

# Determination of Pharmaceuticals in Coastal Systems using Solid Phase Extraction (SPE) followed by Ultra Performance Liquid Chromatography – tandem Mass Spectrometry (UPLC-MS/MS)

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**Abstract:** This paper describes the optimization and validation of an analytical method for the determination of 83 pharmaceutically active compounds (PhACs) in aqueous samples using solid-phase extraction (SPE) followed by ultra performance liquid chromatography-triple quadrupole mass spectrometry (UPLC-QqQ-MS/MS). First, several experiments were conducted to optimize different SPE extraction parameters such as pH, elution solvents, and Na<sub>2</sub>EDTA addition. Extraction recovery percentages were between 17 and 146%, being higher than 70% for 47 target analytes. The method limits of detection (LOD) and quantification (LOQ) were below 1 ng L<sup>-1</sup> for most compounds (> 90%), and the precision of the method, calculated as the relative standard deviation (RSD) of replicate extractions and analyses, was less than 20%. The optimized method was successfully applied to the analysis of real water samples in estuarine and coastal systems from SW Spain (Cadiz Bay and Huelva Estuary). 49 out of 83 target compounds were found in 75% of samples. Ibuprofen, atenolol, gemfibrozil and caffeine were the most commonly substances detected, reaching concentrations up to 195 ng L<sup>-1</sup>. These are among the first data available on the occurrence of a wide range of pharmaceuticals in European coastal waters.

**Keywords:** pharmaceuticals; antibiotics; seawater; mass spectrometry; solid phase extraction; estuary

## 1. INTRODUCTION

Recent studies have demonstrated that a combination of the widespread use of pharmaceuticals (PhACs) and their relative inefficient removal in wastewater treatment plants (WWTPs) leads to the detection of low concentrations of these chemicals (sub-ppb levels) in most sewage-impacted aquatic systems [1-4]. The presence of pharmaceutically active compounds (PhACs) in the receiving waters is concerning as it can represent a threat not only for humans through drinking water intake [5-6] or development of antibiotic-resistant bacteria [7], but also for aquatic organisms [8]. At the same time, chronic and acute toxicity caused by PhACs and other organic micro-contaminants is an open question. Most recent studies have reported behavioral and physiologic alterations in aquatic organisms exposed to sub-lethal concentrations of PhACs during short periods of time [9-11]. The occurrence of long-term effects, however, is still widely unknown, as are the synergism and/or antagonism in toxicity of mixtures and the role of secondary products that could be even more harmful than parent PhACs [12].

The presence of a wide range of PhACs in surface waters at very low concentrations has led to the development of several multiresidue methods for their analysis over the last decade, most of them relying on a preconcentration stage and later determination of target compounds by liquid chromatography-mass spectrometry (LC-MS) [13-16]. The preconcentration step is mandatory not only for achieving lower detection limits but also in order to minimize matrix effects during LC-MS analysis [18-19]. The most commonly used extraction technique for isolation of PhACs is solid phase extraction (SPE) [20-22]. This technique offers the possibility of automation (e.g., online SPE) to minimize sample manipulation and induced errors [5, 23]. Several studies have tested different cartridge types –octadecylsilica (C18), Isolute ENV+, Oasis MCX, Lichrolut EN, and Oasis HLB, among others- for isolating PhACs, all of them showing positive results for some groups such as most anti-inflammatories, lipid regulators or sulfonamides [24-26, 15]. Extraction recoveries reported for the non-polar sorbent C18 have been acceptable for most compounds, but these values are generally 20% below those obtained with Oasis HLB. Isolute ENV+, on the other hand, is only adequate for a narrow range of substances, mostly polar acidic organic compounds. It is also able to retain some neutral analytes such as macrolide antibiotics, achieving similar recoveries (80%) than those for Oasis HLB cartridges [25, 26]. Lichrolut EN has been

55 successfully applied for extraction of aqueous samples at neutral pH but recoveries are poor (< 50%) for  
56 some antibiotics [24, 26]. Oasis MCX cartridges are not effective [25] unless samples are acidified first,  
57 showing an enhancement in recovery percentages between 20 and 40% for basic drugs such as  
58 glibenclamide, trimethoprim and metronidazole [15]. Overall, some sorbents are better for specific  
59 compound families such as penicillins, where an improvement of up 36% for amoxicillin can be observed  
60 using Oasis MCX cartridges instead of other sorbents [24]. Psychiatric drugs like fluoxetine show  
61 recoveries up to 100% when employing octadecylsilica cartridges [25]. Better recoveries, however, are  
62 often achieved for the majority of tested analytes and conditions (water samples at acid and basic pH  
63 values) using Oasis HLB cartridges, the most popular option when developing multiresidue methods [15].

64 Regarding LC-MS analysis of PhACs, the most successful technique over the last decade has been the  
65 triple quadrupole (QqQ) detector coupled either with high- or ultra-performance liquid chromatography  
66 (HPLC/UPLC). This instrument can determine environmentally relevant concentrations (sub-ppb) of  
67 organic trace substances such as PhACs via target analysis in multiple reaction monitoring mode (MRM)  
68 [25, 27, 15]. However, with increasing sensitivity, high resolution mass spectrometry (HR-MS)  
69 instruments (Orbitrap and time-of-flight mass analyzers, or ToF) are used increasingly to analyze a wide  
70 range of target and non-target micro-pollutants at trace levels [28]. Several methods using ToF-MS have  
71 been recently developed by our group for the analysis of surfactants such as secondary alkane sulfonates  
72 (SAS) and nonionic compounds at detection limits below 30 ng L<sup>-1</sup> in coastal waters [29, 30]. In addition,  
73 Hollender and co-workers [31] have analyzed 220 micropollutants in water using HR-MS, including a  
74 variety of pharmaceuticals (sulfonamides, antiinflammatories, and lipid regulators) and pesticides, with  
75 detection limits as low as 0.1 ng L<sup>-1</sup> for specific chemicals such as trimethoprim, being lower than 120 ng  
76 L<sup>-1</sup> for most PhACs.

77 In spite of a significant increase in the number of reports on the environmental distribution and  
78 concentrations of PhACs in aquatic systems [32], most of the information is heavily biased towards  
79 freshwater systems and WWTP removal efficiency [33, 34]. Coastal environments have usually received  
80 less attention in the research not only on PhACs but also on other polar organic micropollutants [35-39].  
81 So far, concentrations of up to 50 ng L<sup>-1</sup> have been reported for lipid regulators (clofibric acid) and anti-  
82 inflammatories (diclofenac and ibuprofen) in coastal waters from Taiwan [38]. In addition, near 200 ng L<sup>-1</sup>  
83 were detected for carbamazepine in the Baltic Sea and for atenolol in samples from Aegean Sea and  
84 Dardanelles in Greece and Turkey, respectively [39]. The behavior and removal of these compounds in  
85 coastal systems, however, is not clear, especially in more complex systems such as estuaries, where many  
86 processes can influence the reactivity of PhACs [40]. For instance, microbial [41] or sunlight degradation  
87 [42] of compounds such as propranolol and indomethacin can shorten their half-lives to < 24 hours. On the  
88 other hand, a constant concentration with no decline has been reported for some substances such as  
89 sulfamethoxazole and carbamazepine [37, 43, 39] which can be considered as highly persistent  
90 wastewater markers in coastal water. The aim of this paper is therefore to provide a better knowledge of  
91 the occurrence, concentrations and distribution of a wide range of PhACs in coastal systems. First, we  
92 have explored several aspects of the extraction and preconcentration of pharmaceuticals in aqueous  
93 samples using SPE under different conditions. Separation, identification and quantification of target  
94 pharmaceuticals have been then carried out by a new generation UPLC-QqQ-MS/MS system. Once  
95 optimized, the developed method has been applied to the analysis of pharmaceutical residues in surface  
96 water samples taken from two different systems located in SW Spain (Cadiz Bay and Huelva Estuary)  
97 where no information on these compounds was available.

## 98 99 **2. EXPERIMENTAL SECTION**

### 100 **2.1. Material and standards**

101 Methanol and acetonitrile were of chromatography quality and purchased from Scharlau (Barcelona,  
102 Spain); formic acid (98%), ammonia (25%), ammonium formate (97.8%), ammonium acetate (97%),  
103 hydrochloric acid (37%), sodium hydroxide (98%), acetic acid (99%) and Na<sub>2</sub>EDTA (99%) were  
104 purchased either from Sigma Aldrich (Madrid, Spain) or Panreac (Barcelona, Spain). Water was Milli-Q  
105 quality and the solid-phase extraction (SPE) mini-columns used (60 and 500 mg) were supplied by  
106 Waters (Oasis HLB cartridges, Waters Corp., Milford, MA). Analytical standards (> 95% purity) and  
107 deuterated or <sup>13</sup>C-labelled surrogates used for quantification were obtained from several suppliers listed  
108 in Table 1.

### 109 110 **2.2. Sampling areas**

111 Surface water samples were taken from Cadiz Bay and Huelva Estuary (SW Spain) in October 2011  
112 using 2.5 L pre-washed amber glass bottles. Samples were kept < 4°C during their transport to the

laboratory, where they were filtered using 1  $\mu\text{m}$  glass fiber filters (Pall Corporation, Madrid, Spain) and processed immediately. Huelva Estuary is located at the confluence of the Odiel and Tinto rivers (128 and 100 km long, respectively), which forms a coastal wetland known as Ría of Huelva Estuary. This estuary is within a natural protected area (Marismas del Odiel) and flows into the Atlantic Ocean ( $37^{\circ}7'47.22''\text{N}$ ,  $6^{\circ}50'51.59''$ ). The province of Huelva has 142 284 inhabitants and the estuary system covers more than 7000  $\text{hm}^2$  between counties of Huelva and Punta Umbría (Fig. 1a). This area is also strongly affected by acid mine drainage due to ancient mining activity that produces an important background metal pollution [44]. Cadiz Bay is also situated in the southwest of the Iberian Peninsula and contains five different counties (Cadiz, Chiclana, El Puerto de Santa María, Puerto Real, and San Fernando) with nearly 435000 inhabitants. The area is characterized by coastal marshes, estuaries and tidal creeks such as Río San Pedro and Sancti Petri. In addition, the bay hosts five ports and several shipyards. Most of this marshy area is part of a natural park (Bahía de Cádiz). Guadalete river (157 km long) flows across the province of Cadiz entering the sea in the northern part of the Bay of Cadiz at El Puerto de Santa María (89 068 inhabitants) (Fig. 1b). Most of the terrain adjacent to the river is used for agriculture, and there are also wastewater discharges from a WWTP located upstream [45, 46].

