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Multi-residue analysis of pharmaceutical compounds in aqueous samples☆

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Abstract

Pharmaceutical compounds are nowadays an emerging group of organic pollutants in aquatic systems. Several methodologies have already been published to measure these pollutants in the environment, showing the difficulties to take into account the various compounds belonging to numerous therapeutical and chemical groups. In order to develop environmental monitoring, there is a need for a less costly and time-consuming multi-component procedure. The work presented here deals with the development of an extraction procedure which enables the measurement of a wide spectrum of pharmaceuticals at trace levels ($ng\,l^{-1}$) with quite simple equipment (i.e. GC–MS with single quadruple as analyzer). The analyzed compounds comprise anti-inflammatories, antidepressants and hypolipidic drugs. The reliability and sensitivity have been tested on 18 different compounds (7 basic compounds and 11 acidic drugs) extracted simultaneously and analyzed by GC–MS. The optimized procedure has been successfully applied to the analysis of wastewaters, surface waters and drinking waters from the following areas: first the Cortiou rocky inlet, in the Mediterranean Sea (South coast of France), highly impacted by the Marseilles wastewater treatment plant effluent and secondly the Hérault watershed by studying drinking water, surface water and wastewater. In both cases, the level of pharmaceuticals was totally unknown. Results obtained have demonstrated the suitability of the method for multi-residue analysis of different types of water matrices.
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Keywords: Gas chromatography–mass spectrometry (GC–MS); Pharmaceuticals; Multi-residue analysis; Urban wastewaters; Marine waters; Drinking waters; Surface waters; Solid-phase extraction (SPE)

1. Introduction

The presence of pharmaceuticals in the environment, classified as the so-called emerging contaminants, has raised great concern among the scientific community during the last few years. Unfortunately, as a result of their growing use, these compounds have been found in aquatic systems, in sewage treatment plant effluents [1,2] as well as in surface waters [3] even detected in drinking waters [4].

Their ubiquity in the environment has prompted researchers to identify the effects that these compounds could have on non-target species [5,6] and to develop chronic exposure risk assessment on aquatic organisms [7] as well as on human beings [8,9].

Their monitoring is necessary to provide wider knowledge about their occurrence in the environment, to understand their fate, partition and organism exposure levels [10].

The quantification of pharmaceuticals in human biological matrix such as blood, plasma or urine [11] has been developed for a long time. But similar developments concerning pharmaceuticals in natural waters present more difficulties: these compounds are present at low levels and as very complex mixtures of dozens of different molecules. Simultaneous extraction of different therapeutic groups is particularly focused on antibiotics and steroids with HPLC–MS–MS or GC–MS analyses [12].

Some studies have already presented very efficient analytical procedures designed for specific pharmaceutical classes [13]. Analyzing simultaneously a wide spectrum of pharmaceuticals with different physico-chemical properties is rather difficult and requires a compromise, sometimes not resulting in the obtention of the best conditions for all analytes. Some procedures have allowed to measure pharmaceuticals at trace levels but

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with very tedious extraction procedures [14] and large extraction volumes, reducing the number [8] of analyzed samples which makes the environmental screening difficult to achieve [15]. Nowadays, multi-residue analytical methods are required in order to provide wider knowledge about the presence of pharmaceuticals in the environment. The work presented here deals with the development of an extraction procedure that enables the measurement of a wide spectrum of pharmaceuticals at trace level (ng l^{-1}), including anti-inflammatory d rugs, antidepressants, hypolipidic drugs, etc. The reliability and sensitivity have been tested on 18 different compounds (7 basic compounds and 11 acidic drugs) extracted simultaneously by off-line SPE and analyzed with GC–MS. In order to validate the applicability of the method, it has been applied to the analysis of wastewaters, surface waters and drinking waters from the Hérault watershed and the Cortiou rocky inlet. First, the Hérault watershed has been studied including wastewaters as well as surface waters and spring waters dedicated to human consumption, with low pharmaceutical levels. On the other hand, seawaters, highly contaminated by the Marseilles wastewater treatment plant effluent [16] and with a high organic matter content, have been monitored in the Cortiou rocky inlet, in the Mediterranean Sea (Marseilles area, South coast of France).

2. Materials and methods

2.1. Chemicals and reagents

Pharmaceutical products (presented in Table 1) as well as pyrene and 1-hydroxypyrene used as recovery determination standards were purchased from Sigma–Aldrich (St. Quentin Fallavier, France; purity >98%). Deuterated products (diazepam *d*5, amitryptiline *d*6 and nordiazepam *d*5) were purchased from Euriso-Top (St. Aubin, France, purity >98%). Acetone, ethyl acetate and methanol (HPLC reagent grade, Scharlau) were purchased from ICS (Belin-Beliet, France). Hydrochloric acid 37% (reagent grade) and phosphoric acid 85% (reagent grade) were obtained from Atlantic Labo (Eysines, France). Ultrapure water was obtained with a Milli-Q system (Millipore, Molsheim, France). Sixty-milligram Oasis MCX cartridges were purchased from Waters (St. Quentin en Yvelines, France).

