Fast analysis of relevant contaminants mixture in commercial shellfish

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Abstract

One of the major challenges currently faced is to develop systematic ways of addressing chemical mixtures in environmental assessment. With this purpose, a simple, rapid, and sensitive method for the detection and quantification of a mixture of relevant contaminants in molluscs has been developed. The method is based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) and Ultra-High Performance Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS). It includes a mixture of 23 compounds formed by pesticides, endocrine disruptors and pharmaceuticals (metolachlor, simazine, desethylatrazine, atrazine, thiabendazole, diazinon, malathion, bentazone, MCPA, propanil, acetamiprid, imidacloprid, caffeine, bisphenol A, triclosan, triclocarban, methylparaben, ethylparaben, propylparaben, 1H-benzotriazole, sulfamethoxazole, venlafaxine and carbamazepine). The method was developed and validated in 4 different types of shellfish of high commercial interest such as mussel (*Mytilus galloprovincialis*), oyster (*Crassostrea gigas*), cockle (*Cerastoderma edule*) and razor shell (*Solen marginatus*). The mean percentage of recoveries obtained for all the compounds in each mollusc type (intra-specie) ranged from 96% to 107% showing the good performance of the method developed. The relative standard deviation was under 10% for the intra-day and 17% inter-day analyses. Method detection limits and method quantification limits were below 10 ng/g dry weight for all the species and compounds targeted. Finally, the method was applied to aquaculture samples, oysters and cockles, from Ebro Delta (Spain), after some episodes of mortality occurred in 2017. A high level of bisphenol A was detected in *C. edule* which may explain the mortality suffered by this organism. *C. gigas* presented low levels of metolachlor, bentazone, acetamiprid, and methylparaben.

1. Introduction

Multitude of contaminants derived from daily human activities are present in the aquatic environment and can interact with organisms such as molluscs. They can be simultaneously accumulated making a "cocktail" of hazardous substances with potential negative effects for the organism and for humans through the food chain. One of the major challenges currently faced is to develop systematic ways of addressing chemical mixtures in environmental assessment [1] and to identify priority mixtures of potential concern.

The analysis of contaminants mixtures in environmental samples is a difficult task. Concretely in seafood, when different groups of contaminants were targeted, the approach traditionally used was to apply as many different analytical methods as needed to the sample [2]. This strategy although useful is time- and money-consuming which is not suitable for use in regular analysis derived from quality control monitoring. Therefore, alternative fast and cheaper methods are needed for covering the analysis of relevant contaminants mixtures in seafood. Currently, very few methods are available that allow to extract and quantify a certain mixture of contaminants in molluscs. Most of them are focused on the analysis of persistent organic pollutants (POPs) mixtures, containing chemicals from different groups, mainly polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), but also some polychlorinated naphthalenes (PCNs) and organochlorinated pesticides (OCPs) [3-5]. The development of methods for the analysis of contaminants mixtures containing contaminants of emerging concern (CECs) in shellfish is scarce. Bayen et al. in 2015 [6] developed a method for the extraction and identification of a mixture composed by a long list of pharmaceuticals compounds (PhACs) and some endocrine disrupting chemicals (EDCs) in molluscs [6]. Recently, Mijangos at al. [7] has published a method for the determination of multiclass organic pollutants including artificial sweeteners, industrial products, hormones, PhACs, personal

care products, pesticides and phytoestrogens in tissues and biofluids of mussel and fish. Robust multi-residue analytical methods for accurate determination of contaminants mixtures are essential for effective biomonitoring of environmental quality. Target analysis offers good sensitivity and reliable identification of the compounds, but it has a significant disadvantage as it always misses all compounds not included in the method. The use of High-Resolution Mass Spectrometry (HRMS) permits to overcome this issue through non-target analysis of the sample. Therefore, the selection of a high-resolution mass analyser when developing a target analytical method gives the opportunity of digging in the complexity of the contaminants mixture accumulated in a certain organism. Consequently, the present work aimed to develop and validate a simple, rapid, and sensitive method for the detection and quantification of a mixture of relevant contaminants in molluscs by using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) and High-Resolution Mass Spectrometry. The mixture of contaminants selected was formed by pesticides (organonitrogen and organophosphorus pesticides, herbicides and insecticides), EDCs (stimulant, plasticiser, antibacterials, preservatives and triazole) and PhACs (psychiatric drugs and antibiotic) based on their occurrence and levels in the marine environment [8-11]. Twenty-three compounds were included and it was developed and validated in 4 different types of shellfish of high commercial interest such as mussel, oyster, cockle and razor shell. The method was successfully applied to aquaculture samples, oysters and cockles, from Ebro Delta (Spain) after some episodes of mortality occurred in 2017 in order to find out if there was any relationship with chemical contamination.

2. Material and methods

2.1. Chemicals and reagents

See supporting information for details.

2.2. Sampling

Organisms corresponding to different species of shellfish were collected from Ebro Delta (Catalonia, Spain) between April and Nov 2017. Spring and summer are the seasons when the highest load of contaminants are released to the Delta mainly due to agriculture (discharge of drainage channels from rice crops) and urban activities (industry and tourism).

Mussels (*Mytilus galloprovincialis*) and razor shell (*Solen marginatus*) were sampled from Alfacs bay (located at the south of the delta), oyster (*Crassostrea gigas*) and cockle (*Cerastoderma edule*) were taken from Fangar Bay (located at the north of the delta). For the method development the samples were collected from a shellfish farm allocated outside of Alfacs Bay and considered as clean site. The target bivalve's species were selected due to their high consumption by the population. Thirty organisms were collected per species, their shells were discarded, and a pool was made by homogenising their tissues. The composite sample was freeze-dried, grounded in a mortar, and kept at -20ºC until its analysis.

2.3. Extraction and purification

One-gram dry weight (dw) of the sample was extracted by using QuEChERS Bekolut Citrat-Kit-01. Prior to the extraction, the internal standards mixture was added at a concentration of 50 ng/g dw (Table 1), vortexed and left to equilibrate overnight in refrigerated conditions. Procedural blank extractions were also simultaneously carried out. The next day, 10 mL of ACN and 5 mL of HPLC water were added together with a mixture of salts containing 4g of MgSO4, 1g of NaCl, 1g of sodiumcitrate and 0.5g of disodium citrate sesquihydrate. Then the sample was vortexed (1 min) and centrifuged (5 min at 3220 RCF and 15ºC). Immediately after, the supernatant liquid (6 mL) was transferred to a centrifuge tube to perform the dispersive solid phase extraction (dSPE)

by adding QuEChERS Bekolut PSA-Kit-04A that consists of 4 mg of primary secondary amine (PSA), 400 mg of octadecylsilane (C18e) and 1200 mg of MgSO4. It was vortexed (1 min) and centrifuged again (5 min at 3220 RCF and 15ºC). The supernatant liquid was transferred to a glass tube to evaporate under a gentle stream of nitrogen until complete dryness. Then it was re-dissolved in 1 mL of ACN and filtered through Ostro™, a phospholipids removal plate. The filtered sample was then transferred to an appropriate vial for its injection in UHPLC-HRMS.

2.4. Analysis by UHPLC-HRMS

For the identification and quantification of the target compounds, the shellfish extracts were injected in an UHPLC-HRMS instrument. Chromatographic separations were carried out with an Acquity Ultra-Performance™ Water liquid chromatograph system from Waters (Milford, MA, USA), equipped with two binary pumps systems using a Purospher STAR RP-18 end-capped column (150 mm x 2.1 mm, 2 μm particle size) (Merk, Darmstadt, Germany) for both positive and negative electrospray ionization (Table 1). The mobile phase consisted of acetonitrile (A) and water (B) at a flow rate of 0.2 mL/min. A gradient elution was applied for chromatographic separation: 0 min: 10%A; 2.5 min: 50%A; 12.5 min: 80%A, 13.5 min: 100%A; 16.5 min: 10%A; 25 min: 10%A. The column temperature was set at 25 ºC; the injection volume was 20 μL. Detection was performed using an Orbitrap Q-Exactive™ mass spectrometer (Thermo Fischer Scientific, San Jose, CA, USA). Full scan data in both positive and negative mode were acquired at a resolving power of 70,000 FWHM. Ion source parameters in positive electrospray mode (ESI+) were: spray voltage 3.5 kV, sheath gas (N2 > 95%) 35, auxiliary gas (N2 > 95%) 10, capillary temperature 350 °C, S-lens RF level 60, auxiliary gas heater temperature 250 °C. Ion source parameters in negative mode (ESI-) were: spray voltage -3.5 kV, sheath gas (N2 > 95%) 25, auxiliary gas (N2 > 95%) 10, capillary

temperature 350 °C, S-lens RF level 60, auxiliary gas heater temperature 250 °C. For the compounds of interest, a scan range of m/z 70–1000 was selected; the automatic gain control (AGC) was set at 1×10^6 and the maximum injection time was set to 50 ms. Data analysis and processing were done using Thermo Xcalibur Software v. 3.1.

