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1 **Simultaneous determination of 42 pharmaceuticals in seafood samples by solvent**
2 **extraction coupled to liquid chromatography - tandem mass spectrometry**

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16 **Keywords:** pharmaceuticals / emerging pollutants / seafood / fish tissue / multi-residue
17 method / liquid chromatography / tandem mass spectrometry

18 **Highlights**

- 19 • A multi-residue determination of 42 pharmaceuticals in seafood is presented
- 20 • A very simple methanol extraction combined with an LC-MS/MS analysis is proposed
- 21 • Validation data including hake, red mullet, sole and shrimp matrices are provided
- 22 • Commercial seafood muscles from the Bay of Biscay (France) have been analysed
- 23 • 4 compounds were detected, Caffeine exhibited the highest concentration

24 **Abstract**

25 An efficient and sensitive analytical method based on liquid chromatography - tandem mass
26 spectrometry (LC-MS/MS) has been developed and validated for the simultaneous
27 determination of 42 pharmaceuticals belonging to different therapeutic classes (i.e.
28 antibiotics, analgesics, anti-inflammatories, cardiovascular agents, anxiolytics and human
29 indicators) in seafood samples. **The very simple sample preparation included analytes**
30 **extraction with acidified methanol, concentration by evaporation and filtration of the final**
31 **extract prior to LC-MS/MS analysis.** Analytical performances were evaluated in muscles of four
32 commercial species (hake, red mullet, sole and shrimp) and showed good recoveries at two
33 spiked concentration levels, with relative standard deviations below 45%. Limits of
34 quantification ranged from 0.1 to 40.2 ng/g. This procedure has been successfully applied to
35 the determination of the target analytes in seafood collected from the Bay of Biscay (Southern
36 France) and 4 of these 42 pharmaceuticals were detected at low ng/g levels, suggesting a very
37 limited contamination.

38 **1. Introduction**

39 Pharmaceuticals are a large group of chemicals that are daily used for human and veterinary
40 medicine. This group of anthropogenic chemicals is among the ones with the largest input into
41 the environment (Petrovic, Perez, & Barcelo, 2013). As pharmaceuticals consumption is
42 continuously increasing (Nikolaou, Meric, & Fatta, 2007), it raises concerns about their impact
43 on the environment and undesired physiological effects they can cause to aquatic organisms
44 (Althakafy, Kulsing, Grace, & Marriott, 2018; Besse & Garric, 2008; Fent, Weston, & Caminada,
45 2006; Länge & Dietrich, 2002; Ramirez et al., 2009; Zeilinger et al., 2009). Residues of several
46 pharmaceuticals have been found in surface and ground waters, soils and animal tissues
47 across the world at concentrations depending upon the pharmaceutical and the nature and
48 proximity of sources (Álvarez-Muñoz et al., 2015; Gaw, Thomas, & Hutchinson, 2014). Certain
49 painkillers, antimicrobials, antidepressants, contraceptives and antiparasitics are commonly
50 found (European Commission, 2019). Some antibiotic residues detected in food can have
51 negative effects on consumer health and safety (Chiesa et al., 2018). In addition, several
52 pharmaceuticals, such as diclofenac (anti-inflammatory), have been identified as priority
53 substances for regulation at EU level via the Water Framework Directive (Official Journal of
54 the European Union, 2013).

55 Consequently, there is a growing need to develop reliable analytical methods that enable
56 rapid, robust, sensitive and selective determination of these emerging pollutants at trace
57 levels in seafood.

58 In recent decades, pressurized liquid extraction (PLE) (Huerta, Jakimska, Gros, Rodríguez-
59 Mozaz, & Barceló, 2013), ultrasonic-assisted extraction (UAE) (Liu, Hu, Bao, & Yin, 2018),
60 solvent-based extraction (SLE) (Bayen, Estrada, Juhel, & Kelly, 2015), microwave-assisted

61 extraction (MAE) (Guedes-Alonso, Sosa-Ferrera, & Santana-Rodríguez, 2017), matrix solid-
62 phase dispersion (MSPD) (Hertzog, Soares, Caldas, & Primel, 2015) or QuEChERS (Lopes,
63 Reyes, Romero-González, Vidal, & Frenich, 2012) have been used for the extraction of organic
64 pollutants from solid complex samples. After extraction, pharmaceuticals are commonly
65 analysed by liquid chromatography - tandem mass spectrometry (LC-MS/MS) methods using
66 an **electrospray ionization source (ESI)** for the simultaneous determination of a wide-range of
67 polar compounds.

68 Previous studies used protocols which allowed to characterize pharmaceuticals with method
69 detection limits (MDL) reaching the low ng/g range (Guidi et al., 2018; Luo et al., 2018).
70 However, most of the published methods only focused on a short range of compounds,
71 antibiotics being the most frequent class reported. So, there is still a need to develop universal
72 methods applicable to different matrices and able to cover a large range of compounds with
73 different physicochemical properties, which is challenging as it generally requires a
74 compromise in the selection of experimental conditions (Petrovic et al., 2010).

75 The presence of undesirable sample components that co-elute with the analytes, altering the
76 ionization process and thus the signal is the main drawback associated to LC-MS/MS methods
77 (Gracia-Lor, Sancho, & Hernández, 2011). Matrix effects may lead to a suppression or an
78 enhancement of the signal, which can result in a wrong analytes quantification. **Matrix effects**
79 **depend on each analyte/matrix combination, but also on the sample preparation, the**
80 **chromatographic separation, mass spectrometry instrumentation and the ionization**
81 **conditions (Gosetti, Mazzucco, Zampieri, & Gennaro, 2010). The evaluation of matrix effects**
82 **should be included in the validation process of the method considering the different matrices**
83 **studied. Several strategies were proposed to solve matrix effects, including modifications of**

84 sample pre-treatment, chromatographic or MS conditions and calibration techniques (Gosetti
85 et al., 2010). The use of deuterated internal standards is, by far, the most used in the
86 pharmaceutical residues analysis field (Gracia-Lor et al., 2011; Gros, Petrović, & Barceló,
87 2006).

88 The objective of the present study was to develop a multi-residue analytical methodology
89 based on a simple solvent extraction protocol followed by LC-MS/MS detection for the
90 simultaneous analysis of 42 pharmaceuticals commonly used for human and veterinary
91 purposes (including antibiotics, analgesics, anti-inflammatories, cardiovascular agents,
92 anxiolytics and human indicators) in seafood, in order to provide a routine method for the
93 monitoring of these emerging contaminants. The sample preparation was optimized by testing
94 different extraction solvent and studying target compounds retrieval after several evaporation
95 techniques and filtration of the final extract with different kinds of filters. Matrix effects and
96 analytical performances of the optimal procedure were evaluated. Finally, the developed
97 method was successfully applied to investigate occurrence of target pharmaceuticals in
98 muscles of four seafood species with commercial interest collected from the Bay of Biscay
99 (Southern France). Since several compounds were detected in the water leaving the local
100 wastewater treatment plant (Miossec, Lancelleur, & Monperrus, 2019), the present study
101 allowed to investigate the potential bioaccumulation of these pharmaceuticals in the marine
102 biota.

103 2. Materials and Methods

104 2.1 Reagents and materials

105 Reference standards of pharmaceuticals were purchased from Sigma Aldrich (Saint-Louis,
106 USA). All standard references were of analytical grade (>98%). 42 compounds were studied:
107 acetaminophen, acetazolamide, acetylsalicylic acid, amiodarone, amoxicillin, ampicillin,
108 atenolol, azithromycin, caffeine, carbamazepine, ciprofloxacin, clarithromycin,
109 cyclophosphamide, diclofenac, erythromycin A, flumequine, gemfibrozil, hydrochlorothiazide,
110 ibuprofen, josamycin, ketoprofen, lorazepam, losartan, metoprolol, metronidazole, niflumic
111 acid, nordiazepam, 19-norethindrone, norfloxacin, ofloxacin, oxazepam, oxolinic acid,
112 phenazone, piperacillin, roxithromycin, spiramycin, sulfadiazine, sulfamethazine,
113 sulfamethoxazole, tetracycline, trimethoprim and tylosine. Isotopically labelled Atenolol-d7,
114 carbamazepine-d10, ibuprofen-d3, nordiazepam-d5 and ofloxacin-d3 were used as internal
115 standards and purchased from Sigma Aldrich. **These five internal standards have been chosen**
116 **because they were deuterated analog of compounds of interest. They were very chemically**
117 **similar as a majority of compounds as they had the same functional groups.**

118 MeOH, acetonitrile (ACN) (LC-MS grade) and acetone (laboratory reagent, 99.5%) were
119 supplied by Fisher (Hampton, USA). Formic acid (98-100%) and acetic acid (99.8-100.5%) were
120 purchased from Sigma Aldrich. Ultrapure water was obtained with a PURELAB Classic water
121 purification system from Veolia (Paris, France).

122 Standards stock solutions were prepared at 1,000 mg/L for each compounds in methanol
123 (MeOH) and stored in the dark at -20°C. A multicomponent solution containing the 42
124 compounds was prepared monthly at 10 mg/L in MeOH and stored in the dark at -20°C. A
125 solution containing internal standards (atenolol-d7, carbamazepine-d10, ibuprofen-d3,
126 nordiazepam-d5 and ofloxacin-d3) at 2, 2, 50, 2 and 10 mg/L respectively was prepared by
127 diluting the stock solutions in MeOH, stored in the dark at -20°C and prepared monthly.