### 2.3. Sample extraction and purification by SPE

Target compounds were extracted from water samples by SPE. Different operational conditions were compared to optimize the extraction method by spiking water aliquots at different concentrations (1-2.5  $\text{ng L}^{-1}$ ) using a standard mixture of target substances. First, two Oasis HLB cartridge types were tested (60 and 500 mg), as well as five different pH values (2, 4, 6, 8 and 10) by adding hydrochloric acid and/or sodium hydroxide to water samples. Additionally, four different amounts of  $\text{Na}_2\text{EDTA}$  (0, 0.5, 1 and 2.5  $\text{g L}^{-1}$ ) and five different elution solvents (methanol, acetonitrile, methanol 1% formic acid, methanol 1% ammonium acetate and ethylacetate-acetone 1:1) were also tested. All recovery experiments were performed by analyzing spiked water samples in triplicate ( $n=3$ ).

Once the SPE methodology was optimized, samples from Cadiz Bay and Huelva Estuary were processed by taking two 200 mL aliquots (one of them acidified to pH 2.5) from each surface water sample and spiking them to 50  $\mu\text{g L}^{-1}$  using surrogates (Table 1) prior SPE. HLB cartridges (500 mg) were conditioned using 8 mL of methanol and 8 mL of water. Thereafter, the samples were passed through the SPE columns at 2  $\text{mL min}^{-1}$ . The sorbent was washed with 10 mL water and air-dried for 20 min. Elution was performed with pure methanol (10 mL). The extracts were evaporated under a gentle stream of nitrogen, reconstituted in a methanol/water mixture (25:75) and filtered using 0.22  $\mu\text{m}$  polytetrafluoroethylene (PTFE) filters (Teknochroma, Barcelona, Spain).

### 2.4. Triple quadrupole mass spectrometry detection

Analysis of compounds was carried out by UPLC-QqQ-MS/MS using a Bruker EVOQ Elite system (Bruker, Billerica, MA). The injection volume was set to 10  $\mu\text{l}$ . The chromatographic separation was performed on a reverse-phase C18 analytical column (Intensity Solo HPLC Column) of 100 mm x 2.1 mm and 2  $\mu\text{m}$  particle size. Several mobile phases and additives at different concentrations were tested to optimize peak shapes and intensities, as well as chromatographic separation for compounds acquire under both positive and negative electrospray modes (ESI+/-). Aqueous mobile phase additives consisted in formic acid (0.1, 0.01%, and 10 mM) and ammonium formate (10 mM) for positive ionization and acetic acid (0.1% and 0.01%), ammonium acetate and ammonia (5 mM) for negative ionization combined with methanol and acetonitrile as organic solvents. The best results were obtained using methanol (solvent A) as organic phase and water with 10 mM of formic acid and ammonium formate or 5mM of ammonium acetate and ammonia as aqueous phases (solvent B) for ESI- and ESI+, respectively (flow rate = 0.4  $\text{mL min}^{-1}$ ). The elution gradient for positive mode started at 5% of solvent A. The percentage of methanol was then increased linearly to 100% during the first 5 min, and kept at 100% over 3 min. Total run time was 10 min including a re-equilibration time of 2 min. Initial conditions were similar operating in negative mode increasing the amount of methanol linearly to 100 % in 2 min, keeping it at 100% during 4 min and then, back to initial conditions within 2 min (overall run time = 8 min).

The MS system used the following settings: source temperature = 250°C, probe temperature = 450 °C, cone gas flow = 20  $\text{mL min}^{-1}$ , probe gas flow = 50  $\text{mL min}^{-1}$ , nebulizer gas flow = 60  $\text{mL min}^{-1}$ , collision gas pressure = 2.0 mTorr. The ion spray voltage was 4500 V and 4200 V for ESI+ and ESI- mode, respectively. Standard solutions (1  $\text{g L}^{-1}$ ) of each individual substance were infused to get the optimal collision energy. Optimization of cone voltages was not required unlike in other MS/MS systems [27] as the EVOQ instrument includes a special feature (flat-tuning) that maximizes the sensitivity. MRM transitions were monitored in 2 min windows to get the highest sensitivity and enough points per chromatographic peak (15). Scan time was at least 12 ms for each compound, achieving a total scan time

172 between 0.6 (ESI-) and 1 s (ESI+). Identification of compounds was based on comparing their retention  
173 times to those previously obtained using standards. In addition, two MRM transitions were used to  
174 confirm the compound identity, considering also a deviation in the ion ratio between both transitions  
175 lower than 20%. However, only one product ion could be obtained for tetracycline, azithromycin and  
176 amitriptyline (Table 1). Quantification was performed using the MRM transition showing the highest  
177 intensity and using a calibration curve prepared with standards at concentrations ranging from 0.1 to 100  
178  $\mu\text{g L}^{-1}$ . Deuterated or  $^{13}\text{C}$ -labelled compounds were also used to correct for losses during the extraction  
179 process and matrix effects. The method limits of detection (mLOD) and quantification (mLOQ) were  
180 determined from 200 mL spiked water samples as the minimum detectable amount of analytes with a  
181 signal to noise ratio of 3 and 10 respectively. Instrument limits of detection (iLOD) were also calculated  
182 taking into account the amount of sample injected (10  $\mu\text{L}$ ). The repeatability and reproducibility of the  
183 method was checked through three successive injections of the same sample and re-analyzing a batch of  
184 samples and standards one week after their first analysis. All the data were acquired and processed using  
185 MS Workstation 8.1. Software.

### 186 187 3. RESULTS AND DISCUSSION

#### 188 3.1. Solid phase extraction optimization for aqueous samples

189 Figure 2 shows the SPE extraction efficiencies for some of the most representative target compounds  
190 during different experiments. First, Oasis HLB cartridges were chosen based on numerous studies that  
191 consider that sorbent optimal when developing a multi-residue method for PhACs [47, 25, 48]. Two  
192 different cartridges were compared (60 and 500 mg), and lower recoveries were found for those having 60  
193 mg of sorbent. This could be due to lower retention of target compounds proportional to the sorbent  
194 amount. Two examples are sulfamethazine and indomethazine, whose recovery percentages were reduced  
195 by 40% when using 60 mg cartridges instead of 500 mg (Figs. 2a and b). Once 500 mg Oasis HLB  
196 cartridges were considered for the next experiments, the effect of pH in water samples was tested by  
197 selecting a wide range of pH values (from 2 to 10). Better extraction efficiencies were obtained in  
198 samples at acid (pH 2-3) and neutral (pH 7) conditions. These results can be explained by considering that  
199 many antibiotics present acidic functional groups, therefore lowering pH by 2 units under their pKa  
200 values enhances the presence of neutral forms and their interaction with the HLB sorbent [49, 1, 22] (Fig.  
201 2c). In this sense, many antibiotic groups considered here showed better results at acid conditions,  
202 particularly fluoroquinolones (pKa values between 5 and 6) [20, 50, 16, 51]. The importance of acidic  
203 media for the extraction was also reflected for tetracyclines, increasing their recoveries up to 60% when  
204 comparing with neutral conditions [22]. Accordingly, acid conditions produced an extraction  
205 improvement in flumequine (15%) and tetracycline (27%) in this study (Figs. 2 b and c). Nevertheless,  
206 there is a significant fraction of target PhACs where no pH adjustment yields higher extraction  
207 efficiencies [25] (Table 2). Keeping neutral pH proved to be very critical for some substances such as  
208 macrolides (erythromycin) and sulfonamides (sulfadimethoxine) [51, 25, 1] as extraction efficiency  
209 decreased  $\geq 40$  and 90%, respectively, at lower pH values (Figs. 2 b and c).

210 Previous studies pointed out the importance of a cation complexing agent ( $\text{Na}_2\text{EDTA}$ ) addition to  
211 avoid chelation of metals and to minimize interferences for some antibiotics such as macrolides [52] or  
212 tetracyclines [53, 17]. The use of acidic elution solvents was also explored. However, extraction  
213 efficiencies did not increase significantly ( $p < 0.05$ ) when  $\text{Na}_2\text{EDTA}$  was added in our case. In fact, there  
214 was a decrease up to 15% in recoveries for tetracycline and albuterol at pH 2-3 and 7, respectively.  
215 Recoveries stayed similar or were even lower for other compounds when EDTA was added. Two  
216 examples are erythromycin and indomethacin, whose extraction efficiencies decreased between 46 and  
217 62% (Fig 2 b to e). At the end, addition of chelating agent, acid and neutral pH conditions and methanol  
218 as elution solvent was not chosen to achieve higher recovery percentages for most of the PhACs. Table 2  
219 shows the SPE extraction efficiencies for all target compounds once the method was optimized. At least  
220 half of the target compounds exhibit recoveries that exceed 80 %, and about 80 % of PhACs show  
221 extraction efficiencies  $\geq 50\%$ . The choice of two different pH values was justified because of the wide  
222 range of compounds selected and their very different physicochemical properties (especially in terms of  
223  $\text{pK}_a$  values). This can be illustrated by considering flumequine and chloramphenicol, whose extraction  
224 recoveries were 29% and 50% higher at acid and neutral pH values, respectively (Figs. 2b and d). Many  
225 of the tested drugs, however, were not affected by pH changes (e.g., trimethoprim, atenolol,  
226 amitriptyline), presenting a RSD in their extraction efficiencies  $\leq 15\%$  when comparing both acid and  
227 neutral conditions (Figs. 2c and b). The same tendency was observed for other chemicals when  
228 considering adding  $\text{Na}_2\text{EDTA}$ , such as tetracycline or chloramphenicol (Figs. 2c and e). Compared to  
229 previous studies, the relatively lower recoveries obtained by our group for cephalosporines are  
230 nevertheless comparable to those obtained by other researchers that report similar values for this group  
231 (around 40%) when extracting aqueous samples at neutral pH [26]. Regarding penicillins, our method has

232 improved the extraction efficiency up to 45% with respect to previous studies using HLB cartridges.  
233 Nevertheless, better results for amoxicillin (from 18 to 36%) and oxacillin (from 17 to 76%) could be  
234 obtained using Isolut ENV+ cartridges and water at pH 5 instead [54].