The selectivity and specificity of the method were verified by analysing samples and standard solutions. The peaks of the target compounds in the samples were confirmed by comparing their retention times with those in the standard solutions and also by identifying the precursor ion with a mass error below 5 ppm. Blank samples (100% ACN) were run every 3 samples on the sample queue both between standards, spiked and non-spiked samples in order to detect any possible carryover effect.

2.5. Recovery study

Mussel, oyster, cockle and razor shell (1 g of dw sample per triplicate (n=3), for each matrix) were spiked with the contaminants mixture (Table 1) in order to study the extraction efficiency of the proposed methodology. The spike concentration was 50 ng/g dw. The internal standards mixture was also added at the same concentration prior to the extraction. The samples were immediately vortexed after addition of every mixture, and they were kept at 4 ºC overnight before the extraction process was carried out. Triplicate control samples of each matrix were also analysed to determine possible background levels of the target compounds. Total recoveries were calculated by comparing the concentrations measured in the sample after the analytical procedure with the initial spiked concentration. The concentrations measured in the sample were determined by using internal calibration. For this purpose, an 8 points calibration curve (between 0.05 and 50 ng/mL) of the target compounds was made up in acetonitrile.

2.6. Statistical analysis

The mean percentage of recovery and its relative standard deviation (RSD) were calculated for each compound and matrix. Besides, statistical analysis of the percentages of recoveries obtained for all compounds in every matrix was carried out with "R" software v 3.5.1. The median, percentile 25^{th} and 75^{th} are shown by the box plot (see Fig. S1). Whiskers above and below the box indicate the 10^{th} and 90^{th} percentiles. Points above and below the whiskers indicate outliers.

3. Results and discussion

3.1. Extraction and purification

QuEChERS was proposed as extraction and purification technique because it is simple, fast, it offers high sample throughput and the possibility of obtaining high recoveries for a wide variety of organic chemicals, from polar to non-polar compounds in biological matrices [12]. It was compared with Pressurised Liquid Extraction (PLE) using different solvents mixtures (acetonitrile, methanol:water (1:2) and dichloromethane:acetone (1:1)). For the purification stage Solid Phase Extraction (SPE), on different cartridges such as HLB and ENV+ and dispersive SPE (dSPE) were evaluated. The results indicated that PLE (performing the extraction at 50ºC, 1500 psi and 3 static cycles) followed by SPE was not appropriate because most of the target chemicals were either non-detected or there was co-extraction of other matrix compounds that interfered with the analysis (data not shown).

The total recoveries obtained for the four-target species *M. galloprovincialis*, *C. gigas*, *C. edule* and *S. marginatus* at the spiking level chosen are shown in Fig. 1. The numeric values and their respective RSD are also presented in the supporting information (Table S1). The spike concentration used was 50 ng/g dw, which was selected considering that levels of pesticides, EDCs and pharmaceuticals in marine biota usually ranged in the low ppb levels [8]. Even so, sometimes depending on the specific compound (mainly

pesticides and EDCs), type of sample and origin, the concentrations can be much higher reaching the ppm levels [9, 13]. The extracted ion chromatograms of the 23 compounds included in the method in a standard and in the 4 different matrices studied are presented in Fig. 2, Fig. S2, S3 and S4. They show the elution order in both positive and negative ionisation modes.

Recoveries ranged between 62% and 148%. However, when the variation of the dataset was studied, the box plot diagram (Fig. S1) identified both percentages as extremes outliers. The mean percentage of recoveries obtained for all the compounds in each mollusc type (intra-specie) ranged from 96% to 107%, being very close to the medians, which ranged between 96 and 105% (Fig. S1). Their RSD reached a maximum of 17%, although in the majority of the cases it was below 10% which indicates a high degree of repeatability (Table S1). The recoveries obtained for the four mollusc types (inter-species) ranged in a very narrow interval, which shows the robustness of the proposed methodology. This interval was particularly close for *M. galloprovincialis*, *C. gigas* and *C. edule*. In the case of *S. marginatus* slightly lower recoveries were achieved for the majority of the compounds (Fig. 1).

As a limitation of the method, it was found that the extractions of BPA from *C. gigas* and VEN from *S. marginatus* were not possible due to the co-extraction of others matrix compounds that interfered with the analysis. Changes in the chromatography were tested in order to improve their separation (using different solvents and elution gradient), but this was a detriment to the others compounds analysed. Anyhow, considering the good performance of the original method for BPA and VEN in the other target organisms, and the importance of these contaminants from an environmental point of view, it was decided to keep them.

3.2. Matrix effects

The effects caused by the 4 different matrices studied (mussel, oyster, cockle and razor shell) on the analysis of the target compounds were checked. For this purpose, the peak areas of the shellfish's extracts (first subtracted by the peak areas corresponding to the native analytes present) spiked at 1, 5, 10, 25 and 50 ng/g were compared to those of the analytes in solvent (100% ACN) spiked at the same levels. The percentages of signal reduction or enhancement are shown in Table 2 and Fig. S5. Ion suppression was the effect mostly observed with few exceptions (parabens in *M. galloprovincialis*, VEN in *C. gigas* and BPA and TCS in *C. edule*). It ranged in a wide interval from practically not suppressed (i.e. METO in *M. galloprovincialis*) to strongly suppressed (i.e. BEN in *S. marginatus*). It was noted that *M. galloprovincialis* was the shellfish presenting less ion suppression (mean percentage of ion suppression 16%) followed by *C. gigas* (42 %), *C. edule* (51%) and finally *S. marginatus* (70%). Matrix effects, typically matrix suppression, are a well-known problem in LC-MS analysis and need to be evaluated together with recoveries [12]. In order to minimize matrix interferences and avoid any under or overestimation during quantification internal sample calibration can be performed [14]. In the present work, the use of internal sample calibration (also known as matrix-matched calibration) together with isotopically labelled internal standards was initially considered, and 4 calibration curves (one per target species) were made up in the corresponding shellfish extracts. However, the recoveries obtained were not significantly different to the ones obtained with solvent calibration (t-test, $p>0.05$), and this approach was discarded.

3.3 Method validation

Method detection limits (MDLs) and method quantification limits (MQLs) for *M. galloprovincialis*, *C. gigas*, *C. edule* and *S. marginatus* are presented in Table 3. They were determined in spiked samples (n=3) of the four shellfish types. The calculation was

based on signal-to-noise ratio (S/N) for those compounds and matrices which exhibited baseline noise. The acceptance criteria were S/N=3 for MDL and S/N=10 for MQL. For those compounds and matrices which baseline was completely flat (and therefore N was equal to 0), the MDL and MQL were established by visual inspection of the calibration curve made up in the corresponding matrix extract [15]. The concentration of the lowest standard that could be clearly distinguished was used as MDL and double it as MQL. Calibration curve range was between 0.01 and 50 ng/g, they were generated using linear regression (regression coefficients shown in Table S2). MDLs and MQLs were below 10 RCFng/g dw for all the species and compounds targeted. Similar MQLs were described by Baduel et al. [12] for the extraction of a mixture of contaminants from fish muscle using LC-QTOF-MS/MS. Bayen et al. [6] reported MDLs for a mix of contaminants in mussel generally below 1 ng/g but expressed as wet weight. According to our previous experience [16], the conversion factor from wet weight to dry weight concentration for mussels ranged from 4 to 7. Therefore, MDLs in the same range (expressed in dw) than the ones reported here were obtained for similar compounds.

Accuracy and precision for the developed method were determined for the same spiked sample (*M. galloprovincialis*) intra-day (n=5 injections the same day) and inter-day (n=3 injections performed in 3 different days, one per day). Accuracy was defined as the deviation of the measured mean concentration from the spiked concentration, expressed in percentage, as described by Bogialli et al. [17]. Accuracy values are expressed as percentage of error and they ranged between -17.1 and 18.8 % (which correspond to an accuracy of 82.9% and 118.8%) (Table 4). Precision was expressed as the relative standard deviation of the measured concentration. RSD values for the intra-day analysis (repeatability) ranged between 0.3 and 10 %, and between 0.1 and 16.9 % for the inter-day analysis (reproducibility) (Table 4). This demonstrates the repeatability and

reproducibility of the method with an error below 17% and therefore its effectiveness for quantification purposes.

