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Development of on-line solid-phase extraction-liquid chromatography coupled with tandem mass spectrometry method to quantify pharmaceutical, glucuronide conjugates, and metabolites in water.

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Abstract:

The present work describes the development of an analytical method, based on automated on-line solid phase extraction followed by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (SPE-LC-MS/MS) for the quantification of 37 pharmaceutical residues, covering various therapeutic classes, and some of their main metabolites, in surface and drinking water. A special attention was given to some glucuronide conjuguates and metabolites of active subtances. Multiple Reaction Monitoring (MRM) was chosen and two transitions per compound are monitored (quantification and confirmation transitions). Quantification is performed by standard addition approach to correct matrix effect. The method provides limit of quantification inferior to 20 $ng.L^{-1}$ for all compounds. The methodology was successfully applied to the analysis of surface water and drinking water of 8 drinking water treatment plant in west of France. The highest drug concentrations in surface water and drinking water were reported for ketoprofen, hydroxyibuprofen, acetaminophen, caffeine and danofloxacin.

Key words: *pharmaceuticals, automated on-line solid phase extraction, liquid chromatography, tandem mass spectrometry, water analysis*

31 **1. Introduction**

32 Pharmaceuticals are an important group of emerging contaminants in the environment [1].
33 In recent years many reports have been made on the occurrence of the large, differentiated
34 group of pharmaceuticals in wastewater, surface water, ground water and drinking water in
35 many countries [2-9]. After administration, most pharmaceuticals are not completely
36 metabolized. The unmetabolized parent pharmaceutical and some metabolites are
37 subsequently excreted from the body via urine and faeces [10]. Reports have shown that
38 many pharmaceuticals do not totally degrade during conventional wastewater treatment
39 [11,12]. The concentrations of individual compounds in wastewater, surface water, ground
40 water and drinking water are typically in the range of ng/L to µg/L. The effect on long-term
41 pharmaceutical residues in aquatic environments remains largely unknown. In addition, the
42 risks to the environment are evaluated for a particular drug, while we find a mixture of all
43 these compounds in aquatic environments. Studies have shown that combinations of drugs
44 may be more powerful than the simple addition of two drugs individually toxic effects [13-
45 14].

46 Wastewater effluent is a major source for the input of pharmaceuticals to the environment
47 [11;12], which can then migrate through water systems and into source water intended for
48 drinking water supplies. Advanced wastewater treatment processes have been shown to
49 significantly reduce the concentrations of emerging contaminants. However, some
50 compounds are not completely removed even if treatment techniques are used [15].
51 Moreover, most of the WWTP do not include these specifically designed treatment units.

52 In this context, sensitive analytical methods allowing the quantification of many pollutants at
53 trace concentration is essential. Solid Phase Extraction (SPE) is the most commonly used
54 technique to prepare sample before analysis. SPE allows the concomitance of analyte

concentration and interferences removal [16;17]. To date, most of the published multi-residue methods for the determination of ultra traces of pharmaceuticals compounds in surface and drinking water use off-line SPE followed by gas chromatography mass spectrometry (GC–MS) or by liquid chromatography-tandem mass spectrometry (LC–MS/MS) [2-5;7;9;12]. However, On-line Solid Phase Extraction is an emerging method for analysis of the trace compounds of organic micropollutants (reactive drugs, pesticides…). This technique has many advantages: saving time, automated method, reproducibility, very low solvent consumption, small sample handling, SPE cartridges reuse… [17]. The cartridges used to concentrate pharmaceuticals residues are usually Oasis^{TM} HLB or hydrophobic resins. [18;19]. This technique is generally coupled to liquid chromatography with UV, MS or MS/MS detector with reversed phase column [20-24].

The objectives of this work has been to develop a fully automated method to analyze a number of target compounds belonging to different therapeutical classes and some by product using on-line SPE directly coupled to liquid chromatography tandem mass spectrometry (LC–MS/MS). This analytical technique limits matrix effect impact. However remaining, interfering species can affect the analytical train, especially natural organic matter may coeluate with targeted compounds which leads to a signal disturbance causing over/underestimation or false positive results, or some compounds may react with targeted molecules during sampling and storage [25].

This method was evaluated in different water matrices: UltraPure Water (UPW) to develop the analytical method, surface water and drinking water for validation.

2. Material and methods

2.1. Compound selection

81 32 pharmaceuticals and 3 metabolites and 2 glucuronide conjugates were selected for this
82 study (Table 1 and Table S1). These molecules were chosen based on the following criteria: i)
83 selected compounds should exhibit a variety of physical properties, such as functional
84 groups and polarity, ii) they should represent of a diversity of pharmaceutical classes, iii)
85 high frequencies of environmental occurrence, iv) low removal efficiencies by drinking water
86 and wastewater treatment techniques in France or others countries [2-9]. Table 1 lists the 37
87 molecules selected for our study and their optimized parameters for quantification, chemical
88 structure is provided in the figure S1 in Supporting Information. Thereafter, the molecules
89 will be called by the short identifiers which are given in the table 1. The pharmaceutical
90 classes represented are cardiovascular drugs, anticancer agents, human or veterinary
91 antibiotics, neuroleptics, non-steroidal anti-inflammatory drugs and hormones.

92

93 *2.2. Pharmaceutical standards and reagents*

94 All pharmaceutical compounds have minimum 90% purity, used as received in solid form and
95 were obtained from Sigma Aldrich (FRANCE). Ultra pure water (UPW) was delivered by a
96 ElgaPureLab System (resistivity 18.2 MΩ.cm, COT< 50µg C/L). Chromatographic and SPE
97 solvents, acetonitrile (ACN) with or without 0.1 % formic acid (FA) and methanol (MeOH)
98 were purchased from JT Baker (LC-MS grade) and were used in association with UPW in also
99 or not with 0.1 % formic acid.

100 All concentrated stock solution of individual pharmaceuticals were prepared in methanol
101 with a concentration of 500 $mg.L^{-1}$ and stored at −20 ◦C. The mixed spiking solutions were
102 prepared in methanol at 500 $\mu g.L^{-1}$ and stored at 4◦C during 15 days maximum. This mixed
103 spiking solution is daily diluted in water to obtained 500 $ng.L^{-1}$ before use for standard

104 addition. Concentrations prepared for analytical development and to quantify the target
105 compounds in the different matrices are: 5, 10, 20, 50, 100, 250 and 500 ng.L^{-1}.
106
107 *2.3. On-line solid phase extraction and liquid chromatography*

108 The analytical system consists of an automated SPE sampler coupled with an LC-MS/MS. The
109 online extraction was carried out using a 2777 autosampler equipped with two parallel
110 Oasis^{TM} HLB cartridge (Direct Connect HP 20µm, 2.1x30mm) working sequentially. The
111 switching from the loading flow pattern, to elution, then conditioning and back to loading is
112 performed using two six positions Everflow^{TM} valves. Loading eluent (UPW) and conditioning
113 eluent (methanol) were provided by a quaternary pump (Acquity^{TM} QSM). Elution of the
114 analytes from the SPE cartridge to LC system was achieved by connected the cartridge to the
115 inlet of the separation column and using the initial chromatographic elution solution.
116 Separation was carried out using a reversed phase column (Acquity^{TM} BEH C18, 100 mm x 2.1
117 mm ID, 17µm) placed in an oven (45°C). The elution gradient was produced by a binary
118 pump (Acquity^{TM} BSM) and was optimized and will be described later in the manuscript.
119
120 *2.4. Mass spectrometry*

