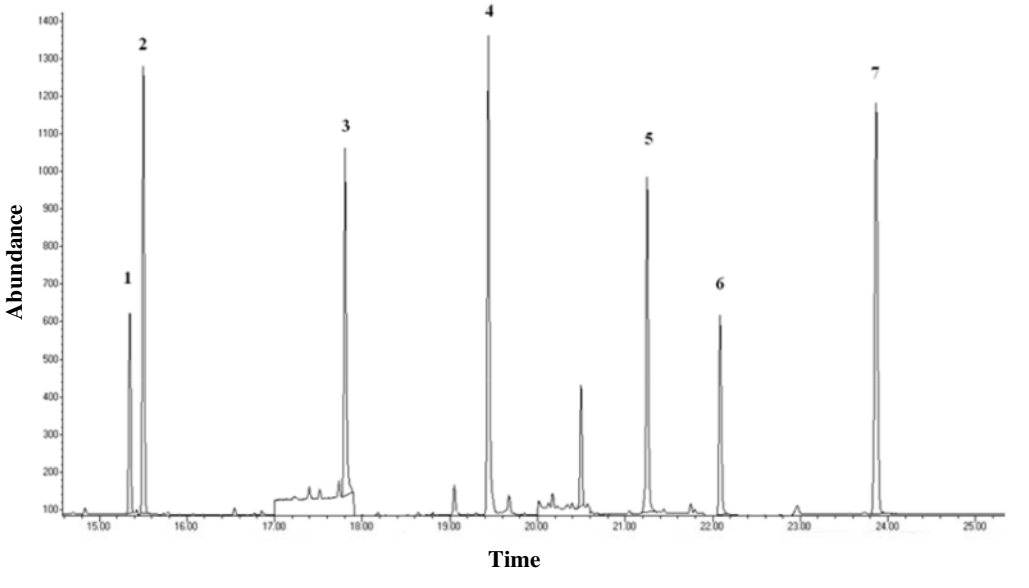

Figure 4. Chromatogram in SIM mode of selected compounds (1. ibuprofen; 2. paracetamol; 3. caffeine; 4. pyrene; 5. ketoprofen; 6. diclofenac; 7. Idroxypyrene).

The results showed that the optimum time for derivatisation was 35 min. Different solvents have been used for derivatisation in the literature [24,25]; in this study, toluene, ethyl acetate and no solvent (dry condition) were tested to determine the suitable reaction medium. The results reported in Figure 3 show that ethyl acetate was the most suitable solvent for MSTFA derivatisation, whereas when the reaction was carried out without solvent (dry condition) the amounts of derivatised products were very low.

A typical chromatogram of the TMS derivates of the selected pharmaceuticals and internal standard compounds is showed in Figure 4.

The mass spectrum of each compound was characterised in full-scan mode and the selected ion mode was used for all quantitative measurements. Typical m/z ratios for quantitative and qualitative analysis and the analytical detection limit are shown in Table 1.

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Table 1. Molecular weight, molecular weight of trimethylsilyl derivates, quantification and qualification ions of pharmaceutical compounds and analytical limits of detection.

Analyte	Mx	Mx-TMS	Quantification ion (m/z)	Qualification ion (m/z)	Analytical LOD $\cdot$ pg^{-1} injected
Ibuprofen	206	278	160	117; 263	8.5
Paracetamol	151	295[a]	206	280; 295	2.5
Caffeine	194	–	194	109; 193	12.5
Ketoprofen	254	326	282	105; 311	17.5
Diclofenac	295	367	214	242; 367	12.1

Note: [a]Paracetamol di-TMS. LOD, limits of detection; Mx, molecular weight; Mx-TMS, molecular weight of trimethylsilyl derivates; TMS trimethylsilyl derivative.

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The linearity of the calibration curves was tested using a standard mixture in ethyl acetate at different concentrations of 1–500 $\mu g \cdot L^{-1}$. Depending on the compound, the correlation coefficient ranged from 0.988 (for ketoprofen) to 0.999 (for caffeine). Repeatability expressed as a coefficient of variation was in the range 4–12%.

3.2. *Optimisation of the extraction conditions*

The SPE were effected using a WCX cartridge. In recent years, SPE technology has expanded to offer the use of mixed-mode, polymeric ion-exchange media, which combine the attributes of reversed-phase chemistry and ion-exchange interactions into a single material. Mixed-mode ion-exchange sorbents are designed to interact with ionic species, but they can also retain non-charged species effectively through hydrophobic or hydrophilic interactions. The WCX sorbent is a mixed-mode weak cation-exchange and reversed-phase sorbent resin [i.e. a co-polymer of poly(divinylbenzene)-*co*-*N*-vinylpyrrolidone] containing carboxylic acid groups. These sorbents combine a polar monomer, which promotes hydrophilic interactions, and a cross-linking monomer, which helps to increase the specific surface area and enhance lipophilic interactions. The SPE step was optimised by studying several conditions and each test was performed in triplicate for calculation of RSD (relative standard deviation) values. Because of the different acid properties of the selected compounds, the best extraction pH was determined experimentally. One litre of Milli-Q water was spiked with a mixture of standard in methanol to obtained a solution of 20 $ng \cdot L^{-1}$ and extracted at pH 2.0, 3.5 and 7.0.

