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1 **STANDARD ADDITION METHOD FOR THE DETERMINATION OF**
2 **PHARMACEUTICAL RESIDUES IN DRINKING WATER BY SPE-**
3 **LC-MS/MS**

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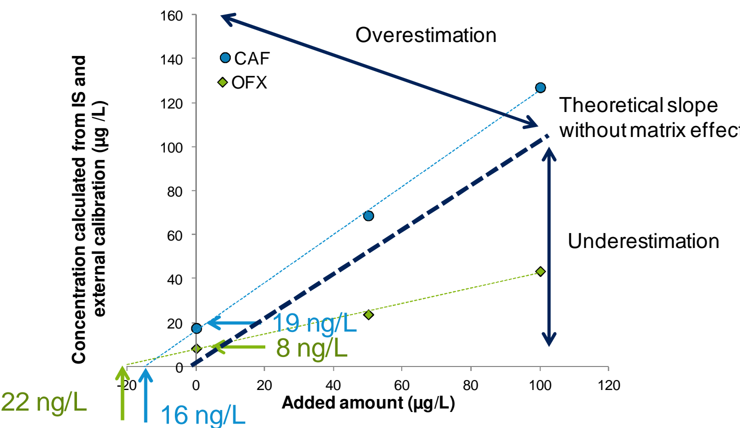
19 **Graphical Abstract**



20

21 **Abstract**

22 The study of the occurrence and fate of pharmaceutical compounds in drinking or waste
23 water processes has become very popular in recent years. LC-MS/MS is a powerful
24 analytical tool often used to determine pharmaceutical residues at trace level in water.
25 However, many steps may disrupt the analytical procedure and bias the results. A list of 27
26 environmentally relevant molecules, including various therapeutic classes and
27 (cardiovascular, veterinary and human antibiotics, neuroleptics, non-steroidal anti-
28 inflammatory drugs, hormones and other miscellaneous pharmaceutical compounds) was
29 selected. In this work, a method was developed using Ultra Performance Liquid
30 Chromatography coupled to tandem Mass Spectrometry (UPLC-MS/MS) and solid phase
31 extraction (SPE) to determine the concentration of the 27 targeted pharmaceutical
32 compounds at the nanogram per liter level. The matrix effect was evaluated from water
33 sampled at different treatment stages. Conventional methods with external calibration and
34 internal standard correction were compared to the standard addition method. An accurate
35 determination of pharmaceutical compounds in drinking water was obtained by the

standard addition method associated with UPLC-MS/MS. The developed method was used to evaluate the occurrence and fate of pharmaceutical compounds in some drinking water treatment plants (DWTPs) in the west of France.

Key words: Pharmaceutical compounds; Multiresidue analysis; Ultra Pressure Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS); drinking water; standard addition method.

1. Introduction

Human and veterinary uses of pharmaceutical compounds lead to the releasing of bioactive compounds into the aquatic environment. Metabolization rates depend on the nature of the drugs and may range from 1 – 96 % [1]. Non-metabolized drugs are thus excreted in urine as free or conjugated forms [2,3], and collect in the waste water network. Pharmaceutical compounds are not completely removed during waste water treatment [4-7]. The efficiency of the process depends on the operating conditions and the nature of the molecule [6]. For example, conventional treatment with activated sludge effectively eliminates ibuprofen, and benzafibrate while diclofenac, carbamazepine and sulfamethoxazole are scarcely removed [8]. Some pilot scale studies have been carried out using a membrane bioreactor in an attempt to improve the outcome; these processes were found to be more efficient than activated sludge reactors at removing pharmaceutical compounds [8,9]. The disinfection of treated waste water can also improve the elimination rate of pharmaceutical compounds [10]. An indirect way of introducing these compounds into the environment is via agricultural activities. Sludge from waste water treatment plants may be spread on fields as

59 a fertilizer and the pharmaceutical compounds which can be immobilized in this sludge
60 may then contaminate the soil [11]. The veterinary use of drugs can lead to a direct
61 environmental contamination by the discharge of untreated effluent from intensive animal
62 farming. Direct soil contamination can occur by the excretion of urine and feces by farm
63 animals onto fields [12]. Rainfall and soil leaching may then transport pharmaceutical
64 compounds from the soil to the aquatic compartment [13]. Intensive livestock farming is
65 one of the main economic sectors of Brittany area (north-west France). Moreover, a large
66 proportion of the population uses a non-collective waste water treatment to clean household
67 effluent, so drinking water treatment specialists are beginning to be concerned about the
68 potential contamination of water resources by pharmaceutical compounds. Some recent
69 study shows that
70 Recently the French Agency for Food Health Safety (AFSSA) determined, the main
71 relevant molecules to examine in drinking water from the total amount consumed and their
72 properties in the aqueous phase [14]. Based on this work, a wide measurement campaign
73 was carried out by the French Agency for Environmental and Occupational Health Safety
74 (ANSES) [15]. From the 150 molecules included in the ANSES study, only 20 were found
75 at concentrations above the the limit of quantification (LOQ) and 11 between the limit of
76 detection (LOD) and the LOQ. Only these molecules were selected for the present work.
77 Accurate trace determination of emerging contaminants in the environment is an important
78 analytical challenge. The first obstacle is associated with the gap between environmental
79 concentrations and the quantification limits of analytical systems. Pharmaceutical
80 compounds concentrations range from LOQ of 2000 ng L^{-1} to LOQ of 200 ng L^{-1} in surface
81 water and drinking water, respectively [16-19] while the LOQ of conventional MS-MS

82 apparatus (without a pre-concentration step), are typically in the µg L^{-1} range.
83 Consequently, a concentration step is needed before analysis; solid phase extraction (SPE)
84 is the method of choice for the determination of emerging contaminants in water [20-22].
85 The second obstacle, resulting from the use of SPE, is the concomitant extraction of
86 interfering species and the target molecules. Polar organic pollutants are commonly
87 determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS).
88 However, interfering species may affect the analytical procedure at different stages: i) some
89 compounds may react with targeted molecules during the sampling and storage periods, ii)
90 organic or inorganic solutes may affect the yield of SPE extraction, iii) natural organic
91 matter may coeluate with targeted compounds which leads to a signal disrupting with
92 under/overestimation or false positive samples [23]. The study presented here deals with
93 the development of the method including an evaluation of the matrix effect. Accurate
94 determination of pharmaceutical compounds in drinking water was performed by the
95 standard addition method associated with ultra pressure liquid chromatography and tandem
96 mass spectrometry. The method was used to evaluate the occurrence and fate of
97 pharmaceutical compounds in some drinking water treatment plants (DWTP) in the west of
98 France.