121 The mass spectrometer (Quattro Premier, Micromass^{TM}) operates with the following
122 conditions: cone gas (N2, 50 L.h^{-1}, 120 °C), desolvation gas (N2, 750 L.h^{-1}, 350 °C), collision
123 gas (Ar, 0.1 mL.min^{-1}), capillary voltage (3000V). The ionization source of the mass
124 spectrometer is an electrospray (ESI) used either in the positive or the negative mode
125 according to pharmaceutical compounds structure (table 1). All the analysis, are made in
126 "multiple reaction monitoring" (MRM) mode, the parent ion from the ESI source is selected
127 in the first quadrupole (pseudomolecular ion in most cases) and fragmented in the collision

128 cell. One or more fragments (quantification ion and, when available, confirmation ions) are
129 then selected by the third quadrupole before being detected by a photomultiplier. This
130 mode allows high sensitivity and selectivity.

131 **Table 1: List of the 35 pharmaceuticals with pharmaceutical class Molecule (short identifier), N°CAS, MW (g/mol), formula, mass parameter and retention time**

Pharmaceutical class	Molecule (short identifier)	N°CAS	MW (g/mol)	formula of the active substance	ESI	Parents ion	Daughter ion(Q)	Cones (V)	Collisions (V)	Confirmation ion	Collisions (V)	Dwell time (ms)	Tr (min)
Cardiovascular drugs	Amlodipin (AML)	111470-99-6	567.05	$C_{20}H_{25}ClN_2O_5$	+	409.6	238.1	18	11	409.6	13	50	4.03
	Atenolol (ATE)	29122-68-7	266.34	$C_{14}H_{22}N_2O_3$	+	267	145	34	26	74	23	50	1.18
	Losartan (LOS)	124750-99-8	461	$C_{22}H_{23}ClN_6O$	+	423.6	405.2	30	12	207	22	50	4.25
	Naftidrofuryl (NAF)	03200-6-4	473.56	$C_{24}H_{33}NO_3$	+	384.6	99.7	40	21	84.7	25	50	4.29
	Pravastatin (PRA)	81131-70-6	446.51	$C_{23}H_{36}O_7$	-	423.2	100.6	34	23	321.1	16	50	2.63
	Propanolol (PRO)	525-66-6	259.4	$C_{16}H_{21}NO_2$	+	260.2	116	34	18	183	18	50	3.33
	Gemfibrozil (GEM)	25812-30-0	250.33	$C_{15}H_{22}O_3$	-	249	121	34	23			50	4.95
	Trimetazidin (TRI)	13171-25-0	339.26	$C_{14}H_{24}Cl_2N_2O_3$	+	267.4	180.9	21	16	165.8	26	50	1.18
anticancer agent	Tamoxifen (TAM)	10540-29-1	371.5	$C_{26}H_{29}NO$	+	372.5	72	45	14			50	5.42
	Hydroxytamoxifen (OH-TAM)	68047-06-3	387.2	$C_{26}H_{29}NO_2$	+	388.2	72	45	14			50	4.58
	Ifosfamide (IFO)	3778-73-2	261	$C_7H_{15}Cl_2N_2O_2P$	+	261.02	153.95	25	22	92.04	25	75	3
Human Antibiotic	Doxycycline (DOX)	24390-14-5	512.94	$C_{22}H_{24}N_2O_8$	+	445.5	428.2	30	18	153.8	28	50	2.95
	Erythromyicin (ERY)	114-07-8	769.96	$C_{37}H_{67}NO_{13}$	+	734.2	158	28	30	576.2	19	50	3.68
	Ofloxacin (OFX)	82419-36-1	361.37	$C_{18}H_{20}FN_3O_4$	+	362	318	34	19	261	28	80	1.35
	Sulfaméthoxazole (SUL)	723-46-6	253.278	$C_{10}H_{11}N_3O_3S$	+	254	92	26	28	156	16	50	2.74
	Trimetoprime (TRP)	738-70-5	290.3	$C_{14}H_{18}N_4O_3$	+	291.2	230	24	24	261.1	26	50	1.18
Veterinarian Antibiotic	Danofloxacin (DANO)	112398-08-0	357.38	$C_{19}H_{20}FN_3O_3$	+	358.5	314	35	19	283	25	50	1.53
	Lincomycin (LINCO)	859-18-7	461.37	$C_{18}H_{34}N_2O_6S$	+	407.6	125.9	40	28	359.3	18	50	1.23
	Sulfadimerazine (SFZ)	57-68-1	278.33	$C_{11}H_{12}N_4O_2S$	+	279.4	185.9	29	16	91.7	26	50	1.91
	Tylosin (TYL)	74610-55-2	1066.19	$C_{46}H_{77}NO_{17}$	+	917	174	60	37	773	29	50	3.84
Neuroleptic	Carbamazepine (CBZ)	298-46-4	236.27	$C_{15}H_{12}N_2O$	+	237.1	194	28	19	179	39	50	3.85
	Epoxycarbamazepine (Ep-CBZ)	36507-30-9	252.27	$C_{15}H_{12}N_2O_2$	+	253.3	179.9	28	28	236	12	50	3.2
	Oxazepam (OZP)	604-75-1	286.71	$C_{15}H_{11}ClN_2O_2$	+	287.4	241	34	20	269.1	14	50	4.08
	Oxazepam (Glu-OZP)	6801-81-6	462.84	$C_{21}H_{19}ClN_2O_8$	+	463.2	287.1	26	15	269	26	15	3.34
Non-steroidal anti-inflammatory drugs (NSAID)	Diclofenac (DICLO)	15307-79-6	294.14	$C_{14}H_{11}Cl_2NO_2$	+	296.1	250	22	10	214.1	25	100	5.5
	Ibuprofen (IBU)	15687-27-1	206.28	$C_{13}H_{18}O_2$	-	205	161	17	7			50	4.06
	Hydroxyibuprofen (OH-IBU)	51146-55-5	222.28	$C_{13}H_{18}O_3$	-	221.2	177	19	9	158.7	13	50	1.2
	Ketoprofen (KETO)	22071-15-4	254.28	$C_{16}H_{14}O_3$	+	255	209	29	12	105	22	100	4.14
	Salicylic acid (SCA)	69-72-7	138.12	$C_7H_6O_3$	-	137	92.6	30	14	64.7	28	70	1.16
Miscellaneous	Acetaminophen (PARA)	103-90-2	151.16	$C_8H_9NO_2$	+	152	110	25	15	90	10	50	1.24
	Acetaminophen Glucuronide (Glu-PARA)	16110-10-4	327.29	$C_{14}H_{17}NO_8$	+	350	173.8	33	15				1.64
	Caffeine (CAF)	58-08-2	194.19	$C_8H_{10}N_4O_2$	+	195.1	137.7	37	18	109.7	22	50	1.35
	Hydrochlorothiazide (HCTZ)	58-93-5	297.74	$C_7H_8ClN_3O_4S_2$	-	296.2	77.6	42	28	204.8	22	50	1.5
Hormone	Ethyinylestradiol (EE)	57-63-6	296.4	$C_{20}H_{24}O_2$	-	295.2	144.9	54	40	183	35	50	4.07
	17β-Estradiol (βE)	50-28-2	272.38	$C_{18}H_{24}O_2$	-	271.1	145	50	38	183	41	70	3.89
	Estrone (EO)	53-16-7	270.37	$C_{18}H_{22}O_2$	-	269.1	145	53	35	183	36	70	4.14
	Progesterone (PGT)	57-83-0	314.46	$C_{21}H_{30}O_2$	+	315.2	97	32	24	109	26	50	5.77