MSTFA (*N*-methyl-*N*-(trimethylsylil)trifluoroacetamide, purity >97% from Acros Organics (Noisy-le-Grand, France)) was used as the derivatizing reagent for GC–MS analyses. Whatman GFF glass fibre filters (pore size 0.7 μm) were purchased from VWR International (Fontenay-sous-Bois, France) and Atlantic Labo (Eysines, France).

2.2. Sample pretreatment and solid-phase extraction optimization

Water samples have been collected in amber glass bottles, previously detergent washed, acid rinsed and heated at 450 °C for 6 h. The samples were filtered on GFF fibre filters immediately after collection and pharmaceutical extraction was done during the day of sampling.

Raw water was filtered on GFF filters to separate dissolved phase and particles. For natural waters, 1 l was filtered whereas for Wastewater Treatment Plant Effluent (WWTP effluent), 500 ml was used for each extraction. Sample pH was adjusted prior to extraction at a value of 2 with HCl (3.5 M). Moreover, internal standards (25–50 μl of a methanolic mixture containing 1 μg g^{-1} of each standard depending on the type of waters: the least for surface waters, the most for wastewaters) were added to the samples.

Before sample loading, SPE cartridges were conditioned with 3 ml of ethyl acetate and 3 ml of Milli-Q-water at adjusted pH 2. Water was percolated under vacuum onto the cartridges at a flow rate of 12–15 ml min^{-1} and afterwards dried for 1 h under vacuum. After elution with three successive solvents, 3 ml of ethyl acetate, 3 ml of ethyl acetate/acetone (50/50; v/v) and 3 ml of ethyl acetate/acetone/ammonium hydroxide (48/48/2; v/v/v), respectively, the samples were completely evaporated under nitrogen and transferred into GC injection vials in 50–100 μl of ethyl acetate. For recovery control, pyrene was added to the final extracts for basic compounds before GC–MS analysis and 1-hydroxypyrene was added to the final extracts for acidic compounds before the derivatization step, consisting in adding 30 μl of MSTFA before incubation at 65 °C for 35 min.

All additions of matrix, standards, solvents or reagents were gravimetrically controlled. Blanks were performed for each batch experiment in order to prevent any contamination. No compounds have been found in blank samples.

2.3. GC–MS analysis

GC–MS analyses were carried out using an HP 6890 gas chromatograph from Agilent Technologies (Palo Alto, CA, USA). The capillary column was an HP5/MS (30 m × 0.25 mm × 0.25 μm film thickness; phase: 5% diphenyl, 95% dimethylsiloxane) from Bios Analytique (L'Union, France). Samples were injected (1 μl) into the GC in splitless mode at 250 °C using an HP 6890 series injector. The carrier gas was ultrapure helium (99.99990%, Linde Gas, Bassens, France) set at constant flow mode (1.3 ml/min). For GC separation, the temperature program started at 70 °C (held for 2 min), set at 10 °C/min to 250 °C and then was held isothermally at 250 °C for 5 min. The gas chromatograph was coupled to an HP 5973N mass selective detector (LMSD, Agilent Technologies, Palo Alto, CA, USA), operated under electronic impact (EI) mode at 70 eV using scan mode (from 50 to 600 amu, 2.69 scan s^{-1}) and single ion monitoring mode at 1.67 scan s^{-1} (dwell time 70 ms). The transfer line, source and quadruple temperatures were 280, 230 and 150 °C, respectively.

Each compound has been first characterized individually in scan mode in order to identify the main ions (*m*/*z* ratio) constituting the mass spectrum and to choose the ions for quantification and for confirmation (Table 1). For acidic compounds, detection has also been investigated after the derivatization step with *N*-methyl-*N*-(trimethylsylil)trifluoroacetamide (MSTFA). For this purpose, the solution has been kept for 35 min in an oven at 65 °C after adding 30 μl of MSTFA.