Interpretation of the extraction recoveries is quite complex for the different structures of the compounds and the absorption phase. The pH affects both the exchange capacity of the sorbent and the ionisation of the compounds, especially those with an acidic character. Paracetamol and caffeine do not have carboxylic groups. Moreover, extraction recovery also depends on hydrophobic interactions and therefore differs between compounds that have different lipophilicity and water solubility.

As showed in Figure 5, with the exception of paracetamol, extraction recovery at pH 2.0 was better for most of the target compounds and ranged from 82% for ibuprofen to 122% for diclofenac. Extraction recovery of paracetamol was very low at all pH values (14–22%). The reason for this is still unclear and paracetamol has therefore not been determined in real samples.

WCX sorbent was then compared with Oasis HLB sorbent, containing a polymeric water-wettable reversed-phase sorbent, which has been widely used for the extraction of acid drugs. The results obtained, and shown in Figure 6, showed that at pH 2.0 the extraction was more or less similar for the acid compounds, particularly for ibuprofen. For neutral compounds, such as caffeine, the HLB phase did not achieve recoveries >50% with eluents such as ethyl acetate and/or acetone [13]. The best recoveries using this phase were obtained by Weigel et al. [16] using

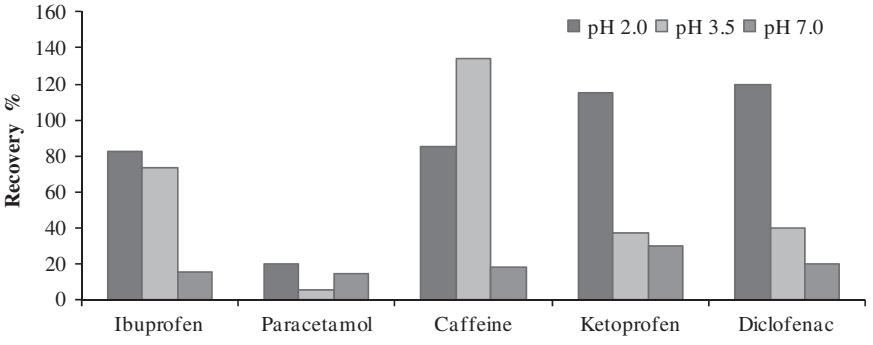

Figure 5. Extraction recovery obtained at different pH of the spiked Milli-Q water (20 ng · L^{-1} for each compound).

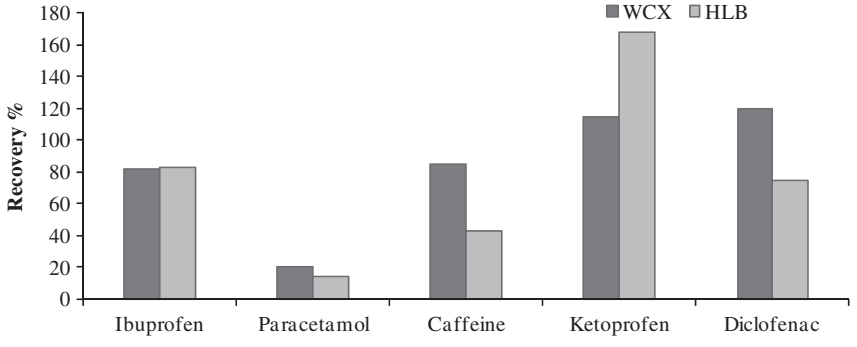

Figure 6. Comparison between different solid-phase extraction sorbents of the spiked Milli-Q water (20 ng · L^{-1} for each compound).

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methanol as the eluent and larger volumes. The WCX cartridge achieves satisfactory recoveries (82%) for caffeine also.

3.3. *Recovery of analysis of real samples*

Relative recoveries of pharmaceuticals were determined by analysing mineral water spiked with 20 ng · L^{-1} of each compound. Typical recoveries ranged from 80% for ibuprofen to 125% for diclofenac, while the RSD values were <20% for all compounds examined. The limits of detection of individuals compounds were calculated as three times the standard deviation of blank and varied according to the properties of compounds and water samples. They vary between 0.8 ng · L^{-1} (caffeine) and 2.8 ng · L^{-1} (ketoprofen). These values enable use of this method to determine pharmaceutical products in natural waters which were found generally at ng · L^{-1} levels [13,26–28]. Levels of the pharmaceutical compounds determined in this study are reported in Table 2. Caffeine was found in all samples, except in the Battendieri River water, with values from not determinable to 10.2 ng · L^{-1}; because of its almost ubiquitous presence, caffeine can be used as a chemical marker for surface water pollution by domestic wastewater [29]; diclofenac and ketoprofen, by contrast, were absent in all natural samples.