99 ## 2. Materials and methods

100 ### *2.1. Stock solution and standard preparation.*

101 Stock solutions of individual pharmaceutical compounds were prepared by diluting reagent-
102 grade chemicals (Sigma Aldrich) in methanol (Fisher). Ultra pure water (UPW) was
103 provided by an ElgaPureLab System (18.2 MΩ.cm). Chromatographic solvents (MeCN;

104 MeCN with 0.1 % formic acid) were purchased from JT Baker (LC-MS grade) and were
105 used in association with UPW or UPW with 0.1 % formic acid. A standard mix solution (5
106 and 10 mg L^{-1} in MeOH) was prepared from individual stock solutions including all the
107 targeted molecules except amoxicillin, caffeine, oxazepam and internal standards. The
108 solution was then divided into a series of vial and stored at -20°C in the dark. The vial
109 containing the standard mix was placed at room temperature before use and the unused
110 amount was discarded. The standard mix was used to prepare both injections standard for
111 the external calibration and spiked solutions for the standard addition method. Amoxicillin,
112 caffeine, and oxazepam stock solution were prepared in UPW, MeOH and MeCN,
113 respectively. Calibration curves were plotted using eight-level standard solutions (1, 2, 5,
114 10, 25, 50, 100, 200 µg L^{-1} and up to 2000 µg L^{-1} for caffeine). The chromatographic
115 sequence consisted of the injection of standards and samples as follows: calibration curve -
116 first samples analysis - calibration curve - second samples analysis - calibration curve. In
117 addition a middle-range standard solution was injected every 10 injections in order to verify
118 the absence of significant signal deviation.

119 ***2.2. Sample preparation***

120 On arrival at the laboratory, water samples stored in 2-L amber glass bottles were filtered
121 through a 0.45 µm cellulose acetate membrane to remove suspended matter and colloids.
122 Samples were then stored in the dark before preparation and analysis. All the analyses were
123 carried out within a maximum storage period of 5 days. Solid phase extraction was
124 performed by filtering 200 mL of sample into a 6 mL Oasis HLB cartridge (6 cc, 150 mL,
125 Waters). HLB cartridges were conditioned with 5 mL of MeCN and rinsed with 5 mL of
126 UPW prior to the extraction step. Extraction was conducted by the filtering 200 mL of

127 sample (acidified at pH = 2 with sulfuric acid or not) under reduced pressure at a flow rate
128 of approximately 3 mL min^{-1}. The cartridge was cleaned with 5 mL UPW or UPW acidified
129 at pH = 2 (depending on the extraction method used) and then eluted with 4 mL MeCN.
130 The extract was evaporated under nitrogen flow to obtain a final volume of 100 µL. 100 µL
131 of internal standard (caffeine-$^{13}C_3$ and ibuprofene-d_3, 100 µg L^{-1} in MeCN/UPW 10/90)
132 was added prior to LC-MS/MS analysis.

133 ***2.3. Liquid Chromatography Tandem Mass Spectrometry***

134 All samples were analyzed using LC/MS/MS equipped with an electrospray ionization
135 source (ESI). The analytical equipment consisted of an ultra pressure liquid
136 chromatography system (Acquity, Waters) equipped with a reversed phase UPLC column
137 from Waters (Acquity C18 BEH, 100 mm x 2.1 mm ID, 1.7µm) and thermostated at 45°C.
138 The autosampler temperature was set at 4°C, and the injection volume was 5 µL in the full-
139 loop mode. The mass spectrometer (Quattro Premier; Micromass) general operating
140 conditions were : cone gas (N_2, 50 L h^{-1}, 120°C) -desolvation gas (N_2, 750 L h^{-1}, 350°C);
141 collision gas (Ar, 0.1 mL min^{-1}); capillary voltage (3000 V). The advanced mass parameters
142 (cone and collision cell voltage) are further described in Table 1.

143 **3. Results and discussion**

144 ***3.1. Optimization of mass spectrometry***

145 Infusion is the first step of method development by liquid chromatography tandem mass
146 spectrometry. It consists of a direct analysis of a pure diluted solution without separation in
147 order to record the mass spectrum of each selected compound and to determine the MRM
148 transitions. During this step the MS parameters such as cone voltage, and collision cell
149 energy were optimized for each compound in order to achieve the maximum sensitivity.

150 Table 1 shows the results obtained for the 29 molecules studied here; 3 internal and
151 recovery standards are also included. ESI is soft ionization technique which allows the
152 selection of a pseudo-molecular ion as the parent ion for MRM transitions; ESI was used in
153 both the negative and positive mode. The positive mode was selected for most of the
154 molecules while 9 analytes were ionized under the negative mode. The pseudo-molecular
155 ion ($[M+H]^+$ or $[M-H]^-$) was selected as the parent ion. When possible, simple fragment
156 loss, such as water or carbon dioxide, was selected for the quantification or confirmation
157 transition (parent ion → daughter ion for the quantification and second daughter ion for
158 confirmation). Only 1 transition was found for ibuprofen and ibubuprofen-d_3.

159 ***3.2. Chromatographic conditions and calibration***

160 UPLC with a BEH C18 column was performed with a gradient of ultra-pure water /
161 acetonitrile at 400 µL min^{-1}. The effect of formic acid addition on the chromatographic
162 separation was also evaluated. The starting eluent composition consisted of 19 %
163 acetonitrile for 1 minute, which was then linearly increased to reach 95.5 % at 7.5 minutes.
164 A final eluent containing 95.5 % acetonitrile for 2 minutes was used to clean the column
165 and prevent any parasite peaks. In order to obtain an acceptable detection of all the
166 molecules, 2 chromatographic conditions, with and without formic acid addition, to
167 promote ionization, were needed (Figure 1). Separation was achieved in 6 minutes with a
168 complete chromatographic run of 12 minutes. Caffeine-$^{13}C_3$ (CAF-$^{13}C_3$) and ibuprofen-d_3
169 (IBU-d_3) were used as the internal standard for quantification under the positive and
170 negative ionization modes, respectively. Moreover, a recovery standard (ketoprofen-d_3) was
171 added prior to the solid phase extraction; no correction relative to ketoprofen-d3 was made

172 and its use was only indicative. External calibration curves were used for the determination

173 of relative response factors (RRF) for each analyte according to the following equations:

$$\mathrm{RRF\ (positive)} = \frac{\mathrm{Analyte\ slope}}{\mathrm{CAF} - {}^{13}\mathrm{C}_3\ \mathrm{slope}} \qquad \mathrm{(negative)} = \frac{\mathrm{Analyte\ slope}}{\mathrm{IBU} - \mathrm{d}_3\ \mathrm{slope}} \qquad \mathrm{(Eq.\,1)}$$

174 ***3.3. Linearity and quantification limits***

175 Recovery rates (RR), linearity and quantification limits were determined at environmentally

176 relevant concentrations; the results are summarized in Table 1. Because those obtained with

177 UPW and surface water samples cannot be easily compared, the evaluation of the basic

178 parameters of the validation method was carried out without organic interfering species

179 (UPW or Evian water). Linearity was validated between 5 – 200 µg L^{-1} in the vial (injected

180 volume = 5 µL) which corresponds to 5 – 200 ng L^{-1} in the starting sample if the RR is

181 considered equal to 100 %. External calibration curves (8 levels + 1 blank) were also used

182 to determine the standard deviation on the instrumental method; the SD presented here does

183 not include the deviation on the SPE step. The results show that acceptable relative

184 standard deviations lower than 10 % were obtained for most of the pharmaceutical

185 compounds. However poor-quality results were obtained for hormones with a relative

186 standard deviation ranging from 40 to 60 %.

