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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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*

1. Introduction

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

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

In this context, sensitive analytical methods allowing the quantification of many pollutants at trace concentration is essential. Solid Phase Extraction (SPE) is the most commonly used 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

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

2.2. Pharmaceutical standards and reagents

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

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

addition. Concentrations prepared for analytical development and to quantify the target compounds in the different matrices are: 5, 10, 20, 50, 100, 250 and 500 ng.L^{-1}.

2.3. On-line solid phase extraction and liquid chromatography

The analytical system consists of an automated SPE sampler coupled with an LC-MS/MS. The online extraction was carried out using a 2777 autosampler equipped with two parallel Oasis^{TM} HLB cartridge (Direct Connect HP 20µm, 2.1x30mm) working sequentially. The switching from the loading flow pattern, to elution, then conditioning and back to loading is performed using two six positions Everflow^{TM} valves. Loading eluent (UPW) and conditioning eluent (methanol) were provided by a quaternary pump (Acquity^{TM} QSM). Elution of the analytes from the SPE cartridge to LC system was achieved by connected the cartridge to the inlet of the separation column and using the initial chromatographic elution solution.

Separation was carried out using a reversed phase column (Acquity^{TM} BEH C18, 100 mm x 2.1 mm ID, 17µm) placed in an oven (45°C). The elution gradient was produced by a binary pump (Acquity^{TM} BSM) and was optimized and will be described later in the manuscript.

2.4. Mass spectrometry

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

cell. One or more fragments (quantification ion and, when available, confirmation ions) are then selected by the third quadrupole before being detected by a photomultiplier. This 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

3. Results and discussion

3.1. Mass spectrometry optimization

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

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 µg.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

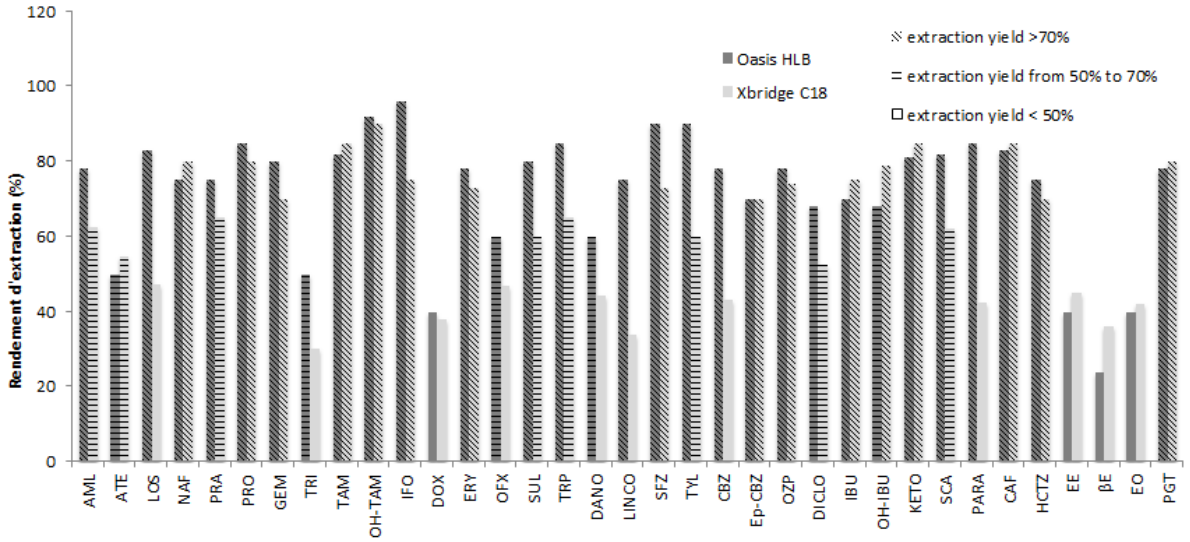
176 the targeted compounds were efficiently extracted at neutral pH values. The recovery yields
177 of thirteen molecules (LOS, GEM, TAM, OH-TAM, IFO, TYL, DICLO, PARA, CAF, CBZ, OZP, PGT
178 and ERY) do not show significant pH dependence. ATE, NAF and LINCO were comparatively
179 more recovered under 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	$3.50\ 10^{-10}$	