The sample from d'Aiedda Channel, which also collects effluent arising from treatment plant wastewater relative to the smaller municipalities of the province of Taranto, showed not determinable values. The absence of drugs in these waters may be due to a dilution effect that occurs along the canal, largely as a result of rain.

Table 2. Concentration ($ng \cdot L^{-1}$) of selected pharmaceuticals in water samples.

	Sampling station					
Analyte	1	2	3	4	5	Tap water
Ibuprofen	3.0 ± 0.3	n.d.	n.d	n.d	235.2 ± 37.6	1.5 ± 0.2
Caffeine	10.2 ± 1.2	n.d.	5.2 ± 0.8	2.0 ± 0.3	60.1 ± 8.4	6.0 ± 0.7
Ketoprofen	n. d.	n.d.	n.d	n.d.	589.3 ± 94.2	n.d.
Diclofenac	n. d.	n.d.	n.d	n.d.	983.3 ± 177.0	n.d.

Note: n.d., not detected. $\pm$ SD (standard deviation, $n = 3$).

Table 3. Concentration of selected pharmaceuticals detected in (a) European surface water and tap water (in bold) and (b) wastewater treatment plant effluent.

		Concentration ($ng \cdot L^{-1}$)			
Site	Ref	Ibuprofen	Caffeine	Diclofenac	Ketoprofen
(a)					
France	[30]	n.d. to 5	13–107	1–33[a]	n.d. to 15
		n.d. to 1	**n.d. to 23**	**n.d. to 3**	**n.d. to 3**
France	[13]	8–176	56–91	n.d.	4–10
Slovenia	[24]	n.d.	–	n. d. to 282	n.d.
		n.d	–	**n.d**	**n.d**
Germany	[16]	5–32	98–176	26–67	n.d.
Germany	[35]	–	–	**35**[a]	–
Germany	[34]	**3**[a]	–	**6**[a]	–
Hungary	[31]	13–109	85–440	n.d. to 141	n.d
Finland	[33]	**9**[a]	–	–	**8**[a]
Italy	[6]	13–20[a]	–	–	–
Sweden	[36]	13–97	–	25–170	10–163
United Kingdom	[23]	349–846	n.d.	n.d.	n.d.
(b)					
France	[30]	18–219	255–2213	211 – 486	22–1081
France	[13]	37–70	114–684	–	149–337
Germany	[32]	370[a]	–	810[a]	200[a]
Hungary	[31]	n.d. to 600	n.d. to 1550	1950 – 3650	n.d. to 1390
Italy	[5]	73[a]	–	2466[a]	n.d.
Sweden	[36]	31–191	–	174 – 1852	174 – 556
United Kingdom	[23]	250–385	–	–	–

Note: [a]Average concentrations; n.d.: not detected.

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In wastewater samples from WWTP effluent, drug concentrations were significant, with a median value that ranged from 60.1 $ng \cdot L^{-1}$ for caffeine to 983 $ng \cdot L^{-1}$ for diclofenac.

Table 3 reports the values for ibuprofen, caffeine, diclofenac and ketoprofen in natural waters, tap waters and STEP effluents for some other European countries [30–36]. The data reported in this study are generally lower (natural and tap water) or comparable with (wastewater) the literature data. In particular, the higher concentrations of pharmaceuticals in wastewater confirm that WWTPs are the main source of these compounds in aquatic environment and that removal of these drugs from water treatment plants is not entirely effective.

4. Conclusions

The developed method applied to analysis of aqueous samples containing the selected pharmaceuticals is accurate, sensitive and reliable, with the exception of paracetamol. WCX sorbent is an

innovative phase for the simultaneous extraction of acid and neutral pharmaceutical compounds from environmental samples. This method proved to be quite fast and inexpensive. Further investigation to better characterised pharmaceutical compounds in environmental waters, and also in biota and sediment, is recommended. Actually, risk assessment does not indicate toxic risk, especially for human exposure [37,38]. For aquatic organism exposure, further research advances are needed before a real risk assessment can be made. The amounts of drugs in rivers, and especially streams, are several magnitudes lower than those applied in medicine; but it cannot be ruled out that the number of drugs present in waters have adverse effects on aquatic organisms. With these low environmental concentrations, the toxic effects may be chronic rather than acute [32]. Moreover, regarding the by-products of pharmaceutical compounds, their toxicity and the potential impact for aquatic ecosystems and human health cannot excluded, therefore, the degradation routes, and the occurrence and fate of these compounds in aquatic systems must be also investigated for better ecotoxicological risk assessment.

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