187 The evaluation of instrumental detection (S/N = 3) and quantification limits (S/N = 10)

188 (IDL and IQL) was performed by the injection of 10 blank samples (Evian water). From the

189 29 targeted compounds, IQL lower than 4 µg L^{-1} were obtained for 27 of them,

190 demonstrating that determination in the nanogram per liter range requires a concentration

191 factor of up to 1000. Higher IQL values were obtained for ethinylestradiol and salicylic

192 acid (8 and 24 µg L^{-1}, respectively). It should be underlined that the evaluation of the limit

193 of quantification (LOQ) by this method (apparatus LOQ without the SPE step and in the
194 absence of interfering compounds) is not directly transposable for the determination of
195 pharmaceutical residues in surface water. Nevertheless, this quick approach demonstrates
196 that our method enables pharmaceuticals in surface and drinking water to be determined at
197 an environmentally relevant concentration.

198 ***3.4. SPE extraction***

199 Solid phase extractions were performed with Oasis HLB cartridges by filtering 200 mL of
200 0.45 µm pre-filtered sample in order to obtain a concentration factor of 1000. Because the
201 selected molecules can be assumed to be weakly basic or weakly acid compounds, the
202 effect of sample the acidification on the extraction yield was evaluated in UPW. Standard
203 solutions each containing 100 ng L^{-1} of analyte were filtered onto an HLB cartridge as
204 previously described. Recovery rates were determined using the internal standards caffeine-
205 $^{13}C_3$ and ibuprofen-d_3 for the analysis under ESI+ and ESI-, respectively (Figure 2).

206 The results of the extraction experiments are summarized in Table 1. Acetaminophen,
207 caffeine, carbamazepine, and oxazepam were almost quantitatively (80–120%) recovered in
208 conditions all investigated. These analytes are assumed to be neutral drugs, which explains
209 their high recovery yields under acidic and neutral extractions. In spite of a pKa value of
210 4.16, a similar result was obtained for losartan. Amphoteric drugs such as danofloxacin and
211 ofloxacin exhibited higher recovery yields under acidic extraction than under neutral
212 conditions. Thus, for these compounds, the SPE is controlled by the carboxylic function
213 and the amino group does not affect the extraction yield. The opposite effect was observed
214 for amoxicillin where no acceptable recovery yields were obtained under acidic or neutral
215 conditions. In this case, the controlling group should be the amino acid function and

216 extraction under basic conditions could increase the recovery yield. Extraction under acidic
217 conditions was selected for most of the carboxylic acids, for example ibuprofen, ketoprofen
218 and salicylic acid. In contrast to acidic drugs, basic drugs containing an amino group (i.e.
219 atenolol, naftidrofuryl and lincomycin) had comparatively higher recoveries under neutral
220 conditions due to the formation of ammonium derivatives at low pH values. Except for
221 amoxicillin, the combination of both acidic and neutral extractions provided acceptable
222 recovery rates for all the analytes. However the recovery rates determined in UPW
223 experiments could be dramatically affected by the presence of interfering species (i.e.
224 natural organic matter).

225 ***3.5. Evaluation of the matrix effect***

226 The presence of organic or inorganic substances could lead to an analytical bias. Natural
227 Organic Matter (NOM) is a complex mixture of polyfunctional macromolecules [24] which
228 may disturb the SPE step, or MS ionization. From the various effects attributable to the
229 presence of NOM some phenomena can be described such as competitive adsorption on the
230 HLB phase [25], the formation of NOM-analyte complexes [26] and the modification of the
231 analyte ionization efficiency in the MS source [27]. Although the presence of NOM is
232 frequently associated with an underestimation of the targeted analytes (decreasing the
233 extraction yield and/or the ionization efficiency), the opposite effect may also occur,
234 despite not being well documented.

235 In order to evaluate the effect of NOM, the recovery rates obtained in pure water were
236 compared with those obtained in surface water. Four surface waters (used to supply
237 drinking water treatment plants) were spiked with stock solutions of pharmaceutical
238 compounds to obtain a concentration of 100 ng L^{-1} of each targeted analyte. Because

239 surface water may initially contain some pharmaceutical residues, unspiked samples were
240 also analyzed to determine the signal contribution due to the presence of analyte in surface
241 water; signal was then corrected to be specific to the added amount of analyte. Figure 3
242 shows the comparison between the recovery rates obtained in pure water and those obtained
243 in raw water (surface water) from the drinking water treatment plant A and B (DWTPA-
244 RW ; DWTPB-RW). These results demonstrate that the determination of pharmaceutical
245 compounds at trace level is very influenced by the water quality. For some compounds,
246 such as tylosin, atenolol, losartan, ibuprofen and amoxicillin, no significant matrix effect
247 was observed. The recovery rate determined for amoxicillin in surface water was quite
248 similar to that observed in pure water. However, due to its very low value, a possible matrix
249 effect may be masked. The absence of a detectable matrix effect on ibuprofen can be
250 explained by the fact that this compound was quantified relative to ibuprofen-d_3. Figure 3
251 shows a significant underestimation of diclofenac and β-estradiol in surface water. In
252 contrast, many compounds such as carbamazepine and epoxy-carbamazepine were
253 overestimated. The recovery rate observed for oxazepam in pure water (105 %) was not
254 significantly different from that observed in DWTPA-RW (104%) but a significant
255 overestimation was observed in DWTPB-RW (145 %). In the case of ethinylestradiol,
256 recovery rates in pure water and DWTPA-RW (108 and 89 %, respectively) were quite
257 similar whereas a significant underestimation was measured in DWTPB-RW (59 %).
258 Clearly, the recovery rates determined with pure water are not transposable to surface
259 water. The recovery rates obtained with surface water differ depending on the nature of the
260 NOM. Therefore, a classical approach with external calibration and internal/external

261 standard correction is not sufficiently accurate for the multi-residue analyses of
262 pharmaceutical compounds at trace level in water.