235

### 236 3.2. UPLC-MS/MS separation and determination of PhACs

237 Fig. 3 shows two total ion current chromatograms obtained under optimized LC-MS conditions.  
238 Several solvents were tested as mobile phase to enhance the separation of target compounds by UPLC  
239 and their signal intensity in the mass analyzer. As many of the target compounds are characterized by  
240 basic behavior, the acid addition to aqueous mobile phase is commonly accepted [27, 51, 15].  
241 Specifically, we could measure a signal improvement >70% for ceftiofur and tetracycline (ESI +) when  
242 using 10 mM of formic acid and ammonium formate buffer (pH 3.2) as aqueous phase, which ended up  
243 being the most appropriate solvent (Fig 3a). Most of the compounds analyzed under positive ionized  
244 conditions are usually protonated at low pH as the interaction between molecules and protons from the  
245 aqueous phase that leads to the formation of quasimolecular ion  $[M+H]^+$  is enhanced. Sometimes, the  
246 most abundant species  $[M+H]^+$  were accompanied by adducts such as  $[M+NH_4]^+$  (e.g., ivermectin) and  
247  $[M+Na]^+$  (e.g., penicillin and monensin) or double charge molecules  $[M+2H]^{2+}$  (e.g., spyramicin) (Table  
248 1). On the other hand, sensitivity considerably decreased when the pH was lowered by adding weak acids  
249 in negative ionization mode phase as other studies have already reported [15]. Slightly basic pH values  
250 improve deprotonation of molecules and enhance production of quasimolecular ions  $[M-H]^-$  when  
251 working in ESI- mode. Thus, we observed a signal decrease of >50% and 30% for triclosan and  
252 acetaminophen, respectively, when 0.1% of acetic acid was added. Increasing pH in the aqueous mobile  
253 phase yielded an appreciable increment in the intensity of peaks (between 50 and 60% for some  
254 compounds such as pravastatin and indomethacin) and improved their shape [27]. Therefore, 5mM of  
255 ammonium acetate and ammonia buffer (pH 8) was finally selected as the most favorable aqueous  
256 solution when operating in negative ionization mode (Fig. 3b). Although peak shapes were enhanced  
257 using acetonitrile as organic solvent, methanol led to further enhancement in the signal intensity for most  
258 compounds (up to 70% for azithromycin and metronidazole) so this solvent was preferred over  
259 acetonitrile.

260 Calibration curves from UPLC-QqQ-MS/MS had strong linearity ( $r^2 > 0.9$ ) for all target analytes. The  
261 instrumental limits of detection (iLOD) were <50 pg of the injected amount in 68% of cases and near  
262 90% of PhACs considered in this study showed values <1 ng L<sup>-1</sup> for both detection (mLOD) and  
263 quantification (mLOQ) limits in real samples (Table 1). Detection and quantification limits were in the  
264 same range in other studies showing slightly better results in the present research. Anti-inflammatories  
265 mLOD in our study ranged from <0.1 to 1 ng L<sup>-1</sup>, reaching 2.4 ng L<sup>-1</sup> for ketoprofen in a previous study  
266 [15]. Other groups, such as beta-blockers or psychiatric drugs followed the same trend being <0.1 ng L<sup>-1</sup>  
267 for 99% of PhACs studied. A notable example could be the mLOD of 7.2 ng L<sup>-1</sup> for tetracycline [15],  
268 quite separate from that found by this study (1 ng L<sup>-1</sup>). The reproducibility and repeatability of the method  
269 generated RSD of < 20%.

270

### 271 3.3. Occurrence of PhACs in Huelva Estuary and Cadiz Bay

272 To validate the applicability of the optimized analytical method, surface water samples from Huelva  
273 estuary and Cadiz bay (both located along the Gulf of Cadiz in SW Spain) were taken to the laboratory  
274 and analyzed. Tables 3 and 4 show the concentrations (in ng L<sup>-1</sup>) of target compounds. Overall, 49 out of  
275 83 pharmaceuticals were detected in 75% of all collected samples. Their concentrations are usually  
276 between one and two orders of magnitude lower than those reported in river waters. For example,  
277 atenolol, salicylic acid and trimethoprim [34] were detected in Spanish rivers at levels between 234-1162  
278 ng L<sup>-1</sup>, significantly higher than those found in our sampling areas (<0.1-40.9 ng L<sup>-1</sup>). These differences  
279 are mostly due to frequent WWTP discharges in most European fresh water systems -often streams  
280 impacted by adjacent settlements along their courses- and the enhanced dilution experienced by chemicals  
281 once they reach coastal ecosystems. Other factors include weather, number of inhabitants and  
282 currents/tides. A more detail study on this topic was published by Benotti and Brownawell [37], where  
283 the authors developed a model to estimate the dilution effect in estuarine systems under wet and dry  
284 conditions, concluding that dilution is further increased in coastal system as a consequence of heavy rain  
285 events. In this sense, the concentration ratio of dry weather/wet weather was 3 and 20 for caffeine and  
286 trimethoprim, respectively, whereas other compounds such as nicotine and acetaminophen were below  
287 LOD during heavy rain episodes. Despite the dilution effect along the different sampling sites in all these  
288 studies, is also evident that concentrations of PhACs significantly increase in those stations located  
289 nearby sewage discharge outlets or areas where water circulation is restricted (e.g., M9 and M11 stations  
290 in Huelva Estuary).

291 Figure 4 summarizes the average concentrations of different groups of antibiotics and other  
292 pharmaceuticals (see Table 1 for details on specific compounds per group) in Huelva Estuary and Cadiz  
293 Bay. Data on the stimulant caffeine was not included in the figure because of the considerably higher  
294 average concentrations measured for this compound (19.4-41.4 ng L<sup>-1</sup>) compared to the rest of analytes.  
295 These values for caffeine are in agreement with other data from studies in coastal waters (7-87 ng L<sup>-1</sup>)  
296 [36]. In fact, this compound has been considered by many authors as an excellent sewage markers that  
297 can be detected even in open waters from North Atlantic/Arctic oceans (7-9 ng L<sup>-1</sup>). Among non-  
298 antibiotic PhACs, anti-inflammatories (6.7-9.6 ng L<sup>-1</sup>), beta-blockers (0.6-3.8 ng L<sup>-1</sup>), lipid regulators  
299 (1.1-5.9 ng L<sup>-1</sup>), and diuretics (0.6-16 ng L<sup>-1</sup>) were the most prominent groups of compounds in our  
300 sampling areas (Fig. 4b,d). Huelva Estuary usually showed higher concentrations for most of these  
301 chemicals than Cadiz Bay. As an example, concentrations near 200 ng L<sup>-1</sup> for ibuprofen and in a range of  
302 1.1-69.7 ng L<sup>-1</sup> for naproxen, 1.1 to 69.2 ng L<sup>-1</sup> for gemfibrozil, and 1.8 to 167.6 ng L<sup>-1</sup> for  
303 hydrochlorothiazide are in contrast with those measured for these PhACs in Cadiz Bay, which were  
304 below 20 ng L<sup>-1</sup> at all sampling stations. These differences also occurred for the stimulant caffeine, which  
305 presented concentrations over 500 ng L<sup>-1</sup> in Huelva and less than 50 ng L<sup>-1</sup> in Cadiz. This disparity could  
306 be explained by the lower dilution in Huelva Estuary as opposed to the higher volume of water in Cadiz  
307 Bay, also more heavily affected by tidal currents. Nevertheless, there were some compounds, especially  
308 antibiotics, such as tetracyclines that were only identified in the Cadiz area (0.7-3.5 ng L<sup>-1</sup>) and  
309 quinolones, that were more prevalent in Huelva Estuary (up to 40 ng L<sup>-1</sup> for norfloxacin, ciprofloxacin,  
310 and enrofloxacin), suggesting different consumption patterns and/or uses (e.g., aquaculture in Cadiz).