3.4. Application to aquaculture samples

The method developed was applied to samples of *C. gigas* and *C. edule* collected from Ebro Delta during several mortality episodes occurred in 2017. In May, when temperatures were around 20 ºC, *C. gigas* cultured at Fangar bay, experimented a massive first mortality event (up to 85% accumulative mortality) of commercial size oyster. This event repeated later on in June and October (12 and 30 % of mortality, water temperature 28 ºC and 22 ºC respectively). In these events, the mortality was significantly lower, especially in June considered as normal mortality for that time of the year. This is noteworthy because June was the month that registered the highest water temperature. In September, a massive mortality event of *C. edule* was also registered (100% of mortality, water temperature 20 ºC). The results obtained after the analysis of these samples are presented in Table 5. Ten out of the 23 contaminants included in the method were determined at concentrations above their respective MDLs. Concretely 4 pesticides (METO, ATRA, BEN and ACET) and 6 EDCs (CAF, BPA, MP, EP, PP and BENZOT). Their levels ranged from below MQL up to 4277.40 ng/g dw of BPA in *C. edule* dead in September. Unfortunately, water samples were not taken during this mortality event, and the concentration of BPA in water was not measured. Anyhow, considering the high level of BPA bioaccumulated in the shellfish a very high concentration in water would be expected (probably due to a direct spill). Based on reported EC50 and LC50 BPA is classified as "moderately toxic" and "toxic" to aquatic biota by the European Commission and the United States Environmental Protection Agency (US EPA), respectively [18]. Therefore, its occurrence and level in *C. edule* could explain the mortality suffered at that specific moment. However, pathogens associated with cockle massive mortality events

such as *Marteilia cochillia* [19] have been previously reported in the same area and they might be also present in the samples. On the contrary, the mortality events suffered by *C. gigas* were not related to any particular compound included in the method since their levels ranged in the low ng/g dw. Eight years ago Köck et al. [9] found very high levels of malathion in shellfish from the same area (53 mg/kg) which could explain the mortalities registered by then. However, in the present research malathion was not detected because this pesticide was banned in 2007 by the European Commission [20]. Therefore, there must be other reasons that may explain such high mortalities percentages like the presence of potential pathogens, for example *Vibrio aestuarianus* and *Vibrio splendidus* that have been recently reported for the first time in Pacific oyster cultured in the Spanish Mediterranean [21].

4. Conclusions

A simple, rapid, and sensitive method has been developed and validated for the simultaneous detection and quantification of a mixture of relevant contaminants in molluscs by using QuEChERS and UHPLC-HRMS. The mixture of contaminants selected was formed by 23 compounds including pesticides (organonitrogen and organophosphorus pesticides, herbicides and insecticides), EDCs (stimulant, plasticiser, antibacterials, preservatives and triazole) and PhACs (psychiatric drugs and antibiotic). The mean percentage of recoveries obtained for all the compounds in each mollusc type (*M. galloprovincialis*, *C. gigas*, *C. edule* and *S. marginatus*) ranged from 96% to 107%. As a limitation of the method, it was found that the extractions of BPA from *C. gigas* and VEN from *S. marginatus* were not possible due to the co-extraction of others matrix compounds that interfered with the analysis. Other than that, RSD values for the intra-day analysis were below 10 %, and below 17 % for the inter-day analysis. This demonstrates the repeatability and reproducibility of the method and therefore its

effectiveness for quantification purposes. MDLs and MQLs were below 10 ng/g dw. Ion suppression was the effect mostly observed with few exceptions, and it was compensated by the use of internal calibration. The method developed was applied to samples of *C. gi*gas and *C. edule* collected from Ebro Delta during several mortality episodes occurred in 2017. Ten out of the 23 contaminants included in the method were determine at concentrations above their respective MDLs. Concretely 4 pesticides (METO, ATRA, BEN and ACET) and 6 EDCs (CAF, BPA, MP, EP, PP and BENZOT). The occurrence and level of BPA in *C. edule* could explain the mortality suffered at that specific moment. On the contrary, the mortality events suffered by *C. gigas* were not related to any particular compound included in the method since their levels ranged in the low ng/g dw. No unknown large peaks potentially behind the mortality were neither observed in the scan screening of chromatograms.

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Declarations of interest

None.

Figure captions

Fig. 1. Mean percentages recoveries (n=3) of the target compounds in a) *M. galloprovincialis* (mussel), b) *C. gigas* (oyster), c) *C. edule* (cockle) and d) *S. marginatus* (razor shell) spiked at 50 ng/g dry weight.

Fig. 2. Extracted ion chromatograms of the compounds analysed in a) a standard b) *M. galloprovincialis* spiked at 50 ng/g in positive and negative electrospray ionisation.

References

[1] E. Commission, The combination effects of chemicals. Chemical mixtures, Communication from the Commission to the Council. , Brussels, 2012.

[2] (!!! INVALID CITATION !!! [2-5]).

[3] M.I. Helaleh, A. Al-Rashdan, A. Ibtisam, Simultaneous analysis of organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) from marine samples using automated pressurized liquid extraction (PLE) and Power Prep clean-up, Talanta 94 (2012) 44-9.

[4] S. Pizzini, R. Piazza, G. Cozzi, C. Barbante, Simultaneous determination of halogenated contaminants and polycyclic aromatic hydrocarbons: a multi-analyte method applied to filter-feeding edible organisms, Anal Bioanal Chem 408(28) (2016) 7991-7999.

[5] M. Breitwieser, E. Vigneau, A. Viricel, V. Becquet, C. Lacroix, M. Erb, V. Huet, C. Churlaud, S. Le Floch, B. Guillot, M. Graber, H. Thomas, What is the relationship between the bioaccumulation of chemical contaminants in the variegated scallop Mimachlamys varia and its health status? A study carried out on the French Atlantic coast using the Path ComDim model, Sci Total Environ 640-641 (2018) 662-670.

[6] S. Bayen, E.S. Estrada, G. Juhel, B.C. Kelly, Direct injection of tissue extracts in liquid chromatography/tandem mass spectrometry for the determination of pharmaceuticals and other contaminants of emerging concern in mollusks, Anal Bioanal Chem 407(19) (2015) 5553-8.

[7] L. Mijangos, H. Ziarrusta, I. Zabaleta, A. Usobiaga, M. Olivares, O. Zuloaga, N. Etxebarria, A. Prieto, Multiresidue analytical method for the determination of 41 multiclass organic pollutants in mussel and fish tissues and biofluids by liquid chromatography coupled to tandem mass spectrometry, Anal Bioanal Chem (2018).

[8] D. Álvarez-Muñoz, Llorca, M., Blasco, J., Barceló D., Contaminants in the Marine Environment, Elsevier, London, UK, 2016.

[9] M. Köck, Farré, M., Martínez, E., Gajda-Schrantz K.,, Ginebreda A., Navarro A., López de Alda M., Barceló D., Integrated ecotoxicological and chemical approach for the assessment of pesticide pollution in the Ebro River delta (Spain), Journal of Hydrology 383 (2010) 73-82.

[10] G. Vandermeersch, H.M. Lourenço, D. Alvarez-Muñoz, S. Cunha, J. Diogène, G. Cano-Sancho, J.J. Sloth, C. Kwadijk, D. Barcelo, W. Allegaert, K. Bekaert, J.O. Fernandes, A. Marques, J. Robbens, Environmental contaminants of emerging concern in seafood - European database on contaminant levels, Environmetnal Research (2015).

[11] D. Alvarez-Munoz, S. Rodriguez-Mozaz, A.L. Maulvault, A. Tediosi, M. Fernandez-Tejedor, F. Van den Heuvel, M. Kotterman, A. Marques, D. Barcelo, Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe, Environ Res 143(Pt B) (2015) 56-64.

[12] C. Baduel, J.F. Mueller, H. Tsai, M.J. Gomez Ramos, Development of sample extraction and clean-up strategies for target and non-target analysis of environmental contaminants in biological matrices, J Chromatogr A 1426 (2015) 33-47.

[13] F. Ferrara, Ademollo, N., Delise, M., Fabietti, F., Funari, E., Alkylphenols and their ethoxylates in seafood from the Tyrrhenian Sea, Chemosphere 72(9) (2008) 1279-85.

[14] D. Alvarez-Muñoz, Huerta, B., Fernandez-Tejedor, M., Rodríguez-Mozaz, S., Barceló, D., Multi-residue method for the analysis of pharmaceuticals and some of their metabolites in bivalves Talanta 136 (2015) 174-182.

[15] A.G. Shrivastava, V.B., Methods for the determination of limit of detection and limit of quantitation of the analytical methods, Chronicles of Young Scientist 2(1) (2011) 21-25.

[16] D. Alvarez-Munoz, S. Rodriguez-Mozaz, S. Jacobs, A. Serra-Compte, N. Caceres, I. Sioen, W. Verbeke, V. Barbosa, F. Ferrari, M. Fernandez-Tejedor, S. Cunha, K. Granby, J. Robbens, M. Kotterman, A. Marques, D. Barcelo, Pharmaceuticals and endocrine disruptors in raw and cooked seafood from European market: Concentrations and human exposure levels, Environ Int 119 (2018) 570-581.