187

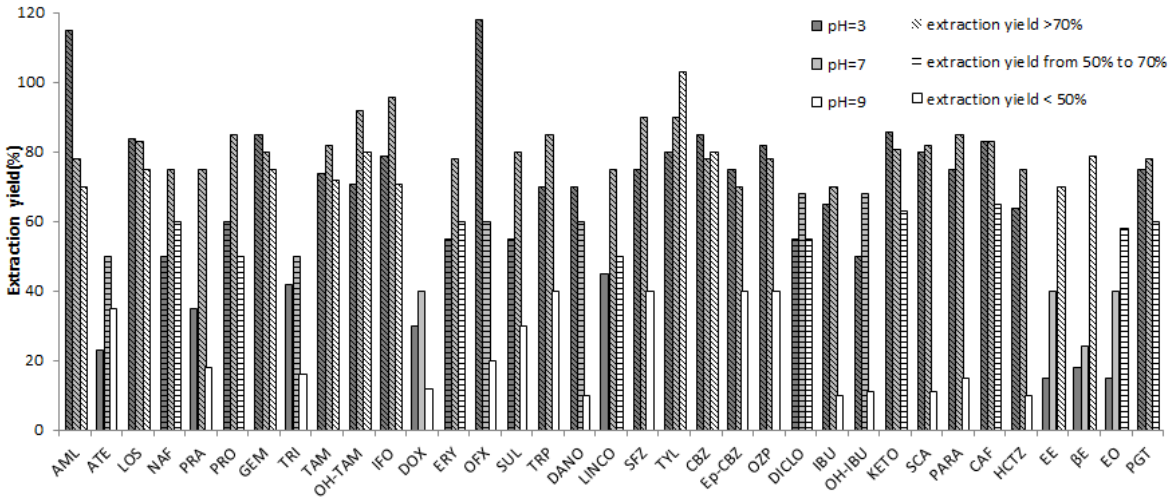


188

189 **Figure 1: Extraction yields calculated for the two cartridges (Oasis HLB and Xbridge C18) tested for all**

190 **molecules in neutral pH**

191



192

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

194 *3.3. Chromatographic conditions*

195 Three chromatographic columns packed with different stationary phases were studied, two
196 using the reversed phase mode: Acquity BEH C18 (100 mm x 2.1 mm ID, 1.7 µm) and Acquity
197 HSST3 (100 mm x 2.1 mm ID, 1.7 µm). These two columns have the same stationary phase
198 but Acquity HSST3 should allow for better separation of polar molecules due to the greater
199 proportion of residual silanol groups. The third column has a polar stationary phase: BEH
200 amide (100 mm x 2.1 mm ID, 1.7 µm) in order to separate the analyte using hydrophilic
201 interaction liquid chromatography (HILIC). Comparing the chromatograms obtained for the
202 C18 and HSST3 column, the results are quite similar. Seven minutes are required to obtain
203 sufficient separation. It should be underlined that the resolution between two consecutive
204 peaks was quite low. However, because the quantification was done using different MRM
205 channels this poor resolution does not affects the analytical performances.
206 Figure 3 summarizes the results by plotting the polarity (log Kow) as function of the capacity
207 factor of the molecule, molecules with k'<1 form the unretained groups with no log(kow)
208 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.

