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Highlights

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

Abstract

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

1. Introduction

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

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

In recent decades, pressurized liquid extraction (PLE) (Huerta, Jakimska, Gros, Rodríguez-Mozaz, & Barceló, 2013), ultrasonic-assisted extraction (UAE) (Liu, Hu, Bao, & Yin, 2018), 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, Lanceleur, & 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

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

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

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

2.2 Samples collection and pre-treatment

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

2.3 Sample preparation

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

2.4 LC-MS/MS analysis

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

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

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

2.5 Quantification and quality control

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

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

3. Results and discussion

3.1 Sample preparation optimization

3.1.1 Extraction solvent

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

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

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

3.2 Method validation

3.2.1 Matrix effects evaluation

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

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

$$\mathrm{MEx}\ (\%) = \left(\frac{\mathrm{Ax}(matrix) - \mathrm{Ax}(blank)}{\mathrm{Ax}(solvent)} - 1\right) x100$$

Where Ax*(matrix)* is the area of the compound x in the spiked matrix, Ax*(blank)* is the area of the compound x in the non-spiked matrix and Ax*(solvent)* is the area of the compound x in the spiked procedural blank.

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

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

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

3.2.2 Method performances

Analytical performances of the optimized method are reported in Table 3. Concerning linearity, equations and R^2 were calculated in the range 0-500 ng/g. Limits of quantification (LOQ) were determined as lowest injected compound concentrations in matrix that yielded a signal-to-noise (S/N) ratio of 10.

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

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

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

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

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

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

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

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

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

3.3 Application to real seafood samples

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

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

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

4. Conclusions

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

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

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

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

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Conflict of interest statement

Authors have declared no conflict of interest.

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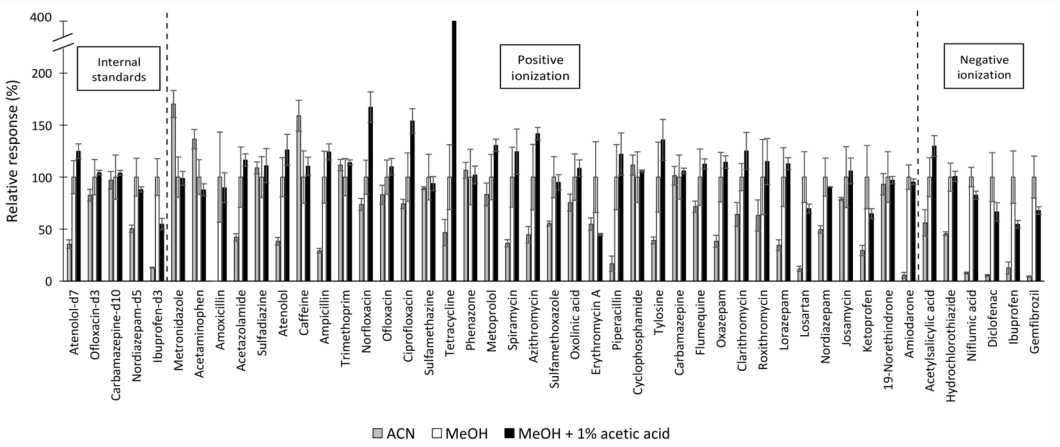
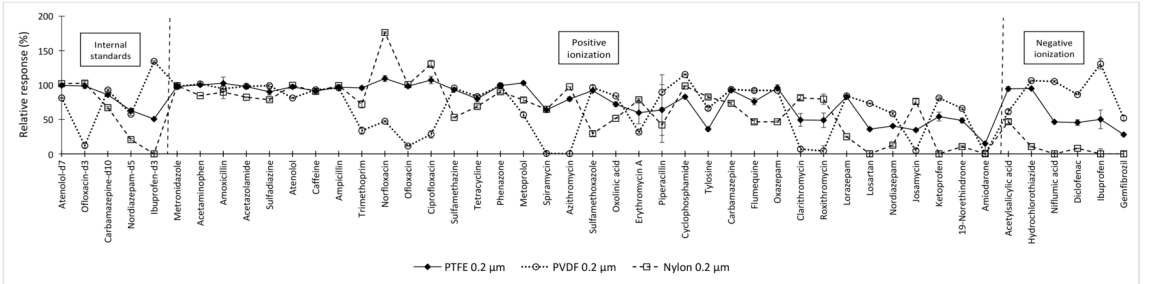
485