128 **2.2 Samples collection and pre-treatment**

129 Three fish species hake (*Merluccius merluccius*), red mullet (*Mullus surmuletus*) and sole
130 (*Solea solea*) and one crustacean species shrimp (*Palaemon serratus*) were collected from the
131 coastal area of the Bay of Biscay (Southern France). For fish, the white dorsal muscle was
132 separated (skin and bone excluded) and for shrimp, the abdomen muscle was separated.
133 Muscles were then freeze-dried with a VaCo2 lyophilizer (Zirbus, Bad Grund, Germany),
134 grinded to a homogeneous powder using a glass mortar and stored at -80 °C prior to extraction
135 and analysis.

136 **2.3 Sample preparation**

137 An aliquot of 0.2 g of freeze-dried sample was weighed into a 50 mL polypropylene tube.
138 Internal standards atenolol-d7, carbamazepine-d10, ibuprofen-d3, nordiazepam-d5 and
139 ofloxacin-d3 were added at concentrations of 400, 400, 10,000, 400, and 2000 ng/g,
140 respectively, to each sample, by adding 40 µL of the multicomponent solution. After addition
141 of 10 mL of extraction solvent (MeOH +1% acetic acid), the tube was shaken by vortex for 1
142 min. The tube was then centrifuged for 5 min at 4,500 rpm and the supernatant was
143 transferred into 10 mL glass tubes, evaporated to dryness under a gentle air stream using a
144 TurboVap LV Evaporator system (Zymark, Hopkinton, USA), and dissolved in 1mL MeOH/water
145 (5/95 v/v). Finally, extracts were vortexed a few seconds, filtered through 0.2 µm
146 polytetrafluoroethylene (PTFE) syringe filters, transferred into vials and kept at -20°C until
147 analysis (Fig. S1).

148 **2.4 LC-MS/MS analysis**

149 Analysis were performed by LC-MS/MS using an Acquity UPLC system (Waters) connected to
150 a Xevo TQ MS triple quadrupole with an electrospray source (ESI) interface (Waters). A C18
151 Acquity UPLC HSS T3 (1.8 μ m particle size, 50mm x 2.1mm i.d.,) column (Waters) preceded by
152 a guard column (1.8 μ m particle size, 5mm x 2.1mm i.d.,) of the same packing material was
153 used at a flow rate of the mobile phase 0.4 mL/min. The column temperature was fixed at
154 40°C and sample manager was maintained at 15°C. Sample injection volume was 5 μ L. Two
155 injections were used for the quantification of all compounds both in positive and negative
156 ionization mode. The analysis in positive mode was performed using ultrapure water with
157 0.1% formic acid as eluent (A) and ACN as eluent (B). In negative mode, eluent (A) was
158 ultrapure water with 0.01% formic acid and eluent (B) was ACN. For both modes, the initial
159 composition was 2% (B) during 2 min and increased linearly to reach 60% at 4 min and 100%
160 at 6 min. It held 1 min before returning to the initial composition (2% B) at 7.1 min and held
161 for 3 min. The total analysis run time was then 10 min (See example of chromatograms in
162 Supporting Information Fig. S2).

163 For the mass spectrometer, cone gas and desolvation gas flows were set at 10 and 600 L/h,
164 respectively. Drying gas, as well as nebulizing gas were nitrogen, generated by pressurized air
165 in a Nitrocraft nitrogen generator (Air Liquide). Source temperature was set to 150°C and
166 desolvation temperature to 600°C. Capillary voltages of 0.5 kV (positive ionization mode) and
167 -1.0 kV (negative ionization mode) were applied. Collision gas was Argon with a purity >
168 99.999% (Linde). Waters MassLynx software was used for the instrument control, data
169 acquisition and data treatment. Quantification was carried out in Multiple Reaction
170 Monitoring (MRM) mode, selecting two characteristics transitions for each compound. Table

171 1 presents MRM transitions, retention times, ion ratios and internal standards for each
172 compound.

173 **2.5 Quantification and quality control**

174 Matrix-matched calibrations with deuterated analogs of the target analytes were used to
175 quantify target compounds. Six-point calibration curves were performed with 0.2 g of
176 different seafood species spiked with increasing pollutants concentration levels ranging from
177 0 to 500 ng/g as well as internal standards at various concentration (described in 2.3), and the
178 optimized procedure was carried out.

179 Analytical performances and quality control were evaluated for each run by solvent blanks
180 (MeOH/water 5/95 v/v) and procedural blanks to evaluate contamination and detection
181 limits. Accuracy was also evaluated using matrix spikes at 2 levels (20 and 200 ng/g) to
182 determine compound recoveries.

183 **3. Results and discussion**

184 **3.1 Sample preparation optimization**

185 **3.1.1 Extraction solvent**

186 The choice of the appropriate solvent is a crucial step in the sample pretreatment procedure
187 to extract the desired analytes with minimum coextraction of matrix interferences (Kung, Tsai,
188 Ku, & Wang, 2015). To date, various extraction solvents have been used for the extraction of
189 pharmaceuticals: MeOH (Hertzog et al., 2015), ACN (Freitas et al., 2014; Saxena et al., 2018),
190 MeOH/ACN mixture (Bayen et al., 2015; Kim, Lee, & Oh, 2017; Ondarza, Haddad, Avigliano,
191 Miglioranza, & Brooks, 2019) and some organic solvents with acetic acid acidification (Mu et
192 al., 2016; Yao et al., 2016). The present approach aims to develop a method for the extraction

193 of a maximum of target compounds (which includes weakly basic and weakly acidic molecules)
194 in one single step.

195 Effects of several extraction solvents such as ACN, MeOH and MeOH + 1% acetic acid on
196 extraction efficiencies were evaluated. A lyophilized hake muscle was spiked with 200 ng/g of
197 all target compounds (by adding 40 μ L of a 1 mg/L multicomponent solution) prior to the
198 extraction step. Comparison of peak areas indicated that extraction with ACN led to poor
199 recoveries for some compounds, and especially for the least polar, in accordance with the fact
200 that ACN is more polar than MeOH (Fig. 1). Recoveries of target molecules were higher for 34
201 compounds out of 42 when extracted with MeOH compared to extraction with ACN. **Among**
202 **MeOH and acidified MeOH, even if extraction rates were globally similar, acidification**
203 **provided slightly higher responses. This result was expected, as the extraction of polar**
204 **compounds is pH dependent, and compounds which are carrying a carboxylic group (i.e.**
205 **ampicillin, norfloxacin, ofloxacin, ciprofloxacin...) are expected to present best recoveries in**
206 **acidic conditions.** Consequently, MeOH + 1% acetic acid was selected as the optimal solvent
207 to extract all target analytes in one single step.

208 **3.1.2 Evaporation and filtration**

209 The evaporation stage could be a critical step, especially for the most volatile compounds,
210 which may be lost. Retrievals of our target compounds after evaporation under different
211 conditions has previously been investigated (Miossec et al., 2019), and evaporation to dryness
212 under air stream at room temperature was chosen as a gentle evaporation method in order
213 to concentrate while preserving molecules as much as possible. The quantification with a
214 matrix-matched calibration using internal standards allows to correct for losses observed for
215 some analytes.

216 When injecting tissues extracts into an HPLC column, suspended particles may affect
217 chromatographic performances, by creating interferences with target compounds, or by
218 fouling even clogging the column. Therefore, the filtration of the final extract is an essential
219 step. To date, different filtration materials have been indifferently used : PTFE (Bayen et al.,
220 2015; Rodrigues et al., 2019; Saxena et al., 2018), polyvinylidene difluoride (PVDF) (Carmona,
221 Andreu, & Picó, 2017; Freitas et al., 2014) and nylon (Lopes et al., 2012; Zhao et al., 2017).
222 Various types of syringe filters were tested in order to study the potential loss by adsorption
223 of target compounds, which may occur during the filtration step (and eventually leading to an
224 underestimation of final results). A solution containing all the target compounds at 100 µg/L
225 in MeOH/water (5/95 v/v) was filtered through 0.2 µm PTFE, PVDF and nylon syringe filters
226 and then analysed following the above described LC-MS/MS method. Responses obtained for
227 pharmaceutical compounds were compared with those obtained from the analysis of the
228 same spiked non-filtered solution (Fig. 2).

229 It appeared that six compounds (losartan, ketoprofen, amiodarone, niflumic acid, ibuprofen
230 and gemfibrozil) were completely retained by the nylon syringe filter, and that three
231 compounds (spiramycin, azithromycin and amiodarone) were totally retained by the PVDF
232 syringe filter, meanwhile none of them was fully retained when using the PTFE filter.
233 Antibiotics presented global low recoveries after PVDF filtration (ofloxacin, clarithromycin,
234 roxithromycin and josamycin below 20%). In summary, PTFE (0.2 µm) syringe filters seems to
235 be the best choice for the filtration of these final extracts intended for the analysis of this kind
236 of pharmaceuticals, showing satisfactory results for 30 out of the 42 tested compounds
237 (recovery >50%).

238 **3.2 Method validation**

239 3.2.1 Matrix effects evaluation

240 Matrix effects causing signal suppression or enhancement are mainly due to matrix
241 compounds eluted with the same retention time as the target compounds (Rogatsky & Stein,
242 2005; Stüber & Reemtsma, 2004). They also depend on the matrix nature, the efficiency of the
243 sample preparation step, the detection response and the chromatographic behaviour.
244 Therefore, interfering compounds should be eliminated during the sample preparation while
245 the analytes should be conserved. In this work, in order to evaluate the matrix effects,
246 different seafood matrices (hake, red mullet, sole and shrimp) were spiked by adding 200 ng/g
247 of the target compounds and subjected to the optimized procedure.