263 ### *3.6. Standard addition method*

264 The standard addition method (SAM) is very efficient for correcting the matrix effect and
265 providing an overall evaluation of this effect on both the SPE step and MS ionization.
266 Moreover, it can be used even if the molecules were not initially present in water. All
267 samples were spiked with stock solutions containing the 29 targeted pharmaceuticals (not
268 spiked; 50 and 100 ng L^{-1}). The conventional quantification method (external calibration
269 with internal standard correction) was compared with SAM results according to the
270 following equations:

$$\text{conventional method: [Analyte]} = \frac{\text{Analyte Area}}{\text{IS Area}} \times \frac{[\text{IS}]}{\text{RRF}} \text{ with } [\text{IS}] = 100\ \mu\text{g L}^{-1} \quad (\text{Eq. 2})$$

$$\text{SAM method: [Analyte]} = \frac{\text{Measured signal in unspiked sample}}{\text{slope of the standard addition calibration curve}} \quad (\text{Eq. 3})$$

271 Figure 4.a shows an example of matrix effect evaluation for some molecules not detected in
272 the raw water of DWTP A. In the absence of a matrix effect, a theoretical slope equal to 1
273 should be obtained; in the present case (DWTP A-RW), some compounds such as estrone
274 and sulfadimerazine were weakly affected by water quality and interfering species.
275 Conversely, the low recovery rate obtained for diclofenac could be attributed to a decrease
276 in the extraction yield and/or signal suppression caused by a modification of ionization in
277 the ESI source. The inverse effect was observed for naftidrofuryl, for which conventional
278 quantification leads to an overestimation. Because no signal attributable to naftidrofuryl
279 was observed in the non-spiked sample, the overestimation could not be due to the co-

280 elution of a false-positive compound, but it could be caused by an ion enhancement effect.
281 This type of matrix effect has previously been reported in the literature [23] with similar
282 compounds (basic drugs) in surface water. Moreover, Dams et al. [28] underlined that ESI
283 was especially susceptible compared to APCI. The same approach was adopted with
284 compounds initially observed in the non-spiked sample (Figure 4.b). In the case of caffeine,
285 similar results were obtained with the conventional method (19 ± 3 ng L^{-1}) and SAM (16 ±
286 3 ng L^{-1}). However, the quantification of ofloxacin by the conventional method (8 ± 2 ng L^{-}
287 ^{1}) led to a significant underestimation (SAM: 22 ± 3 ng L^{-1}) of its concentration in drinking
288 water.
289 The standard addition method was used to determine the concentration of pharmaceuticals
290 at different treatment stages from raw water to drinking water in four drinking water
291 treatment plants (DWTPs). The matrix effect was evaluated on a total of 16 samples. The
292 slopes of the curves, obtained with the 29 targeted compounds in the different samples
293 (example given in Figure 4), are summarized as a box plot (Figure 5.). These results
294 underline that the chromatographic method proposed here fails to determine the 29 targeted
295 compounds accurately. Recovery rates obtained for amoxicillin were lower than 3 %, which
296 could be explained by the extraction step (SPE yield lower than 7 % in pure water).
297 Moreover, in some cases amoxicillin was not detected in the spiked samples (50 and 100 ng
298 L^{-1}), so a competitive effect on the adsorption step and/or signal suppression could be
299 suggested in addition to poor SPE efficiency. Not only was salicylic acid dramatically
300 affected by the matrix effect, but antagonistic effects (signal suppression and enhancement)
301 were also observed with similar water qualities: large signal suppression was observed in
302 the raw water of DWTP A while signal enhancement occurred after the sand filtration step

303 of the same DWTP. A review of the chromatographic data also reveals an abnormally large
304 area associated with salicylic acid. In some cases, the calculated concentrations with both
305 the conventional and standard addition methods reach the milligram per liter range, so a
306 cross-talk effect could be suggested. As smaller deviations between the conventional
307 method and SAM were observed for compounds which were quantified relative to their
308 analogous IS (ibuprofen, caffeine), the results obtained here demonstrate that the correction
309 of the matrix effect with internal standards cannot easily be transposed to other compounds.
310 In spite of the efficiency of the SAM to correct the matrix effect, amoxicillin and salicylic
311 acid were removed from the quantifiable list of compounds; thus only the 27 of the 29
312 pharmaceutical compounds initially targeted were accurately quantified by the method
313 proposed here.

314 ***3.7. Application to drinking water analysis***

315 Concentrations of pharmaceutical compounds in the samples from DWTP were calculated
316 from Equation 3. The results obtained during the sampling campaign show that only 13
317 molecules were observed at concentrations above the LOD at least once. Figure 6
318 summarizes the occurrence and fate of the detected compounds in the four sampled
319 DWTPs. Concentrations observed ranged from the LOQ to 95 ng L^{-1} (hydroxy-ibuprofen in
320 DWTP D). From the 13 detected molecules, only 3 pharmaceutical compounds were
321 quantified in all samples (caffeine, ofloxacin, hydroxy-ibuprofen). 9 molecules were
322 detected with concentrations lower than the LOQ and 3 of these were only observed in raw
323 water (losartan, epoxy-carbamazepine and ketoprofen). Erythromycin, tylosin,
324 progesterone, hydrochlorothiazide and ibuprofen were detected (<LOQ) at different stage
325 of the water treatment. Finally 6 targeted compounds were never detected during the

326 sampling campaign (lincomycin, diclofenac, estrone, pravastatin, atenolol and
327 doxycycline). It should be underlined that the SAM approach identified significant signal
328 inhibition of danofloxacin and ofloxacin in the raw water of DWTP B and in the
329 chlorinated water of DWTP A. Since the spiking of danofloxacin and ofloxacin does not
330 lead to a significant increase in peak area associated with these compounds, their
331 quantification was not possible. Nevertheless, ofloxacin and danofloxacin were accurately
332 determined after sand filtration at a concentration ranging from 5 – 10 ng L^{-1}, so it could be
333 suggested that they were initially present in the raw water. This particular case reinforces
334 the efficiency of the SAM approach for identifying matrix effects and facilitating the
335 interpretation of results. Only the quantified compounds were considered when examining
336 the effect of the water treatment process on the removal of pharmaceuticals (Figure 6).
337 From the results obtained, 3 classes of pharmaceuticals can be defined. Several compounds,
338 such as caffeine, trimethazine and oxazepam were partially removed during the treatment
339 process. The clarification step (coagulation-flocculation-sand filtration) seemed to be most
340 efficient for eliminating pharmaceutical compounds. In fact, acetaminophen,
341 carbamazepine, amlodipine, sulfamethazine, β-estradiol and ethinylestradiol were
342 completely removed after this step. These data are consistent with the work of Vieno et al.
343 who demonstrated that coagulation of surface water with ferric sulfate could efficiently
344 remove some pharmaceutical residues [29]. A second class of compounds can be defined as
345 refractory pollutants; ofloxacin, danofloxacin and naftidrofuryl were not significantly
346 eliminated during drinking water production. The third group of molecules consists of
347 metabolites formed during water treatment; only hydroxy-ibuprofen in the present study. A
348 large increase in hydroxy-ibuprofen concentration was observed in all the DWTPs

349 considered although ibuprofen was never observed at a concentration level above the LOQ.
350 The gulcuronide conjugate of ibuprofen is the main metabolite from ibuprofen metabolism
351 [3]. Cleavage of this conjugate could occur during water treatment releasing the free form
352 of ibuprofen, which could then be oxidized to produce hydroxy-ibuprofen. A similar
353 mechanism has previously been proposed by Ternes et al. to explain the formation of
354 estrone from the glucuronide conjugate of β-estradiol in a waste water treatment plant
355 [[30]].