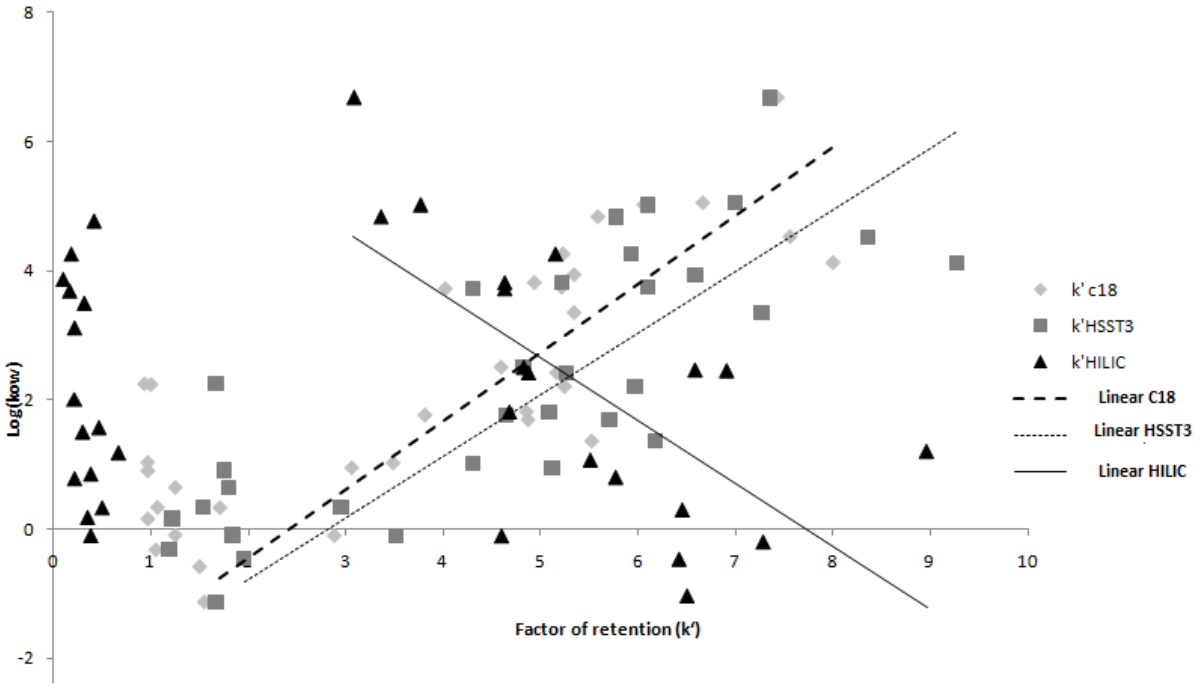


Figure 3: Polarity (log Kow) as function of the capacity factor for all molecules and for 3 chromatographic columns

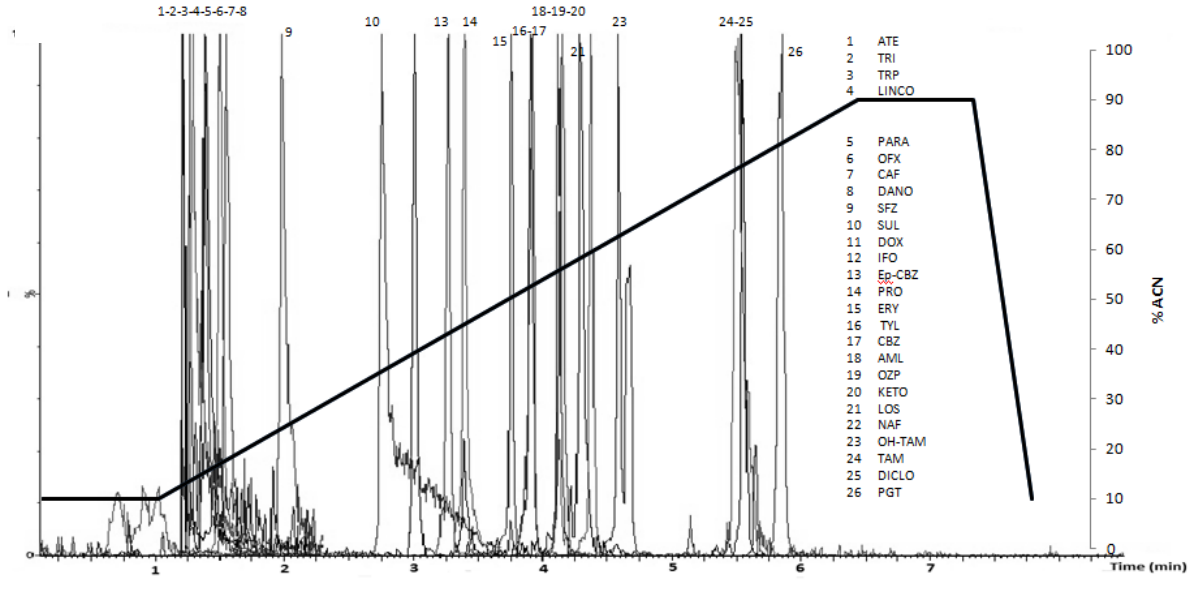
The mobile phase flow rate was 0.4mL.min^{-1}, corresponding to the optimum zone of the Van Deemter curve with this column [26]. The elution conditions were optimized. Two chromatographic separation methods were needed to quantify all the target analytes.