Table 1
Studied compounds with various parameters (structure, molecular weight, *m/z* ratio (quantification and confirmation), internal standards and linearity)

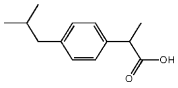
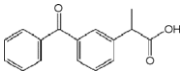
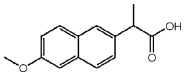
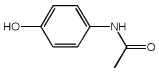
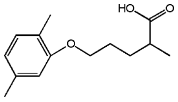
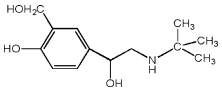
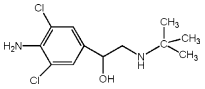
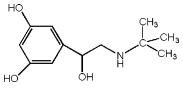
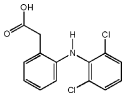
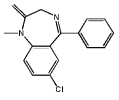
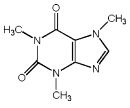
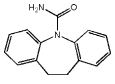
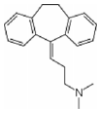
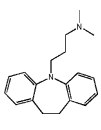
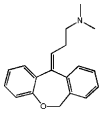
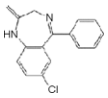
Compound	Retention time (min)	Therapeutic group	Chemical structure	MW ($g\,mol^{-1}$)	*m/z* ratio	Internal standards	R^2
Aspirin (ASP)	14.0	Non-steroidal anti-inflammatory drugs (NSAID)		180	195 [M_{TMS}–COO–CH_3]	Diazepam *d*5	0.9958
Ibuprofen (IBU)	16.4			206	160 [M_{TMS}–COO–TMS] (confirmation: 263)	Diazepam *d*5	0.9953
Ketoprofen (KETO)	16.8			254	282 [$M_{di\text{-}TMS}$–COO–TMS] (confirmation: 311)	Diazepam *d*5	0.9963
Naproxen (NAP)	22.9			230	185 [M_{TMS}–COO–TMS] (confirmation: 302)	Diazepam *d*5	0.9934
Paracetamol (PARA)	23.5			151	206 [M_{TMS}–NH_2] (confirmation: 295)	Diazepam *d*5	0.9916
Gemfibrozil (GEMF)	24.9	Lipid regulator		250	201 [M_{TMS}–$(CH_3)_2$–C_6H_3–O]	Diazepam *d*5	0.9996
Salbutamol (SALB)	25.7	Bronchodilator		239	369 [$M_{tri\text{-}TMS}$–CH_2–$NHC(CH_3)_3$] (confirmation: 86)	Diazepam *d*5	0.9863
Clenbuterol (CLENB)	26.2	Bronchodilator		276	335 [$M_{di\text{-}TMS}$–$CH_2(CH_3)_3$] (confirmation: 86)	Diazepam *d*5	0.9908
Terbutalin (TERB)	27.9	Bronchodilator		225	356 [$M_{tri\text{-}TMS}$–CH_2–$NHC(CH_3)_3$] (confirmation: 86)	Diazepam *d*5	0.9854
Diclofenac (DICLO)	29.4	NSAID		295	214 [M_{TMS}–COO–TMS–Cl] (confirmation: 367)	Diazepam *d*5	0.9987
Diazepam (DZP)	32.4	Antidepressant		284	256 [M–CH_2N] (confirmation: 221)	Diazepam *d*5	0.9989
Caffeine (CAF)	21.8	Stimulant		194	194 [M^+] (confirmation: 109)	Diazepam *d*5	0.9943
Carbamazepine (CBZ)	28.9	Sedative		236	193 [M–NHCO] (confirmation: 165)	Diazepam *d*5	0.9949
Amitryptiline (AMI)	29.0	Antidepressant		277	58 fragment [–CH_2–$N(CH_3)_2$] (confirmation: 202)	Amitryptiline *d*6	0.9503
Imipramine (IMIP)	29.3			280	58 fragment [M–$HN(CH_3)_2$], [–CH_2–$N(CH_3)_2$] (confirmation: 234)	Amitryptiline *d*6	0.9558

Table 1 (*Continued*)

Compound	Retention time (min)	Therapeutic group	Chemical structure	MW ($g\,mol^{-1}$)	*m/z* ratio	Internal standards	R^2
Doxepine (DOX)	31.1			279	58 fragment [$–CH_2–N(CH_3)_2$] (confirmation: 280)	Amitryptiline *d*6	0.9677
Nordiazepam (NDZP)	34.2	Benzodiazepines active metabolite		270	242 [*M*–HCO] (confirmation: 270)	Nordiazepam *d*5	0.9858

2.4. Method validation

To allow the quantification of the different compounds, the use of several internal standards has also been investigated. The accuracy of the quantification method and the recovery of internal standard extraction have been evaluated with direct injections of solutions in ethyl acetate and on spiked samples.

2.5. Analytical development

After preparing individual mother standard solutions in methanol (10 $\mu g\,g^{-1}$) stored at 4 °C, diluted mixtures have been prepared in ethyl acetate (1 $\mu g\,g^{-1}$ for each compound).

Elution on MCX cartridges has been tested with three successive elutions, respectively, 3 ml of ethyl acetate, 3 ml of ethyl acetate/acetone (50/50; v/v) and 3 ml of ethyl acetate/acetone/ammonium hydroxide (48/48/2; v/v/v). This experiment has been compared with optimized C18 and Oasis HLB extraction procedures (elution with 9 ml of ethyl acetate/acetone (50/50; v/v)) already developed and validated [17].