248 Matrix effects (ME, %) were calculated according to:

$$249 \text{ME}_x (\%) = \left(\frac{A_x(\text{matrix}) - A_x(\text{blank})}{A_x(\text{solvent})} - 1 \right) \times 100$$

250 Where $A_x(\text{matrix})$ is the area of the compound x in the spiked matrix, $A_x(\text{blank})$ is the area of
251 the compound x in the non-spiked matrix and $A_x(\text{solvent})$ is the area of the compound x in the
252 spiked procedural blank.

253 Table 2 gives the results for all the target analytes. An enhancement of the signal leading to a
254 positive value indicates a positive matrix effect. A signal suppression leading to a negative
255 value corresponds to a negative matrix effect.

256 Matrix effects were different within the evaluated compounds and also within the different
257 types of seafood. ME ranged from -83%, the highest signal suppression (acetaminophen and
258 acetylsalicylic acid in shrimp), to +2,191%, the highest signal enhancement (amiodarone in
259 hake). As expected, signal suppression has been observed to be higher in red mullet muscle

260 than in the three other matrices, probably because of higher fat content level. As the 5
261 selected internal standards didn't cover the behaviour of the 42 molecules, both the addition
262 of deuterated internal standards and the realization of matrix-matched calibrations are
263 therefore mandatory to balance matrix effects and correctly quantify all the molecules.

264 3.2.2 Method performances

265 Analytical performances of the optimized method are reported in Table 3. Concerning
266 linearity, equations and R² were calculated in the range 0-500 ng/g. Limits of quantification
267 (LOQ) were determined as lowest injected compound concentrations in matrix that yielded a
268 signal-to-noise (S/N) ratio of 10.

269 Recoveries (R, %) were also determined at two concentration levels according to:

$$270 R (\%) = \frac{C(spiked) - C(blank)}{C(ref)} \times 100$$

271 Where C(spiked) is the concentration in the spiked matrix, C(blank) is the concentration in the
272 non-spiked matrix and C(ref) is the theoretical added concentration. Precision was expressed
273 as the relative standard deviation (RSD) of 3 replicates.

274 Coefficients of determination were higher than 0.99 for all compounds except amoxicillin in
275 red mullet, acetylsalicylic acid and ibuprofen in sole, amoxicillin and acetylsalicylic acid in
276 shrimp, demonstrating that the method is linear in the range assayed.

277 Limits of quantification ranged from 0.1 to 40.2 ng/g with most of the molecules between 0.1
278 and 5.0 ng/g. Amoxicillin, acetylsalicylic acid and ibuprofen exhibited lower sensitivities in the
279 four biologic matrices related to their lower MS detection sensitivities.

280 Recoveries achieved for all target compounds at spiking level 20 ng/g ranged from 29% to
281 164% in hake muscle, from 33% to 128% in red mullet muscle, from 28% to 188% in sole

282 muscle and from 26% to 132% in shrimp muscle. Recoveries achieved for all target compounds
283 at spiking level 200 ng/g ranged from 69% to 131% in hake muscle, from 51% to 114% in red
284 mullet muscle, from 45% to 119% in sole muscle and from 64% to 117% in shrimp muscle.

285 RSDs at spiking level 20 ng/g ranged from 1.4% (atenolol) to 62.9% (piperacillin) in hake, from
286 1.2% (sulfamethazine) to 25.5% (ibuprofen) in red mullet, from 0.1% (erythromycin A) to
287 52.7% (amoxicillin) in sole and from 2.8% (cyclophosphamide) to 87.4% (acetylsalicylic acid) in
288 shrimp with mean RSDs at 17.7, 11.2, 15.6, and 17.1 respectively. RSDs at spiking level 200
289 ng/g ranged from 1.3% (nordiazepam) to 39.4% (ibuprofen) in hake, from 0.6% (losartan) to
290 38.2% (piperacillin) in red mullet, from 1.2% (sulfamethoxazole) to 43.1% (erythromycin A) in
291 sole and from 3.4% (metronidazole) to 26.8% (acetylsalicylic acid) in shrimp with mean RSDs
292 at 10.7, 10.6, 13.7, and 11.2 respectively. High variability was observed for the precision
293 between compounds and between matrices. In a general way, RSD were found lower at
294 spiking level 200 ng/g compared to 20 ng/g. No trend was observed according to compounds
295 family. Compounds exhibiting the highest RSDs were generally the compounds which had the
296 lower sensitivity.

297 As a result, a sensitive, reliable and repeatable analytical method for the quantitative
298 determination of pharmaceutical residues in seafood samples was established and validated.

299 **3.3 Application to real seafood samples**

300 The optimized and validated methodology was applied to analyse seafood muscles (hake, red
301 mullet, sole and shrimp) collected from the coastal area of the Bay of Biscay (Southern France).
302 Four of the 42 molecules (azithromycin, clarithromycin, acetaminophen and caffeine) were
303 detected at concentrations above the LOQ at least once (Table 4). Caffeine (human indicator)

304 was found in all samples, which is in accordance with literature (Álvarez-Muñoz et al., 2015).
305 Concentrations ranged from the LOQ to 11.4 ng/g (caffeine in shrimp muscle). The two
306 antibiotics (azithromycin and clarithromycin) were only measured in red mullet and sole at
307 concentrations below 1.0 ng/g. The analgesic (acetaminophen) was measured once in hake
308 with a very low concentration (1.4 ng/g).

309 As seafood species considered in this study were caught in the open sea, these low
310 occurrences and concentrations (low ng/g range) were expected and are in agreement with
311 previous studies (Chiesa et al., 2018; Hertzog et al., 2015; Wang & Gardinali, 2012; Zhao et al.,
312 2017). In addition, several pharmaceuticals such as diclofenac and ibuprofen undergo an
313 efficient biotransformation into glucuronide metabolites in the fish bile before being excreted.
314 Therefore, muscle is not the target organ for bioconcentration and metabolism of these
315 contaminants (Lahti, Brozinski, Jylhä, Kronberg, & Oikari, 2011) that coupled with the high
316 dilution effect in the open sea, could explain these obtained results.

317 **4. Conclusions**

318 An efficient and sensitive LC-MS/MS method was successfully developed for the simultaneous
319 quantification of 42 pharmaceutical compounds in seafood. Sample preparation is easy and
320 fast, making it a perfect routine method for the monitoring of those emerging contaminants
321 in seafood samples.

322 Matrix effects were calculated for hake, red mullet, sole and shrimp muscles and resulted in a
323 higher signal suppression in red mullet, the matrix with the highest fat content. Matrix-
324 matched calibrations using deuterated internal standards were used for quantification, which
325 allowed to correct for the matrix effects and obtain acceptable recoveries. Analysis by LC-

326 MS/MS in positive and negative ionization modes provided good sensitivity and selectivity,
327 with limits of quantification ranging from 0.1 to 40.2 ng/g.

328 This method was successfully applied to the determination of target compounds in seafood
329 samples collected from the Bay of Biscay (Southern France). We also suggest that this
330 analytical method could be used as routine method for future environmental and safety
331 monitoring.

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336 **Conflict of interest statement**

337 Authors have declared no conflict of interest.

338 **References**

- 339 Althakafy, J. T., Kulsing, C., Grace, M. R., & Marriott, P. J. (2018). Determination of selected emerging
340 contaminants in freshwater invertebrates using a universal extraction technique and liquid
341 chromatography accurate mass spectrometry. *Journal of Separation Science*, *41*(19), 3706–
342 3715. <https://doi.org/10.1002/jssc.201800507>
- 343 Álvarez-Muñoz, D., Rodríguez-Mozaz, S., Maulvault, A. L., Tediosi, A., Fernández-Tejedor, M., Van den
344 Heuvel, F., ... Barceló, D. (2015). Occurrence of pharmaceuticals and endocrine disrupting
345 compounds in macroalgae, bivalves, and fish from coastal areas in Europe. *Environmental*
346 *Research*, *143*(Pt B), 56–64. <https://doi.org/10.1016/j.envres.2015.09.018>
- 347 Bayen, S., Estrada, E. S., Juhel, G., & Kelly, B. C. (2015). Direct injection of tissue extracts in liquid
348 chromatography/tandem mass spectrometry for the determination of pharmaceuticals and
349 other contaminants of emerging concern in mollusks. *Analytical and Bioanalytical Chemistry*,
350 *407*(19), 5553–5558. <https://doi.org/10.1007/s00216-015-8760-9>
- 351 Besse, J.-P., & Garric, J. (2008). Human pharmaceuticals in surface waters. Implementation of a
352 prioritization methodology and application to the French situation. *Toxicology Letters*,
353 *176*(2), 104–123. <https://doi.org/10.1016/j.toxlet.2007.10.012>
- 354 Carmona, E., Andreu, V., & Picó, Y. (2017). Multi-residue determination of 47 organic compounds in
355 water, soil, sediment and fish-Turia River as case study. *Journal of Pharmaceutical and*
356 *Biomedical Analysis*, *146*, 117–125. <https://doi.org/10.1016/j.jpba.2017.08.014>
- 357 Chiesa, L., Panseri, S., Pasquale, E., Malandra, R., Pavlovic, R., & Arioli, F. (2018). Validated multiclass
358 targeted determination of antibiotics in fish with high performance liquid chromatography-
359 benchtop quadrupole orbitrap hybrid mass spectrometry. *Food Chemistry*, *258*, 222–230.
360 <https://doi.org/10.1016/j.foodchem.2018.03.072>
- 361 European Commission. (2019). *Communication from the commission to the european parliament, the*
362 *council and the european economic and social committee. Brussels, 11.3.2019.*