356 **4. Conclusion**

357 In this study, a multiresidue analysis of pharmaceuticals at trace level in surface and
358 drinking water involving a solid phase extraction followed by UPLC-MS/MS determination
359 was developed. Matrix effects were examined for 29 pharmaceuticals in 16 samples. Matrix
360 effects were severe, even with internal standard correction, so the standard addition method
361 was necessary for an accurate determination. The analytical method developed here was
362 then used to evaluate the occurrence and fate of drug residues in drinking water treatment
363 plants. Further studies will be conducted to confirm the effect of the water treatment
364 process on the elimination of pharmaceutical residues.

365

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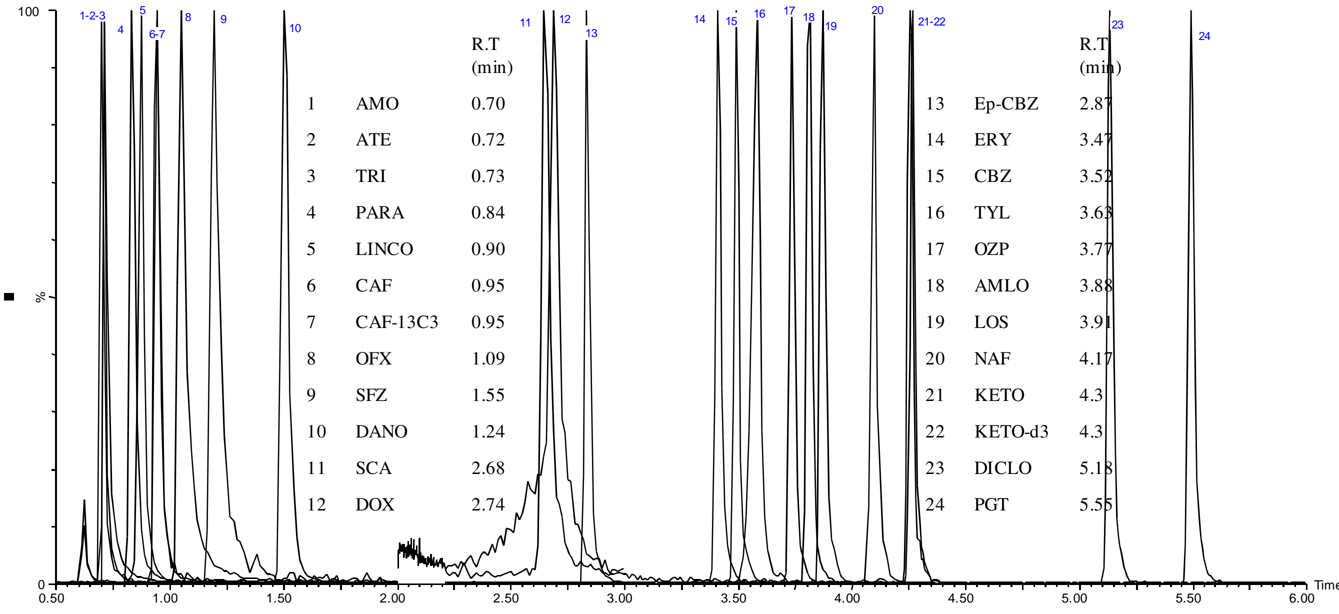
Table 1. Summary of the method development

Therapeutic class		Molecule	MW (g/mol)	ESI (+ / -)	MRM transitions (m/z) Parent ion. (CV[a])	Quant. ion (CE[b])	Conf. ion (CE)	r^2	SD	LOD (µg L^{-1})	LOQ (µg L^{-1})	SPE	UPLC
Cardiovascular		Amlodipine (AML)*	567.05*	+	409.6 (18)	238.1 (11)	292.2 (13)	0.994	0.109	0,3	1,0	[c]A	A
		Atenolol (ATE)	266.34	+	267.0 (34)	145.0 (26)	74.0 (23)	0.986	0.118	0,2	1,0	[d]N	A
		Losartan (LOS)*	461.00*	+	423.6 (30)	405.2 (12)	207.0 (22)	0.987	0.100	0,4	1,0	[e]N+A	A
		Naftidrofuryl (NAF)*	473.56	+	384.6 (40)	99.7 (21)	84.7 (25)	0.992	0.106	1,4	2,0	N	A
		Pravastatin (PRA)*	446.51	-	423.2 (34)	100.6 (23)	321.1 (16)	0.988	0.119	0,1	1,0	N	N
		Trimetazidine (TRI)*	339.26	+	267.4 (21)	180.9 (16)	165.8 (26)	0.995	0.082	0,2	1,0	N	A
Antibiotics	Human	Amoxicillin (AMO)	365.40	+	366.5 (16)	113.7 (24)	349.0 (10)	0.980	0,176	0,4	1,0	A	A
		Doxycycline (DOX)*	512.94	+	445.5 (30)	428.2 (18)	153.8 (28)	0.986	0,103	0,7	2,0	A	A
		Erythromycin (ERY) *	769.96*	+	734.2 (28)	158.0 (30)	576.2 (19)	0.987	0,123	0,0	1,0	N	A
		Ofloxacin (OFX)	361.37	+	362.0 (34)	318.0 (19)	261.0 (28)	0.985	0,155	0,9	2,0	A	A
	Veterinary	Danofloxacin (DANO)	357.38	+	358.5 (35)	314.0 (19)	283.0 (25)	0.976	0,210	1,8	4,0	A	A
		Lincomycin (LINCO)*	461.01	+	407.6 (40)	125.9 (28)	359.3 (18)	0.984	0,125	0,0	1,0	N	A
		Sulfadimerazine (SFZ)	278.33	+	279.4 (29)	185.9 (16)	91.7 (26)	0.979	0,133	0,3	1,0	N	A
		Tylosin (TYL) *	1066.19*	+	917.0 (60)	174.0 (37)	773.0 (29)	0.994	0,088	0,3	1,0	N	A
Neuro.		Carbamazepine (CBZ)	236.27	+	237.1 (28)	194.0 (19)	179.0 (39)	0.976	0.122	0,1	1,0	N+A	A
		Epoxycarbamazepine (Ep-CBZ)	252.27	+	253.3 (28)	179.9 (28)	236.0 (12)	0.988	0.111	0,3	1,0	A	A
		Oxazepam (OZP)	286.71	+	287.4 (34)	241.0 (20)	269.1 (14)	0.985	0.109	0,5	2,0	N+A	A
NSAID		Diclofenac (DICLO)	294,14	+	296,1 (22)	250,0 (10)	214,1 (25)	0.987	0.120	0,2	1,0	N	A
		Ibuprofen (IBU)	206,28	-	205,0 (17)	161,0 (7)	/	0.965	0.188	0,2	1,0	A	N
		Hydroxyibuprofen (OH-IBU)	222,28	-	221,2 (19)	177,0 (9)	158,7 (13)	0.994	0.103	0,8	2,0	A	N
		Ketoprofen (KETO)	254,28	+	255,0 (29)	209,0 (12)	105,0 (22)	0.989	0.085	0,3	1,0	A	A
		Salicylic acid (SCA)	138,12	-	137,0 (30)	92,6 (14)	64,7 (28)	0.984	0.100	9,1	24,0	A	A
Misc.		Acetaminophen (PARA)	151,16	+	152,0 (25)	110,0 (15)	90,0 (10)	0.986	0.094	1,3	4,0	N+A	A
		Caffeine (CAF)	194,19	+	195,1 (37)	137,7 (18)	109,7 (22)	0.987	0.129	1,8	3,0	N+A	A
		Hydrochlorothiazide (HCTZ)	297,74	-	296,2 (42)	77,6 (28)	204,8 (22)	0.981	0.170	0,1	1,0	A	N
Hormones		Ethinylestradiol (EE)	296,40	-	295,2 (54)	144,9 (40)	183,0 (35)	0.873	0.406	2,8	8,0	A	N
		17β-Estradiol (βE)	272,38	-	271,1 (50)	145,0 (38)	183,0 (41)	0.741	0.611	0,4	1,0	A	N
		Estrone (EO)	270,37	-	269,1 (53)	145,0 (35)	183,0 (36)	0.875	0.393	0,5	1,0	A	N
		Progesterone (PGT)	314,46	+	315,2 (32)	97,0 (24)	109,0 (26)	0.992	0.097	0,2	1,0	N	A
IS		Ketoprofen-d3 (KETO-d3)	257,30	+	258,4 (25)	212,0 (15)	179,8 (23)					N+A	A
		Caffeine-$^{13}C_3$ (CAF-13C3)	195,19	+	198,2 (35)	139,7 (20)	111,7 (22)					/	A
		Ibuprofene-d_3 (IBU-d3)	209,30	-	208,2 (18)	163,9 (7)	/					/	N