2.6. Environmental analysis

A sampling campaign was conducted in April 2004 in the Hérault watershed. This area has been chosen in order to compare with other works concerning the potential contamination of the waters of this area by anthropic effluents characterized for some of them by the occurrence of gadolinium, a pharmaceutical residue of Magnetic Resonance Imaging (MRI) analysis [18]. Three kinds of water have been sampled: three sewage treatment plant wastewater samples, six surface water samples and six tap water samples. All sampling stations are presented in Fig. 1. Spring water samples have been collected just before the chlorination process. The limits of detection of water have been calculated on the three kinds of water.

The Cortiou rocky inlet is located near Marseilles (France), in the Mediterranean Sea. The effluent of the Marseilles city

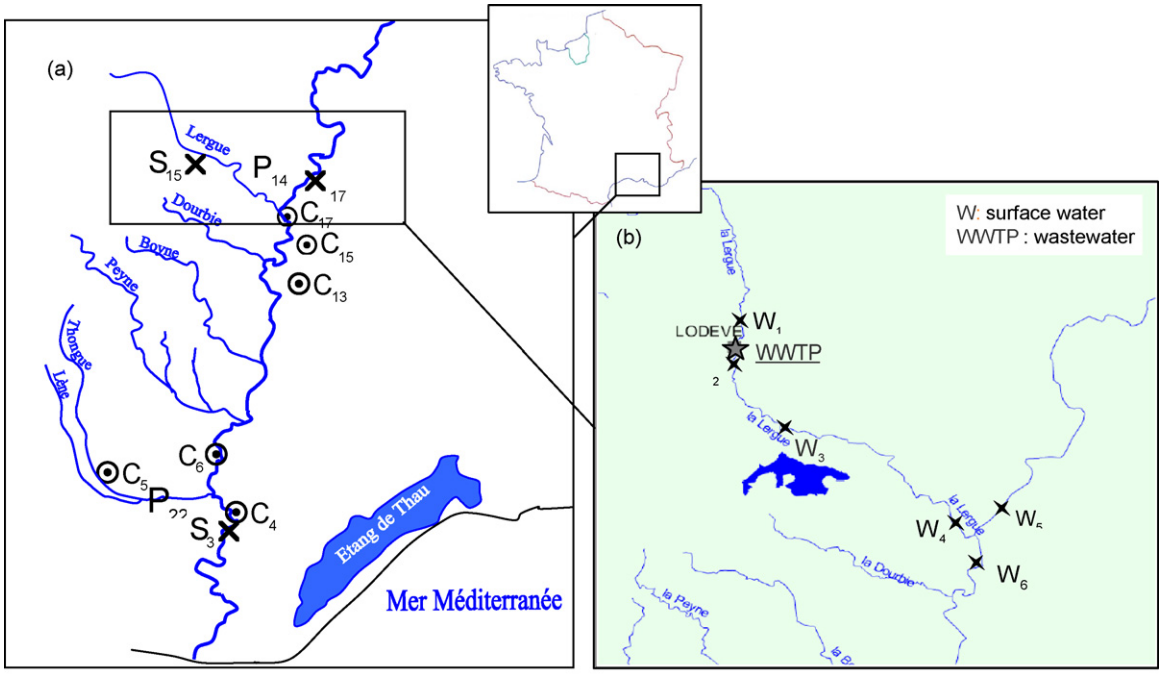


Fig. 1. Map of the Herault watershed (a) with sampling sites and zoom on the Lergue river for the study of surface water stations (b).