132

133 **3. Results and discussion**

134 *3.1. Mass spectrometry optimization*

135 The selection of optimum detection parameters (collision energy, cone voltage, ionization
136 mode) for each targeted compound was carried out by introducing a standard diluted single
137 solute solution at 5 $mg.L^{-1}$ directly in the mass spectrometer (without separation). The
138 pseudo-molecular ion ($[M+H]^+$ or $[M-H]^-$) was selected as the parent ion. Acetaminophen-
139 glucuronide was ionized as sodium adducts ($[M+Na]^+$) and the daughter ion correspond to
140 the sodium adduct of paracetamol obtained by the loss of glucuronic acid. Similar
141 fragmentation pattern with loss of carbohydrate group was observed with Glu-OZP ($[M+H]^+$
142 $\rightarrow$ $[M-Glu+H]^+$). In some cases, the standard molecules were purchased as sodium or
143 chloride salt so molecular weight of the commercial product indicated in the table 1 does
144 not correspond to the formula of active compounds. So the molecular weights indicated in
145 the table 1 do not correspond to the mass of the pseudo molecular ion (AML, LOS, NAF, PRA,
146 TRI, DOX, ERY, LINCO and TYL). Positive mode was selected for most of the molecules and 8
147 analytes were ionized under negative mode because of their tendancy to loose a proton.
148 Two transitions are chosen for quantification and confirmation. If possible transition
149 corresponding to the loss of simples fragments (ie. $-H_2O$ or $-CO_2$) has been prefered for
150 quantification or confirmation transition. Only one transition could be found to 4 molecules:
151 Ibuprofen, Gemfibrozil, Tamoxifen and Hydroxy-Tamoxifen. The results are presented in
152 table 1.

153 *3.2. On-line SPE method development*

154 The efficiency of the SPE step was studied using two different types of SPE cartridge phases :
155 Oasis HLB (Direct Connect HP 20µm, 2.1x30mm) and XBridge C18 (Direct Connect HP 10µm,
156 2.1x30mm). The low energie interactions are predominant with the C18 phases, unlike for
157 HLB phases where the dipole-dipole interactions are brought into play. Table 2 presents
158 characteristics (log(Kow), pka, coefficient of dissociation, dipolar moment) of molecules. The
159 extraction yield was then calculated according to the following equation:

$$Extraction\ yield\ (\%) = 100 * \frac{\mathrm{Area}_{\mathrm{SPE\ mode}}}{\mathrm{Area}_{\mathrm{conventional\ mode}}}$$

160 For each compounds, the area obtained with the injection of 5mL of solution at 100 $ng.L^{-1}$ in
161 SPE mode was compared to the area obtain in conventional mode (Vinj=5µL; C=100 $\mu g.L^{-1}$).

162 The results are presented in figure 1. In a global overview the extraction yields are better
163 with the Oasis HLB phase in comparison to the C18 phase. 11 molecules have slightly better
164 extraction yields with the XBridge C18 media. Given these results, Oasis HLB phase was
165 chosen for the SPE cartridges. The extraction yields are between 24% and 96%. Six
166 molecules, among them three hormones (ATE, TRI, DOX, EE, βE and EO) have extraction
167 yields inferior or equal to 50% but the signal is sufficient for our analysis given the
168 reproducibility of the extraction step. The loading time and flow rate influence the analyte
169 retention onto the preconcentration cartridge. If the loading time is too short, a part of the
170 molecules of interest will not be collected in the cartridge. MeOH is used for the cartridge
171 conditioning during 3 minutes and UPW for the loading sample during 5.5 minutes at
172 2mL/min. 5mL of sample are injected onto the cartridge. Elution of our compounds is made
173 using the initial chromatographic conditions. The preconcentration method takes 8.5
174 minutes. The pH of samples and eluents was also optimized to try to improve the extraction
175 yields. The figure 2 shows the effect of pH (3, 7 and 9) on molecule's recovery yields. Most of

176 the targeted compounds were efficiently extracted at neutral pH values. The recovery yields
177 of thirteen molecules (LOS, GEM, TAM, OH-TAM, IFO, TYL, DICLO, PARA, CAF, CBZ, OZP, PGT
178 and ERY) do not show significant pH dependence. ATE, NAF and LINCO were comparatively
179 more recovered under neutral condition due to the amine/ammonium repartition for the
180 low pH values. DANO and OFX are amphoteric molecules and exhibit higher recovery yields
181 under acid extraction than under neutral conditions. AML and OFX have extraction yields
182 superior to 100%, the differences may be included within the experimental errors. Three
183 hormones have a better extraction yields at basic pH while below 23% for an acid pH. The
184 SPE appears globally controlled by the carboxylic functions. The best compromise to our
185 analytical method is the neutral pH.

186 **Tableau 2: log(Kow), pka, coefficient of dissociation and dipolar moment of molecules**

Molecule	Log(Kow)	pka	coefficient of dissociation	dipolar moment
AML	3	8.6	$5.00\ 10^{-5}$	
ATE	0.16	9.6	$1.50\ 10^{-5}$	5.71
LOS	1.19	5,5	$8.80\ 10^{-3}$	
NAF	4.56	8.7	$4.70\ 10^{-5}$	2.83
PRA	1.35	4.5	$5.60\ 10^{-3}$	
PRO	3.48	9.5	$1.70\ 10^{-5}$	
GEM	4.77	4.7	$4.40\ 10^{-3}$	
TRI	1.04	4.3/8.9	$7.00\ 10^{-3}$	
TAM	3.24	8.76	$4.20\ 10^{-5}$	
OH-TAM	4.74	3.2/6.4	$6.30\ 10^{-4}$	
IFO	0.86	13.2	$2.50\ 10^{-7}$	
DOX	2,37	3.5/7.7	$1.40\ 10^{-4}$	
ERY	3,02	8.8	$3.90\ 10^{-5}$	
OFX	0.65	6.1	$9.40\ 10^{-4}$	7.2
SUL	0.79	5.7	$1.40\ 10^{-3}$	
TRP	0.91	7.1	$2.80\ 10^{-4}$	
DANO	0,44	6.0	$9.90\ 10^{-4}$	
LINCO	0,56	7.6	$1.60\ 10^{-4}$	
SFZ	0.19	7	$3.20\ 10^{-4}$	7.34
TYL	1.63	7.7	$1.40\ 10^{-4}$	
CBZ	2,77	7	$1.00\ 10^{-7}$	3.66