311 In spite of the scarce information available, we can compare our data with those reported in a few  
312 coastal systems in United States and Europe. In general, we can observe that the same groups of PhACs  
313 are also predominant in Long Island Sound Estuary (LISE, NY), where average concentrations for anti-  
314 inflammatories (0.1-50 ng L<sup>-1</sup>), beta-blockers (0.5-13 ng L<sup>-1</sup>), lipid regulators (0.2-29 ng L<sup>-1</sup>), and the  
315 diuretic hydrochlorothiazide (10 ng L<sup>-1</sup>) were in a similar range of those measured in SW Spain [40].  
316 More specifically, some compounds such as bezafibrate (0.1-1.1 ng L<sup>-1</sup>), clofibrac acid (0.5-0.6 ng L<sup>-1</sup>),  
317 and diclofenac (2.5-11.8 ng L<sup>-1</sup>) were within the same order of magnitude in Cadiz Bay, Huelva Estuary  
318 and LISE (0.7 ng L<sup>-1</sup>, 0.2 ng L<sup>-1</sup> and 4 ng L<sup>-1</sup> respectively). Regarding the occurrence of antibiotics,  
319 similar concentrations were also found in other estuary systems [43, 40, 39], being between 0.4 and 4 ng  
320 L<sup>-1</sup> for quinolones (e.g., ciprofloxacin) and trimethoprim. On the other hand, there are also significant  
321 differences between US and EU regarding the ratios and occurrence of some specific PhACs, which could  
322 be attributed to different consumption/prescription patterns and authorized uses (veterinary vs health  
323 care). One example is the relatively high concentrations of some anti-inflammatories (e.g., naproxen up to  
324 50 ng L<sup>-1</sup>) and beta-blockers (e.g., metoprolol up to 150 ng L<sup>-1</sup>) that were measured in US West and East  
325 coasts [43, 39, 40], whereas these compounds are not detected in our study area. Antibiotics such as  
326 trimethoprim could not be found by Nödler and co-workers [39] but it was detected in more than 50% of  
327 our sampling stations (0.1-2 ng L<sup>-1</sup>). Regarding Asia, Fang and collaborators [38] analyzed surface coastal  
328 waters in Northern Taiwan, screening for some antiinflammatories such as ibuprofen (<2.5-57.1 ng L<sup>-1</sup>)  
329 and diclofenac (<2.5-53.6 ng L<sup>-1</sup>). These results are in contrast to those reported here from Huelva and  
330 Cadiz coastal waters, where concentrations of these compounds are significantly higher for ibuprofen and  
331 much lower for diclofenac (0.8-11.8 ng L<sup>-1</sup>).

#### 332 333 4. CONCLUSION

334 This study has contributed to expand the limited information available on the occurrence and  
335 distribution of pharmaceuticals in coastal waters. We have presented the optimization of an analytical  
336 method for the extraction and determination of 83 pharmaceuticals that includes the use of isotopically  
337 labelled compounds, SPE and triple quadrupole mass spectrometry detection. Due to the wide range of  
338 analytes considered and their different physicochemical properties, their extraction from aqueous samples  
339 was preferred at acid and neutral pH values to achieve proper recoveries (more than 80% for half of the  
340 compounds). Separation and quantification of target PhACs were also performed in two different runs as  
341 different ionization modes (ESI+ and -) were required. Two different buffers (10 mM of formic acid and  
342 ammonium formate, and 5mM of ammonium acetate and ammonia) were also used as mobile aqueous  
343 phases to achieve highest sensitivity and better peak shapes. Once optimized, the method detection limits  
344 were within a few ng L<sup>-1</sup> or below for all analytes. The application of the method for the analysis of  
345 surface water samples from Huelva Estuary and Cadiz Bay (SW Spain) has revealed the predominance of  
346 some compounds such as metronidazole (a nitroimidazol at an average concentration of 7-7.8 ng L<sup>-1</sup>),  
347 ofloxacin (a quinolone at 2-3.5 ng L<sup>-1</sup>), atenolol (a beta-blocker at 0.8-3.7 ng L<sup>-1</sup>) and several anti-  
348 inflammatories: ibuprofen (4.3-15.6 ng L<sup>-1</sup>), mefenamic acid (5.2-16.8 ng L<sup>-1</sup>), fenoprofen (1.9-12.2 ng L<sup>-1</sup>)  
349 and diclofenac (0.8-11.8 ng L<sup>-1</sup>). Concentrations of many of the target compounds were consistent with  
350 previous studies in other European and American coastal and estuarine systems, but some differences  
351 among specific compounds reveals different consumption/uses patterns.

352 **ACKNOWLEDGEMENTS**

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354 de Innovación, Ciencia y Empresa (Junta de Andalucía), who also provided a FPI fellowship.

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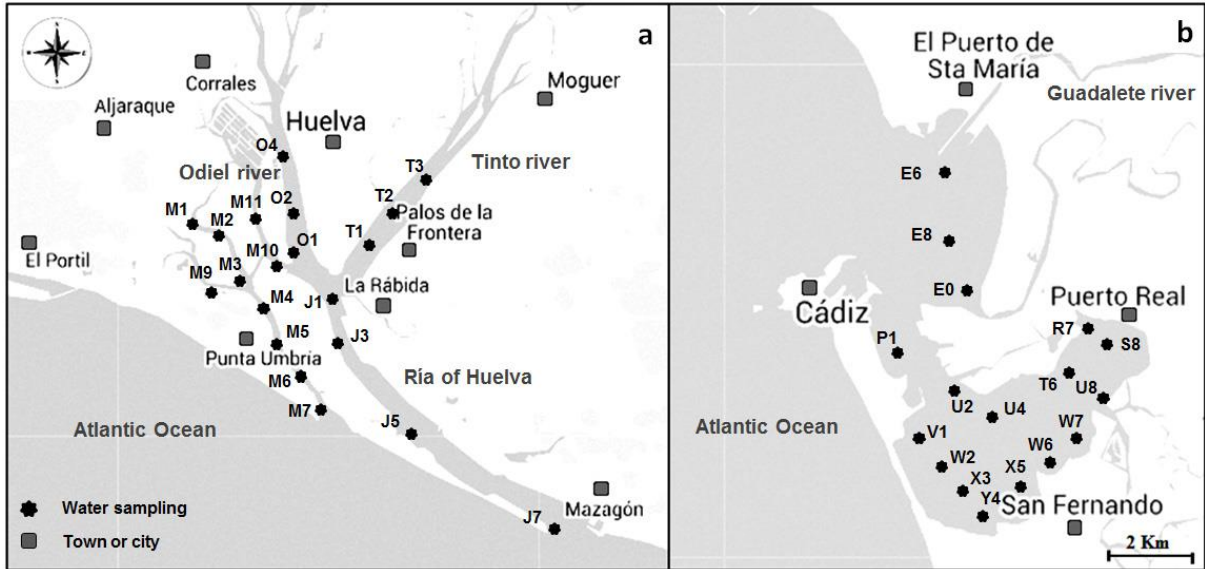
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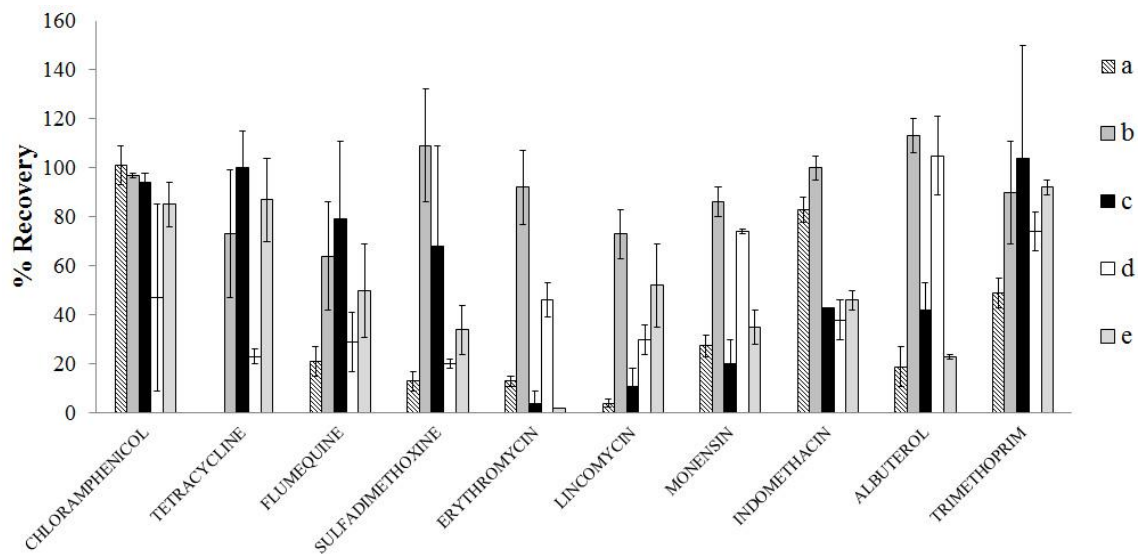
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Figure 1. Map showing the location of the sampling stations along the Gulf of Cadiz in Huelva Estuary (Fig.1a) and the Cadiz Bay (Fig.1b).



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Figure 2. Optimized SPE parameters, a) pH 7 using 60 mg cartridge and elution using methanol; b) pH 7 using 500 mg cartridge and elution using methanol; c) pH 2 using 500 mg cartridge and elution using methanol; d) pH 7 using 500 mg cartridge, adding 1g of EDTA and elution using methanol 1% formic acid; e) pH 2 using 500 mg cartridge, adding 1g of EDTA and elution using methanol with 1% formic acid.