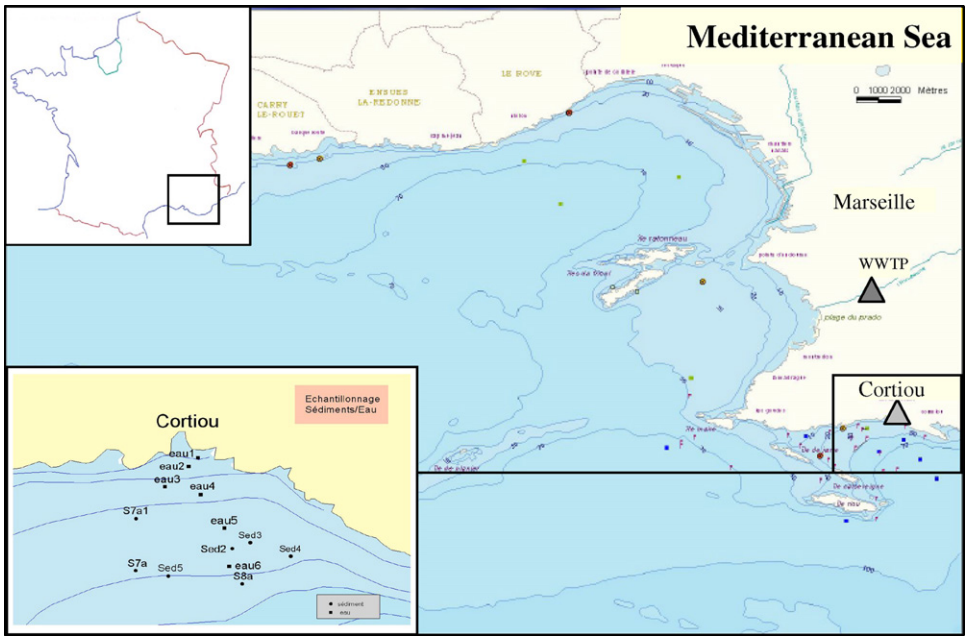


Fig. 2. Map of the rocky Cortiou inlet with sampling stations.

wastewater treatment plant (1,300,000 population equivalents) which undergoes no biological treatment pollutes this area. Several samples have been collected in the effluent plume (Fig. 2) to investigate the influence area of the effluent.

3. Results and discussion

3.1. Sample pretreatment and solid-phase extraction optimization

3.1.1. Recoveries for MCX extraction procedures

Combined extraction using MCX cartridges has given high recovery values, presented in Table 2. The obtained values range between 54 ± 12% (for terbutaline) and 120 ± 6% (carbamazepine), with an average at 88%.

3.1.2. Comparison with C18 and HLB extraction

The previous procedure used previously [17] allowed only to extract separately the acidic and basic groups, by using two kinds of cartridges (C18 for the basic group and HLB for the acidic one). The new methodology is also more interesting from a practical point of view. Furthermore, basic compound recovery rates have been widely enhanced, more particularly as regards doxepine and imipramine that were not extracted at all with the previous procedure using C18 cartridges. Table 2 shows recovery rates depending on solvents and sorbents. Fig. 5 shows compared results between two similar wastewater samples coming from a WWTP effluent analyzed by the two methods: the procedure using C18 and HLB cartridges and the combined procedure with MCX cartridges. Results show a good similarity between both measured concentrations, which allows the use of the new procedure, easier to manage, more efficient for some compounds and less costly and time consuming than the previous one.

3.2. GC–MS analysis

GC–MS tests have been done in SCAN mode in order to choose the quantification ions. The compounds have been separated into two groups: basic and acidic compounds. The basic compound group (caffeine, carbamazepine, amitryptiline, imipramine, doxepine, nordiazepam and diazepam) is directly injected. Compounds with one or more acidic functional groups require a derivatization step in order to improve chromatographic performances.

Among the tested derivatizing products and derivatization conditions [19], it has been chosen to use 30 μl of MSTFA per

Table 2
Comparison between different solid-phase extraction sorbents in the case of spiked water (500 ng l^{-1} for each compound) ($n = 6$)

Compounds	HLB extraction	C18 extraction	MCX extraction
DICLO	91 ± 10	–	105 ± 5
KETO	89 ± 14	–	106 ± 6
NAP	79 ± 12	–	90 ± 3
ASP	84 ± 18	–	71 ± 12
GEMF	78 ± 11	–	81 ± 1
IBU	84 ± 11	–	80 ± 3
CAF	–	70 ± 6	68 ± 11
DOX	–	2 ± 0	98 ± 9
CBZ	–	99 ± 11	120 ± 6
IMIP	–	1 ± 1	95 ± 5
AMI	–	–	95 ± 9
PARA	–	–	76 ± 4
DZP	–	–	87 ± 3
SALB	–	–	62 ± 14
CLEN	–	–	90 ± 17
TERB	–	–	54 ± 12
DZP	–	–	104 ± 2
NDZP	–	–	101 ± 4

Abbreviations are given in Table 1.

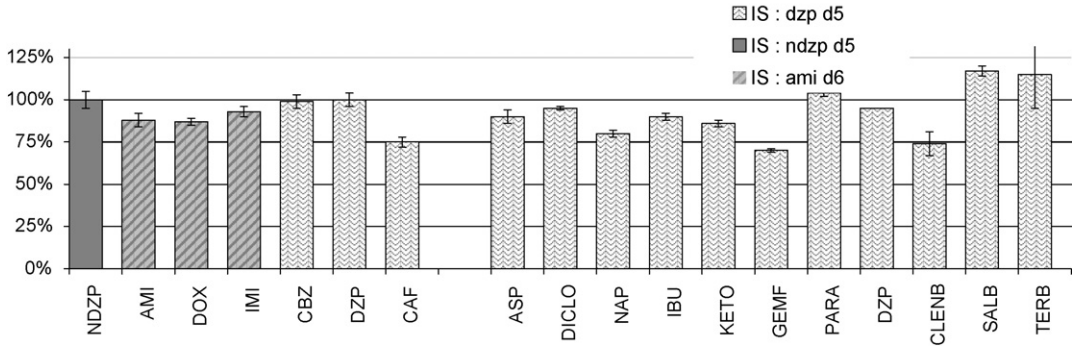


Fig. 3. Quantification of pharmaceutical compounds for standard solutions, using internal standards ($n = 3$). Abbreviations are given in Table 1.

sample and to keep the samples for 35 min at 65 °C before injection, considering the abundance of ions, the repeatability and robustness of this procedure.