Ep-CBZ	1.58	15.9	$1.00\ 10^{-8}$	
OZP	2,24	1.7/11.6	$1.30\ 10^{-1}$	
DICLO	4,51	4	$8.00\ 10^{-3}$	4.55
IBU	3,79	4.5	$5.30\ 10^{-3}$	4.95
OH-IBU	3,97	4.8	$3.90\ 10^{-3}$	
KETO	3.12	4.45	$6.00\ 10^{-3}$	
SCA	1,19	3	$3.10\ 10^{-2}$	
PARA	0,49	9.5	$1.80\ 10^{-5}$	4.55
CAF	-0.091	14	$2.10\ 10^{-1}$	3.71
HCTZ	-0,07	7.9	$1.00\ 10^{-4}$	
EE	3,67	10.3	$7.00\ 10^{-6}$	
βE	3.57	10.71	$4.40\ 10^{-6}$	1.56
EO	3.69	10.4	$6.00\ 10^{-6}$	3.45
PGT	4	18.9	$3.50\ 10^{-10}$	

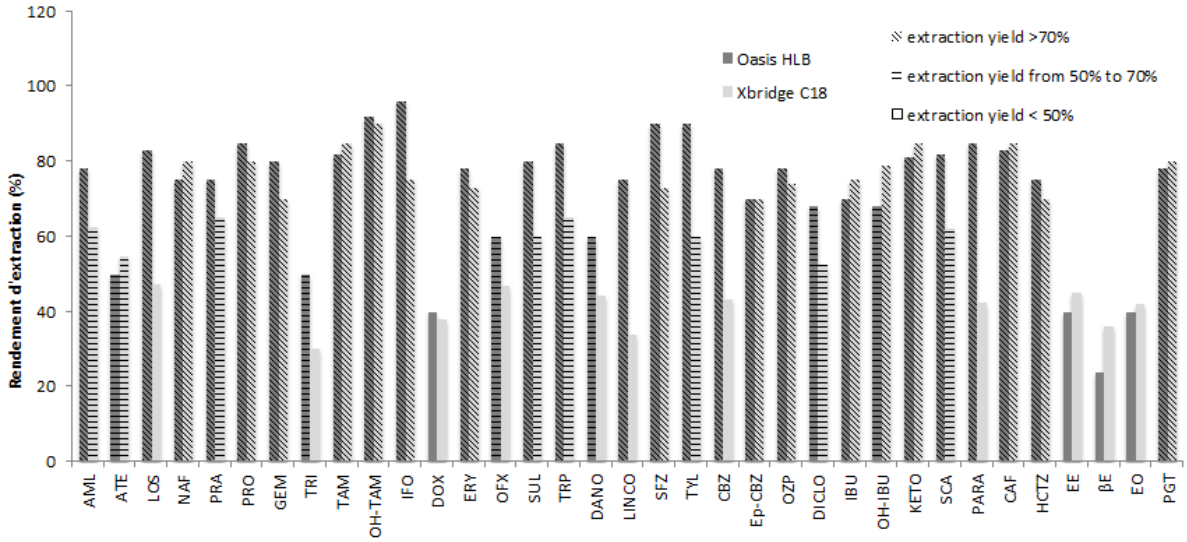


Figure 1: Extraction yields calculated for the two cartridges (Oasis HLB and Xbridge C18) tested for all molecules in neutral pH

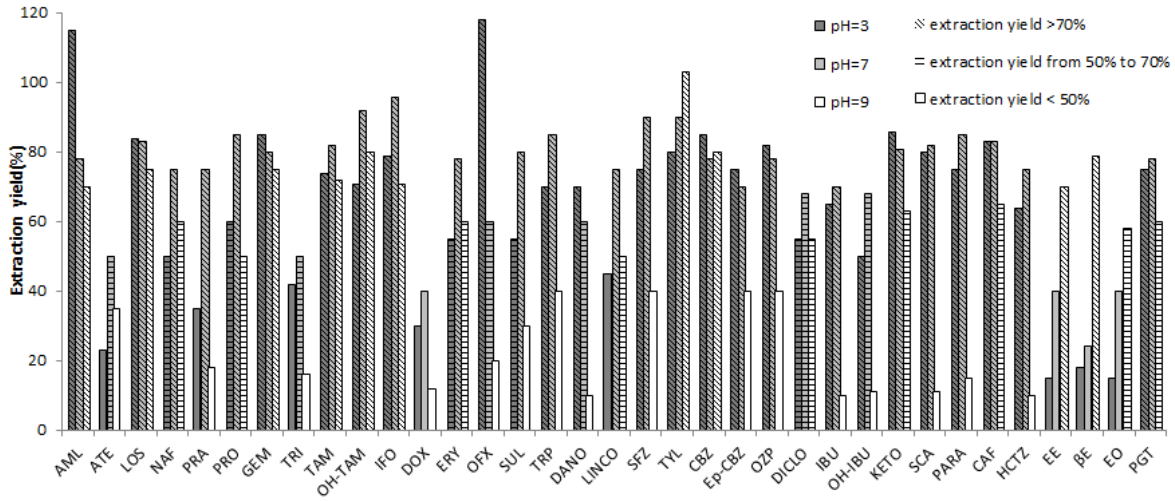


Figure 2: Extraction yields calculated for the 3 pH (3, 7 and 9) for all analytes

3.3. Chromatographic conditions

Three chromatographic columns packed with different stationary phases were studied, two using the reversed phase mode: Acquity BEH C18 (100 mm x 2.1 mm ID, 1.7 µm) and Acquity HSST3 (100 mm x 2.1 mm ID, 1.7 µm). These two columns have the same stationary phase but Acquity HSST3 should allow for better separation of polar molecules due to the greater proportion of residual silanol groups. The third column has a polar stationary phase: BEH amide (100 mm x 2.1 mm ID, 1.7 µm) in order to separate the analyte using hydrophilic interaction liquid chromatography (HILIC). Comparing the chromatograms obtained for the C18 and HSST3 column, the results are quite similar. Seven minutes are required to obtain sufficient separation. It should be underlined that the resolution between two consecutive peaks was quite low. However, because the quantification was done using different MRM channels this poor resolution does not affects the analytical performances.