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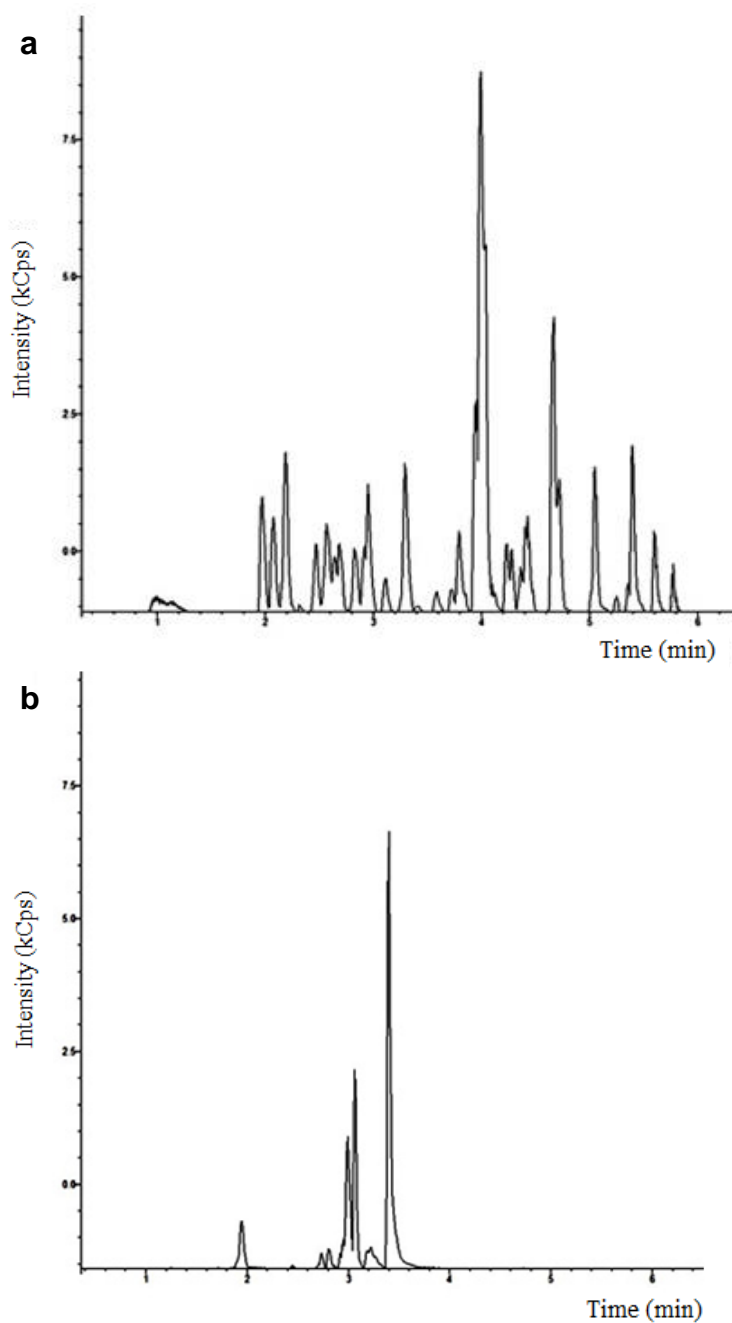


Figure 3. Representative total ion current (TIC) chromatograms of a 25 ng mL<sup>-1</sup> standard mixture of the compounds analyzed under positive (a) and negative (b) ionization.

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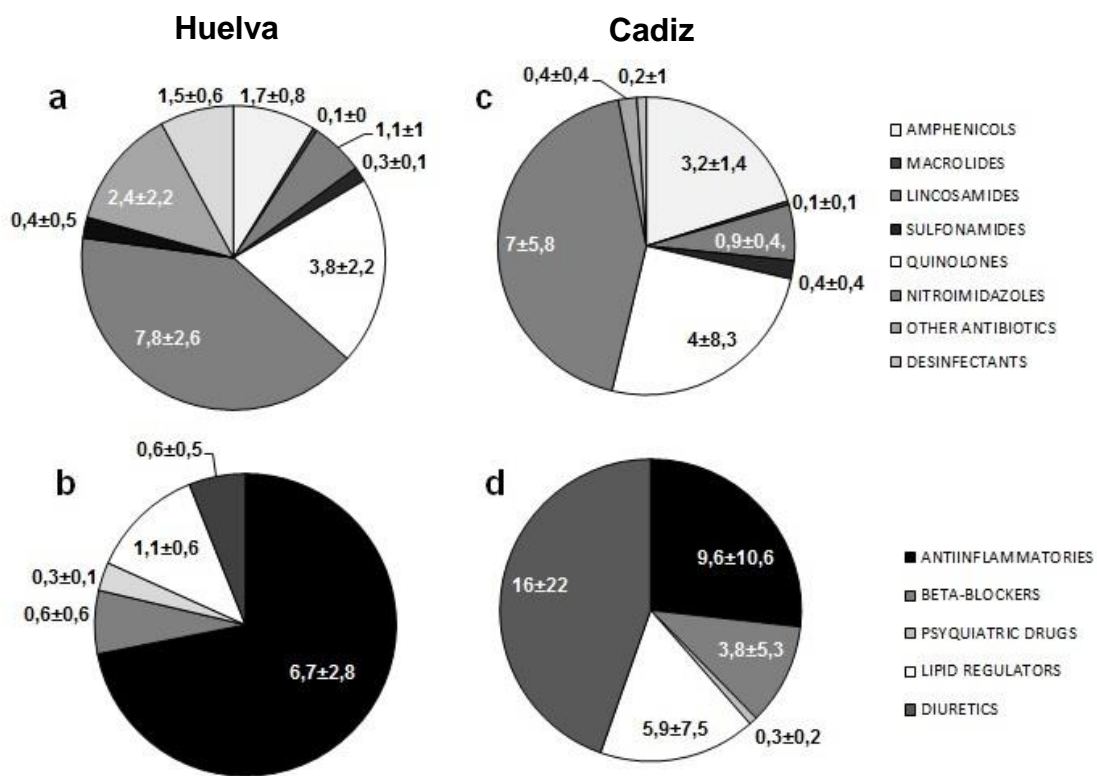


Figure 4. Average concentrations (in ng L<sup>-1</sup>) of (a,c) antibiotics and (b,d) PhACs in Huelva Estuary and the Bay of Cadiz, respectively.

**Table 1. UPLC-MS/MS parameters (ionization mode, retention time, MRM transitions and limits of detection) for the analysis of target PhACs and their corresponding isotopically labelled compounds. Suppliers are also indicated.**

PhACs group	Compound	t <sub>R</sub> (min)	MRM	Collision Energy	iLOD (pg)	mLOD (ng L <sup>-1</sup> )	mLOQ (ng L <sup>-1</sup> )
Penicillins	Amoxicillin <sup>a1</sup> (+)	1.77	366.10>349.20 366.10>114.30	6 20	309,3	0,9	2,9
	Penicillin-G <sup>a1</sup> (+)	3.64	357.00>198.20*	12	681,8	0,4	1,3
	Oxacillin <sup>a1</sup> (+)	3.89	357.00>182.00 402.10>160.40	14 11	16,1	<0.1	0,2
	Ampicillin <sup>a1</sup> (+)	2.72	402.10>243.40 350.10>106.30	11 15	5,0	<0.1	<0.1
				350.10>160.40	11		
Cephalosporins	Cefaclor <sup>a1</sup> (+)	2.58	368.00>174.40 368.00>106.40	13 19	10,3	<0.1	<0.1
	Cefdinir <sup>a1</sup> (+)	2.31	396.00>227.10 396.00>126.10	11 26	69,8	0,1	0,3
	Ceftiofur <sup>a1</sup> (+)	3.34	524.04>241.20 524.04>209.90	15 22	15,5	<0.1	0,1
	Cefadroxil <sup>a1</sup> (+)	1.82	363.30>114.30 363.30>208.30	13 8	21,5	<0.1	0,1
	Cefquinome <sup>a1</sup> (+)	2.39	529.00>134.40 529.00>396.40	13 9	34,1	<0.1	<0.1
Tetracyclines	Doxycycline <sup>a2</sup> (+)	2.91	445.20>410.40 445.20>267.60	21 35	142,6	0,2	0,5
	Oxytetracycline <sup>a2</sup> (+)	2.99	461.10>425.20 461.10>426.90	20 19	72,8	<0.1	0,1
	Chlortetracycline <sup>a2</sup> (+)	3.43	479.10>444.10 479.10>462.10	20 15	24,0	<0.1	0,1
	Tetracycline <sup>a2</sup> (+)	3.67	445.20>428.20	10	2097,9	1,0	3,5
Amphenicols	Tiamulin <sup>a1</sup> (+)	4.05	494.30>191.40 494.30>193.10	19 19	4,4	<0.1	<0.1
	Chloramphenicol <sup>a1</sup> (-)	2.73	321.00>152.10 321.00>257.50	16 8	14,3	<0.1	<0.1
Macrolides	Erythromycin <sup>a3</sup> (+)	4.16	734.50>158.20 734.50>575.40	31 20	66,6	<0.1	0,1
	Clarithromycin <sup>a3</sup> (+)	4.49	748.48>158.10 748.48>590.20	26 18	0,4	<0.1	<0.1
	Azithromycin <sup>a3</sup> (+)	4.43	748.90>591.40	26	1,0	<0.1	<0.1
	Roxithromycin <sup>a3</sup> (+)	4.51	837.50>158.30 837.50>679.50	32 20	1,7	<0.1	<0.1
	Spiramycin <sup>a3</sup> (+)	3.40	422.00>174.30* 422.00>101.20	20 18	74,3	0,1	0,5
	Tylosin <sup>a3</sup> (+)	4.08	916.50>174.20 916.50>771.80	39 29	6,8	<0.1	<0.1
Lincosamides	Lincomycin <sup>a1</sup> (+)	2.66	407.20>126.30 407.20>359.40	24 16	0,6	<0.1	<0.1
	Clindamycin <sup>a1</sup> (+)	3.83	425.20>126.30 425.20>377.40	26 16	5,4	<0.1	<0.1
	Sulfamethazine <sup>a4</sup> (+)	2.72	279.10>186.20 279.10>92.30	19 32	26,8	<0.1	0,1
	Sulfamethizole <sup>a4</sup> (+)	2.64	271.03>156.10 271.03>92.40	13 29	25,0	<0.1	0,1
	Sulfathiazole <sup>a4</sup> (+)	2.26	256.02>156.20 256.02>108.30	14 23	2,2	<0.1	<0.1
	Sulfadiazine <sup>a4</sup> (+)	2.09	251.06>155.20 251.06>92.30	15 26	11,5	<0.1	<0.1