Figure captions

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

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


Relative response (%)
400
200
150
100
50
0
Internal standards
Positive ionization
Negative ionization
Atenolol-d7
Ofloxacin-d3
Carbamazepine-d10
Nordiazepam-d5
Ibuprofen-d3
Metronidazole
Acetaminophen
Amoxicillin
Acetazolamide
Sulfadiazine
Atenolol
Caffeine
Ampicillin
Trimethoprim
Norfloxacin
Ofloxacin
Ciprofloxacin
Sulfamethazine
Tetracycline
Phenazone
Metoprolol
Spiramycin
Azithromycin
Sulfamethoxazole
Oxolinic acid
Erythromycin A
Piperacillin
Cyclophosphamide
Tylosine
Carbamazepine
Flumequine
Oxazepam
Clarithromycin
Roxithromycin
Lorazepam
Losartan
Nordiazepam
Josamycin
Ketoprofen
19-Norethindrone
Amiodarone
Acetylsalicylic acid
Hydrochlorothiazide
Niflumic acid
Diclofenac
Ibuprofen
Gemfibrozil
ACN
MeOH
MeOH + 1% acetic acid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Ibuprofen-d3
Metronidazole
Acetaminophen
Amoxicillin
Acetazolamide
Sulfadiazine
Atenolol
Caffeine
Ampicillin
Trimethoprim
Norfloxacin
Ofloxacin
Ciprofloxacin
Sulfamethazine
Tetracycline
Phenazone
Metoprolol
Spiramycin
Azithromycin
Sulfamethoxazole
Oxolinic acid
Erythromycin A
Piperacillin
Cyclophosphamide
Tylosine
Carbamazepine
Flumequine
Oxazepam
Clarithromycin
Roxithromycin
Lorazepam
Losartan
Nordiazepam
Josamycin
Ketoprofen
19-Norethindrone
Amiodarone
Acetylsalicylic acid
Hydrochlorothiazide
Niflumic acid
Diclofenac
Ibuprofen
Gemfibrozil
PTFE 0.2 µm
PVDF 0.2 µm
Nylon 0.2 µm

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 R^2 coefficient of determination), limits of quantifications (LOQ), recoveries (n=3) and precisions (RSD%, n=3).

Compounds	Hake 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
Metronidazole	0.6 - 500	y = 0.428x - 0.01 (0.992)	0.6	83	7.4	78	7.3
Acetaminophen	1.2 - 500	y = 0.272x - 0.0062 (0.993)	1.2	83	4.8	83	6.1
Amoxicillin	24.3 - 500	y = 0.0086x - 0.0004 (0.995)	24.3	N/A	N/A	69	8.2
Acetazolamide	2.3 - 500	y = 0.0357x - 0.0002 (0.999)	2.3	65	11.7	103	9.8
Sulfadiazine	1.5 - 500	y = 0.058x - 6E-05 (0.999)	1.5	128	18.5	126	21.8
Atenolol	0.3 - 500	y = 1.1211x - 0.0196 (0.997)	0.3	81	1.4	87	5.0
Caffeine	0.3 - 500	y = 0.0842x - 0.0002 (0.990)	0.3	104	25.2	97	6.2
Ampicillin	2.4 - 500	y = 0.0397x - 0.0004 (0.994)	2.4	82	33.1	100	8.4
Trimethoprim	0.3 - 500	y = 0.2956x - 0.0065 (0.992)	0.3	90	12.0	85	10.2
Norfloxacin	2.9 - 500	y = 0.3297x - 0.0008 (0.999)	2.9	42	29.5	74	5.4
Ofloxacin	0.9 - 500	y = 1.3986x - 0.0028 (0.995)	0.9	74	10.1	88	5.7
Ciprofloxacin	0.9 - 500	y = 0.3931x - 0.0019 (0.997)	0.9	50	23.3	76	3.6
Sulfamethazine	0.5 - 500	y = 0.3762x - 0.0056 (0.996)	0.5	87	8.5	87	8.6
Tetracycline	3.3 - 500	y = 0.1913x + 0.0005 (0.991)	3.3	66	42.5	81	17.7
Phenazone	0.9 - 500	y = 0.2279x - 0.0044 (0.996)	0.9	93	7.7	87	5.7
Metoprolol	1.0 - 500	y = 0.4285x - 0.0093 (0.993)	1.0	85	4.4	84	3.8