Figure 3 summarizes the results by plotting the polarity (log Kow) as function of the capacity factor of the molecule, molecules with k'<1 form the unretained groups with no log(kow) dependances. For the others, correlation between k' and log(kow) shows two adverse

behaviours in relation with the different stationary phase, BEH and HSST3 on the one part and HILIC on the second part. Reversed phase HPLC columns (BEH C18 and HSST3) provide a satisfactory separation with k' ranging from 0.93 to 9.91 according to the polarity of the considered compounds. However numerous analytes exhibit a high polarity and were poorly retained using reversed-phase HPLC. Normal phase HPLC column (BEH Amide) provides separation with k' ranging from 0.1 to 9.6. Molecules retained by the reversed phase HPLC column are not retained in normal phase HPLC with k'<1. Moreover, peak tailing are observed for some molecules with HSST3 (SUL, GEM, DOX) and with HILIC column (PARA, DANO, HCTZ, TRI). The best compromise for our analyses is to use the BEH C18 column.

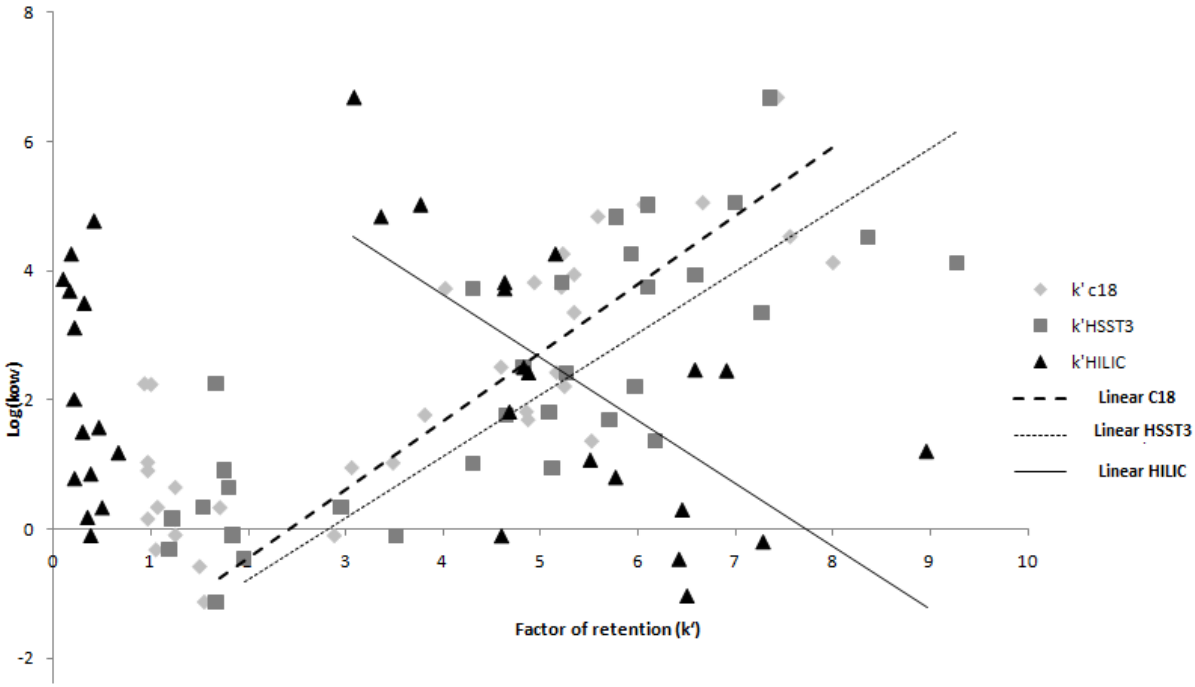


Figure 3: Polarity (log Kow) as function of the capacity factor for all molecules and for 3 chromatographic columns

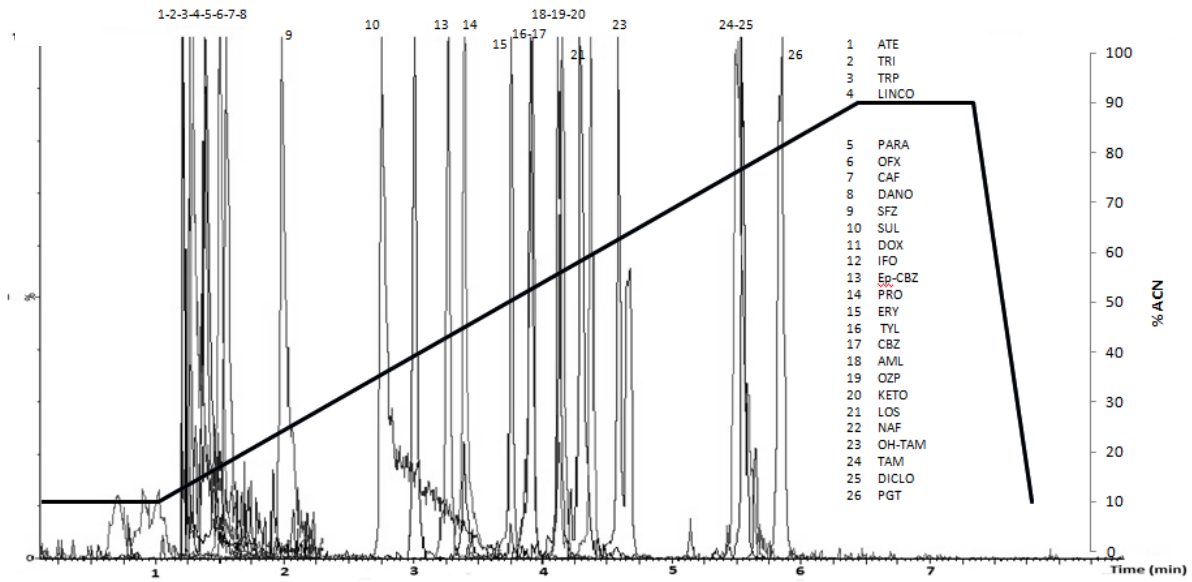
The mobile phase flow rate was 0.4mL.min^{-1}, corresponding to the optimum zone of the Van Deemter curve with this column [26]. The elution conditions were optimized. Two chromatographic separation methods were needed to quantify all the target analytes.

226 Indeed, analytes with ESI+ detection have better sensitivity with acidified eluents (with 0.1%
227 of formic acid) unlike molecules with ESI- detection which have better sensitivity with
228 neutral eluents. Moreover, the combination of both positive and negative ionization mode
229 during the same run does lead to a decrease of the sensibility.

230 The elution conditions start with 20% ACN/80% UPW during 1 minute followed by a gradient
231 90% ACN within 6 minutes and remain constant for 1 min before returning to initial
232 conditions, details of the method are presented in Supporting information (Section B –
233 Figures S1-S3)

234 Examples of chromatograms obtained with a solution of 50 ng.L^{-1} in UPW and the eluent
235 program are presented in Figure 4. 12 molecules elute within two minutes for the ESI+/acid
236 eluent method. As mentioned above, the detection mode (MRM) allows an accurate
237 quantification even if the resolution is low.

a.