Sulfonamides	Sulfamethoxazole <sup>a4</sup> (+)	2.86	254.06>156.10 254.06>91.40	15 16	13,6	<0.1	<0.1
	Sulfamethoxypyridazine <sup>a4</sup> (+)	2.75	281.07>156.10 281.07>92.90	16 29	13,0	<0.1	<0.1
	Sulfadimethoxine <sup>a4</sup> (+)	3.35	311.08>156.80 311.08>92.00	24 40	7,7	<0.1	<0.1
	Sulfisoxazole <sup>a4</sup> (+)	2.99	268.07>156.00 268.07>92.10	13 25	12,4	<0.1	<0.1
	Sulfaguanidine <sup>a4</sup> (+)	1.11	215.06>156.40 215.06>92.30	13 24	27,8	<0.1	0,1
	Sulfanilamide <sup>a4</sup> (+)	1.31	173.03>156.20 173.03>92.20	6 18	447,8	0,4	1,2
	Quinolones	Flumequine <sup>a5</sup> (+)	4.00	262.10>243.20 262.10>244.90	17 16	3,1	<0.1
Norfloxacin <sup>a5</sup> (+)		2.77	320.10>302.20 320.10>231.20	19 40	71,4	<0.1	0,1
Ofloxacin <sup>a5</sup> (+)		2.72	362.10>318.90 362.10>317.10	17 17	0,2	<0.1	<0.1
Ciprofloxacin <sup>a5</sup> (+)		2.83	332.10>314.80 332.10>313.30	21 21	140,2	0,1	0,2
Enrofloxacin <sup>a5</sup> (+)		2.83	360.10>342.20 360.10>316.20	19 16	22,1	<0.1	<0.1
Sparfloxacin <sup>a5</sup> (+)		3.15	393.20>349.20 393.20>375.20	17 18	4,8	<0.1	<0.1
Danofloxacin <sup>a5</sup> (+)		2.83	358.10>340.80 358.10>339.10	22 23	88,8	0,1	0,2
Aminocoumarin antibiotic	Novobiocin <sup>a5</sup> (+)	5.06	613.20>189.30 613.20>133.40	27 57	9,1	<0.1	<0.1
Nitroimidazols	Nitrofurantoin <sup>a1</sup> (+)	1.99	239.00>122.20 (-)	19	1071,4	1,5	5,0
	Metronidazole <sup>a1</sup> (+)	2.23	239.00>95.70 172.10>128.80	12 14	44,9	<0.1	0,1
	Ornidazole <sup>a1</sup> (+)	3.02	172.10>82.30 220.05>128.10	27 14	10,4	<0.1	<0.1
Other antibiotics	Trimethoprim <sup>a6</sup> (+)	2.64	220.05>82.40 291.10>229.20	27 24	1,0	<0.1	<0.1
	Monensin <sup>a1</sup> (+)	5.82	291.10>122.40 688.00>636.00*	25 10	3,5	<0.1	<0.1
	Ivermectin <sup>a1</sup> (+)	6.06	688.00>617.00 892.00>569.40*	30 12	48,4	0,2	0,5
	Rifampicin <sup>a1</sup> (+)	4.32	892.00>307.00 823.40>791.50	23 14	51,4	0,1	0,2
Antibacterials	Triclocarban <sup>a7</sup> (-)	3.39	823.40>399.10 315.10>161.90	23 11	9,6	<0.1	0,1
	Triclosan <sup>b8</sup> (-)	3.44	315.10>159.90 286.70>35.80	12 5	1,8	<0.1	<0.1
Antibacterials	Phenazone <sup>a9</sup> (+)	3.02	189.00>77.30 189.00>55.50	44 28	132,2	0,1	0,2
	Phenylbutazone <sup>a10</sup> (-)	2.85	308.00>131.00 308.00>280.20	21 16	1153,8	0,8	2,8
	Acetaminophen <sup>a11</sup> (-)	1.90	150.00>107.80 150.00>106.00	21 13	1209,7	0,5	1,8
	Salicylic Acid <sup>a10</sup> (-)	1.96	137.00>93.40 137.00>65.90	10 19	2777,8	1,3	4,4
	Ketoprofen <sup>a9</sup> (+)	4.40	255.20>209.30 255.20>105.20	11 20	25,5	0,1	0,3

Antiinflammatories	Naproxen <sup>a10</sup> (-)	2.73	229.10>169.10 229.10>185.20	16 4	595,2	0,3	0,9
	Ibuprofen <sup>a12</sup> (-)	2.96	205.10>160.00 205.10>162.10	5 5	2272,7	1,0	3,5
	Fenoprofen <sup>a10</sup> (-)	2.90	241.10>197.10 241.10>93.40	5 32	351,3	0,1	0,5
	Indomethacin <sup>a10</sup> (-)	2.96	358.10>312.10 356.10>297.20	6 14	1239,7	0,6	2,1
	Diclofenac <sup>a10</sup> (-)	2.93	294.00>250.00 294.00>36.00	7 22	113,2	0,1	0,2
	Mefenamic acid <sup>a10</sup> (-)	2.96	240.10>196.90 240.10>180.10	14 23	11,8	<0.1	<0.1
Beta-blockers	Atenolol <sup>a1</sup> (+)	2.01	267.19>145.10 267.19>72.80	28 22	2,5	<0.1	<0.1
	Metoprolol <sup>a1</sup> (+)	3.15	268.40>116.20 268.40>74.20	17 20	94,9	0,1	0,2
	Propranolol <sup>a1</sup> (+)	3.80	260.10116.20 260.10>72.90	17 21	33,3	<0.1	0,1
	Timolol <sup>a1</sup> (+)	3.18	317.10>261.10 317.10>74.40	15 22	0,4	<0.1	<0.1
	Nadolol <sup>a1</sup> (+)	2.72	310.20>254.20 310.20>201.10	16 22	2,8	<0.1	<0.1
	Pindolol <sup>a1</sup> (+)	2.50	249.10>116.90 249.10>115.40	17 16	39,3	<0.1	0,1
Histamine receptor antagonist	Famotidine <sup>a1</sup> (+)	2.07	338.00>189.80 338.00>188.20	19 19	16,5	<0.1	0,1
	Ranitidine <sup>a1</sup> (+)	2.01	315.00>129.40 315.00>130.80	31 31	18,9	<0.1	0,1
Psychiatric drugs and stimulants	Carbamazepine <sup>a13</sup> (+)	4.05	237.10>193.80 237.10>192.40	22 31	7,6	<0.1	<0.1
	Fluoxetine <sup>a14</sup> (+)	4.32	310.10>44.60 310.10>148.40	10 7	3,9	<0.1	<0.1
	Amitriptiline <sup>a13</sup> (+)	4.29	278.20>91.20	27	47,9	<0.1	0,1
	Caffeine <sup>a1</sup> (+)	2.72	195.10>137.30 195.10>138.90	17 18	105,6	0,1	0,3
Lipid Regulators	Clofibrac acid <sup>a15</sup> (-)	2.76	213.03>127.90 213.03>85.50	12 10	106,8	0,1	0,2
	Gemfibrozil <sup>a15</sup> (-)	3.04	249.10>120.70 249.10>121.70	11 11	11,8	<0.1	<0.1
	Fenofibrate <sup>a15</sup> (+)	5.46	361.10>232.00 361.10>139.20	15 27	6,2	<0.1	0,1
	Bezafibrate <sup>a15</sup> (-)	2.82	360.10>274.20 360.10>85.50	14 13	2,3	<0.1	<0.1
	Pravastatin <sup>a15</sup> (-)	2.76	423.00>101.90 423.00>100.40	26 25	50,6	<0.1	0,1
Diuretics	Hydrochloroth. <sup>a18</sup> (-)	1.73	295.90>269.10 295.90>205.20	17 22	4,3	<0.1	<0.1
	Furosemide <sup>a1</sup> (-)	2.47	328.90>205.00 328.90>285.10	20 12	18,6	<0.1	<0.1
Other PhACs	Albuterol <sup>a16</sup> (+)	2.01	240.10>148.20 240.10>222.30	17 7	64,4	<0.1	0,1
	Glibenclamide <sup>a17</sup> (+)	4.76	494.10>369.20 494.10>169.10	12 38	1,8	<0.1	<0.1
	Metotrexate <sup>a1</sup> (+)	2.50	455.18>175.20 455.18>307.10	47 24	29,4	<0.1	0,1
	1. Atenolol-d <sub>7</sub> <sup>d</sup> (+)	2.01	274.20>146.00 274.20>144.50	26 26			



Isotopically labelled compounds	2.Demeclocycline <sup>a</sup> (+)	3.15	465.10>448.10	17
			465.10>430.20	23
	3.Erythromycin-d <sub>3</sub> <sup>a</sup> (+)	4.16	738.90>162.00	33
			738.90>581.10	20
	4.Sulfadimethoxine-d <sub>6</sub> <sup>a</sup> (+)	3.34	317.40>155.80	19
			317.40>92.00	29
	5.Ofloxacin-d <sub>3</sub> <sup>a</sup> (+)	2.72	365.20>261.00	30
			365.20>320.90	18
	6.Trimethoprim-d <sub>9</sub> <sup>a</sup> (+)	2.58	301.00>235.10	25
			301.00>123.90	25
	7.Triclocarban <sup>13</sup> C <sub>6</sub> <sup>f</sup> (-)	3.39	319.00>159.90	12
			321.00>161.90	15
	8.Triclosan-d <sub>3</sub> <sup>b</sup> (-)	3.44	291.60>35.50	5
	9.Phenazone-d <sub>3</sub> <sup>d</sup> (+)	3.02	192.00>59.30	24
			192.00>77.20	34
	10.Naproxen methoxy-d <sub>3</sub> <sup>a</sup> (-)	2.73	232.20>169.90	16
	11.Acetaminophen-d <sub>4</sub> <sup>d</sup> (-)	1.90	154.19>111.00	17
	12.Ibuprofen-d <sub>3</sub> <sup>d</sup> (-)	2.96	208.30>164.00	3
13.Carbamazepine-d <sub>10</sub> <sup>e</sup> (+)	4.05	247.20>204.50	23	
		247.20>203.10	23	
14.Fluoxetine-d <sub>5</sub> <sup>a</sup> (+)	4.32	315.00>153.00	10	
15.Gemfibrozil-d <sub>6</sub> <sup>e</sup> (-)	3.04	255.20>120.50	11	
16.Albuterol-d <sub>3</sub> <sup>a</sup> (+)	2.01	243.33>150.90	18	
		243.33>224.90	8	
17.Glibenclamide-d <sub>3</sub> <sup>e</sup> (+)	4.76	498.03>372.80	16	
		498.03>171.80	37	
18.Hydrochlorothiazide <sup>13</sup> C <sub>6</sub> <sup>a</sup> (-)	1.73	302.70>256.50	4	
		302.70>210.90	22	

<sup>a</sup> Sigma-Aldrich, <sup>b</sup> Dr. Ehrenstorfer GmbH, <sup>c</sup> Clariant Produkte, <sup>d</sup> LGC Standards, <sup>e</sup> CDN Isotopes S.A., <sup>f</sup> Cambridge Isotope Laboratories. Inc.