Therefore, each sample has first been analyzed by GC–MS before the derivatization step, then reinjected after derivatization using a second GC–MS method. Both chromatographic methods are similar; diazepam that presents the same main fragment and responses using both methods are used to compare the two injections.

Ions (m/z ratios) chosen for quantification, molecular weights and linearity coefficients (between 2 pg and 5 ng injected) obtained for GC–MS analyses are presented in Table 1, with the chemical structures of studied pharmaceuticals. For acidic compounds, in the case of multiple possibilities of derivatives, the most abundant and stable derivative has been selected.

In the case of carbamazepine, previous studies have shown that a thermal degradation phenomenon could happen, occurring in the injection system, which could affect carbamazepine quantification by forming stilbene [20]. These degradation phenomena have been checked in our study and were shown to be very small and constant. Degradation rate was constant for all analyses with a variability below 10% and the use of internal standards for quantification that are not influenced by thermal degradation allows to monitor possible degradation (or not) of carbamazepine by following evolution of response coefficient between diazepam *d*5 (as internal standard) and carbamazepine in standard solutions to control the stability of the system. Diazepam *d*5 is proved to be very stable so if the response factors of carbamazepine in comparison with diazepam *d*5 do not vary, it shows that under our GC conditions the potential thermal degradation of carbamazepine is not significant.

3.3. Method validation

All available deuterated standards have been used for quantification. Paracetamol *d*4 and diazepam *d*5 have been tested for acidic compound quantification and diazepam *d*5, amitryptiline *d*6, nordiazepam *d*5 and caffeine C13 for the basic group. Pyrene and 1-hydroxypyrene have been used for quantification of deuterated compounds ("syringe" standards). Use of "syringe standards" added after extraction step and just before GC–MS analysis allows to check internal standard recovery for each sample.

Concerning acidic compounds, the rates of quantification for standard solutions obtained using diazepam *d*5 are between 74 and 115% with RSDs between 0 and 20% (Fig. 3).

Caffeine C^{13} and paracetamol *d*4 have not been selected as internal standards: even if the quantification results are correct, the successive steps, especially the evaporation one, lead to significant and not reproducible losses. For diazepam *d*5, amitryptiline *d*6 and nordiazepam *d*5, recoveries are the same as those of non-labeled compounds.

Some quantification problems on some compounds, as well as high RSDs going up to 20% for salbutamol, are mainly due to derivatization problems. Indeed for clenbuterol, salbutamol, and terbutaline different derivatives are formed [17] but not in reproducible conditions.

In the case of basic compounds, reproducible optimum quantification results have been obtained (see Fig. 3). Different deuterated compounds have been selected depending on best recoveries, that is, nordiazepam *d*5 for nordiazepam quantification ($100 \pm 5\%$), diazepam *d*5 for diazepam, carbamazepine and caffeine (respectively, 100 ± 4, 99 ± 4 and $75 \pm 3\%$), and amitryptiline *d*6 for amitryptiline, imipramine and doxepine (respectively, 88 ± 4, 93 ± 3 and $87 \pm 2\%$).

Pyrene (for basic compounds) and 1-hydroxypyrene (for acidic ones) are used as syringe standards (added just before the injection so not depending on sample preparation) in order to quantify internal standards (diazepam *d*5, amitryptiline *d*6 and nordiazepam *d*5) and calculate recoveries (Fig. 4). The results that have been obtained (between 86 ± 5 and $110 \pm 5\%$) make it possible to use pyrene and 1-hydroxypyrene as standards for controls of pharmaceutical extraction rates.

The different kinds of water studied present various organic matter contents and matrix complexity. The limits of detection

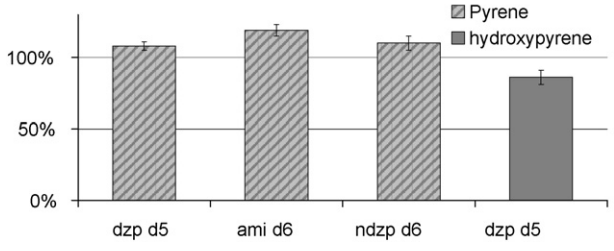


Fig. 4. Quantification of internal standards by "syringe" standards ($n = 3$). Abbreviations are given in Table 1.