[17] S. Bogialli, R. Curini, A. Di Corcia, M. Nazzari, R. Samperi, A liquid chromatography-mass spectrometry assay for analyzing sulfonamide antibacterials in cattle and fish muscle tissues, Anal Chem 75(8) (2003) 1798-804.
[18] S. Flint, T. Markle, S. Thompson, E. Wallace, Bisphenol A exposure, effects, and policy: a wildlife perspective, J Environ Manage 104 (2012) 19-34.
[19] M.H.P. Carrasco N., Durfort M., Andree K.B., Malchus N., Lacuesta B., González M., Roque A., Rodgers C., Furones M.D., Marteilia cochillia sp. nov., a new Marteilia species affecting the edible cockle Cerastoderma edule in European waters, Aquaculture 412-413 (2013) 223-230.
[20] E. Commission, Decision 2007/389/EC concerning the non-inclusion of malathion in Annex I to Council Directive 91/414/EEC., Journal of European Community L 146 (2007) 19.
[21] V. Carrasco N., L., Bertomeu E., Álvarez-Muñoz D., Aranguren R., Fernández M., Roque A., Andree K., Solé M., Furones M.D. , *Vibrio aestuarianus* associated to pacific oyster *Crassostrea gigas* mortality in the Ebro Delta, in the Catalan Mediterranean coast, AQUA 2018, Montpellier (France) (2018).

Table 1. Target compounds organized by chemical family, ESI mode, m/z and isotopically labeled internal standard assigned for their quantification.

Chemical family	Compound	ESI	m/z	RT (min)	Internal Standard	m/z	RT (min)
Organonitrogen pesticides	**Atrazine (ATRA)**	POS	216.1010	7.13	Atrazine-d5	221.1324	7.09
	Desethylatrazine (DEA)	POS	188.0697	4.65	Atrazine-d5	221.1324	7.07
	Metolachlor (METO)	POS	284.1411	10.77	Metoalachlor-d6	290.1789	10.66
	Simazine (SIMA)	POS	202.0853	5.97	Simazine-d10	212.1481	5.87
Organophosphorus pesticides	**Diazinon (DIAZ)**	POS	305.1083	12.98	Diazinon-d10	315.1710	12.82
	Malathion (MALA)	POS	331.0433	10.42	Malathion-d7	338.0875	10.31
	Thiabendazole (THIA)	POS	202.0433	5.10	Thiabendazole-C13	208.0636	5.1
Herbicides	**Bentazone (BEN)**	NEG	239.0487	3.49	Bentazon-d7	246.0926	2.48
	MCPA	NEG	199.0155	4.06	MCPA-d3	202.0342	4.04
	Propanil (PROP)	NEG	217.9948	8.44	Propanil-d5	223.0261	8.39
Insecticides	**Acetamiprid (ACET)**	POS	223.0748	4.70	Acetamiprid-d3	226.0935	4.69
	Imidacloprid (IMIDA)	POS	256.0599	4.62	Imidacloprid-d4	260.0850	4.6
EDCs	**1H-benzotriazole (BENZOT)**	POS	120.0556	4.18	Benzotriazole-d4	124.0814	4.15
	Bisphenol A (BPA)	NEG	227.1067	6.49	Bisphenol A d-4	231.1325	6.45
	Caffeine (CAF)	POS	195.0876	3.60	Caffeine-d3	198.1064	3.59
	Ethylparaben (EP)	NEG	165.0543	5.90	Ethylparaben-C13	171.0743	5.9
	Methylparaben (MP)	NEG	151.0385	5.12	Ethylparaben-C13	171.0743	5.9
	Propylparaben (PP)	NEG	179.0713	6.95	Ethylparaben-C13	171.0743	5.9
	Triclocarban (TCC)	NEG	312.9707	12.87	Triclosan-d3	289.9622	12.92
	Triclosan (TCS)	NEG	286.9438	12.99	Triclosan-d3	289.9622	12.92
PhACs	**Carbamazepine (CBZ)**	POS	237.1022	5.53	Carbamazepine-d10	247.1653	5.46
	Sulfamethozaxole (SMX)	POS	254.0593	4.84	Sulfamethoxazole-d4	258.0844	4.83
	Venlafaxine (VEN)	POS	278.2114	9.98	Venlafaxine-d6	284.2491	9.97

Table 2. Matrix effect (%) expressed as ion suppression (-) or enhancement (+) for the target compounds tested in *M. galloprovincialis*, *C. gigas*, *C. edule* and *S. marginatus*.

	Matrix effect (%)							
Compound	***M. galloprovincialis***	**RSD(%)**	***C. gigas***	**RSD(%)**	***C. edule***	**RSD(%)**	***S. marginatus***	**RSD(%)**
Atrazine	-6.62	9.74	-36.74	4.12	-58.12	12.70	-59.89	18.25
Desethylatrazine	-38.09	27.68	-45.82	31.82	-85.33	6.70	-82.18	2.44
Metolachlor	-0.41	9.22	-17.76	3.95	-50.34	18.90	-58.45	7.20
Simazine	-15.92	10.45	-59.15	4.19	-71.68	7.48	-76.96	3.82
Diazinon	-15.35	3.29	-30.07	1.84	-63.18	12.71	-62.31	6.90
Malathion	-6.31	16.81	-18.15	11.60	-52.16	16.87	-57.12	7.78
Thiabendazole	-4.19	8.11	-48.58	6.17	-58.58	15.03	-72.41	2.57
Bentazone	-45.39	11.32	-47.65	23.88	-94.80	1.63	-97.24	0.88
MCPA	-14.57	17.58	-39.11	9.59	-85.45	8.90	-92.97	3.77
Propanil	-5.25	4.42	-46.73	2.98	-9.42	8.98	-70.60	1.19
Acetamiprid	-34.91	14.07	-71.85	18.13	-76.01	5.67	-61.24	14.16
Imidacloprid	-9.30	38.28	-2.48	2.23	-83.56	7.04	-70.94	4.88
1H-benzotriazole	-74.17	8.79	-86.79	4.41	-89.62	7.48	-86.83	4.16
Bisphenol A	-6.97	13.39	-75.57	1.88	14.08	5.63	-91.99	0.21
Caffeine	-73.53	10.37	-85.81	1.42	-55.54	2.73	-77.02	2.94
Ethylparaben	5.74	10.04	-28.96	7.03	-29.73	4.21	-62.02	21.67
Methylparaben	25.30	22.54	-10.26	17.13	-29.40	8.47	-89.42	1.60
Propylparaben	2.83	7.88	-37.93	3.97	-23.70	7.68	-69.24	10.63
Triclocarban	-8.52	4.49	-41.43	2.63	-51.23	2.08	-84.02	2.10
Triclosan	-2.91	7.02	-40.02	9.07	32.88	5.76	-46.97	14.00
Carbamazepine	-6.02	9.79	-33.81	3.28	-53.41	15.11	-43.37	11.52
Sulfamethozaxole	-32.09	25.30	-85.79	1.90	-82.80	7.94	-75.54	5.69
Venlafaxine	-7.40	2.53	5.77	5.48	-36.18	28.62	-39.68	13.27

Table 3. Method detection and quantification limits (MDL, MQL) of the target compounds in *M. galloprovincialis*, *C. gigas*, *C. edule* and *S. marginatus*.

	MDL (ng/g dw)				**MQL (ng/g dw)**			
Compound	***M. galloprovincialis***	***C. gigas***	***C. edule***	***S. marginatus***	***M. galloprovincialis***	***C. gigas***	***C. edule***	***S. marginatus***
Atrazine	0.33	0.04	0.05	0.05	1.09	0.12	0.10	0.10
Desethylatrazine	0.07	0.22	0.50	0.10	0.23	0.75	1.00	0.50
Metolachlor	0.02	0.05	0.05	0.03	0.08	0.16	0.10	0.11
Simazine	0.02	0.28	0.02	0.05	0.08	0.95	0.05	0.10
Diazinon	0.10	0.28	0.05	0.11	0.33	0.93	0.10	0.37
Malathion	0.25	0.50	0.25	0.10	0.50	1.00	0.50	0.50
Thiabendazole	0.02	0.07	0.05	0.16	0.08	0.25	0.10	0.55
Bentazone	0.10	0.05	0.01	0.02	0.50	0.16	0.02	0.05
MCPA	0.05	0.03	0.10	0.08	0.25	0.09	0.50	0.27
Propanil	0.25	0.50	0.10	0.22	0.50	1.00	0.50	0.73
Acetamiprid	0.02	0.07	0.50	0.05	0.07	0.24	1.00	0.10
Imidacloprid	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00
1H-benzotriazole	0.16	0.10	0.50	0.10	0.52	0.25	1.00	0.50
Bisphenol A	3.00	10.00	0.50	10.00	10.00	10.00	1.00	10.00
Caffeine	3.00	2.00	2.50	5.00	10.00	4.00	5.00	5.00
Ethylparaben	0.01	0.15	0.01	0.50	0.01	0.50	0.02	1.00
Methylparaben	0.01	0.29	0.02	0.02	0.01	0.98	0.05	0.05
Propylparaben	0.49	0.50	0.05	0.35	1.65	1.66	0.10	1.18
Triclocarban	0.50	0.50	0.25	1.24	1.00	1.00	0.50	4.13
Triclosan	0.50	0.50	0.25	1.07	1.00	1.00	0.50	3.57
Carbamazepine	0.09	0.20	0.02	0.20	0.30	0.66	0.05	0.66
Sulfamethozaxole	0.05	0.25	1.00	0.10	0.17	0.50	1.50	0.50
Venlafaxine	0.07	0.20	0.50	10.00	0.24	0.65	1.00	10.00