\* Quasimolecular ions different from [M+H]<sup>+</sup>: penicillin and monensin: [M+Na]<sup>+</sup>, spyramycin: [M+2H]<sup>2+</sup>, ivermectin: [M+NH<sub>4</sub>]<sup>+</sup>.

Isotopically labelled compounds are indicated by numerated superscript for each compound.

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**Table 2. Extraction recovery percentages for PhACs by SPE**

<b>Compound</b>	<b>Recovery (%)</b>	<b>Compound</b>	<b>Recovery (%)</b>
Amoxicillin	18±3	Nitrofurantoin*	36±10
Penicillin-G	86±34	Metronidazole	78±22
Oxacillin	17±3	Ornidazole	60±16
Ampicillin	51±12	Trimethoprim	90±21
Cefaclor	41±11	Monensin	86±6
Cefdinir	34±13	Ivermectin	15±4
Ceftiofur	45±15	Rifampicin	36±5
Cefadroxil	27±11	Triclocarban	20±1
Cefquinome	49±19	Triclosan	53±0
Doxycycline	47±11	Phenazone	110±17
Oxytetracycline*	84±24	Phenylbutazone	68±12
Chlortetracycline*	57±20	Acetaminophen	113±5
Tetracycline*	100±15	Salicylic Acid	106±11
Tiamulin	70±16	Ketoprofen	17±4
Chloramphenicol	97±1	Naproxen	115±7
Erythromycin	92±15	Ibuprofen	109±9
Clarithromycin	69±17	Fenoprofen	117±17
Azithromycin	81±27	Indomethacin	100±5
Roxithromycin	81±27	Diclofenac	106±8
Spiramycin	27±4	Mefenamic acid	101±13
Tylosin	68±12	Atenolol	97±6
Lincomycin	73±10	Metoprolol	64±16
Clindamycin	44±11	Propranolol	70±5
Sulfamethazine	69±9	Timolol	68±15
Sulfamethizole	76±17	Nadolol	77±9
Sulfathiazole	93±14	Pindolol	66±19
Sulfadiazine	89±31	Famotidine	38±17
Sulfamethoxazole	93±18	Ranitidine	22±11
Sulfamethoxyp.	81±19	Carbamazepine	104±15
Sulfadimethoxine	109±23	Fluoxetine*	80±18
Sulfisoxazole	78±22	Amitriptiline*	100±7
Sulfaguanidine	65±5	Caffeine	68±12
Sulfanilamide	62±0	Clofibric acid	73±3
Flumequine*	79±32	Gemfibrozil	97±5
Norfloxacin*	94±24	Fenofibrate	17±4
Ofloxacin*	81±18	Bezafibrate	64±6
Ciprofloxacin*	111±27	Pravastatin	65±3
Enrofloxacin*	80±26	Albuterol	113±7
Sparfloxacin*	101±12	Glibenclamide	146±9
Danofloxacin*	87±12	Metotrexate	72±17
Novobiocin	31±10	Hydrochlorot.	112±5
		Furosemide	108±22

\* pH2

**Table 3. Concentrations of target PhACs in Huelva water samples (ng L<sup>-1</sup>). Only those compounds that were detected are shown here.**

Compound	O1	O2	O4	M1	M2	M3	M4	M5	M6	M7	M9	M10	M11	T1	T2	T3	J1	J3	J5	J7
Tiamulin	0.5	0.6	0.8	0.6	0.6	0.6	0.6	0.6	0.6	1.3	0.7	0.7	0.7	0.4	0.5	0.9	0.7	0.8	0.7	0.8
Chloramphenicol	8.1	5.0	10.6	3.5	5.6	4.0	5.6	4.1	4.7	3.6	3.6	7.0	2.9	13.8	6.8	3.2	3.8	8.3	3.5	5.9
Erythromycin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Lincomycin	0.8	0.4	0.5	0.7	0.4	0.4	0.4	0.4	0.3	0.5	0.6	0.6	0.4	0.5	0.8	0.7	0.6	0.5	0.6	0.6
Clindamycin	1.0	0.9	1.1	0.9	1.0	1.6	1.1	0.9	1.0	2.2	1.4	1.3	1.5	1.0	1.3	3.8	2.4	<LOD	1.8	1.4
Sulfamethazine	0.8	0.3	0.4	0.5	0.4	0.4	0.5	0.5	0.4	0.6	0.5	0.8	0.8	0.7	0.4	0.4	0.7	0.6	1.0	0.9
Sulfamethizole	0.2	<LOD	<LOD	<LOD	<LOD	0.1	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	0.2	<LOQ	0.3	<LOD	0.1	<LOD	<LOD	<LOD
Sulfathiazole	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4
Sulfadiazine	0.3	0.1	0.1	<LOQ	0.1	0.1	0.1	0.1	<LOQ	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	<LOQ	0.1	0.2
Sulfamethoxazole	0.9	0.1	0.1	0.03	0.3	0.1	<LOD	0.1	<LOD	<LOD	15.5	0.3	4.8	0.7	1.7	0.7	0.6	0.3	0.3	0.4
Sulfamethoxypyridazine	0.3	<LOD	<LOD	0.1	<LOQ	<LOQ	0.1	<LOD	<LOD	0.1	<LOQ	0.1	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	0.1	0.4
Sulfadimethoxine	0.6	0.3	0.3	0.5	0.4	0.3	0.5	0.3	0.4	0.4	0.3	0.4	0.5	0.3	0.4	<LOD	0.4	0.4	0.5	0.5
Sulfanilamide	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	1.8	<LOQ	<LOQ	<LOD	<LOD	<LOD
Flumequine	1.1	0.4	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.7	0.2	0.3	0.1	0.1	0.1	0.5	0.3	0.2	0.3	0.1
Norfloxacin	42.9	8.2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ofloxacin	28.0	6.7	4.4	1.9	2.0	5.7	2.8	2.2	2.0	4.0	1.9	2.6	1.7	1.8	7.5	8.0	4.9	2.6	4.2	2.7
Ciprofloxacin	47.5	7.2	3.3	2.2	1.4	3.0	1.8	<LOD	0.8	3.7	1.6	1.8	1.1	1.4	<LOD	6.4	5.7	<LOD	3.5	<LOD
Enrofloxacin	61.7	14.9	6.2	3.3	4.9	4.4	4.2	2.9	2.5	6.3	7.0	3.8	<LOD	<LOD	<LOD	<LOD	4.4	3.5	3.8	3.0
Sparfloxacin	11.9	2.9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Danofloxacin	77.3	17.1	8.1	3.0	4.6	5.0	3.2	2.0	2.7	6.2	4.7	5.5	3.2	1.0	5.5	<LOD	3.8	2.6	5.0	3.3
Metronidazole	16.3	9.8	9.1	10.4	9.6	9.2	9.9	8.6	9.6	8.6	10.7	9.0	10.0	9.3	10.0	8.4	8.3	8.1	12.1	11.8
Nitrofurantoin	<LOD	<LOD	<LOD	<LOD	<LOD	18.56	<LOD	<LOD	<LOD	<LOD	<LOD	46.88	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	17.6
Trimethoprim	0.5	<LOD	0.3	<LOD	<LOD	0.1	0.8	0.7	<LOD	1.1	0.1	1.6	1.0	0.8	2.0	0.2	2.0	0.3	1.8	0.6
Monensin	0.3	<LOQ	<LOD	0.1	<LOD	<LOD	<LOD	<LOQ	-	<LOD	<LOD	0.2	<LOQ	<LOQ	0.1	<LOD	0.3	<LOQ	0.4	0.1
Triclocarban	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.3	<LOD	<LOD
Triclosan	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	7.9	<LOD	<LOD
Acetaminophen	<LOD	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	2.0	2.2	33.7	11.4	75.6	13.6	<LOQ	<LOD	<LOQ	<LOD
Diclofenac	1.9	<LOD	7.8	<LOD	<LOD	<LOD	<LOD	1.5	<LOD	<LOD	3.1	<LOD	5.5	9.2	10.7	2.2	1.6	6.2	<LOD	0.3
Fenoprofen	5.9	6.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.9	7.5	6.7	<LOD	<LOD	<LOD	8.7	20.6	8.3	7.2
Ibuprofen	<LOD	8.8	<LOD	9.0	8.4	8.5	6.9	8.2	8.7	4.3	181.8	11.3	195.0	33.7	136.0	39.6	15.3	9.3	<LOQ	<LOD
Indomethacin	<LOQ	2.2	2.5	3.2	2.4	<LOQ	2.5	2.3	2.1	<LOQ	<LOQ	2.5	<LOD	7.7	5.8	4.1	3.6	3.6	3.4	<LOQ