Table 3
Limits of detection obtained for natural waters (expressed in ng l^{-1}, for 1 l extracted for tap and surface and saline waters, 500 ml for wastewater effluent)

	Tap water	Surface water	Marine water	Wastewater effluent
Amitryptiline	0.7	2.2	2.6	6.8
Aspirin	0.2	2.1	2.1	15.0
Caffeine	1.5	2.5	2.3	28.6
Carbamazepine	0.8	1.4	2.2	22.3
Clenbuterol	0.6	0.3	1.2	4.0
Diazepam	0.4	1.4	1.9	13.8
Nordiazepam	0.4	1.4	1.9	13.8
Diclofenac	0.9	0.7	2.6	9.0
Doxepine	0.7	2.1	2.4	16.6
Gemfibrozil	0.1	0.3	1.2	3.2
Ibuprofen	0.1	0.1	1.7	4.8
Imipramine	0.7	1.2	1.6	12.7
Ketoprofen	0.3	0.7	1.8	11.6
Naproxen	0.1	1.0	2.1	6.2

Table 4
Minimal and maximal values measured in the case of the Hérault watershed samples, expressed in ng l^{-1} (nd: not detected)

	WWTP effluent (3)	surface water (6)	drinking water (6)
Amitryptiline	nd–6.0	nd	nd–1.4
Aspirin	23.5–51.5	nd	nd
Caffeine	255.1–2212.7	13.0–107.2	nd–22.9
Carbamazepine	157.3–293.4	nd–56.3	nd–43.2
Clenbuterol	nd–5.9	nd	nd
Diazepam	nd	nd	nd
Diclofenac	210.7–486.4	1.36–33.2	nd–2.5
Doxepine	nd	nd	nd
Gemfibrozil	13.3–17.2	nd–2.3	nd
Ibuprofen	17.7–219.0	nd–4.5	nd–0.6
Imipramine	nd	nd	nd
Ketoprofen	21.8–1080.6	nd–14.5	nd–3.0
Naproxen	42.1–289.1	nd–9.1	nd–0.2
Nordiazepam	nd–8.3	nd–2.4	nd
Paracetamol	108.1–11308.9	10.6–72.3	nd–210.1
Salbutamol	nd	nd	nd
Terbutaline	nd–4.1	nd	nd

are very different depending on the origin and kind of water. They vary between 0.1 and 1.5 ng l^{-1} for tap water, between 0.1 and 2.5 ng l^{-1} for surface water and between 3.2 and 28 ng l^{-1} for wastewater, with variability depending on compounds as presented in Table 3. These low detection levels allow to quantify pharmaceuticals in the two studied environments. For the three different types of water (tap water, surface water and waster water), spiked samples have been managed (including all compounds) and have shown the good performances of the protocol: recoveries vary between 70 and 110% depending on the compounds and on the matrix.

Repeatability and reproducibility have been tested in order to assess the extraction procedure reliability. Repeatability measured on six independent replicates, fluctuates between 3 (for aspirin) and 26% (for clenbuterol). Reproducibility fluctuates between 2 and 11% on triplicates for basic compounds and between 1 and 17% for the acidic group, with higher value for clenbuterol (17%). In the case of the spiked samples the RSD were below 20% for all the compounds (below 15% for basic ones).

In both cases, the compounds for which the protocol is the less reliable are paracetamol, clenbuterol, terbutaline and salbutamol. This weakness is partly due to the GC–MS step, due to some difficulties during the derivatization step as explained previously, especially highlighted in natural samples, due to the occurrence of numerous interfering compounds that could disturb derivatization.

This procedure is robust and repeatable, also applicable to environmental samples and allows single determination for each studied sample, which is important considering environmental monitoring at large scale.

3.4. Environmental implementation

The minimal and maximal measured values in the case of the Hérault watershed are presented in Table 4 showing the sensitivity of the developed procedure. Compared with other studies these values are below detection limits generally obtained using GC–MS analyses [20] or even LC–MS–MS [21]. This is partly due to high ratio of reconcentration which is possible for internal standards are used at two levels (for quantification and for control (syringe standard)) and also because gravimetric manipulations are preferred to volumetric ones. Currently, LC–MS–MS and GC–MS–MS analyses give lower detection limits (below 1 ng l^{-1}) [22] but need more method optimization and are more complex to implement. Especially for LC–MS–MS analyses, some difficulties linked to interfering compounds can occur, such as matricial interferences and signal suppression [23].

Comparing with other studies which try to develop multi-residue analytical protocols [12], this optimized procedure has been applied to several kinds of waters showing its multi-matrix property. Using an easy to use GC–MS analyzer allows less costly and easier environmental monitoring than HPLC-MS–MS or GC–MS–MS systems.

Concerning the Hérault watershed, results have shown a real contamination of the WWTP effluents but also of the surface waters and tap waters (Table 4). Close connection between WWTP and the tap water collecting station can explain this occurrence [24], already demonstrated in other publications [25,26]. The use of a single extraction procedure for all samples, sensitive and robust whatever the typed sample, irrespective of concentration levels and water sample physico-chemical properties is an important advance in environmental monitoring. This can allow to monitor environmental areas, without previous knowledge of contamination levels or other characteristics, as is needed for first environmental screening.