[a]Cone Voltage in volt; [b]Collision energy in volt; [c]SPE extraction at pH = 2, [d]SPE extraction at pH = 7; [e]Mean value of the 2 methods. *molecule whose molecular weight of the commercial product purchased does not correspond to the molecular weight of the active compound (i.e. amlodipine besylate – MW = 567.05 versus amlodipine – MW = 408.87)

a)



b)

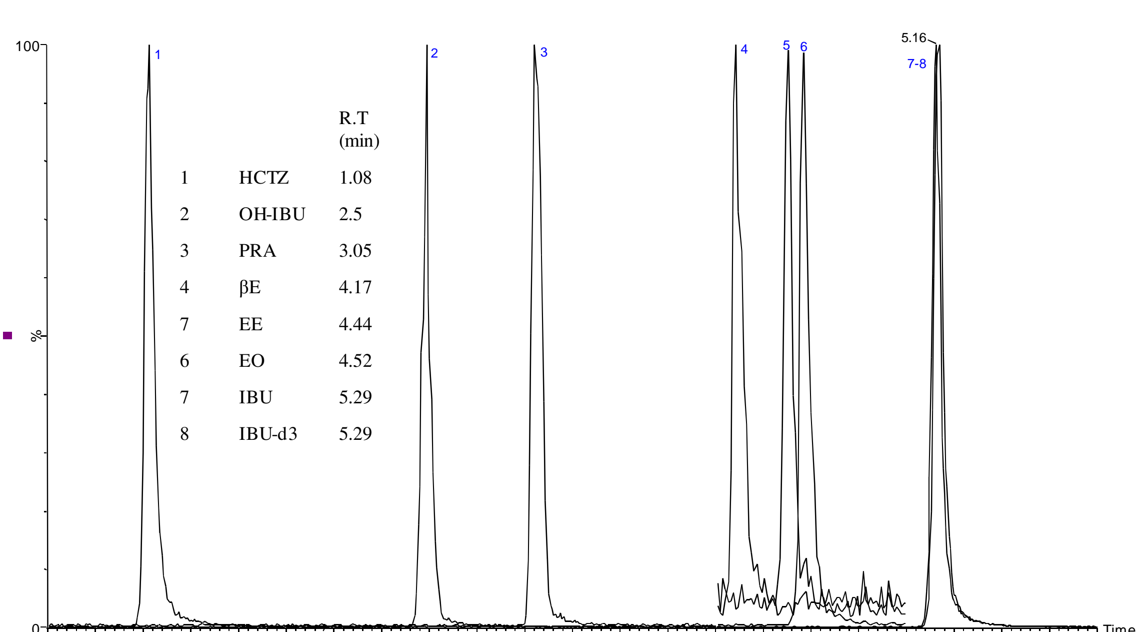


Figure 1. Example of chromatogram obtained with a standard mix solution at 100 µg L^{-1}. Chromatograms obtained with (a) addition of 0.1% formic acid and (b) without acidification.

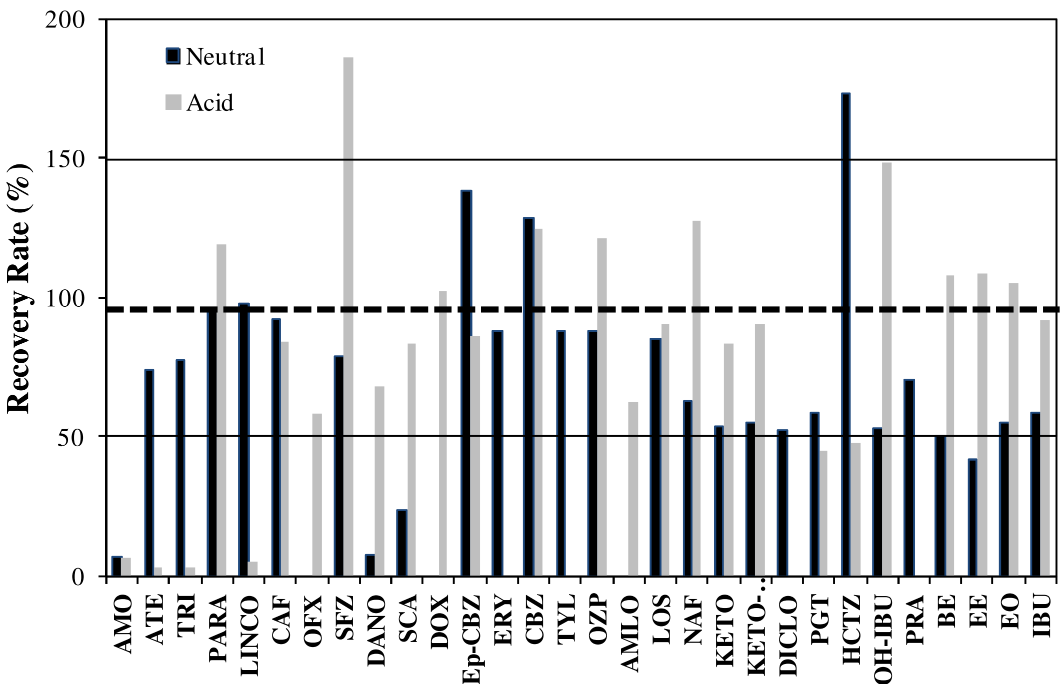


Figure 2. Effect of pH during SPE extraction on the recovery rate in pure water.