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

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

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

a.



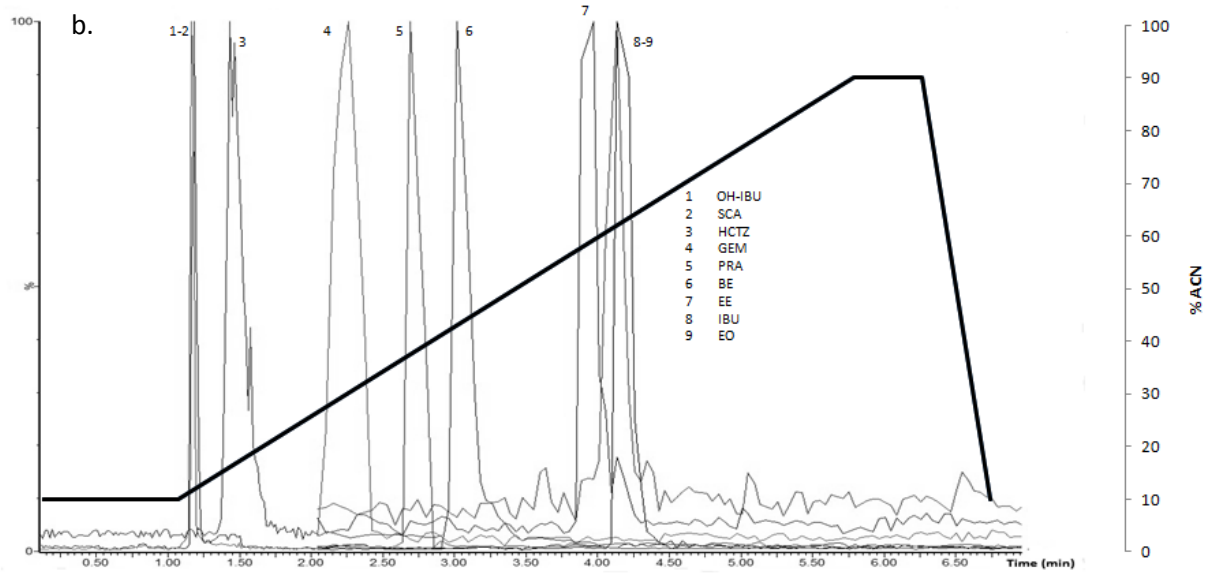


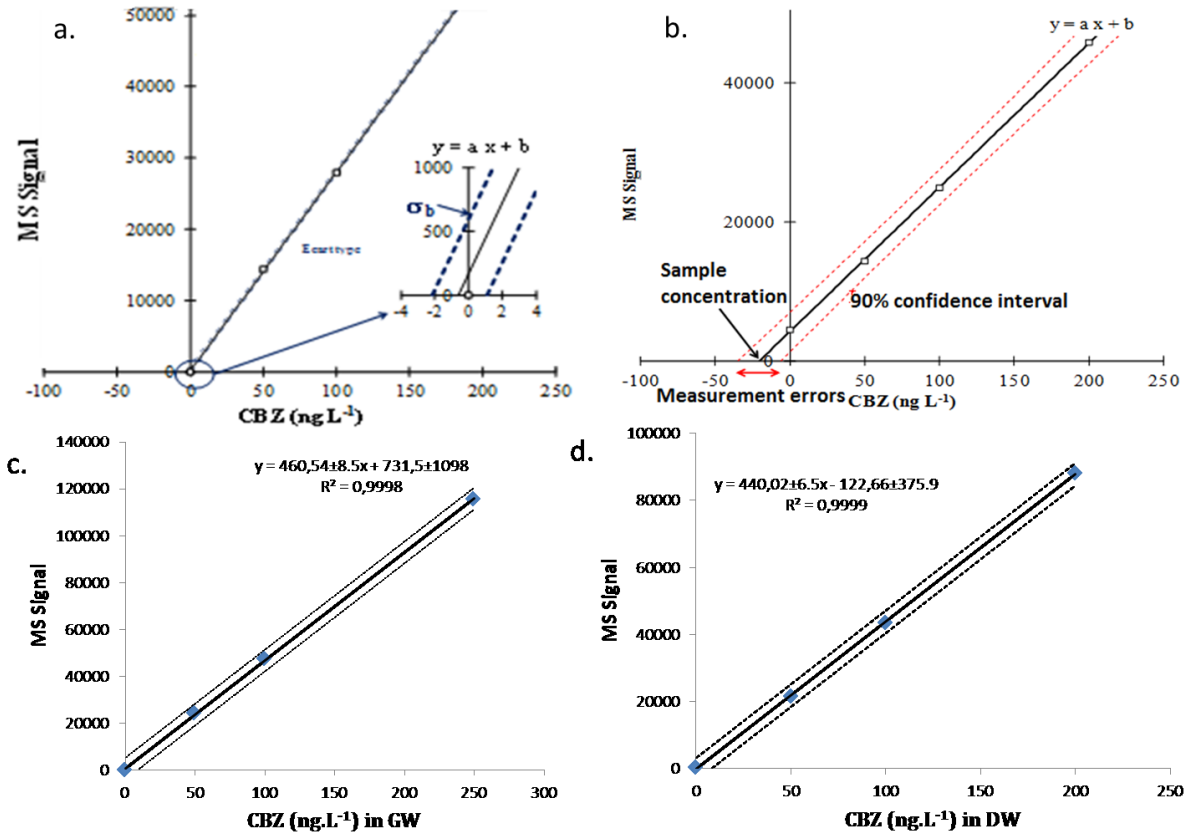
Figure 4: Chromatogram obtained at 50ng/L in UPW. a. first method with ESI+. b. Second method with ESI-