Concerning the Cortiou rocky inlet (Fig. 5), the measured values are interesting for several reasons. Marseilles wastewater treatment plant has an important capacity (85,500,000 m^3 treated and 1,300,000 population equivalents), without secondary treatment. In France, it is the most important station of this kind, allowing evaluation of WWTP effluent in the worst conditions. This station should be renovated in 2007; the results obtained

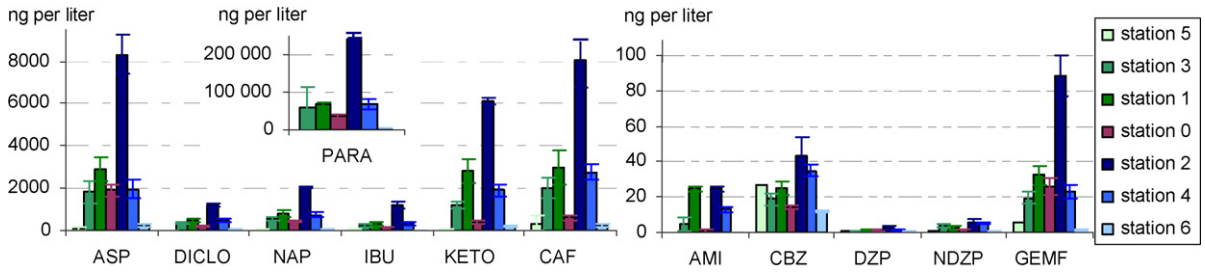


Fig. 5. Pharmaceutical concentrations measured in the Cortiou rocky inlet. Abbreviations are given in Table 1.

here allow future comparison studies and monitoring of remediation processes.

Some compounds, rarely found in surface water as well as in wastewater effluents have been measured around 10 ng l^{-1}, especially for amitryptiline, diazepam and nordiazepam, as antidepressants. The high concentration level of treated wastewaters is explained by the relationship between pharmaceutical consumption and occurrence in effluent. Considering 1,300,000 inhabitants, the number of antidepressant consumers is high, increasing the content of those drugs in effluent and then allowing their detection. For the same reasons, very high amounts of non-prescribed drugs have been measured, such as aspirin (8 μg l^{-1}), caffeine (8 μg l^{-1}), but above all paracetamol (200 μg l^{-1}). The degradation of these three compounds is highly related to biological treatment efficiency in wastewater treatment plants [27–29]. In the absence of biological treatment in the Marseilles WWTP, high concentrations of those compounds are thus measured in the effluent.

Concerning the plume of dilution of the effluent and its impact on the Cortiou rocky inlet, this preliminary study has focused on the area close to the emissary, more or less 500 m from the emissary for the remotest stations (stations 5 and 6). Currents influence the plume of dilution and carry effluents towards one side more than towards the other. This very important effluent, introduced into a semi-open aquatic system, poorly submitted to dilution phenomena can present a high environmental risk for aquatic organisms living in the area. Considering the first results presenting the occurrence of antidepressants in fish living in an effluent-dominated stream [30], studies focusing on fish exposure and toxicological impact related to this exposure need to be undertaken.

4. Conclusions

This procedure, using MCX solid-phase extraction is at the same time quick and semi-automatic, allowing numerous sample processing, reliable, robust and reproducible for drinking water, surface water as well as wastewaters. A protocol allowing to measure 18 different compounds, belonging to 5 therapeutic groups and having important differences in chemical structure has been developed and has been proven to be very interesting for environmental screening.

The obtained results have shown that the developed tool ensures to follow aquatic environment contamination whatever the water physico-chemical characteristics are. The low levels of detection limits (ng l^{-1}) obtained for this optimized procedure make it possible to quantify those compounds in little contaminated environments, such as drinking water, seawater or other environments. Results have highlighted the contamination of two sensitive systems, the Cortiou rocky inlet and the Hérault watershed. These results are among the first showing those high concentration levels in seawater and drinking water. Compared with other studies, this procedure is faster and easier to manage, considering the simultaneous extraction of all pharmaceutical compounds studied. This protocol stands comparison with other extraction processes using multiple extraction steps [13,21,31] or using complex and costly analysis apparatus [12]. By using this process, impacted areas have been discovered, presenting potentially human health risks, if we consider concentration measured in drinking water or environmental risks considering the Cortiou rocky inlet situation.

Within the limits of current knowledge, risk assessment does not indicate toxic risk, especially concerning human exposure [32,33]. As regards aquatic organism exposure, research advances need to be more important before a real risk assessment, particularly if we consider long time exposure at environmental concentration levels.

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