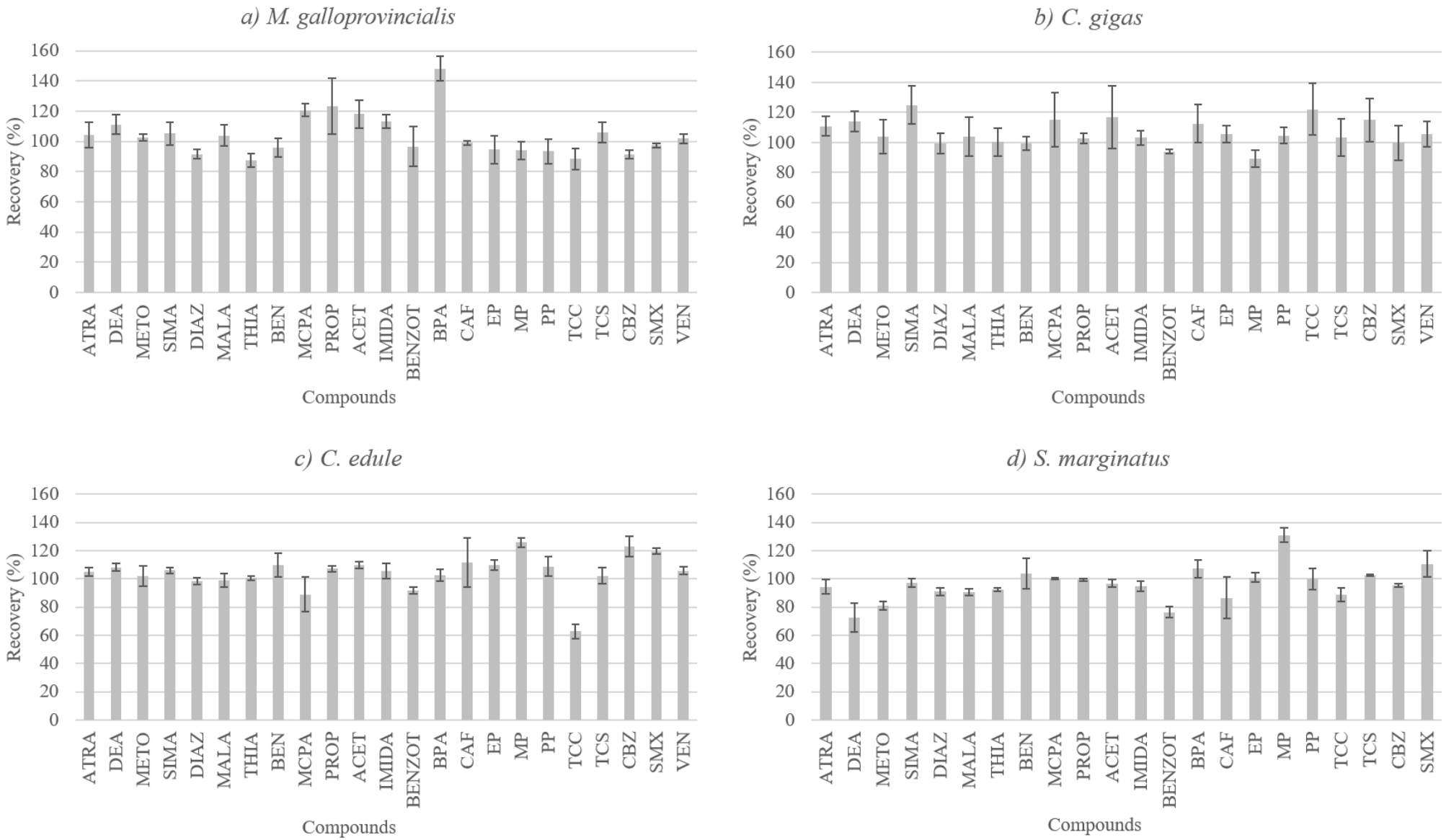
Table 4. Accuracy and precision in *M. galloprovincialis*.

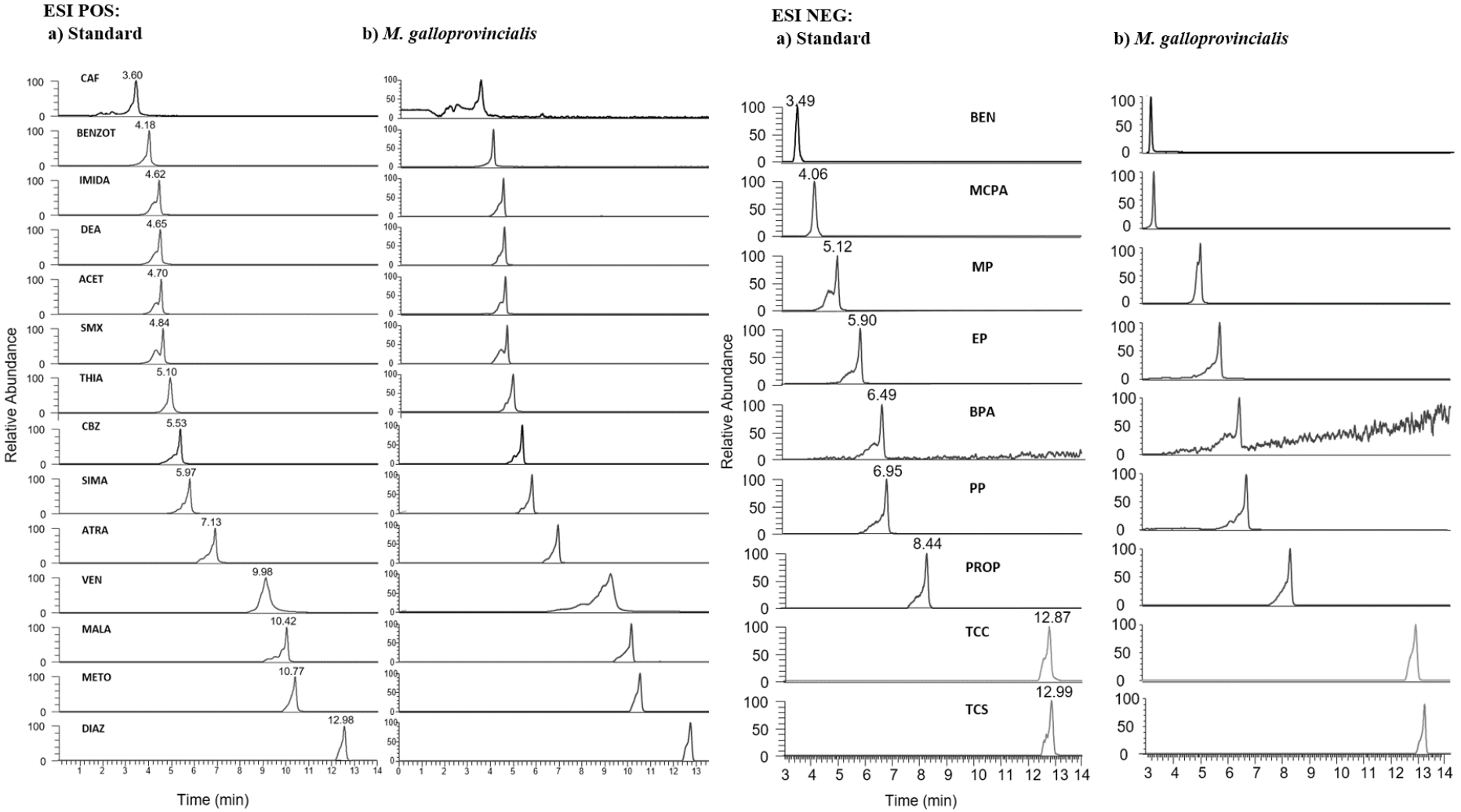
Compound	Intra-day Error (%)	Repeatability RSD (%)	Inter-day Error (%)	Reproducibility RSD (%)
Atrazine	0.2	1.3	-3.0	1.5
Desethylatrazine	2.4	1.3	-12.1	14.9
Metolachlor	-5.8	0.4	-1.5	1.6
Simazine	-3.3	1.6	-5.6	0.1
Diazinon	1.0	0.6	-3.2	1.8
Malathion	-6.3	0.7	-3.3	0.2
Thiabendazole	13.6	1.8	15.8	0.6
Bentazone	1.2	0.5	2.6	0.6
MCPA	-15.0	1.5	-13.1	2.0
Propanil	-15.5	2.6	-12.2	1.1
Acetamiprid	-5.1	1.0	4.6	0.8
Imidacloprid	-6.1	0.3	4.6	2.2
1H-benzotriazole	7.6	1.7	-1.0	1.2
Bisphenol A	-17.1	3.2	-14.1	8.9
Caffeine	13.3	10.0	16.1	10.2
Ethylparaben	18.7	1.1	15.1	1.7
Methylparaben	5.3	3.5	10.6	1.1
Propylparaben	7.0	1.2	8.0	2.2
Triclocarban	2.6	7.4	18.8	16.9
Triclosan	-8.0	0.6	-7.9	1.4
Carbamazepine	-5.2	0.9	17.9	4.8
Sulfamethozaxole	1.3	1.3	2.4	1.9
Venlafaxine	16.7	0.7	6.8	3.4