Compounds	Red mullet 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
Metronidazole	0.3 - 500	y = 0.2171x - 0.0022 (0.997)	0.3	92	5.8	87	8.3
Acetaminophen	3.2 - 500	y = 0.1297x - 0.00005 (0.997)	3.2	104	4.5	91	3.7
Amoxicillin	40.2 - 500	y = 0.0074x - 0.0007 (0.984)	40.2	N/A	N/A	78	15.6
Acetazolamide	2.0 - 500	y = 0.0308x - 0.0007 (0.994)	2.0	72	9.4	81	15.1
Sulfadiazine	3.0 - 500	y = 0.075x - 0.001 (0.997)	3.0	88	8.4	85	4.9
Atenolol	0.8 - 500	y = 1.0497x - 0.0124 (0.998)	0.8	100	4.5	95	5.7
Caffeine	0.1 - 500	y = 0.0715x - 0.0007 (0.994)	0.1	98	5.5	87	13.1
Ampicillin	1.9 - 500	y = 0.0393x - 0.0004 (0.997)	1.9	89	11.1	86	15.8
Trimethoprim	0.1 - 500	y = 0.2117x - 0.0013 (0.997)	0.1	100	4.1	90	3.6
Norfloxacin	1.9 - 500	y = 0.2807x - 0.0013 (0.997)	1.9	42	19.7	89	3.9
Ofloxacin	0.3 - 500	y = 1.1657x + 0.00007 (0.999)	0.3	86	11.9	99	3.3
Ciprofloxacin	0.9 - 500	y = 0.3583x - 0.0011 (0.998)	0.9	33	25.1	95	6.0
Sulfamethazine	0.6 - 500	y = 0.2379x - 0.0014 (0.997)	0.6	112	1.2	90	3.9
Tetracycline	5.1 - 500	y = 0.2002x - 0.0009 (0.998)	5.1	47	6.4	78	22.4
Phenazone	1.1 - 500	y = 0.1603x - 0.0012 (0.997)	1.1	103	5.2	93	14.7
Metoprolol	0.6 - 500	y = 0.335x -	0.6	96	5.6	90	6.7