363 Fent, K., Weston, A. A., & Caminada, D. (2006). Ecotoxicology of human pharmaceuticals. *Aquatic*
364 *Toxicology (Amsterdam, Netherlands)*, 76(2), 122–159.
365 <https://doi.org/10.1016/j.aquatox.2005.09.009>

366 Freitas, A., Leston, S., Rosa, J., Castilho, M. da C., Barbosa, J., Rema, P., ... Ramos, F. (2014). Multi-
367 residue and multi-class determination of antibiotics in gilthead sea bream (*Sparus aurata*) by
368 ultra high-performance liquid chromatography-tandem mass spectrometry. *Food Additives &*
369 *Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 31(5), 817–
370 826. <https://doi.org/10.1080/19440049.2014.891764>

371 Gaw, S., Thomas, K. V., & Hutchinson, T. H. (2014). Sources, impacts and trends of pharmaceuticals in
372 the marine and coastal environment. *Philosophical Transactions of the Royal Society B:*
373 *Biological Sciences*, 369(1656). <https://doi.org/10.1098/rstb.2013.0572>

374 Gosetti, F., Mazzucco, E., Zampieri, D., & Gennaro, M. C. (2010). Signal suppression/enhancement in
375 high-performance liquid chromatography tandem mass spectrometry. *Journal of*
376 *Chromatography. A*, 1217(25), 3929–3937. <https://doi.org/10.1016/j.chroma.2009.11.060>

377 Gracia-Lor, E., Sancho, J. V., & Hernández, F. (2011). Multi-class determination of around 50
378 pharmaceuticals, including 26 antibiotics, in environmental and wastewater samples by ultra-
379 high performance liquid chromatography–tandem mass spectrometry. *Journal of*
380 *Chromatography A*, 1218(16), 2264–2275. <https://doi.org/10.1016/j.chroma.2011.02.026>

381 Gros, M., Petrović, M., & Barceló, D. (2006). Development of a multi-residue analytical methodology
382 based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for screening and
383 trace level determination of pharmaceuticals in surface and wastewaters. *Talanta*, 70(4),
384 678–690. <https://doi.org/10.1016/j.talanta.2006.05.024>

385 Guedes-Alonso, R., Sosa-Ferrera, Z., & Santana-Rodríguez, J. J. (2017). Determination of steroid
386 hormones in fish tissues by microwave-assisted extraction coupled to ultra-high performance
387 liquid chromatography tandem mass spectrometry. *Food Chemistry*, 237, 1012–1020.
388 <https://doi.org/10.1016/j.foodchem.2017.06.065>

389 Guidi, L. R., Santos, F. A., Ribeiro, A. C. S. R., Fernandes, C., Silva, L. H. M., & Gloria, M. B. A. (2018).
390 Quinolones and tetracyclines in aquaculture fish by a simple and rapid LC-MS/MS method.
391 *Food Chemistry*, 245, 1232–1238. <https://doi.org/10.1016/j.foodchem.2017.11.094>

392 Hertzog, G. I., Soares, K. L., Caldas, S. S., & Primel, E. G. (2015). Study of vortex-assisted MSPD and LC-
393 MS/MS using alternative solid supports for pharmaceutical extraction from marketed fish.
394 *Analytical and Bioanalytical Chemistry*, 407(16), 4793–4803. [https://doi.org/10.1007/s00216-](https://doi.org/10.1007/s00216-015-8685-3)
395 015-8685-3

396 Huerta, B., Jakimska, A., Gros, M., Rodríguez-Mozaz, S., & Barceló, D. (2013). Analysis of multi-class
397 pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem
398 mass spectrometry. *Journal of Chromatography. A*, 1288, 63–72.
399 <https://doi.org/10.1016/j.chroma.2013.03.001>

400 Kim, H.-Y., Lee, I.-S., & Oh, J.-E. (2017). Human and veterinary pharmaceuticals in the marine
401 environment including fish farms in Korea. *The Science of the Total Environment*, 579, 940–
402 949. <https://doi.org/10.1016/j.scitotenv.2016.10.039>

403 Kung, T.-A., Tsai, C.-W., Ku, B. C., & Wang, W.-H. (2015). A generic and rapid strategy for determining
404 trace multiresidues of sulfonamides in aquatic products by using an improved QuEChERS
405 method and liquid chromatography–electrospray quadrupole tandem mass spectrometry.
406 *Food Chemistry*, 175, 189–196. <https://doi.org/10.1016/j.foodchem.2014.11.133>

407 Lahti, M., Brozinski, J.-M., Jylhä, A., Kronberg, L., & Oikari, A. (2011). Uptake from water,
408 biotransformation, and biliary excretion of pharmaceuticals by rainbow trout. *Environmental*
409 *Toxicology and Chemistry*, 30(6), 1403–1411. <https://doi.org/10.1002/etc.501>

410 Länge, R., & Dietrich, D. (2002). Environmental risk assessment of pharmaceutical drug substances—
411 conceptual considerations. *Toxicology Letters*, 131(1–2), 97–104.
412 [https://doi.org/10.1016/S0378-4274\(02\)00071-1](https://doi.org/10.1016/S0378-4274(02)00071-1)

413 Liu, Y.-Y., Hu, X.-L., Bao, Y.-F., & Yin, D.-Q. (2018). Simultaneous determination of 29 pharmaceuticals
414 in fish muscle and plasma by ultrasonic extraction followed by SPE-UHPLC-MS/MS. *Journal of*
415 *Separation Science*, 41(10), 2139–2150. <https://doi.org/10.1002/jssc.201701360>

416 Lopes, R. P., Reyes, R. C., Romero-González, R., Vidal, J. L. M., & Frenich, A. G. (2012). Multiresidue
417 determination of veterinary drugs in aquaculture fish samples by ultra high performance
418 liquid chromatography coupled to tandem mass spectrometry. *Journal of Chromatography*.
419 *B, Analytical Technologies in the Biomedical and Life Sciences*, 895–896, 39–47.
420 <https://doi.org/10.1016/j.jchromb.2012.03.011>

421 Luo, Z., Lu, J., Li, H., Tu, Y., Wan, Y., & Yang, Z. (2018). Air-assisted liquid-liquid microextraction
422 integrated with QuEChERS for determining endocrine-disrupting compounds in fish by high-
423 performance liquid chromatography–tandem mass spectrometry. *Food Chemistry*, 260, 174–
424 182. <https://doi.org/10.1016/j.foodchem.2018.04.007>

425 Miossec, C., Lancelur, L., & Monperrus, M. (2019). Multi-residue analysis of 44 pharmaceutical
426 compounds in environmental water samples by solid phase extraction coupled to liquid
427 chromatography - tandem mass spectrometry. *Journal of Separation Science*.
428 <https://doi.org/10.1002/jssc.201801214>

429 Mu, P., Xu, N., Chai, T., Jia, Q., Yin, Z., Yang, S., ... Qiu, J. (2016). Simultaneous determination of 14
430 antiviral drugs and relevant metabolites in chicken muscle by UPLC–MS/MS after QuEChERS
431 preparation. *Journal of Chromatography B*, 1023–1024, 17–23.
432 <https://doi.org/10.1016/j.jchromb.2016.04.036>

433 Nikolaou, A., Meric, S., & Fatta, D. (2007). Occurrence patterns of pharmaceuticals in water and
434 wastewater environments. *Analytical and Bioanalytical Chemistry*, 387(4), 1225–1234.
435 <https://doi.org/10.1007/s00216-006-1035-8>

436 Official Journal of the European Union. (2013). *DIRECTIVE 2013/39/EU OF THE EUROPEAN*
437 *PARLIAMENT AND OF THE COUNCIL of 12 August 2013*.

438 Ondarza, P. M., Haddad, S. P., Avigliano, E., Miglioranza, K. S. B., & Brooks, B. W. (2019).
439 Pharmaceuticals, illicit drugs and their metabolites in fish from Argentina: Implications for
440 protected areas influenced by urbanization. *The Science of the Total Environment*, *649*, 1029–
441 1037. <https://doi.org/10.1016/j.scitotenv.2018.08.383>

442 Petrovic, M., Farré, M., de Alda, M. L., Perez, S., Postigo, C., Köck, M., ... Barcelo, D. (2010). Recent
443 trends in the liquid chromatography-mass spectrometry analysis of organic contaminants in
444 environmental samples. *Journal of Chromatography. A*, *1217*(25), 4004–4017.
445 <https://doi.org/10.1016/j.chroma.2010.02.059>

446 Petrovic, M., Perez, S., & Barcelo, D. (2013). *Analysis, Removal, Effects and Risk of Pharmaceuticals in*
447 *the Water Cycle* (Vol. 62). Retrieved from [https://www.elsevier.com/books/analysis-removal-](https://www.elsevier.com/books/analysis-removal-effects-and-risk-of-pharmaceuticals-in-the-water-cycle/petrovic/978-0-444-62657-8)
448 [effects-and-risk-of-pharmaceuticals-in-the-water-cycle/petrovic/978-0-444-62657-8](https://www.elsevier.com/books/analysis-removal-effects-and-risk-of-pharmaceuticals-in-the-water-cycle/petrovic/978-0-444-62657-8)

449 Ramirez, A. J., Brain, R. A., Usenko, S., Mottaleb, M. A., O'Donnell, J. G., Stahl, L. L., ... Chambliss, C. K.
450 (2009). Occurrence of pharmaceuticals and personal care products in fish: results of a
451 national pilot study in the United States. *Environmental Toxicology and Chemistry*, *28*(12),
452 2587–2597. <https://doi.org/10.1897/08-561.1>