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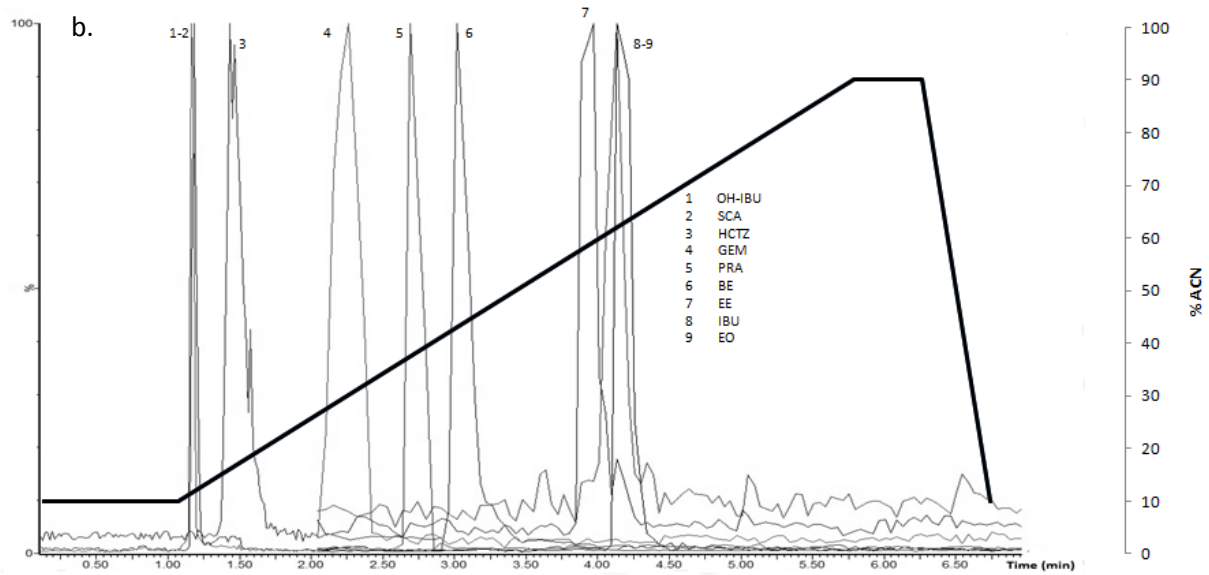

Figure 4: Chromatogram obtained at 50ng/L in UPW. a. first method with ESI+. b. Second method with ESI-

3.4. Quantification limit and matrix effect

Standard addition method was selected for calibration method in order to minimize or eliminated matrix effects. Figures 5 present examples of calibration curve for CBZ in UPW, Groundwater (GW), Drinking water (DW) and Surface water (SW). Limit of quantification (LOQ) were determined for all targeted compounds in UPW and GW with the equation given in figure 5a, in accordance with the AFNOR NF-T-90-210 norm for all analytes. GW could be considered free of pharmaceuticals residues because GW is drawn from a well recovering the waters on a small watershed without collective or on-site sanitation water release, and UPW can be considered as a matrix blank. Negatively ionized molecules (EO, BE, EE, HCTZ, SCA, IBU, OH-IBU, GEM, PRA) have higher limits of quantification because the background noise is more important than for ESI^+. The values of the quantification limit of targeted compounds are presented in figure 6a. LOQ values obtained range from 5 to 17ng/L. These limits of quantification are sufficient for our purpose.

255 Measurement errors were incorporated by defining the 90% confidence intervals (figure 5b).
256 Figures 5c and d show standard addition calibration lines of CBZ in GW and DW.
257 Comparisons of the slopes obtained with real waters to the slope obtain in the blank
258 (a_{GW}/a_{UPW} and a_{DW}/a_{UPW}) allow a comprehensive approach of the matrix effects. These slope
259 ratios are presented in figure 6b for all analytes. The matrix effect is a classical phenomenon
260 which can be very important in liquid chromatography coupled with mass spectrometry
261 because of the ionization process may be drastically influenced by the presence of
262 interfering species. Many studies have already described this phenomenon especially with
263 wastewaters. The presence of organic or inorganic substance can cause inhibition (<1) or
264 enhancement (>1) of a compound's signal [27-29]. In our case, natural organic matter may
265 disturb the SPE step or mass ionization so the rationalization of the slopes provides a global
266 overview of matrix effect but do not allow to identify the critical step.
267 In figure 6b, matrix effects are not significant when the ratio is close to 1. In drinking water
268 this ratio was close to 1 for most of the analytes, only AML has a ratio superior to 5.
269

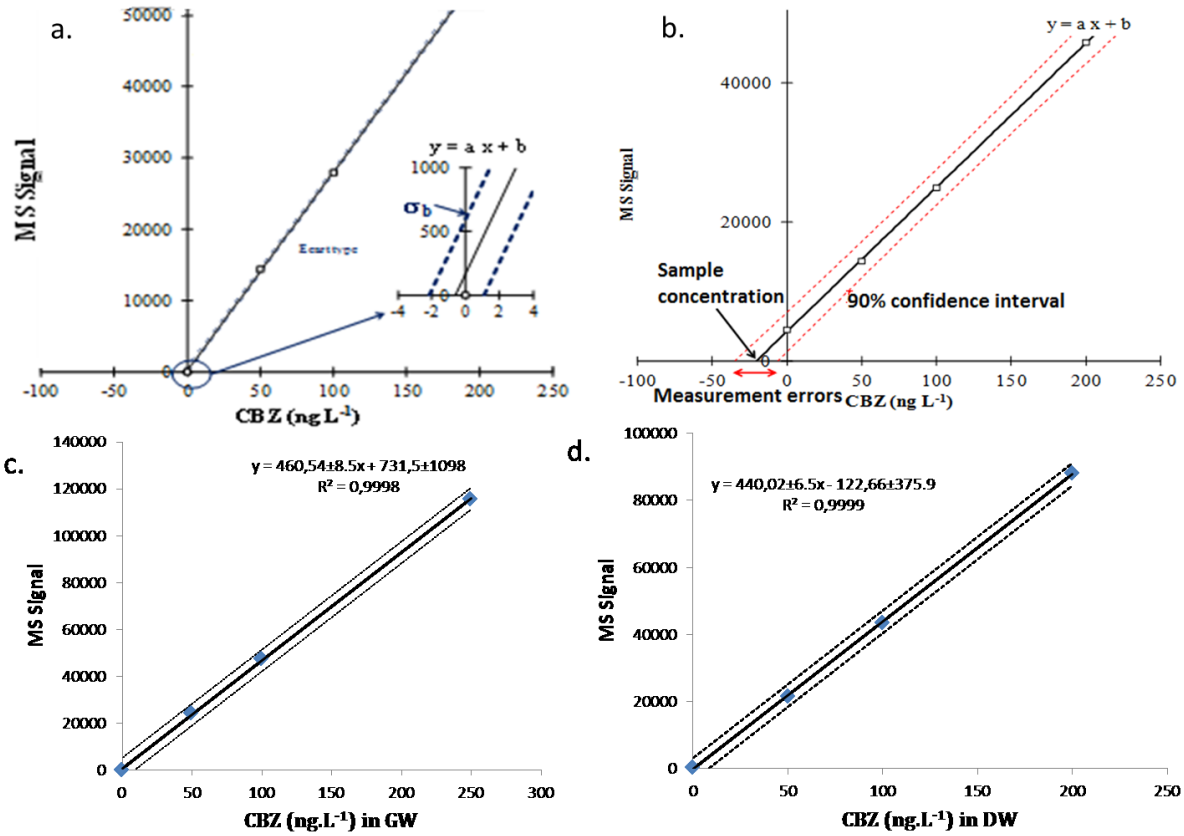

Figure 5: a. equation of LOQ determanation. b. Exemple of standard addition for CBZ with 90% confidence interval. c. and d. Exemple of standard addition in GW and DW for CBZ