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

270



271

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

274

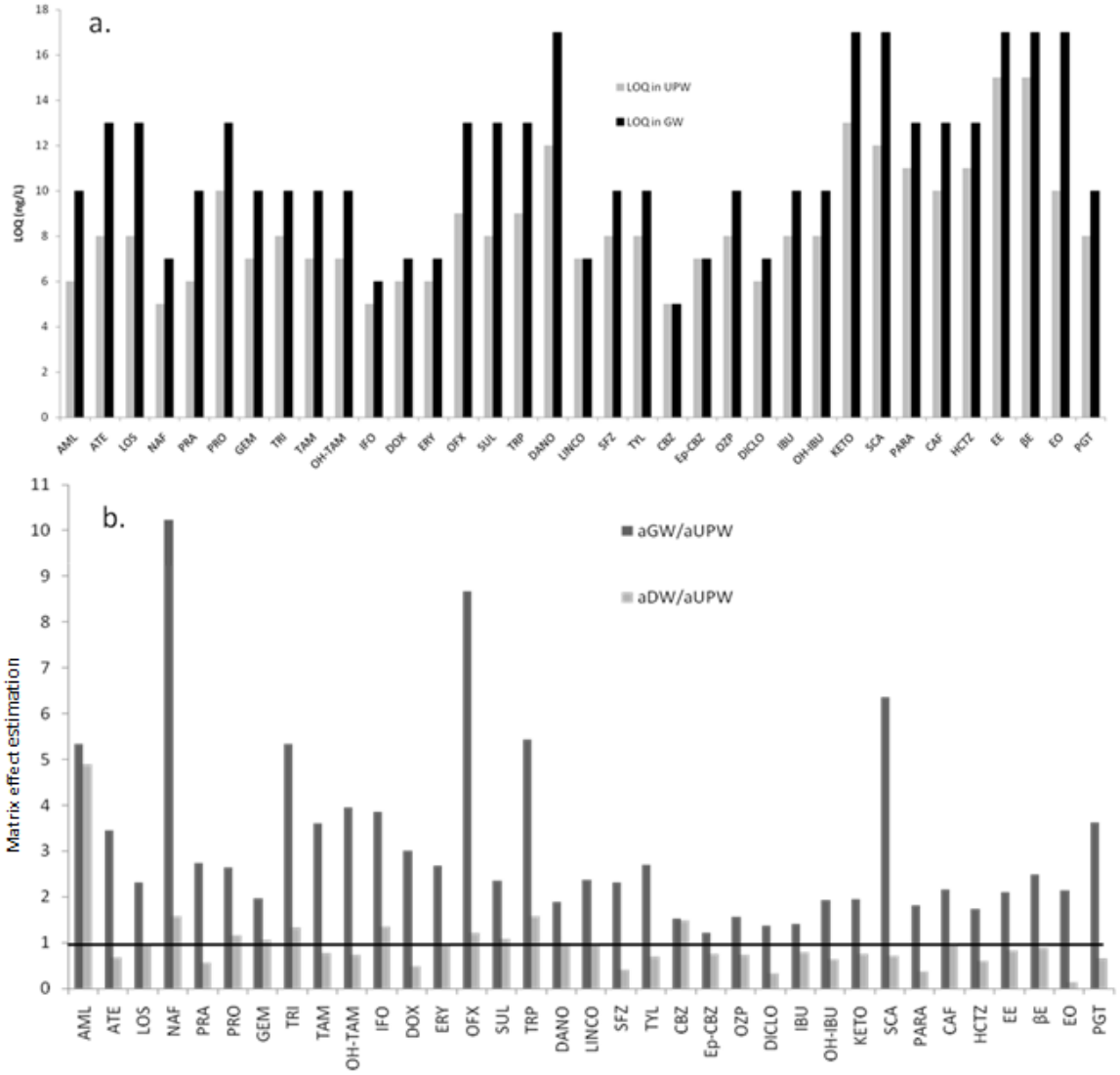


Figure 6: a. LOQ in UPW and GW for all molecules b. Matrix effects of all analytes

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.