453 Rodrigues, J., Albino, S., Silva, S., Cravo, A., Cardoso, V. V., Benoliel, M. J., & Almeida, C. M. M. (2019).
454 Development of a Multiresidue Method for the Determination of 24 Pharmaceuticals in
455 Clams by QuEChERS and Liquid Chromatography-Triple Quadrupole Tandem Mass
456 Spectrometry. *Food Analytical Methods*, *12*(4), 838–851. [https://doi.org/10.1007/s12161-](https://doi.org/10.1007/s12161-018-01418-y)
457 [018-01418-y](https://doi.org/10.1007/s12161-018-01418-y)

458 Rogatsky, E., & Stein, D. (2005). Evaluation of matrix effect and chromatography efficiency: new
459 parameters for validation of method development. *Journal of the American Society for Mass*
460 *Spectrometry*, *16*(11), 1757–1759. <https://doi.org/10.1016/j.jasms.2005.07.012>

461 Saxena, S. K., Rangasamy, R., Krishnan, A. A., Singh, D. P., Uke, S. P., Malekadi, P. K., ... Gupta, A.
462 (2018). Simultaneous determination of multi-residue and multi-class antibiotics in

463 aquaculture shrimps by UPLC-MS/MS. *Food Chemistry*, 260, 336–343.
464 <https://doi.org/10.1016/j.foodchem.2018.04.018>

465 Stüber, M., & Reemtsma, T. (2004). Evaluation of three calibration methods to compensate matrix
466 effects in environmental analysis with LC-ESI-MS. *Analytical and Bioanalytical Chemistry*,
467 378(4), 910–916. <https://doi.org/10.1007/s00216-003-2442-8>

468 Wang, J., & Gardinali, P. R. (2012). Analysis of selected pharmaceuticals in fish and the fresh water
469 bodies directly affected by reclaimed water using liquid chromatography-tandem mass
470 spectrometry. *Analytical and Bioanalytical Chemistry*, 404(9), 2711–2720.
471 <https://doi.org/10.1007/s00216-012-6139-8>

472 Yao, L., Zhao, J.-L., Liu, Y.-S., Yang, Y.-Y., Liu, W.-R., & Ying, G.-G. (2016). Simultaneous determination
473 of 24 personal care products in fish muscle and liver tissues using QuEChERS extraction
474 coupled with ultra pressure liquid chromatography-tandem mass spectrometry and gas
475 chromatography-mass spectrometer analyses. *Analytical and Bioanalytical Chemistry*,
476 408(28), 8177–8193. <https://doi.org/10.1007/s00216-016-9924-y>

477 Zeilinger, J., Steger-Hartmann, T., Maser, E., Goller, S., Vonk, R., & Länge, R. (2009). Effects of
478 synthetic gestagens on fish reproduction. *Environmental Toxicology and Chemistry*, 28(12),
479 2663–2670. <https://doi.org/10.1897/08-485.1>

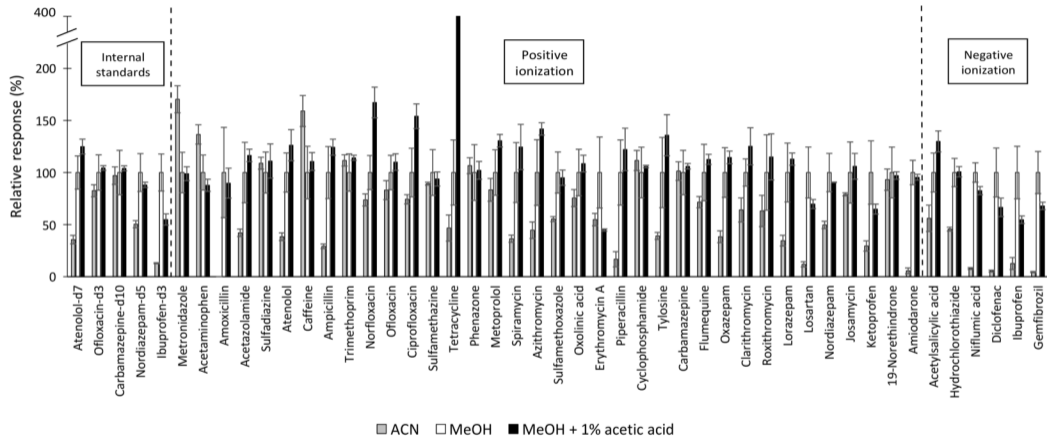
480 Zhao, F., Gao, X., Tang, Z., Luo, X., Wu, M., Xu, J., & Fu, X. (2017). Development of a simple multi-
481 residue determination method of 80 veterinary drugs in *Oplegnathus punctatus* by liquid
482 chromatography coupled to quadrupole Orbitrap mass spectrometry. *Journal of*
483 *Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 1065–1066,
484 20–28. <https://doi.org/10.1016/j.jchromb.2017.09.013>

485

486 **Figure captions**

487 Fig. 1: Influence of extraction solvent (ACN, MeOH, MeOH + 1% acetic acid) on measured areas
488 (matrix: hake muscle, spiking level 200 ng/g, n=3).

489 Fig. 2: Recovery (n=3) of the target compounds after filtration of a spiked solution of
490 MeOH/water (5/95) at 100 µg/L.



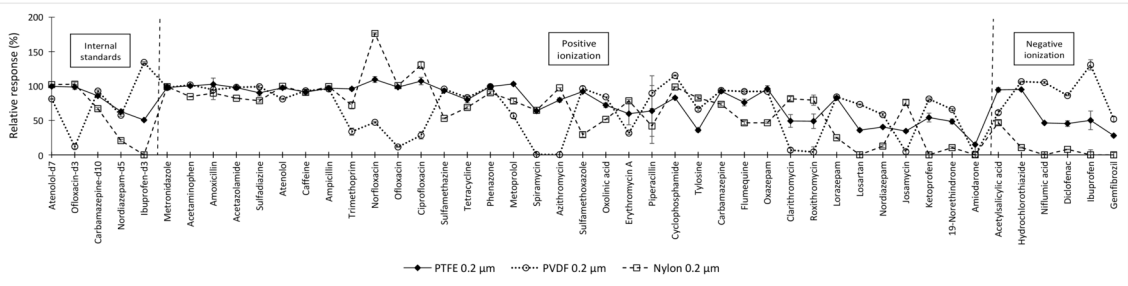


Table 1: MS/MS parameters for the analysis of target analytes by MRM in negative and positive ionization modes.

Compounds	Internal standards	RT (min)	Ionization modes	MRM 1	MRM 2	Ion Ratios (Quant/Qual)
Atenolol-d7	-	3.54	ESI +	274.4 > 145.1	274.4 > 190.3	3.0
Ofloxacin-d3	-	3.91	ESI +	365.4 > 261.1	365.4 > 321.2	1.3
Carbamazepine-d10	-	4.70	ESI +	247.4 > 204.2	247.4 > 186.9	10.7
Nordiazepam-d5	-	4.91	ESI +	276.0 > 140.2	276.0 > 165.1	0.8
Metronidazole	Carbamazepine-d10	2.49	ESI +	172.3 > 128.1	172.3 > 82.0	2.4
Acetaminophen	Carbamazepine-d10	2.69	ESI +	152.0 > 110.1	152.0 > 92.8	4.8
Amoxicillin	Carbamazepine-d10	2.78	ESI +	366.4 > 114.0	366.4 > 349.2	1.3
Acetazolamide	Carbamazepine-d10	3.36	ESI +	223.3 > 180.9	223.3 > 164.0	1.1
Sulfadiazine	Carbamazepine-d10	3.51	ESI +	251.3 > 156.0	251.3 > 92.0	0.8
Atenolol	Atenolol-d7	3.55	ESI +	267.5 > 145.1	267.5 > 190.1	1.8
Caffeine	Carbamazepine-d10	3.83	ESI +	195.4 > 138.1	195.4 > 110.1	3.5
Ampicillin	Carbamazepine-d10	3.84	ESI +	350.4 > 106.0	350.4 > 160.0	2.4
Trimethoprim	Carbamazepine-d10	3.88	ESI +	291.4 > 230.1	291.4 > 123.1	0.9
Norfloxacin	Ofloxacin-d3	3.90	ESI +	320.4 > 276.2	320.4 > 233.1	1.3
Ofloxacin	Ofloxacin-d3	3.91	ESI +	362.4 > 261.2	362.4 > 318.2	1.3
Ciprofloxacin	Ofloxacin-d3	3.93	ESI +	332.4 > 288.1	332.4 > 245.2	1.2
Sulfamethazine	Carbamazepine-d10	4.06	ESI +	279.3 > 186.0	279.3 > 92.0	1.3
Tetracycline	Ofloxacin-d3	4.07	ESI +	445.4 > 410.1	445.4 > 154.0	1.3
Phenazone	Carbamazepine-d10	4.09	ESI +	189.4 > 131.0	189.4 > 55.4	26.1
Metoprolol	Carbamazepine-d10	4.09	ESI +	268.4 > 116.1	268.4 > 121.1	2.7
Spiramycin	Ofloxacin-d3	4.11	ESI +	843.7 > 174.1	843.7 > 142.1	3.4
Azithromycin	Ofloxacin-d3	4.14	ESI +	749.7 > 158.1	749.7 > 591.5	1.1
Sulfamethoxazole	Carbamazepine-d10	4.31	ESI +	254.3 > 92.0	254.3 > 156.0	1.1
Oxolinic acid	Carbamazepine-d10	4.44	ESI +	262.4 > 244.1	262.4 > 216.0	4.7
Erythromycin A	Carbamazepine-d10	4.50	ESI +	734.7 > 158.2	734.7 > 576.5	5.6
Piperacillin	Carbamazepine-d10	4.53	ESI +	518.4 > 143.1	518.4 > 114.7	5.1
Cyclophosphamide	Carbamazepine-d10	4.53	ESI +	261.3 > 140.0	261.3 > 106.0	2.5
Tylosine	Ofloxacin-d3	4.57	ESI +	916.7 > 174.1	916.7 > 100.9	5.1
Carbamazepine	Carbamazepine-d10	4.72	ESI +	237.4 > 194.1	237.4 > 179.1	8.7
Flumequine	Carbamazepine-d10	4.78	ESI +	262.4 > 202.0	262.4 > 244.1	0.9
Oxazepam	Nordiazepam-d5	4.79	ESI +	287.0 > 241.1	287.0 > 269.1	1.2
Clarithromycin	Carbamazepine-d10	4.80	ESI +	748.7 > 158.1	748.7 > 590.4	6.6
Roxithromycin	Ofloxacin-d3	4.82	ESI +	837.8 > 158.1	837.8 > 679.4	2.6
Lorazepam	Nordiazepam-d5	4.84	ESI +	323.1 > 277.0	323.1 > 305.0	2.2
Losartan	Carbamazepine-d10	4.86	ESI +	423.4 > 207.1	423.4 > 405.2	2.1
Nordiazepam	Nordiazepam-d5	4.92	ESI +	271.0 > 140.0	271.0 > 165.0	1.7
Josamycin	Ofloxacin-d3	4.93	ESI +	828.7 > 109.1	828.7 > 174.1	1.3
Ketoprofen	Carbamazepine-d10	5.06	ESI +	255.4 > 105.1	255.4 > 209.1	1.3
19-Norethindrone	Carbamazepine-d10	5.15	ESI +	299.4 > 109.0	299.4 > 91.0	1.8
Amiodarone	Carbamazepine-d10	5.45	ESI +	646.3 > 100.1	646.3 > 86.1	1.2
Ibuprofen-d3	-	5.53	ESI -	207.9 > 163.8	-	-
Hydrochlorothiazide	Ibuprofen-d3	3.68	ESI -	296.1 > 269.0	296.1 > 205.0	1.2
Acetylsalicylic acid	Ibuprofen-d3	4.30	ESI -	179.1 > 137.0	179.1 > 93.0	3.5
Niflumic acid	Ibuprofen-d3	5.46	ESI -	281.2 > 237.0	281.2 > 177.0	5.4
Diclofenac	Ibuprofen-d3	5.47	ESI -	294.1 > 250.0	294.1 > 214.1	18.5
Ibuprofen	Ibuprofen-d3	5.53	ESI -	205.2 > 161.1	-	-
Gemfibrozil	Ibuprofen-d3	5.78	ESI -	249.2 > 121.0	249.2 > 127.1	17.9