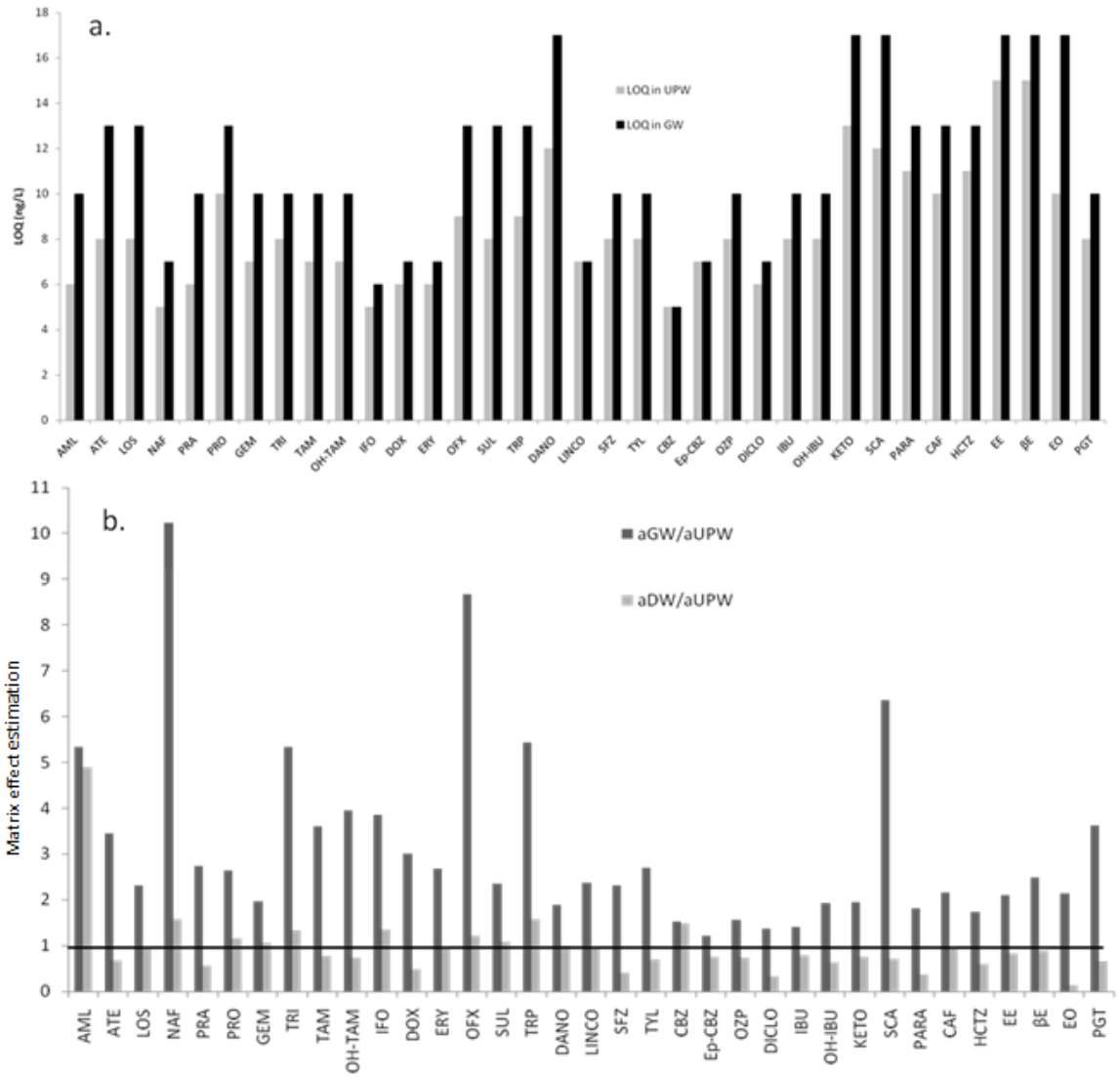

Figure 6: a. LOQ in UPW and GW for all molecules b. Matrix effects of all analytes

3.6. Analysis of surface water and drinking water

The developed method was used to determine the concentration of 37 pharmaceuticals substances in inflow and outflow waters of 8 drinking water treatment plants (DWTP) in west of France. The samples were collected once a month between october 2013 and april 2015, resulting in an average of 100 inflow and 100 outflow concentration values for each molecule. Nine pharmaceuticals have not been detected or with concentrations below the LOQ (AML, TAM, OH-TAM, IFO, ERY, LINCO, EE, βE and PGT). Figure 7 shows the

285 concentrations of 27 pharmaceuticals or metabolites in surface water as a box plot; this
286 statistical representation summarizes the data, for each compound, by the mean values,
287 median value, first and third quartiles and observed extrema. 7 molecules (PARA-GLU, KETO,
288 OH-IBU, DANO, PARA, SCA, CAF) have a mean concentration greater than 50 ng.L-1. 10
289 molecules were quantifieded with mean concentrations higher than 10 $ng.L^{-1}$ (GEM, CBZ,
290 DICLO, OZP, OFX, IBU, HCTZ, ATE, PRO and DOX). The last detected 10 molecules exhibit
291 mean concentration lower than 10 ng.L-1 (SFZ, SUL, TRI, PRA, Ep-CBZ, TRP, EO, NAF, TYL,
292 LOS). For some molecules, large differences between the extrema are observed (PARA-Glu,
293 KETO, OH-IBU, SCA). These differences depend on the sampling date essentially. It should be
294 underlined that median values are close to mean values indicating that extrema values do
295 not play an important role. The maximum observed concentration in surface water was 650
296 $ng.L^{-1}$ for KETO. Detection frequencies depend on compounds and range from 100%
297 occurrence for CAF and PARA and 9% for TYL. 13 molecules (PARA-Glu, KETO, OH-IBU,
298 DANO, PARA, CAF, SCA, DICLO, GEM, CBZ, OZP, OFX and ATE) were quantified in more than
299 50% of surface water samples. In drinking water (figure 8), six molecules (KETO, PARA-Glu,
300 OH-IBU, DANO, PARA and CAF) were quantified in 90% or more of the drinking water
301 samples. These 6 molecules were also the most quantified molecules in surface water. The
302 overall mean concentration values are between 4 (OZP) and 327 ng/L. The maximum
303 concentration found was 650 ng/L for KETO. For drinking water, the same remark than for
304 surface water may be made concerning the gap between minimum and maximum
305 concentrations: the eight drinking water treatment plants operate different treatment
306 chains with different type of water resources.