[Analyte] = 100 ng L^{-1} ; concentration factor = 1000.

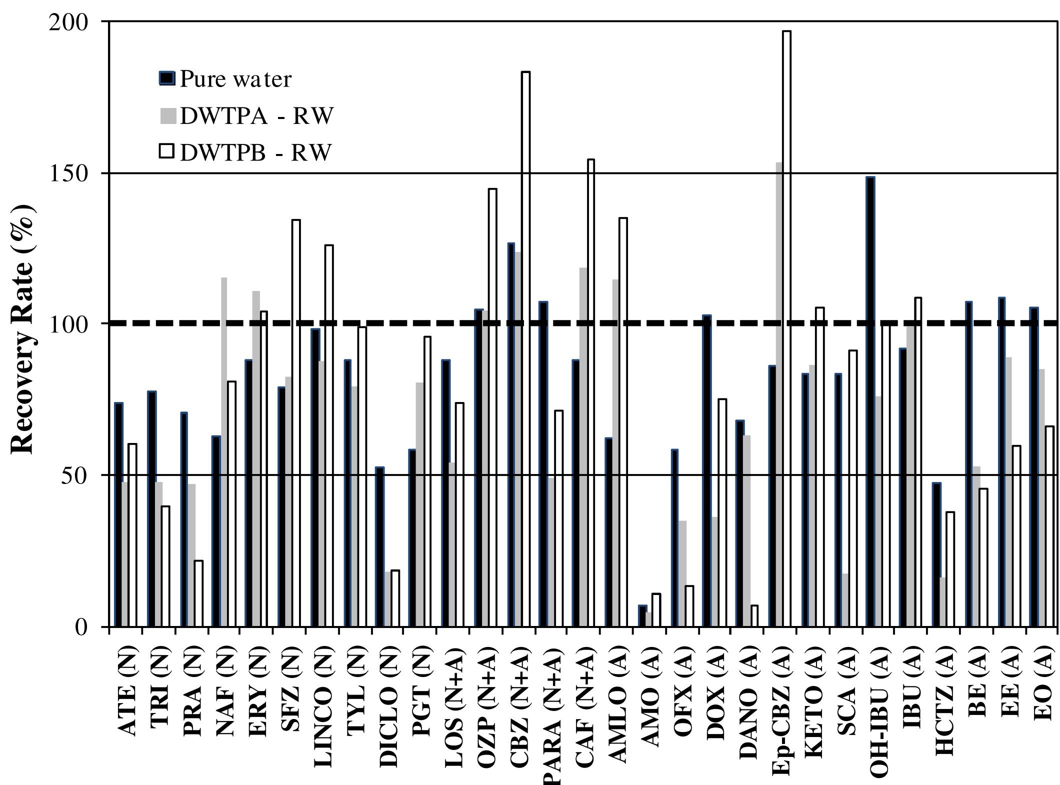


Figure 3. Effect of water quality on the recovery rate. [Analyte] = 100 ng L^{-1} ; concentration factor = 1000 ; letters in parentheses refer to the SPE mode i.e. Acid and/or Neutral conditions.

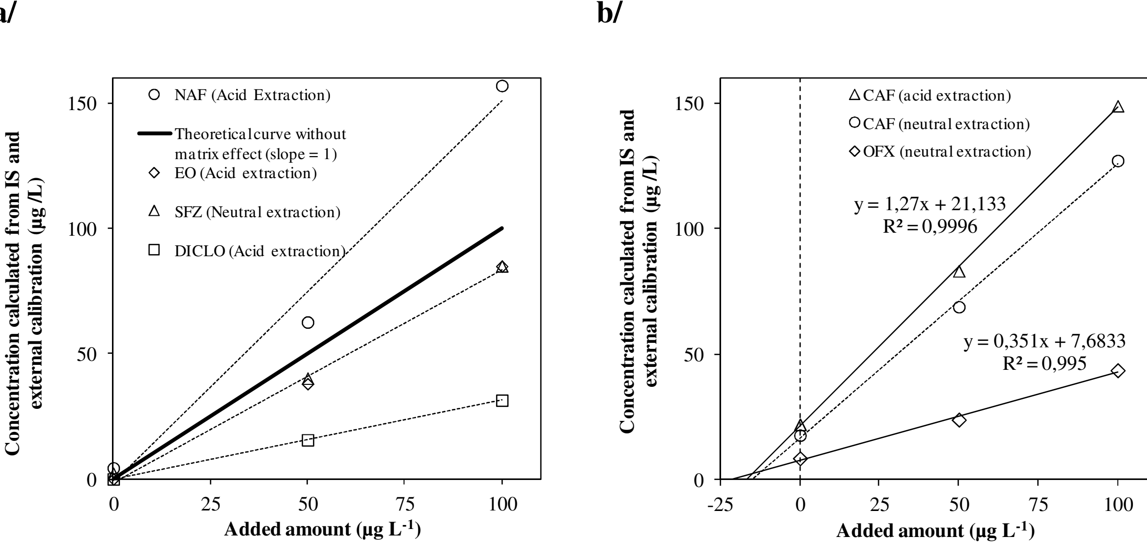


Figure 4. Comparison of concentrations determined by external calibration with internal standard correction and standard addition method. Example of compounds (a) not detected and (b) detected in DWTP A-RW.