MRM 1: transition used for quantification

MRM 2: transition used for confirmation

Table 2: Matrix effects (%) calculated for all target analytes in different seafood matrices.

	ME (%)			
	Hake muscle	Red mullet muscle	Sole muscle	Shrimp muscle
Atenolol-d7	-51	-54	-41	-57
Ofloxacin-d3	-18	-18	-13	-24
Carbamazepine-d10	-42	-52	-23	-23
Nordiazepam-d5	-58	-77	-52	-48
Metronidazole	-66	-67	-66	-79
Acetaminophen	-72	-75	-74	-83
Amoxicillin	-62	-19	-59	-72
Acetazolamide	-58	-56	-56	-70
Sulfadiazine	-82	-79	-75	-82
Atenolol	-58	-55	-43	-66
Caffeine	-48	-48	-49	-63
Ampicillin	-51	-41	-38	-54
Trimethoprim	-56	-53	-50	-64
Norfloxacin	-22	-7	-13	-19
Ofloxacin	-29	-25	-25	-41
Ciprofloxacin	-32	-15	-16	-18
Sulfamethazine	-62	-61	-58	-66
Tetracycline	-82	-73	-75	-83
Phenazone	-20	-22	-16	-34
Metoprolol	-45	-49	-34	-48
Spiramycin	-47	-43	-15	14
Azithromycin	-13	-7	15	53
Sulfamethoxazole	-53	-60	-51	-59
Oxolinic acid	-11	-14	13	-4
Erythromycin A	1009	676	896	680
Piperacillin	-30	-44	-20	-14
Cyclophosphamide	-23	-33	-15	-44
Tylosine	-21	-19	-6	-50
Carbamazepine	-47	-57	-34	-32
Flumequine	-41	-54	-14	1
Oxazepam	-49	-64	-39	-39
Clarithromycin	-48	-52	-41	-37
Roxithromycin	-15	-38	3	4
Lorazepam	-55	-70	-42	-43
Losartan	-69	-82	-61	-47
Nordiazepam	-59	-76	-54	-49
Josamycin	-45	-41	-10	-25
Ketoprofen	-53	-74	-60	-43
19-Norethindrone	-71	-79	-54	-70
Amiodarone	2191	1316	1077	2073
Ibuprofen-d3	-24	-64	-20	-49
Hydrochlorothiazide	-55	-20	-7	-14
Acetylsalicylic acid	-47	-71	-55	-83
Niflumic acid	6	-36	76	-36
Diclofenac	-43	-73	-45	-73
Ibuprofen	11	-53	-21	-34
Gemfibrozil	157	11	139	67

If there is an intense signal suppression due to the presence of the matrix, ME (%) is close to -100

If there is an intense signal enhancement due to the presence of the matrix, ME (%) is close to 100

Table 3: Analytical performances of the analytical procedure: linearity (equations and R² coefficient of determination), limits of quantifications (LOQ), recoveries (n=3) and precisions (RSD%, n=3).