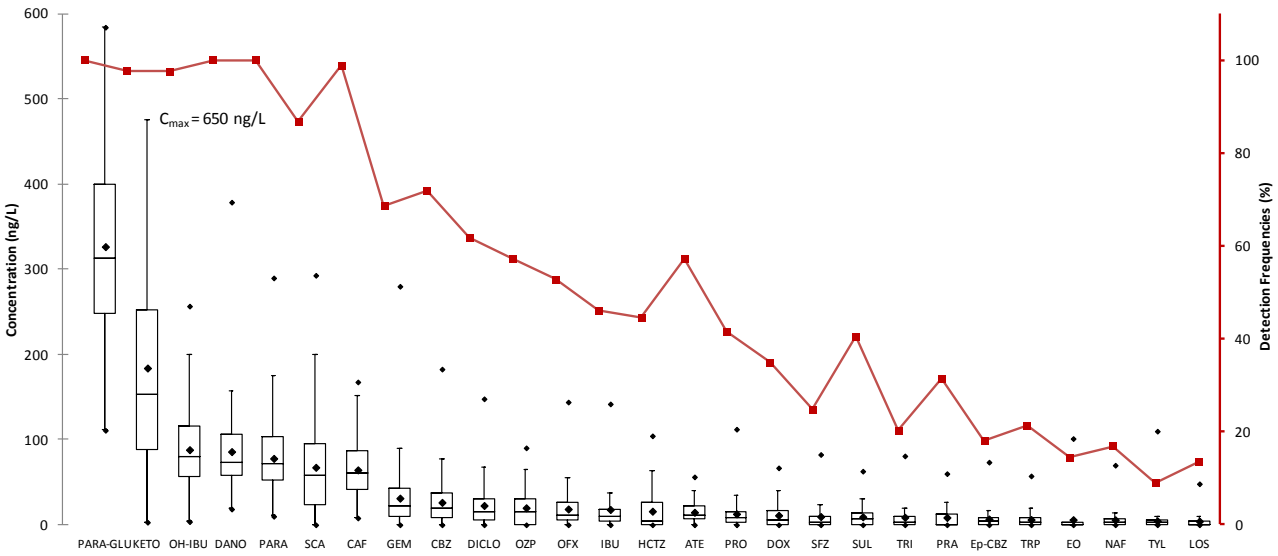

Figure 7: overall mean concentrations (♦), median value, first and third quartiles and extrema of 27 molecules detected on average above LOQ in surface waters and detection frequencies (%, broken line).

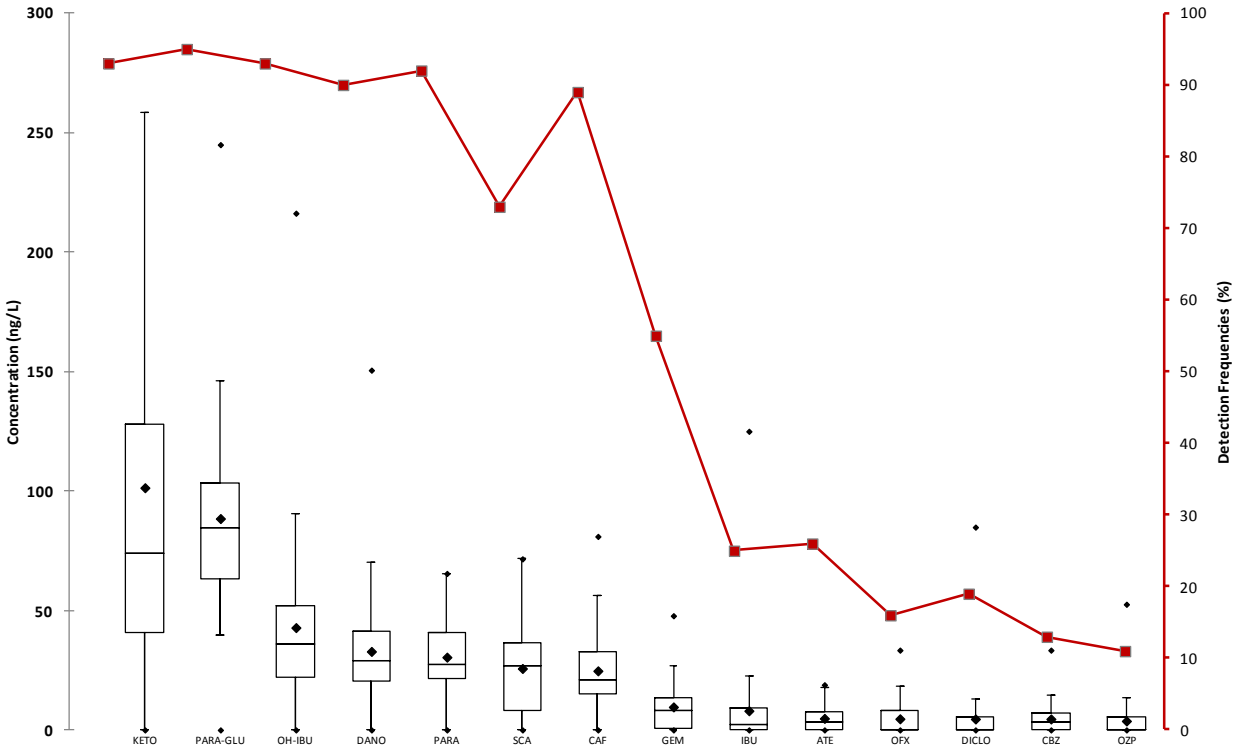

Figure 8: overall mean concentrations(♦), median value, first and third quartiles and extrema for 14 molecules detected in tap waters and detection frequencies (%, broken line).

316 **4. Conclusion**

317 A multiresidue analysis was developed using on-line solid phase extraction connected to
318 liquid chromatography coupled with tandem mass spectrometry in order to quantify residue
319 trace levels 35 pharmaceuticals compounds in surface and drinking water. The short
320 implementation time needed to achieve the preconcentration and the analysis, 17 minutes
321 for the positive mode method and 15 minutes for the negative mode method is among the
322 most significant advantages of this method compared to off-line solid phase extraction. The
323 developed method with a preconcentration factor of one thousand showed detection limits
324 compatible with the study of environmental matrices with very low analyte concentrations.
325 The limits of detection and quantification are between 1.5 and 4 ng/L and 4 and 17ng /L,
326 respectively. Standard addition was chosen for the quantification of molecules in water
327 samples to overcome the matrix effects and provide an accurate determination of targeted
328 compounds. Among all studied substances, doxicycline appeared to be the most affected by
329 a matrix effect. The developed methods were applied to eight surfaces and drinking water.
330 In surface water, 12 molecules could be quantified in almost all analyzed samples with a
331 maximum concentration value of 650ng/L for Ketoprofen. In drinking water, 5 molecules
332 could be regularly detected, with overall mean concentration values between 20 à 120ng/L.

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