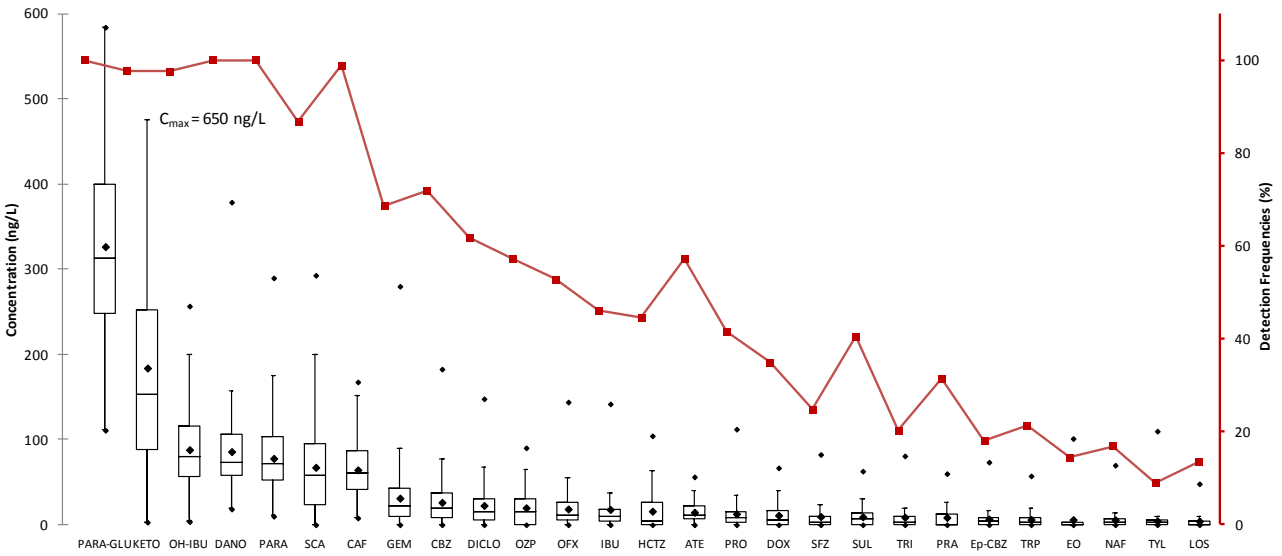


Figure 7: overall mean concentrations (♦), median value, first and third quartiles and extrema of 27 molecules detected on average above LOQ in surface waters and detection frequencies (%, broken line).

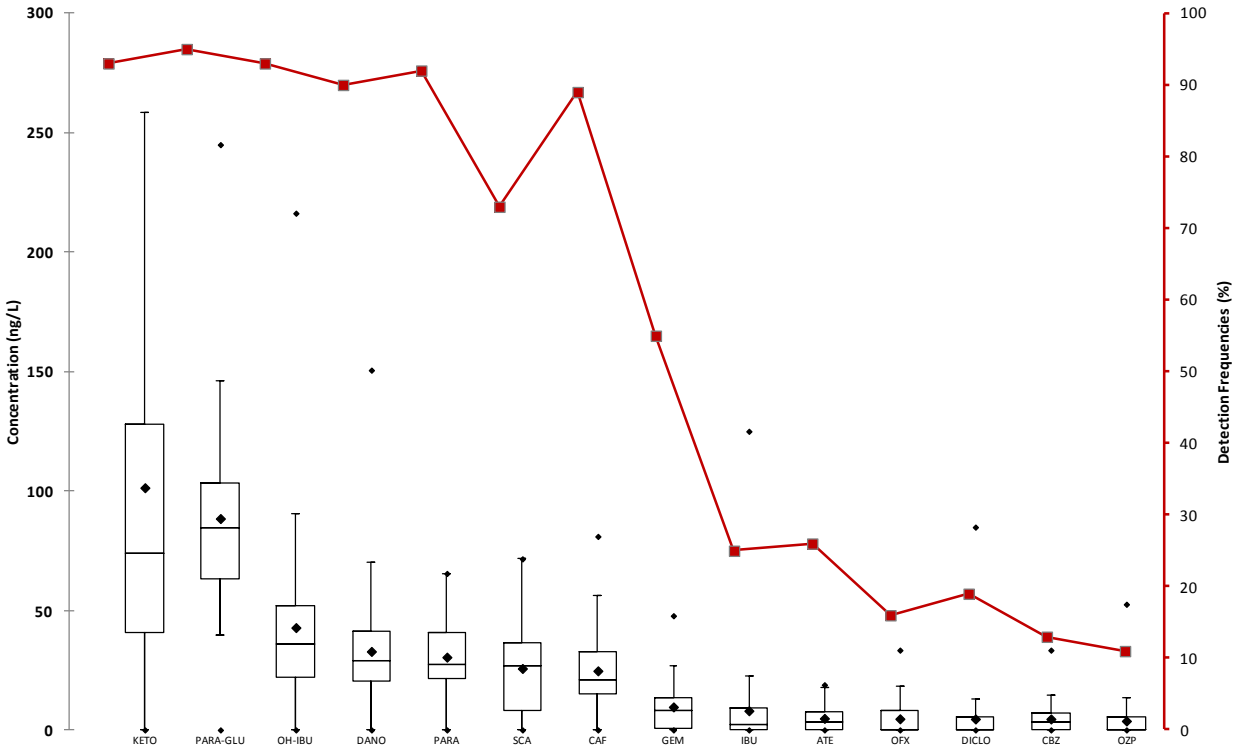


Figure 8: overall mean concentrations(♦), median value, first and third quartiles and extrema for 14 molecules detected in tap waters and detection frequencies (%, broken line).

4. Conclusion

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

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