Compounds	Hake muscle							Red mullet muscle							Sole muscle							Shrimp muscle						
	Linear range (ng/g)	Equations (R ²)	LOQ (ng/g)	Recovery (%) 20 ng/g	Precision (%) 20 ng/g	Recovery (%) 200 ng/g	Precision (%) 200 ng/g	Linear range (ng/g)	Equations (R ²)	LOQ (ng/g)	Recovery (%) 20 ng/g	Precision (%) 20 ng/g	Recovery (%) 200 ng/g	Precision (%) 200 ng/g	Linear range (ng/g)	Equations (R ²)	LOQ (ng/g)	Recovery (%) 20 ng/g	Precision (%) 20 ng/g	Recovery (%) 200 ng/g	Precision (%) 200 ng/g	Linear range (ng/g)	Equations (R ²)	LOQ (ng/g)	Recovery (%) 20 ng/g	Precision (%) 20 ng/g	Recovery (%) 200 ng/g	Precision (%) 200 ng/g
Metronidazole	0.6 - 500	y = 0.428x - 0.01 (0.992)	0.6	83	7.4	78	7.3	0.3 - 500	y = 0.2171x - 0.0022 (0.997)	0.3	92	5.8	87	8.3	0.4 - 500	y = 0.4086x - 0.0005 (0.999)	0.4	79	3.5	87	16.0	0.6 - 500	y = 0.1742x - 0.003 (0.995)	0.6	80	4.9	89	3.4
Acetaminophen	1.2 - 500	y = 0.272x - 0.0062 (0.993)	1.2	83	4.8	83	6.1	3.2 - 500	y = 0.1297x - 0.00005 (0.997)	3.2	104	4.5	91	3.7	3.8 - 500	y = 0.2095x - 0.0021 (0.994)	3.8	84	1.1	89	15.0	2.4 - 500	y = 0.1218x - 0.0022 (0.994)	2.4	78	8.1	89	5.6
Amoxicillin	24.3 - 500	y = 0.0086x - 0.0004 (0.995)	24.3	N/A	N/A	69	8.2	40.2 - 500	y = 0.0074x - 0.0007 (0.984)	40.2	N/A	N/A	78	15.6	11.3 - 500	y = 0.0136x - 0.0002 (0.999)	11.3	28	52.7	84	24.9	38.8 - 500	y = 0.0042x - 0.0004 (0.941)	38.8	N/A	N/A	82	8.1
Acetazolamide	2.3 - 500	y = 0.0357x - 0.0002 (0.999)	2.3	65	11.7	103	9.8	2.0 - 500	y = 0.0308x - 0.0007 (0.994)	2.0	72	9.4	81	15.1	6.8 - 500	y = 0.0394x - 0.0004 (0.999)	6.8	71	4.2	83	11.6	14.0 - 500	y = 0.0229x - 0.0009 (0.992)	14.0	64	10.5	75	6.4
Sulfadiazine	1.5 - 500	y = 0.058x - 6E-05 (0.999)	1.5	128	18.5	126	21.8	3.0 - 500	y = 0.075x - 0.001 (0.997)	3.0	88	8.4	85	4.9	2.1 - 500	y = 0.0948x - 0.0013 (0.994)	2.1	79	6.4	94	15.1	1.4 - 500	y = 0.056x - 0.0015 (0.992)	1.4	70	7.0	85	5.8
Atenolol	0.3 - 500	y = 1.1211x - 0.0196 (0.997)	0.3	81	1.4	87	5.0	0.8 - 500	y = 1.0497x - 0.0124 (0.998)	0.8	100	4.5	95	5.7	0.5 - 500	y = 1.1798x - 0.01 (0.999)	0.5	77	11.7	80	7.5	0.6 - 500	y = 0.9581x - 0.0089 (0.996)	0.6	99	9.1	95	7.9
Caffeine	0.3 - 500	y = 0.0842x - 0.0002 (0.990)	0.3	104	25.2	97	6.2	0.1 - 500	y = 0.0715x - 0.0007 (0.994)	0.1	98	5.5	87	13.1	1.2 - 500	y = 0.107x + 0.0001 (0.999)	1.2	72	9.1	84	16.3	1.2 - 500	y = 0.0474x - 0.0009 (0.995)	1.2	76	18.5	77	6.7
Ampicillin	2.4 - 500	y = 0.0397x - 0.0004 (0.994)	2.4	82	33.1	100	8.4	1.9 - 500	y = 0.0393x - 0.0004 (0.997)	1.9	89	11.1	86	15.8	1.3 - 500	y = 0.0514x + 0.00005 (0.999)	1.3	74	21.2	90	21.0	3.7 - 500	y = 0.0271x - 0.0003 (0.994)	3.7	57	18.8	99	8.0
Trimethoprim	0.3 - 500	y = 0.2956x - 0.0065 (0.992)	0.3	90	12.0	85	10.2	0.1 - 500	y = 0.2117x - 0.0013 (0.997)	0.1	100	4.1	90	3.6	0.5 - 500	y = 0.2432x - 0.0002 (0.999)	0.5	75	7.5	87	5.3	0.7 - 500	y = 0.161x - 0.0017 (0.996)	0.7	87	17.6	102	7.8
Norfloracin	2.9 - 500	y = 0.3297x - 0.0008 (0.999)	2.9	42	29.5	74	5.4	1.9 - 500	y = 0.2807x - 0.0013 (0.997)	1.9	42	19.7	89	3.9	2.7 - 500	y = 0.3423x - 0.001 (0.998)	2.7	40	30.1	70	5.0	1.8 - 500	y = 0.2914x - 0.0009 (0.993)	1.8	39	21.5	89	15.8
Ofloxacin	0.9 - 500	y = 1.3986x - 0.0028 (0.995)	0.9	74	10.1	88	5.7	0.3 - 500	y = 1.1657x + 0.00007 (0.999)	0.3	86	11.9	99	3.3	0.3 - 500	y = 1.6296x - 0.0014 (0.999)	0.3	65	15.1	73	6.2	0.4 - 500	y = 1.1823x - 0.0008 (0.995)	0.4	80	7.4	93	4.8
Ciprofloxacin	0.9 - 500	y = 0.3931x - 0.0019 (0.997)	0.9	50	23.3	76	3.6	0.9 - 500	y = 0.3583x - 0.0011 (0.998)	0.9	33	25.1	95	6.0	0.5 - 500	y = 0.5362x - 0.0016 (0.999)	0.5	47	23.7	66	5.7	0.9 - 500	y = 0.3494x - 0.0005 (0.999)	0.9	47	18.0	97	14.5
Sulfamethazine	0.5 - 500	y = 0.3762x - 0.0056 (0.996)	0.5	87	8.5	87	8.6	0.6 - 500	y = 0.2379x - 0.0014 (0.997)	0.6	112	1.2	90	3.9	0.6 - 500	y = 0.3246x - 0.0023 (0.999)	0.6	67	12.1	82	7.4	0.5 - 500	y = 0.2027x - 0.0009 (0.998)	0.5	99	6.6	102	7.1
Tetracycline	3.3 - 500	y = 0.1913x + 0.0005 (0.991)	3.3	66	42.5	81	17.7	5.1 - 500	y = 0.2002x - 0.0009 (0.998)	5.1	47	6.4	78	22.4	4.3 - 500	y = 0.3585x - 0.0005 (0.999)	4.3	48	12.9	73	8.3	6.2 - 500	y = 0.1854x - 0.0005 (0.990)	6.2	87	35.1	93	16.1
Phenazone	0.9 - 500	y = 0.2279x - 0.0044 (0.996)	0.9	93	7.7	87	5.7	1.1 - 500	y = 0.1603x - 0.0012 (0.997)	1.1	103	5.2	93	14.7	1.6 - 500	y = 0.1749x - 0.0018 (0.994)	1.6	80	2.2	96	9.2	1.3 - 500	y = 0.1277x - 0.0015 (0.997)	1.3	90	11.5	97	9.9
Metoprolol	1.0 - 500	y = 0.4285x - 0.0093 (0.993)	1.0	85	4.4	84	3.8	0.6 - 500	y = 0.335x - 0.0009 (0.997)	0.6	96	5.6	90	6.7	0.4 - 500	y = 0.4427x - 0.0009 (0.997)	0.4	92	5.0	92	11.7	0.8 - 500	y = 0.2699x - 0.0031 (0.997)	0.8	88	7.6	96	7.6

Drug	Min	Max	Y1	X1	X2	X3	Y1	Y2	X1	X2	X3	X4	Y1	Y2	X1	X2	X3	X4	Y1	Y2	X1	X2	X3	X4	X5	X6	
Spiramycin	0.9 - 500	0.9	83	8.8	89	2.2	1.1 - 500	0.0013 (0.999)	1.1	103	9.1	103	6.8	1.2 - 500	0.0053 (0.994)	1.2	88	23.0	79	11.3	1.1 - 500	0.5562x + 0.00005 (0.999)	1.1	93	12.1	108	6.4
Azithromycin	1.1 - 500	1.1	93	7.2	101	4.2	0.7 - 500	0.4406x - 0.0002 (0.999)	0.7	122	13.7	109	5.6	0.2 - 500	0.6146x - 0.0005 (0.999)	0.2	83	6.8	72	14.9	1.0 - 500	0.7826x + 0.0007 (0.999)	1.0	86	11.9	96	12.4
Sulfamethoxazole	0.4 - 500	0.4	83	13.1	91	9.5	2.1 - 500	0.8961x + 0.0004 (0.999)	2.1	89	19.9	88	2.2	1.6 - 500	1.1088x - 0.0012 (0.999)	1.6	67	10.1	84	1.2	2.1 - 500	0.1203x - 0.003 (0.993)	2.1	103	10.0	88	10.2
Oxolinic acid	0.3 - 500	0.3	85	8.2	91	3.5	1.0 - 500	0.0776x - 0.0012 (0.996)	1.0	116	5.1	97	6.0	1.0 - 500	0.1234x - 0.0012 (0.999)	1.0	71	5.3	75	11.4	0.9 - 500	0.667x - 0.0107 (0.997)	0.9	86	5.6	90	9.2
Erythromycin A	1.1 - 500	1.1	53	33.2	69	6.2	0.5 - 500	0.5299x + 0.0078 (0.998)	0.5	54	19.0	59	20.6	0.4 - 500	0.6642x - 0.0087 (0.999)	0.4	64	0.1	45	43.1	1.2 - 500	0.1121x - 0.0031 (0.994)	1.2	56	18.6	74	17.2
Piperacillin	2.1 - 500	2.1	39	62.9	79	11.6	4.9 - 500	0.0732x + 3E-05 (0.998)	4.9	92	20.4	99	38.2	4.3 - 500	0.1248x - 0.0009 (0.995)	4.3	103	18.1	119	5.2	6.0 - 500	0.0294x - 0.0001 (0.997)	6.0	62	12.3	75	11.8
Cyclophosphamide	0.3 - 500	0.3	83	10.4	89	6.5	0.5 - 500	0.0187x - 0.0005 (0.991)	0.5	107	3.1	92	6.1	0.2 - 500	0.0258x - 0.0001 (0.999)	0.2	70	18.6	75	8.2	0.7 - 500	0.3233x - 0.0047 (0.996)	0.7	85	2.8	90	4.6
Tylosine	0.5 - 500	0.5	81	25.6	85	8.8	0.5 - 500	0.2363x - 0.0005 (0.999)	0.5	116	6.2	101	11.2	0.6 - 500	0.3204x - 0.0037 (0.999)	0.6	64	8.6	64	18.6	0.6 - 500	1.1612x + 0.0005 (0.999)	0.6	100	12.1	113	7.3
Carbamazepine	0.1 - 500	0.1	90	6.3	96	1.6	0.1 - 500	0.9321x - 0.0029 (0.998)	0.1	114	6.2	103	6.1	0.2 - 500	1.8692x - 0.0051 (0.999)	0.2	97	10.6	84	6.1	0.1 - 500	0.7743x - 0.005 (0.999)	0.1	101	5.6	94	9.4
Flumequine	0.4 - 500	0.4	88	20.7	93	3.5	0.8 - 500	0.7483x - 0.00003 (0.999)	0.8	97	5.4	87	7.9	0.2 - 500	0.9217x - 0.0006 (0.999)	0.2	62	10.0	62	12.7	0.3 - 500	0.282x - 0.00006 (0.996)	0.3	95	14.1	80	9.8
Oxazepam	0.4 - 500	0.4	96	9.4	96	10.1	0.5 - 500	0.3321x + 0.0009 (0.999)	0.5	109	9.2	114	6.2	0.9 - 500	0.4881x - 0.006 (0.999)	0.9	101	12.9	102	20.9	0.9 - 500	2.6921x - 0.053 (0.994)	0.9	121	15.6	94	20.9
Clarithromycin	0.2 - 500	0.2	83	6.5	79	13.5	0.3 - 500	1.9341x - 0.0091 (0.999)	0.3	95	15.9	88	6.7	0.4 - 500	2.5246x - 0.0254 (0.998)	0.4	64	20.4	66	24.4	1.0 - 500	0.3231x - 0.0019 (0.998)	1.0	98	22.9	92	11.5
Roxithromycin	1.0 - 500	1.0	75	7.6	102	17.5	1.6 - 500	0.3587x - 0.0028 (0.998)	1.6	126	24.1	110	8.4	1.1 - 500	0.6012x - 0.0084 (0.998)	1.1	63	16.8	66	22.5	1.2 - 500	0.2785x + 0.0002 (0.999)	1.2	86	8.4	100	13.1
Lorazepam	2.0 - 500	2.0	99	12.4	103	8.4	1.1 - 500	0.3359x - 0.0005 (0.999)	1.1	108	6.0	105	2.8	1.0 - 500	0.7973x - 0.002 (0.999)	1.0	85	16.3	85	27.6	1.3 - 500	1.7114x - 0.0339 (0.997)	1.3	131	17.2	93	20.7
Losartan	0.7 - 500	0.7	81	31.1	102	22.7	0.8 - 500	1.2736x - 0.0147 (0.997)	0.8	81	21.8	83	0.6	0.4 - 500	1.5684x - 0.0177 (0.999)	0.4	73	24.3	64	30.8	0.4 - 500	0.1177x - 0.0005 (0.999)	0.4	95	18.1	76	22.1
Nordiazepam	0.6 - 500	0.6	92	1.5	102	1.3	0.2 - 500	0.1101x + 0.0004 (0.997)	0.2	102	11.1	97	2.1	0.3 - 500	0.1707x - 0.0019 (0.999)	0.3	84	11.0	81	7.5	0.6 - 500	2.9934x - 0.029 (0.999)	0.6	111	13.7	82	15.1
Josamycin	0.3 - 500	0.3	84	24.0	95	10.9	0.2 - 500	2.5115x - 0.0198 (0.999)	0.2	111	7.9	99	9.2	0.2 - 500	3.1581x - 0.0091 (0.999)	0.2	82	2.7	72	9.1	0.7 - 500	0.9589x + 0.0017 (0.996)	0.7	90	11.1	95	14.3