Table 5. Concentrations measured (ng/g dry weight, n=3) in *C. gigas* and *C. edule* collected from Ebro Delta (Spain).

Compound	Compounds concentrations (ng/g dry weight) ± RSD							
	***C. gigas* control May**	***C. gigas* dead May**	***C. gigas* control June**	***C. gigas* dead June**	***C. gigas* control Nov**	***C. gigas* dead Oct**	***C. edule* control Dec**	***C. edule* dead Sept**
Atrazine	nd[a]	nd	nd	nd	nd	nd	nd	2.64±0.03
Desethylatrazine	nd	nd	nd	nd	nd	nd	nd	nd
Metolachlor	nd	nd	nd	1.15±0.05	nd	nd	nd	nd
Simazine	nd	nd	nd	nd	nd	nd	nd	nd
Diazinon	nd	nd	nd	nd	nd	nd	nd	nd
Malathion	nd	nd	nd	nd	nd	nd	nd	nd
Thiabendazole	nd	nd	nd	nd	nd	nd	nd	nd
Bentazone	nd	nd	1.56±0.13	0.14±0.09	0.61±0.02	1.24±0.14	0.35±0.02	5.11±0.33
MCPA	nd	nd	nd	nd	nd	nd	nd	nd
Propanil	nd	nd	nd	nd	nd	nd	nd	nd
Acetamiprid	3.68[b]	6.71[b]	9.51±0.96	nd	nd	nd	nd	3.50±0.86
Imidacloprid	nd	nd	nd	nd	nd	nd	nd	nd
1H-benzotriazole	nd	nd	nd	nd	nd	nd	nd	2.43±0.89
Bisphenol A	ml[c]	ml	ml	ml	ml	ml	nd	4277.40±13.84
Caffeine	nd	nd	nd	nd	nd	nd	nq[d]	99.85±23.84
Ethylparaben	nd	nd	nd	nd	nd	nd	3.56±0.09	nd
Methylparaben	nd	nd	nd	nd	1.10±0.39	0.29±0.07	6.69±0.25	nd
Propylparaben	nd	nd	nd	nd	nd	nd	0.56±0.05	nd
Triclocarban	nd	nd	nd	nd	nd	nd	nd	nd
Triclosan	nd	nd	nd	nd	nd	nd	nd	nd
Carbamazepine	nd	nd	nd	nd	nd	nd	nd	nd
Sulfamethozaxole	nd	nd	nd	nd	nd	nd	nd	nd
Venlafaxine	nd	nd	nd	nd	nd	nd	ml	ml

[a]nd= non detected (<MDL), [b]low amount of sample, only one replicate analysed, [c]ml=method limitation, [d]nq= non quantified (<MQL).


a) M. galloprovincialis
b) C. gigas
c) C. edule
d) S. marginatus
Recovery (%)
Compounds
0
20
40
60
80
100
120
140
160
ATRA
DEA
METO
SIMA
DIAZ
MALA
THIA
BEN
MCPA
PROP
ACET
IMIDA
BENZOT
BPA
CAF
EP
MP
PP
TCC
TCS
CBZ
SMX
VEN


ESI POS:
a) Standard
b) M. galloprovincialis
ESI NEG:
a) Standard
b) M. galloprovincialis
CAF 3.60
BENZOT 4.18
IMIDA 4.62
DEA 4.65
ACET 4.70
SMX 4.84
THIA 5.10
CBZ 5.53
SIMA 5.97
ATRA 7.13
VEN 9.98
MALA 10.42
METO 10.77
DIAZ 12.98
BEN 3.49
MCPA 4.06
MP 5.12
EP 5.90
BPA 6.49
PP 6.95
PROP 8.44
TCC 12.87
TCS 12.99
Relative Abundance
Time (min)

SUPPORTING INFORMATION

Fast analysis of relevant contaminants mixture in commercial shellfish

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2.1. Chemicals and reagents

All standards were of high purity grade (>90%), and they are summarised in Table 1. All of them were purchased from Sigma Aldrich. Isotopically labelled compounds used as internal standards were also purchased from Sigma Aldrich except for metolachlor-d6, thiabendazole-c13, malathion-d7 and triclosan-d3 that were purchased from Dr Ehrenstorfer, propanil-d5 and sulfamethoxazole-d4 from Toronto Research Chemicals, and caffeine-d3 and bisphenol A d-4 from CDN Isotopes. Individual stock standards and isotopically labelled standards were prepared in methanol at a concentration of 10 µg/mL. Working standards solutions of 1 µg/mL, containing either standards or isotopically labelled internal standards were prepared in 100% acetonitrile (ACN) before each analytical run.

The QuEChERS Bekolut Citrat-Kit-01 and Bekolut PSA-Kit-04A were kindly supplied by Bekolut (Barcelona, Spain). The OstroTM 96 well plate was purchased from Waters (Barcelona, Spain). The HPLC grade water and ACN were supplied by Merck (Darmstadt, Germany).

Table S1. Mean percentage recoveries and relative standard deviation (RSD, n=3) of the target compounds in *M. galloprovincialis* (mussel), *C. gigas* (oyster), *C. edule* (cockle) and *S. marginatus* (razor shell) at the spiking level (50 ng/g dry weight).

Compound	*M. galloprovincialis*		*C. gigas*		*C. edule*		*S. marginatus*	
	Recovery (%)	**RSD**	**Recovery (%)**	**RSD**	**Recovery (%)**	**RSD**	**Recovery (%)**	**RSD**
Atrazine (ATRA)	104.31	8.29	110.91	6.35	105.19	2.99	94.39	5.16
Desethylatrazine(DEA)	111.27	6.42	114.12	6.66	108.24	2.87	72.67	10.08
Metolachlor (METO)	102.61	2.11	103.68	11.25	101.94	7.27	81.01	3.16
Simazine (SIMA)	105.18	7.78	125.00	12.64	105.94	1.90	97.27	3.17
Diazinon (DIAZ)	91.66	3.30	99.26	6.80	98.41	2.54	91.04	2.76
Malathion (MALA)	103.77	7.05	104.03	12.98	98.85	4.79	90.47	2.36
Thiabendazole (THIA)	87.41	4.66	100.43	9.26	100.70	1.52	92.33	1.39
Bentazone (BEN)	95.80	6.02	99.30	4.57	109.75	8.09	103.69	10.80
MCPA	120.69	4.15	114.99	18.10	88.86	12.26	100.27	0.63
Propanil (PROP)	123.32	18.26	102.77	3.34	107.21	2.03	99.48	0.98
Acetamiprid (ACET)	118.06	9.19	116.61	20.89	109.59	2.41	96.69	2.64
Imidacloprid (IMIDA)	113.09	4.49	103.18	4.82	105.78	5.45	94.66	3.69
1H-benzotriazole (BENZOT)	96.64	13.13	93.91	1.56	91.77	2.28	76.41	4.02
Bisphenol A (BPA)	148.24	8.08	ml*	ml	102.51	4.15	107.12	6.32
Caffeine (CAF)	99.02	1.44	112.54	12.56	111.44	17.28	86.52	14.63
Ethylparaben (EP)	94.56	9.11	105.48	5.60	109.59	3.69	101.03	3.24
Methylparaben (MP)	94.08	5.87	89.20	5.72	125.66	3.35	130.83	5.18
Propylparaben (PP)	93.46	8.18	104.68	5.42	108.57	6.89	100.03	7.38
Triclocarban (TCC)	88.37	7.00	122.18	17.14	62.75	5.31	88.74	4.82
Triclosan (TCS)	106.14	6.62	103.18	12.36	102.23	5.73	102.32	0.58
Carbamazepine (CBZ)	91.40	2.93	115.07	14.34	123.15	7.17	95.38	0.94
Sulfamethozaxole (SMX)	97.43	1.36	99.78	11.45	119.49	1.95	110.64	9.46
Venlafaxine (VEN)	101.83	2.98	105.47	8.43	105.85	2.75	ml	ml

*ml=method limitation

Table S2. Linearity (regression coefficient) obtained from calibration curves made the corresponding bivalve extract (mussel, oyster, cockle and razor shell), concentration range from 0.05-50 ng/g.