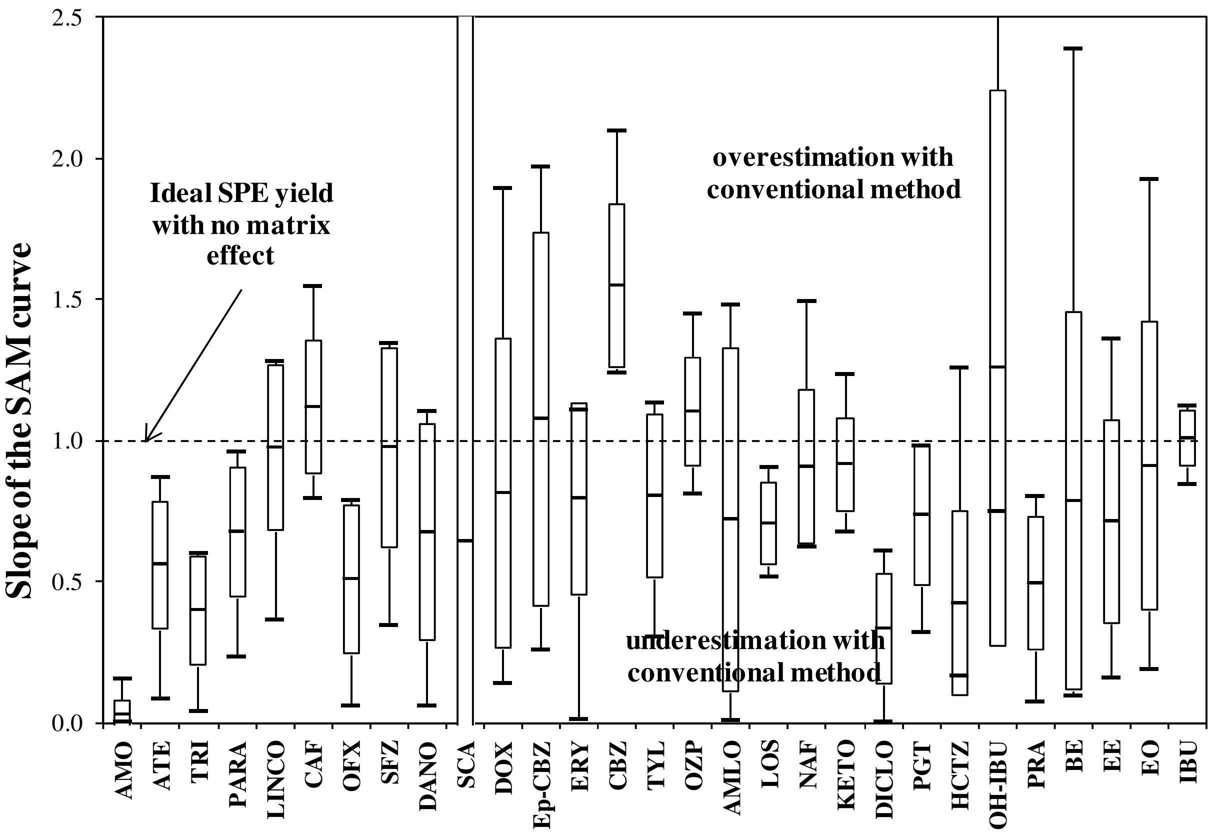


Figure 5. Overall evaluation of the matrix effect in multiresidue analysis of pharmaceutical compounds in surface and drinking water.

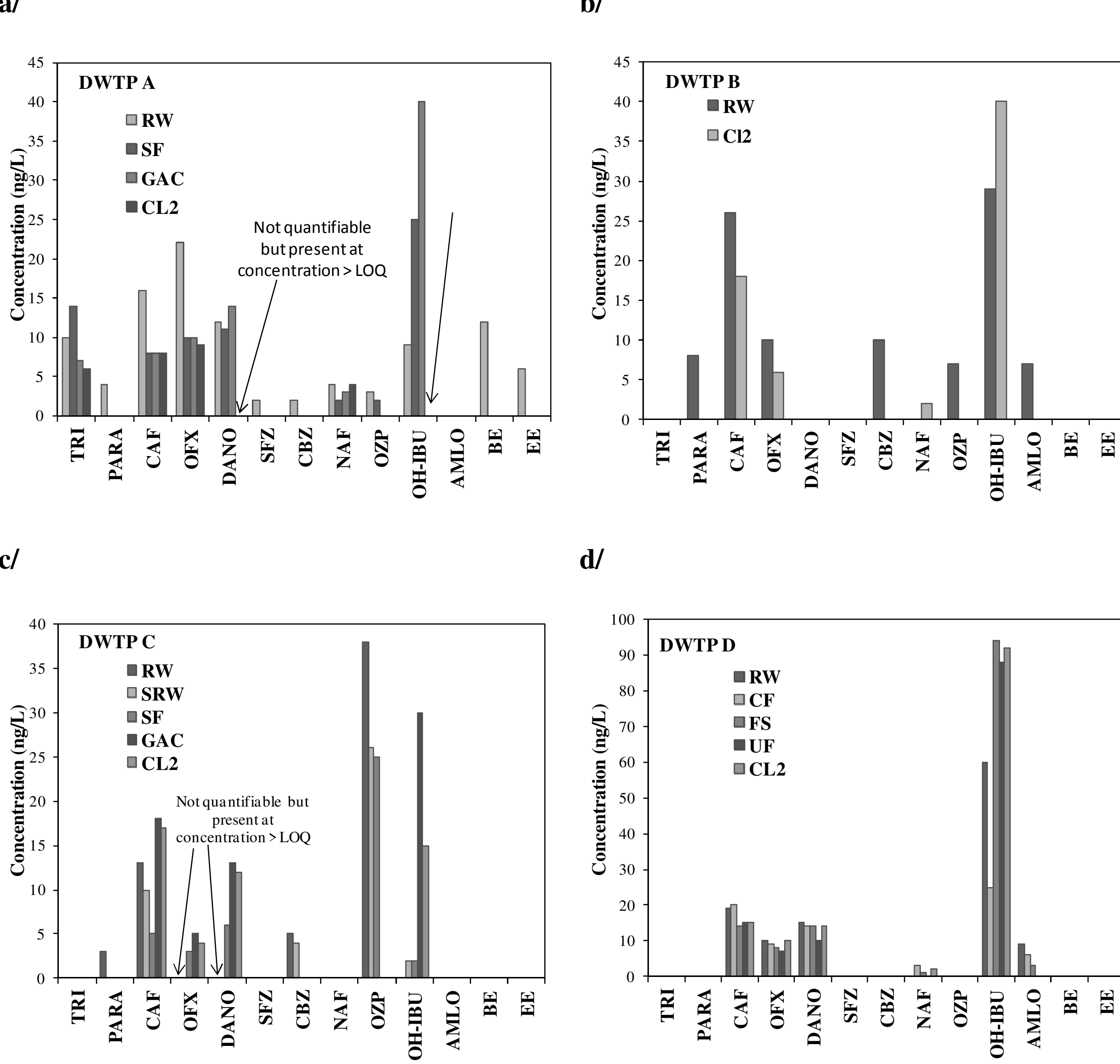


Figure 6. Fate and occurrence of pharmaceutical compounds at different stages of the drinking water process. Raw Water (RW), Stored Raw Water (SRW), Coagulation-Flocculation (CF), Sand Filtration (SF), Granular Activated Carbon (GAC) and Chlorination (CL2). DWTP A and B include an ozonation step before GAC filtration; DWTP B and D include a powder activated carbon reactor in the clarification step and a membrane ultra-filtration (UF) as a polishing treatment before chlorination.