Compounds	Sole 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
Metronidazole	0.4 - 500	y = 0.4086x - 0.0005 (0.999)	0.4	79	3.5	87	16.0
Acetaminophen	3.8 - 500	y = 0.2095x - 0.0021 (0.994)	3.8	84	1.1	89	15.0
Amoxicillin	11.3 - 500	y = 0.0136x - 0.0002 (0.999)	11.3	28	52.7	84	24.9
Acetazolamide	6.8 - 500	y = 0.0394x - 0.0004 (0.999)	6.8	71	4.2	83	11.6
Sulfadiazine	2.1 - 500	y = 0.0948x - 0.0013 (0.994)	2.1	79	6.4	94	15.1
Atenolol	0.5 - 500	y = 1.1798x - 0.01 (0.999)	0.5	77	11.7	80	7.5
Caffeine	1.2 - 500	y = 0.107x + 0.0001 (0.999)	1.2	72	9.1	84	16.3
Ampicillin	1.3 - 500	y = 0.0514x + 0.00005 (0.999)	1.3	74	21.2	90	21.0
Trimethoprim	0.5 - 500	y = 0.2432x - 0.0002 (0.999)	0.5	75	7.5	87	5.3
Norfloxacin	2.7 - 500	y = 0.3423x - 0.001 (0.998)	2.7	40	30.1	70	5.0
Ofloxacin	0.3 - 500	y = 1.6296x - 0.0014 (0.999)	0.3	65	15.1	73	6.2
Ciprofloxacin	0.5 - 500	y = 0.5362x - 0.0016 (0.999)	0.5	47	23.7	66	5.7
Sulfamethazine	0.6 - 500	y = 0.3246x - 0.0023 (0.999)	0.6	67	12.1	82	7.4
Tetracycline	4.3 - 500	y = 0.3585x - 0.0005 (0.999)	4.3	48	12.9	73	8.3
Phenazone	1.6 - 500	y = 0.1749x - 0.0018 (0.994)	1.6	80	2.2	96	9.2
Metoprolol	0.4 - 500	y = 0.4427x -	0.4	92	5.0	92	11.7

Compounds	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
Metronidazole	0.6 - 500	y = 0.1742x - 0.003 (0.995)	0.6	80	4.9	89	3.4
Acetaminophen	2.4 - 500	y = 0.1218x - 0.0022 (0.994)	2.4	78	8.1	89	5.6
Amoxicillin	38.8 - 500	y = 0.0042x - 0.0004 (0.941)	38.8	N/A	N/A	82	8.1
Acetazolamide	14.0 - 500	y = 0.0229x - 0.0009 (0.992)	14.0	64	10.5	75	6.4
Sulfadiazine	1.4 - 500	y = 0.056x - 0.0015 (0.992)	1.4	70	7.0	85	5.8
Atenolol	0.6 - 500	y = 0.9581x - 0.0089 (0.996)	0.6	99	9.1	95	7.9
Caffeine	1.2 - 500	y = 0.0474x - 0.0009 (0.995)	1.2	76	18.5	77	6.7
Ampicillin	3.7 - 500	y = 0.0271x - 0.0003 (0.994)	3.7	57	18.8	99	8.0
Trimethoprim	0.7 - 500	y = 0.161x - 0.0017 (0.996)	0.7	87	17.6	102	7.8
Norfloxacin	1.8 - 500	y = 0.2914x - 0.0009 (0.993)	1.8	39	21.5	89	15.8
Ofloxacin	0.4 - 500	y = 1.1823x - 0.0008 (0.995)	0.4	80	7.4	93	4.8
Ciprofloxacin	0.9 - 500	y = 0.3494x - 0.0005 (0.999)	0.9	47	18.0	97	14.5
Sulfamethazine	0.5 - 500	y = 0.2027x - 0.0009 (0.998)	0.5	99	6.6	102	7.1
Tetracycline	6.2 - 500	y = 0.1854x - 0.0005 (0.990)	6.2	87	35.1	93	16.1
Phenazone	1.3 - 500	y = 0.1277x - 0.0015 (0.997)	1.3	90	11.5	97	9.9
Metoprolol	0.8 - 500	y = 0.2699x - 0.0031 (0.997)	0.8	88	7.6	96	7.6