Chemical family	Compound	Mussel (r^2)	Oyster (r^2)	Cockle (r^2)	Razor Shell (r^2)
Organonitrogen pesticides	**Atrazine (ATRA)**	0.9985	0.9797	0.9999	0.9998
	Desethylatrazine(DEA)	0.9814	0.9884	0.9994	0.9993
	Metolachlor (METO)	0.9999	0.9997	0.9998	0.9997
	Simazine (SIMA)	0.9995	0.9997	0.9944	0.9999
Organophosphorus pesticides	**Diazinon (DIAZ)**	0.9998	0.9982	0.9999	0.9992
	Malathion (MALA)	1.0000	0.9997	0.9994	0.9999
	Thiabendazole (THIA)	0.9986	0.9986	0.9957	0.9989
Herbicides	**Bentazone (BEN)**	0.9947	0.9872	0.9986	0.9998
	MCPA	0.9970	0.9961	0.9988	0.9979
	Propanil (PROP)	0.9971	0.9998	0.9986	0.9997
Insecticides	**Acetamiprid (ACET)**	0.9999	0.9999	0.9986	0.9996
	Imidacloprid (IMIDA)	0.9888	0.9998	0.9999	0.9999
EDCs	**1H-benzotriazole (BENZOT)**	0.9995	0.9994	0.9979	0.9996
	Bisphenol A (BPA)	0.9989	0.9943	0.9887	0.9989
	Caffeine (CAF)	0.9988	0.9981	0.9986	0.8543
	Ethylparaben (EP)	0.9997	1.0000	0.9999	0.9999
	Methylparaben (MP)	0.9998	0.9998	0.9986	0.9996
	Propylparaben (PP)	0.9995	0.9999	0.9994	0.9996
	Triclocarban (TCC)	0.9994	0.9993	0.9996	0.9999
	Triclosan (TCS)	0.9999	0.9992	0.9989	0.9999
PhACs	**Carbamazepine (CBZ)**	0.9998	0.9997	0.9994	0.9994
	Sulfamethozaxole (SMX)	1.0000	0.9999	0.9997	0.9999
	Venlafaxine (VEN)	0.9999	0.9988	0.9999	0.9999

Figure S1. Box plot of shellfish types and percentages of recoveries of the target compounds. The boundary of the box closest to zero indicates the 25th percentile, the black line within the box marks the median and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles.

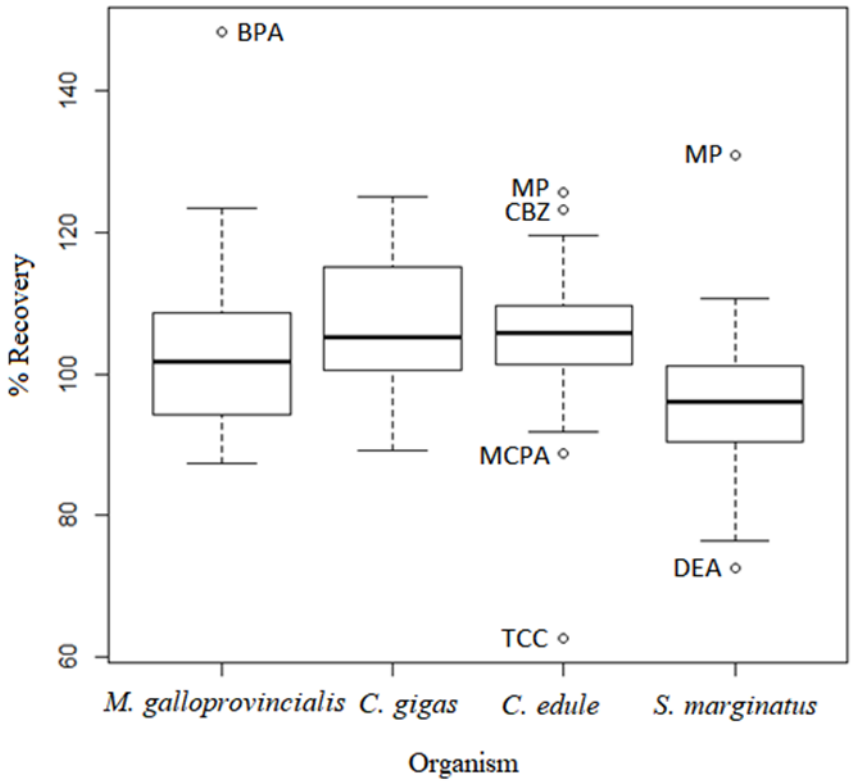

Figure S2. Extracted ion chromatograms of the compounds analysed in *C. gigas* spiked at 50 ng/g in positive and negative electrospray ionisation.

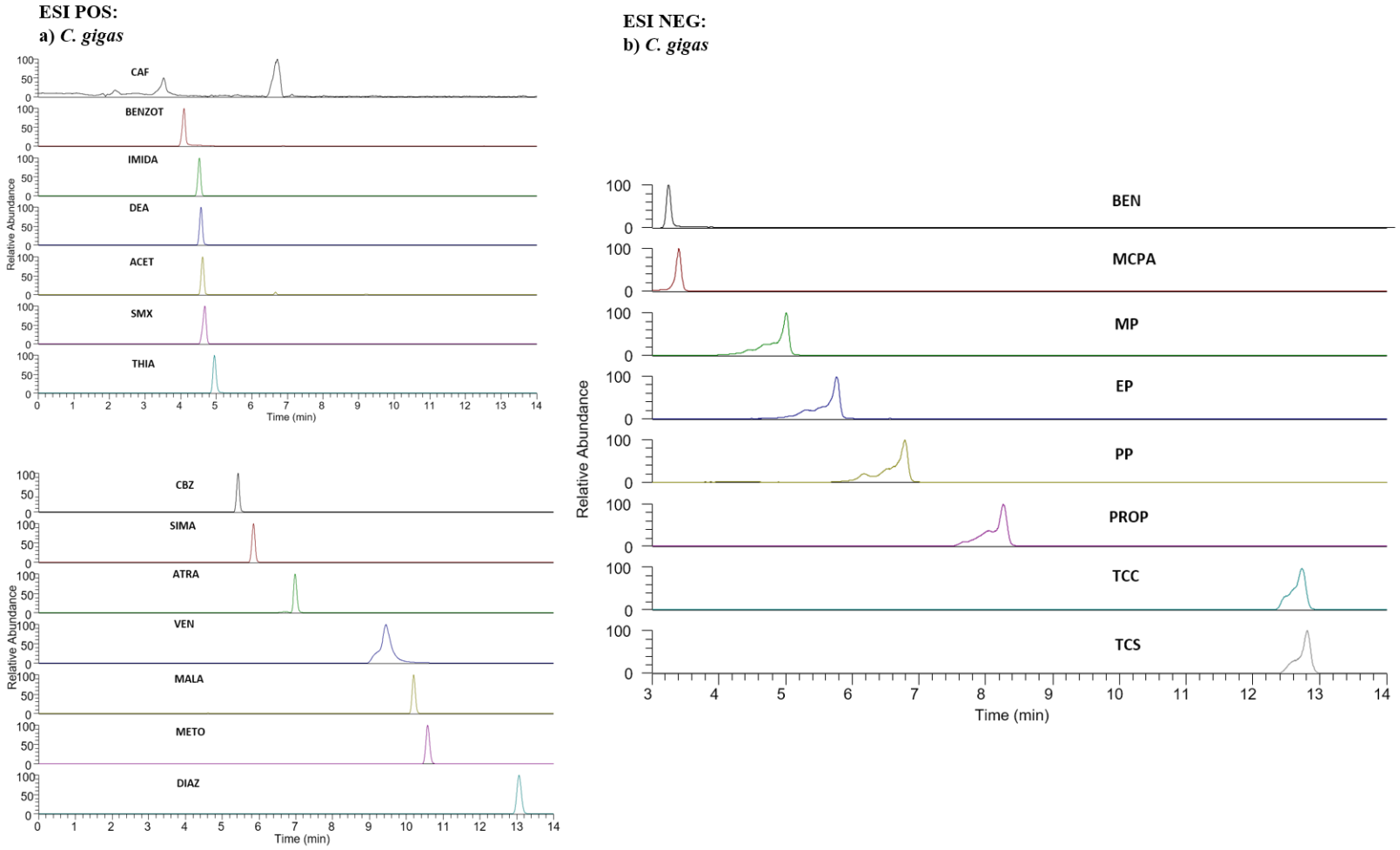

Figure S3. Extracted ion chromatograms of the compounds analysed in *C. edule* spiked at 50 ng/g in positive and negative electrospray ionisation.