Mefenamic acid	7.5	8.9	10.8	12.2	8.0	11.0	8.2	12.7	9.8	14.6	6.5	10.9	8.8	24.6	14.8	7.5	17.6	29.2	12.7	13.3
Naproxen	3.2	2.8	1.8	4.0	3.3	4.7	3.7	3.1	3.3	1.1	69.7	4.8	57.3	15.2	35.9	12.5	4.3	5.7	2.0	<LOD
Phenylbutazone	3.4	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.80	2.89	<LOQ	1.44	2.85	<LOQ	3.07
Salicylic acid	17.5	5.5	13.7	6.0	4.7	<LOQ	6.4	5.1	8.5	6.4	5.5	5.9	15.8	16.3	10.5	5.5	7.8	29.4	4.9	4.3
Atenolol	4.0	3.3	3.3	1.9	2.4	3.4	2.6	2.5	2.5	0.5	24.9	2.7	40.9	9.1	21.7	15.8	4.2	2.8	2.1	0.3
Nadolol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.5	<LOD	0.9	0.	<LOD	0.6	<LOD	0.1	<LOD	<LOD
Carbamazepine	0.6	0.4	0.3	0.4	0.3	0.3	0.2	0.3	0.3	0.1	0.9	0.3	1.6	0.7	1.1	1.0	0.5	0.4	0.4	0.2
Amitriptiline	0.3	0.4	0.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.5	<LOD	<LOD	0.2	0.4	0.3	0.2	<LOD	<LOD	<LOD
Caffeine	39.6	17.4	13.2	20.9	21.8	15.7	11.3	52.1	20.1	10.5	182.3	20.2	522.0	30.2	107.8	59.0	20.7	18.4	25.8	7.2
Gemfibrozil	1.5	6.6	1.1	9.0	6.9	6.7	5.4	6.8	5.1	1.2	46.7	9.1	64.8	29.8	69.2	22.3	12.8	8.9	5.8	1.4
Bezafibrate	<LOD	0.6	<LOD	0.4	0.4	0.6	0.5	0.4	0.3	<LOD	5.6	0.4	5.2	2.2	2.8	1.3	0.5	0.6	0.2	<LOD
Clofibrilic acid	0.5	1.4	<LOD	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.7	0.4	0.8	1.5	2.2	0.9	0.4	0.9	0.3	0.2
Furosemide	8.4	7.4	9.5	1.0	1.0	6.5	6.1	5.3	4.9	1.8	10.6	<LOD	8.2	5.7	29.8	4.5	6.1	11.0	2.3	<LOD
Hydrochlorothiazide	3.8	4.1	1.8	1.8	1.4	3.5	2.1	3.0	2.6	<LOD	20.7	4.1	41.0	63.1	167.6	38.8	7.9	3.8	2.0	<LOD

**Table 4. Concentrations of target PhACs in Cadiz water samples (ng L<sup>-1</sup>). Only those compounds that were detected are shown here.**

Compound	E0	E6	E8	P1	R7	S8	T6	U2	U4	U8	V1	W2	W6	W7	X3	X5	Y4
Doxycycline	<LOD	<LOD	<LOD	3.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oxytetracycline	<LOD	<LOD	<LOD	2.3	<LOD	<LOD	2.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tetracycline	1.4	0.7	1.7	1.6	2.2	1.4	1.9	1.4	0.7	0.9	1.8	1.5	1.5	0.8	1.3	1.1	2.4
Tiamulin	0.7	0.7	0.8	0.6	1.0	1.2	4.5	0.8	1.0	0.8	0.7	0.5	0.6	0.7	0.5	1.1	0.6
Chloramphenicol	3.0	1.9	2.4	2.2	1.7	2.0	<LOD	5.0	2.7	6.0	1.5	<LOD	<LOD	4.3	2.8	2.9	2.1
Erythromycin	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Lincomycin	0.2	0.5	0.2	0.2	0.7	0.3	0.4	0.5	0.3	0.6	0.3	0.3	0.2	0.1	0.5	0.2	0.2
Clindamycin	1.2	1.3	1.4	1.0	1.3	2.0	3.2	1.2	1.5	1.3	1.2	1.0	1.1	1.6	0.9	9.6	0.8
Sulfamethazine	0.3	0.9	0.4	0.3	0.3	0.4	0.3	0.5	0.3	0.2	0.3	0.1	0.2	0.3	0.5	0.1	0.5
Sulfamethizole	<LOD	0.1	<LOD	<LOD	0.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sulfathiazole	0.4	0.5	0.3	0.3	0.4	0.4	0.3	0.4	0.5	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sulfadiazine	0.54	0.3	0.1	0.2	0.3	0.5	0.3	0.2	0.3	0.3	0.3	0.1	0.1	0.1	0.3	0.2	0.1
Sulfamethoxazole	<LOD	0.2	<LOQ	<LOD	0.2	<LOD	<LOD	0.1	0.1	<LOD	<LOD	<LOD	<LOD	<LOD	0.1	<LOD	<LOD
Sulfamethoxypyridazine	<LOD	0.5	<LOD	<LOD	0.4	<LOD	<LOD	<LOQ	0.2	<LOQ	<LOD	<LOD	<LOD	<LOD	0.1	<LOD	<LOD
Sulfadimethoxine	0.4	0.7	0.3	0.4	0.7	0.5	0.3	0.5	0.5	0.5	0.3	0.3	0.3	0.4	0.4	0.3	0.4
Norfloxacin	8.7	5.8	2.9	5.2	1.9	<LOD	8.6	1.2	2.6	6.1	<LOD	2.1	2.3	3.8	<LOD	4.9	1.0
Ofloxacin	3.1	3.2	2.5	4.2	<LOD	2.8	8.5	3.5	3.1	4.8	2.1	1.3	<LOD	1.7	<LOD	4.2	<LOD
Ciprofloxacin	<LOD	<LOD	<LOD	2.2	<LOD	<LOD	6.9	4.6	<LOD	6.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Enrofloxacin	<LOD	<LOD	3.4	3.2	<LOD	<LOD	9.1	<LOD	<LOD	<LOD	3.1	<LOD	15.4	<LOD	1.9	6.7	<LOD
Danofloxacin	1.4	1.8	2.8	5.0	3.0	<LOD	10.0	3.4	4.1	6.0	2.6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Metronidazole	8.2	11.1	7.7	6.5	13.6	9.7	7.8	14.8	11.2	7.5	9.4	7.1	6.0	9.3	7.0	5.4	8.5
Ornidazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.4	0.1	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD
Nitrofurantoin	<LOD	<LOD	<LOD	<LOD	12.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	9.6	<LOD	<LOD
Trimethoprim	<LOD	0.3	<LOD	<LOD	<LOD	<LOD	1.9	0.5	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.3	<LOD
Monensin	0.1	0.1	0.2	0.3	0.3	0.2	0.3	0.3	0.1	0.2	0.6	0.4	0.3	0.2	0.3	1.0	0.3
Triclocarban	0.9	0.4	0.4	1.1	0.8	0.7	<LOD	1.0	0.7	0.3	<LOD	<LOQ	0.2	<LOD	1.3	0.7	0.4
Triclosan	3.6	0.6	<LOQ	3.6	6.3	6.9	4.9	16.1	1.0	2.8	1.3	4.2	1.9	0.02	4.6	11.3	5.7
Acetaminophen	2.6	<LOQ	8.0	5.4	0.6	<LOD	6.4	<LOQ	<LOD	<LOQ	3.9	7.1	2.6	<LOD	2.6	<LOQ	<LOD
Diclofenac	12.5	3.9	3.1	9.0	12.9	16.0	12.1	4.1	5.1	13.5	21.2	<LOD	9.1	4.2	26.0	6.1	27.6
Fenoprofen	31.9	<LOD	7.7	4.4	8.3	29.6	11.7	17.7	2.6	16.7	20.2	<LOD	<LOD	14.8	5.8	<LOD	25.7
Ibuprofen	4.5	<LOQ	18.3	4.4	3.4	<LOQ	4.5	2.8	<LOD	<LOQ	7.8	6.3	3.5	<LOQ	4.9	<LOQ	3.7
Indomethacin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOQ	3.79

Mefenamic acid	25.5	4.7	15.9	12.0	14.9	20.3	24.0	15.7	7.6	15.2	27.6	17.5	16.3	11.2	18.9	13.3	19.3
Naproxen	1.3	<LOD	2.6	2.2	1.0	0.9	2.6	1.2	<LOD	<LOD	2.3	2.5	2.1	<LOD	1.7	1.3	<LOD
Phenylbutazone	3.6	<LOD	<LOQ	<LOQ	<LOQ	<LOQ	4.7	7.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Salicylic acid	7.7	<LOQ	12.6	12.9	6.6	15.8	12.4	5.5	<LOQ	6.3	13.6	13.	12.9	6.6	13.5	11.	14.4
Atenolol	0.4	0.3	0.9	0.6	0.9	0.2	0.8	0.6	0.2	0.3	0.6	0.7	0.4	0.3	0.4	0.2	2.8
Carbamazepine	0.3	0.3	0.3	0.2	0.3	0.4	0.3	0.2	0.4	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.2
Caffeine	11.9	12.3	24.3	15.9	37.9	15.6	20.8	11.7	9.8	14.2	17.3	23.0	42.9	11.8	16.8	14.6	15.21
Gemfibrozil	2.1	0.8	4.2	3.2	2.4	2.1	5.0	1.0	0.7	2.4	3.4	4.8	5.8	1.2	3.9	1.9	1.7
Bezafibrate	<LOD	0.1	0.3	0.3	0.1	0.1	0.3	0.1	0.1	0.2	0.2	0.3	0.1	<LOD	0.2	<LOD	<LOD
Clofibric acid	0.7	0.2	1.3	0.2	0.7	0.4	0.9	0.5	0.4	0.5	0.7	<LOD	0.6	0.4	0.4	0.3	0.8
Furosemide	0.7	<LOD	0.2	<LOD	0.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.0	1.6	<LOD
Hydrochlorothiazide	0.3	<LOD	1.8	1.4	0.6	<LOD	1.4	<LOD	<LOD	<LOD	1.1	1.8	1.6	0.3	1.6	1.3	1.0