									0.0013 (0.999)						0.0053 (0.994)										
Spiramycin	0.9 - 500	y = 0.3138x + 0.0004 (0.999)	0.9	83	8.8	89	2.2	1.1 - 500	y = 0.4406x - 0.0002 (0.999)	1.1	103	9.1	103	6.8	1.2 - 500	y = 0.6146x - 0.0005 (0.999)	1.2	88	23.0	79	11.3	1.1 - 500	y = 0.5562x + 0.00005 (0.999)	1.1	93	12.1	108	6.4
Azithromycin	1.1 - 500	y = 0.7826x + 0.0007 (0.999)	1.1	93	7.2	101	4.2	0.7 - 500	y = 0.8961x + 0.0004 (0.999)	0.7	122	13.7	109	5.6	0.2 - 500	y = 1.1088x - 0.0012 (0.999)	0.2	83	6.8	72	14.9	1.0 - 500	y = 1.3844x - 0.0008 (0.999)	1.0	86	11.9	96	12.4
Sulfamethoxazole	0.4 - 500	y = 0.1203x - 0.003 (0.993)	0.4	83	13.1	91	9.5	2.1 - 500	y = 0.0776x - 0.0012 (0.996)	2.1	89	19.9	88	2.2	1.6 - 500	y = 0.1234x - 0.0012 - (0.999)	1.6	67	10.1	84	1.2	2.1 - 500	y = 0.0734x - 0.001 (0.997)	2.1	103	10.0	88	10.2
Oxolinic acid	0.3 - 500	y = 0.667x - 0.0107 (0.997)	0.3	85	8.2	91	3.5	1.0 - 500	y = 0.5299x + 0.0078 (0.998)	1.0	116	5.1	97	6.0	1.0 - 500	y = 0.6642x - 0.0087 (0.999)	1.0	71	5.3	75	11.4	0.9 - 500	y = 0.5151x - 0.0063 (0.998)	0.9	86	5.6	90	9.2
Erythromycin A	1.1 - 500	y = 0.1121x - 0.0031 (0.994)	1.1	53	33.2	69	6.2	0.5 - 500	y = 0.0732x + 3E-05 (0.998)	0.5	54	19.0	59	20.6	0.4 - 500	y = 0.1248x - 0.0009 (0.995)	0.4	64	0.1	45	43.1	1.2 - 500	y = 0.0703x - 0.002 (0.989)	1.2	56	18.6	74	17.2
Piperacillin	2.1 - 500	y = 0.0294x - 0.0001 (0.997)	2.1	39	62.9	79	11.6	4.9 - 500	y = 0.0187x - 0.0005 (0.991)	4.9	92	20.4	99	38.2	4.3 - 500	y = 0.0258x - 0.0001 (0.999)	4.3	103	18.1	119	5.2	6.0 - 500	y = 0.0136x - 0.0003 (0.993)	6.0	62	12.3	75	11.8
Cyclophosphamide	0.3 - 500	y = 0.3233x - 0.0047 (0.996)	0.3	83	10.4	89	6.5	0.5 - 500	y = 0.2363x - 0.0005 (0.999)	0.5	107	3.1	92	6.1	0.2 - 500	y = 0.3204x - 0.0037 (0.999)	0.2	70	18.6	75	8.2	0.7 - 500	y = 0.1939x - 0.0032 (0.994)	0.7	85	2.8	90	4.6
Tylosine	0.5 - 500	y = 1.1612x + 0.0005 (0.999)	0.5	81	25.6	85	8.8	0.5 - 500	y = 0.9321x - 0.0029 (0.998)	0.5	116	6.2	101	11.2	0.6 - 500	y = 1.8692x - 0.0051 (0.999)	0.6	64	8.6	64	18.6	0.6 - 500	y = 0.7192x - 0.0008 (0.999)	0.6	100	12.1	113	7.3