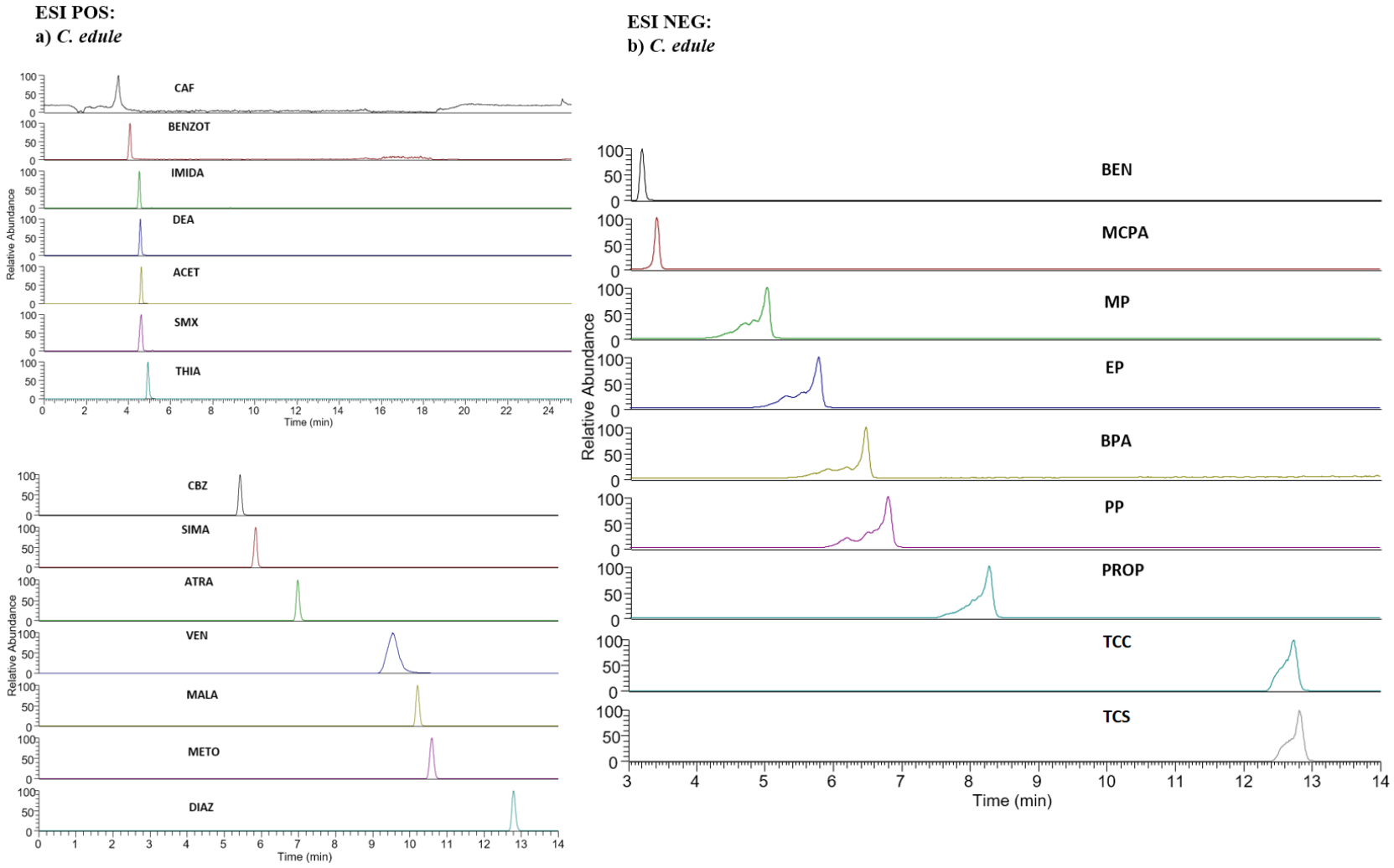

Figure S4. Extracted ion chromatograms of the compounds analysed in *S. marginatus* spiked at 50 ng/g in positive and negative electrospray ionisation.

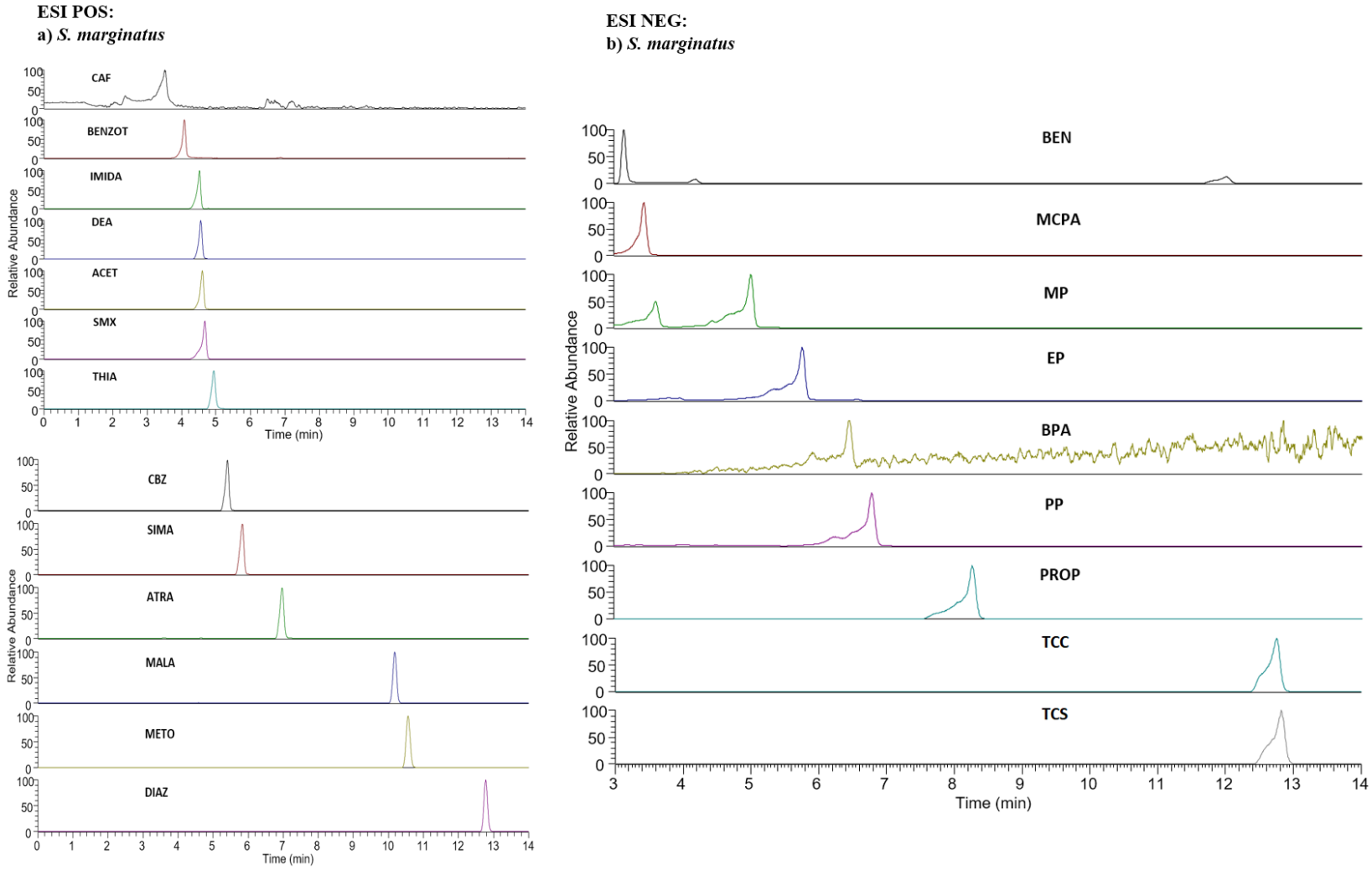

Figure S5. Matrix effect (%) expressed as ion suppression (-) or enhancement (+) for the target compounds tested in M. galloprovincialis (mussel), C. gigas (oyster), C. edule (cockle) and S. marginatus (razor shell)