								0.0054 (0.997)							0.0051 (0.999)													
								y =							y =													
								0.0517x -							0.0618x -													
Ketoprofen	1.3 - 500	y = 0.0471x - 0.0007 (0.997)	1.3	102	26.4	92	12.9	1.9 - 500	0.0008 (0.997)	1.9	97	12.9	85	11.2	1.7 - 500	0.0006 (0.999)	1.7	68	49.5	67	3.9	1.9 - 500	0.0004 (0.997)	1.9	101	11.3	78	12.7
								y =							y =													
								0.0272x -							0.039x -													
19-Norethindrone	1.9 - 500	y = 0.0252x - 0.0003 (0.999)	1.9	78	32.3	114	12.5	3.7 - 500	0.0005 (0.996)	3.7	82	16.6	87	18.2	1.4 - 500	0.0001 (0.997)	1.4	118	34.5	60	18.6	3.5 - 500	0.0004 (0.994)	3.5	103	28.1	79	15.7
								y =							y =													
								0.1903x -							0.1656x +													
Amiodarone	0.9 - 500	y = 0.2827x - 0.0016 (0.996)	0.9	139	N/A	79	N/A	1.3 - 500	0.0048 (0.993)	1.3	82	14.0	51	28.8	0.5 - 500	0.0013 (0.999)	0.5	89	12.4	77	6.4	0.5 - 500	0.0037 (0.998)	0.5	132	40.6	74	26.1
								y =							y =													
								29.501x +							28.323x -													
Hydrochlorothiazide	1.5 - 500	y = 22.29x - 0.0144 (0.996)	1.5	76	N/A	131	N/A	1.8 - 500	0.0132 (0.992)	1.8	102	13.9	112	10.6	0.9 - 500	0.0228 (0.998)	0.9	77	31.4	102	11.7	2.1 - 500	0.0132 (0.995)	2.1	98	23.1	117	14.7
								y =							y =													
								1.4867x +							0.2428x -													
Acetylsalicylic acid	13.9 - 500	y = 0.6272x - 0.0015 (0.991)	13.9	29	N/A	92	N/A	7.5 - 500	0.00003 (0.997)	7.5	106	16.6	102	26.3	37.0 - 500	0.0005 (0.987)	37.0	N/A	N/A	70	34.8	10.7 - 500	0.0012 (0.926)	10.7	26	87.4	64	26.8
								y =							y =													
								318.55x +							y = 358x -													
Niflumic acid	0.3 - 500	y = 263.96x - 0.0662 (0.997)	0.3	109	11.6	100	7.3	0.3 - 500	0.1068 (0.999)	0.3	128	6.0	105	10.7	0.5 - 500	0.1147 (0.999)	0.5	118	16.8	86	11.4	0.5 - 500	0.0153 (0.999)	0.5	80	9.9	92	3.5
								y =							y =													
								7.9887x +							9.223x +													
Diclofenac	4.5 - 500	y = 7.9245x + 0.0227 (0.997)	4.5	N/A	N/A	88	32.2	2.1 - 500	0.0188 (0.997)	2.1	N/A	N/A	109	11.3	4.1 - 500	0.0172 (0.998)	4.1	188	29.3	81	6.8	15.9 - 500	0.0137 (0.992)	15.9	51	28.9	115	4.3
								y =							y =													
								1.4629x -							1.9998x -													
Ibuprofen	8.8 - 500	y = 1.3122x - 0.0016 (0.993)	8.8	164	53.1	81	39.4	9.5 - 500	0.001 (0.999)	9.5	75	25.5	86	19.2	7.3 - 500	0.0037 (0.977)	7.3	85	20.7	53	7.7	20.4 - 500	0.0006 (0.993)	20.4	N/A	N/A	101	8.3
								y =							y =													
								7.8436x -							7.9044x -													
Gemfibrozil	4.0 - 500	y = 7.1567x - 0.0049 (0.996)	4.0	60	4.3	81	36.8	2.1 - 500	0.0063 (0.998)	2.1	71	8.8	88	17.5	1.2 - 500	0.0029 (0.999)	1.2	66	8.9	74	11.7	1.3 - 500	0.0042 (0.999)	1.3	104	38.6	96	7.6

N/A : Not applicable

Table 4: Pharmaceutical concentrations (expressed in ng/g dw) detected in the Bay of Biscay seafood samples.

Therapeutic groups	Compounds	Muscle			
		Hake	Red mullet	Sole	Shrimp
Antibiotics	Amoxicillin	<LOQ	<LOQ	<LOQ	<LOQ
	Ampicillin	<LOQ	<LOQ	<LOQ	<LOQ
	Azithromycin	<LOQ	<LOQ	0.4	<LOQ
	Ciprofloxacin	<LOQ	<LOQ	<LOQ	<LOQ
	Clarithromycin	<LOQ	0.6	1.0	<LOQ
	Erythromycin A	<LOQ	<LOQ	<LOQ	<LOQ
	Flumequine	<LOQ	<LOQ	<LOQ	<LOQ
	Josamycin	<LOQ	<LOQ	<LOQ	<LOQ
	Metronidazole	<LOQ	<LOQ	<LOQ	<LOQ
	Norfloxacin	<LOQ	<LOQ	<LOQ	<LOQ
	Ofloxacin	<LOQ	<LOQ	<LOQ	<LOQ
	Oxolinic acid	<LOQ	<LOQ	<LOQ	<LOQ
	Piperacillin	<LOQ	<LOQ	<LOQ	<LOQ
	Roxithromycin	<LOQ	<LOQ	<LOQ	<LOQ
	Spiramycin	<LOQ	<LOQ	<LOQ	<LOQ
	Sulfadiazine	<LOQ	<LOQ	<LOQ	<LOQ
	Sulfamethazine	<LOQ	<LOQ	<LOQ	<LOQ
	Sulfamethoxazole	<LOQ	<LOQ	<LOQ	<LOQ
	Tetracycline	<LOQ	<LOQ	<LOQ	<LOQ
	Trimethoprim	<LOQ	<LOQ	<LOQ	<LOQ
Tylosine	<LOQ	<LOQ	<LOQ	<LOQ	
Analgesics and NSAIDs	Acetylsalicylic acid	<LOQ	<LOQ	<LOQ	<LOQ
	Acetaminophen	1.4	<LOQ	<LOQ	<LOQ
	Diclofenac	<LOQ	<LOQ	<LOQ	<LOQ
	Ibuprofen	<LOQ	<LOQ	<LOQ	<LOQ
	Ketoprofen	<LOQ	<LOQ	<LOQ	<LOQ
	Niflumic acid	<LOQ	<LOQ	<LOQ	<LOQ
	Phenazone	<LOQ	<LOQ	<LOQ	<LOQ
β -blockers	Atenolol	<LOQ	<LOQ	<LOQ	<LOQ
	Losartan	<LOQ	<LOQ	<LOQ	<LOQ
	Metoprolol	<LOQ	<LOQ	<LOQ	<LOQ
Anti-cancers	Cyclophosphamide	<LOQ	<LOQ	<LOQ	<LOQ
Anti-epileptics	Carbamazepine	<LOQ	<LOQ	<LOQ	<LOQ
Human indicators	Caffeine	1.0	0.3	7.7	11.4
Anxiolytics	Lorazepam	<LOQ	<LOQ	<LOQ	<LOQ
	Nordiazepam	<LOQ	<LOQ	<LOQ	<LOQ
	Oxazepam	<LOQ	<LOQ	<LOQ	<LOQ
Various	Acetazolamide	<LOQ	<LOQ	<LOQ	<LOQ
	Amiodarone	<LOQ	<LOQ	<LOQ	<LOQ
	Gemfibrozil	<LOQ	<LOQ	<LOQ	<LOQ
	Hydrochlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ
	19-Norethindrone	<LOQ	<LOQ	<LOQ	<LOQ