Carbamazepine	0.1 - 500	y = 0.7743x - 0.005 (0.999)	0.1	90	6.3	96	1.6	0.1 - 500	y = 0.7483x - 0.00003 (0.999)	0.1	114	6.2	103	6.1	0.2 - 500	y = 0.9217x - 0.0006 (0.999)	0.2	97	10.6	84	6.1	0.1 - 500	y = 0.7589x - 0.0032 (0.999)	0.1	101	5.6	94	9.4
Flumequine	0.4 - 500	y = 0.282x - 0.00006 (0.996)	0.4	88	20.7	93	3.5	0.8 - 500	y = 0.3321x + 0.0009 (0.999)	0.8	97	5.4	87	7.9	0.2 - 500	y = 0.4881x - 0.006 (0.999)	0.2	62	10.0	62	12.7	0.3 - 500	y = 0.3832x + 0.0016 (0.999)	0.3	95	14.1	80	9.8
Oxazepam	0.4 - 500	y = 2.6921x - 0.053 (0.994)	0.4	96	9.4	96	10.1	0.5 - 500	y = 1.9341x - 0.0091 (0.999)	0.5	109	9.2	114	6.2	0.9 - 500	y = 2.5246x - 0.0254 (0.998)	0.9	101	12.9	102	20.9	0.9 - 500	y = 0.8558x + 0.0068 (0.998)	0.9	121	15.6	94	20.9
Clarithromycin	0.2 - 500	y = 0.3231x - 0.0019 (0.998)	0.2	83	6.5	79	13.5	0.3 - 500	y = 0.3587x - 0.0028 (0.998)	0.3	95	15.9	88	6.7	0.4 - 500	y = 0.6012x - 0.0084 (0.998)	0.4	64	20.4	66	24.4	1.0 - 500	y = 0.2979x - 0.0021 (0.998)	1.0	98	22.9	92	11.5
Roxithromycin	1.0 - 500	y = 0.2785x + 0.0002 (0.999)	1.0	75	7.6	102	17.5	1.6 - 500	y = 0.3359x - 0.0005 (0.999)	1.6	126	24.1	110	8.4	1.1 - 500	y = 0.7973x - 0.002 (0.999)	1.1	63	16.8	66	22.5	1.2 - 500	y = 0.4281x + 0.0004 (0.997)	1.2	86	8.4	100	13.1
Lorazepam	2.0 - 500	y = 1.7114x - 0.0339 (0.997)	2.0	99	12.4	103	8.4	1.1 - 500	y = 1.2736x - 0.0147 (0.997)	1.1	108	6.0	105	2.8	1.0 - 500	y = 1.5684x - 0.0177 (0.999)	1.0	85	16.3	85	27.6	1.3 - 500	y = 0.5528x + 0.0027 (0.996)	1.3	131	17.2	93	20.7
Losartan	0.7 - 500	y = 0.1177x - 0.0005 (0.999)	0.7	81	31.1	102	22.7	0.8 - 500	y = 0.1101x + 0.0004 (0.997)	0.8	81	21.8	83	0.6	0.4 - 500	y = 0.1707x - 0.0019 (0.999)	0.4	73	24.3	64	30.8	0.4 - 500	y = 0.1596x + 0.0004 (0.998)	0.4	95	18.1	76	22.1
Nordiazepam	0.6 - 500	y = 2.9934x - 0.029 (0.999)	0.6	92	1.5	102	1.3	0.2 - 500	y = 2.5115x - 0.0198 (0.999)	0.2	102	11.1	97	2.1	0.3 - 500	y = 3.1581x - 0.0091 (0.999)	0.3	84	11.0	81	7.5	0.6 - 500	y = 1.0758x + 0.0069 (0.993)	0.6	111	13.7	82	15.1
Josamycin	0.3 - 500	y = 0.9589x + 0.0017 (0.996)	0.3	84	24.0	95	10.9	0.2 - 500	y = 1.3304x -	0.2	111	7.9	99	9.2	0.2 - 500	y = 2.5319x -	0.2	82	2.7	72	9.1	0.7 - 500	y = 1.3431x - 0.0004 (0.999)	0.7	90	11.1	95	14.3

Ketoprofen	1.3 - 500	y = 0.0471x - 0.0007 (0.997)	1.3	102	26.4	92	12.9	1.9 - 500	y = 0.0054 (0.997)	1.9	97	12.9	85	11.2	1.7 - 500	y = 0.0051 (0.999)	1.7	68	49.5	67	3.9	1.9 - 500	y = 0.0437x - 0.0004 (0.997)	1.9	101	11.3	78	12.7
19-Norethindrone	1.9 - 500	y = 0.0252x - 0.0003 (0.999)	1.9	78	32.3	114	12.5	3.7 - 500	y = 0.0517x - 0.0008 (0.997)	3.7	82	16.6	87	18.2	1.4 - 500	y = 0.0618x - 0.0006 (0.999)	1.4	118	34.5	60	18.6	3.5 - 500	y = 0.0223x - 0.0004 (0.994)	3.5	103	28.1	79	15.7
Amiodarone	0.9 - 500	y = 0.2827x - 0.0016 (0.996)	0.9	139	N/A	79	N/A	1.3 - 500	y = 0.0272x - 0.0005 (0.996)	1.3	82	14.0	51	28.8	0.5 - 500	y = 0.039x - 0.0001 (0.997)	0.5	89	12.4	77	6.4	0.5 - 500	y = 0.1866x + 0.0037 (0.998)	0.5	132	40.6	74	26.1
Hydrochlorothiazide	1.5 - 500	y = 22.29x - 0.0144 (0.996)	1.5	76	N/A	131	N/A	1.8 - 500	y = 0.1903x - 0.0048 (0.993)	1.8	102	13.9	112	10.6	0.9 - 500	y = 0.1656x + 0.0013 (0.999)	0.9	77	31.4	102	11.7	2.1 - 500	y = 30.085x - 0.0132 (0.995)	2.1	98	23.1	117	14.7
Acetylsalicylic acid	13.9 - 500	y = 0.6272x - 0.0015 (0.991)	13.9	29	N/A	92	N/A	7.5 - 500	y = 29.501x + 0.0132 (0.992)	7.5	106	16.6	102	26.3	37.0 - 500	y = 28.323x - 0.0228 (0.998)	37.0	N/A	N/A	70	34.8	10.7 - 500	y = 0.2524x - 0.0012 (0.926)	10.7	26	87.4	64	26.8
Niflumic acid	0.3 - 500	y = 263.96x - 0.0662 (0.997)	0.3	109	11.6	100	7.3	0.3 - 500	y = 1.4867x + 0.00003 (0.997)	0.3	128	6.0	105	10.7	0.5 - 500	y = 358x - 0.1147 (0.999)	0.5	118	16.8	86	11.4	0.5 - 500	y = 205.72x - 0.0153 (0.999)	0.5	80	9.9	92	3.5
Diclofenac	4.5 - 500	y = 7.9245x + 0.0227 (0.997)	4.5	N/A	N/A	88	32.2	2.1 - 500	y = 318.55x + 0.1068 (0.999)	2.1	N/A	N/A	109	11.3	4.1 - 500	y = 9.223x + 0.0172 (0.998)	4.1	188	29.3	81	6.8	15.9 - 500	y = 7.3104x + 0.0137 (0.992)	15.9	51	28.9	115	4.3
Ibuprofen	8.8 - 500	y = 1.3122x - 0.0016 (0.993)	8.8	164	53.1	81	39.4	9.5 - 500	y = 7.9887x + 0.0188 (0.997)	9.5	75	25.5	86	19.2	7.3 - 500	y = 1.4629x - 0.001 (0.999)	7.3	85	20.7	53	7.7	20.4 - 500	y = 1.9998x - 0.0037 (0.977)	20.4	N/A	N/A	101	8.3
Gemfibrozil	4.0 - 500	y = 7.1567x - 0.0049 (0.996)	4.0	60	4.3	81	36.8	2.1 - 500	y = 1.4629x - 0.001 (0.999)	2.1	71	8.8	88	17.5	1.2 - 500	y = 7.9044x - 0.0029 (0.999)	1.2	66	8.9	74	11.7	1.3 - 500	y = 7.9844x - 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