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Development of a liquid chromatography-quadrupole-time-of-flight-mass spectrometry based method for the targeted and suspect screening of contaminants in the pearl oyster *Pinctada imbricata radiata*☆

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ABSTRACT

A rapid method based on solvent extraction followed by direct injection in liquid chromatography-quadrupole-time-of-flight-mass spectrometry (LC-Q-TOF-MS) was developed for the targeted and suspect screening of contaminants in the soft tissues of the pearl oyster *Pinctada imbricata radiata*. The quantification method was first validated for the targeted analysis of 21 contaminants including some pharmaceutically active compounds, with the relative recoveries ranging from 88 to 123%, and method detection limits generally below 1 ng g^{-1} on the wet weight (ww) basis. This targeted analysis method was then applied to oyster samples collected around the Qatari coast between 2017/2018, and none of the 21 compounds were detected in these samples. The post-acquisition data treatment based on the accurate mass measurement in both full MS scan and All Ions MS/MS was further used for mining other contaminants in oyster extracts, as well as 21 targeted compounds spiked in oyster extracts (suspect screening). The 21 spiked compounds were identified successfully and the estimated limit of identification for the individual 21 compounds ranged from 0.5 to 117 ng g^{-1} ww of oyster tissues. A phthalate, di(2-ethylhexyl) phthalate (DEHP) was identified to be present in oyster extracts from 2018 batches, at a concentration level significantly higher than that in procedure blanks. These results confirmed that high resolution MS data obtained using the targeted method can be exploited through suspect screening workflows to identify contaminants in the tissues of bioindicator mollusks. However, a number of false identifications could be obtained and future work will be on improving the success rate of the correct identifications using this workflow.

1. Introduction

Contaminants of emerging concern (CECs), as firmly defined by Sauvé and Desrosiers, are "naturally occurring, manufactured chemicals or materials which have now been discovered or are suspected present in various environmental compartments and whose toxicity or persistence are likely to significantly alter the metabolism of a living being" (Sauve and Desrosiers, 2014). Chemicals such as current-use pesticides, pharmaceuticals and personal care products (PPCPs), cyanotoxins, nanoparticles, flame retardants are frequently characterized as CECs. Chemicals found in everyday products, such as PPCPs, enter the environment on a large scale mainly through the discharge of municipal wastewater effluents (Petrie et al., 2015). Most of the available studies concentrated on quantification of CECs in water and little is known on the occurrence in biota (Pico et al., 2019). Antibiotics have been detected in intertidal sediments from the northern coastline of Iran (Kafaei et al., 2018). However, to date, there are no other data on the

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occurrence of CECs such as PPCPs in aquatic systems in the Arabian Gulf region, particularly for biota.

Bivalve mollusks are widely used in coastal monitoring programmes due to their ability to accumulate pollutants (Melwani et al., 2013). Generally, the degree of chemical contamination in the environment can be indicated by measuring the levels of contaminants accumulated in the tissues. Species such as mussels have been reported recently for the monitoring of CECs in Singapore (Bayen et al., 2016) or in Hong Kong (Burket et al., 2018). The pearl oyster (*Pinctada imbricata radiata*) is widely distributed throughout the Indo-Pacific and notably in the Arabian Gulf. *P. i. radiata* live in benthic zone with relatively long life cycles and good adaptability to a wide range of environmental changes (Al-Madfa et al., 1998). In Qatar, the pearl oyster is considered being an essential part of the nation's cultural heritage and one of the main economic foundations upon which the nation developed. However, the pearl oyster is not immune to environmental pressures, and as Qatar has prospered and developed coastal urban and industrial areas, oyster beds have been impacted (Smyth et al., 2016).

Analytical approaches for the determination of CECs such as PPCPs in marine organisms traditionally involve an extraction step prior to quantification with high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) (Bayen et al., 2015; Montesdeoca-Esponda et al., 2018). Mass spectrometers such as triple quadrupoles and ion traps are commonly used to analyze and record data for a limited list of target contaminants in each sample. The list of contaminants detected in coastal waters and mollusks keeps on increasing though. In this context, innovative non-targeted tools are highly needed to effectively track known, unknown and unexpected contaminants in marine samples.

Chromatography coupled to high-resolution mass spectrometry (HRMS) is one of the state-of-the-art technologies for the identification of both known and "unknown" chemicals in complex matrices. For example, HRMS has been reported for the non-targeted screening for chemical contaminants in various food, water, wastewater or soil matrices (Blum et al., 2017; Hollender et al., 2017; Knolhoff and Croley, 2016; Tengstrand et al., 2013). With regards to marine organisms, HRMS such as quadrupole-time-of-flight MS (Q-TOF-MS) has been reported for the study of algal toxins in oysters (Kilcoyne and Fux, 2010) and antibiotic metabolites in mussels (Tian and Bayen, 2018). HRMS has also been applied to the multiclass screening of >200 pharmaceutical and other residues in other mollusks species such as mussels (Kong et al., 2018). Turnipseed et al. (2015) concluded in a review that there were no specific examples of HRMS non-targeted analysis for chemotherapeutic residues in seafood, and very few examples for veterinary drug residues in general. To the best of our knowledge, there is still no non-targeted study of anthropogenic contaminants in mollusks such as oysters.

The aim of this study was to develop (i) a rapid method for the targeted analysis of specific contaminants including CECs in oysters using HPLC-Q-TOF-MS, while (ii) simultaneously recording non-targeted information (fast, high-resolution MS scans) for the identification of other environmental contaminants. Twenty-one target compounds were selected to explore the performance of the present approach for different families of contaminants. Some of these compounds have been detected in coastal waters elsewhere (Ali et al., 2018; Bayen et al., 2013; Bayen et al., 2015; Wille et al., 2011). A suspect screening workflow was then applied to real pearl oyster samples as a proof-of-concept. The novelty of this study is the use of direct injection combined with HRMS for the target and suspect screening of contaminants in mollusk tissues.

2. Materials and methods

2.1. Chemicals

Standards of the 21 native target analytes (Table 1), dibutyl phthalate (DBP, 99%) and tris (2-butoxyethyl) phosphate (TBOEP, 95%) and bis(2-ethylhexyl-phthalte) (DEHP) were purchased from Sigma-Aldrich (St Louis, MO, USA). Log K_{ow} (n-octanol/water partition coefficient) ranged from −0.2 to 4.76 for the 21 native target analytes (Kim et al., 2019). Isotopically labeled analogs listed in Table S1 were purchased from C/D/N Isotopes (Pointe-Claire, Canada), Sigma-Aldrich (St Louis, MO, USA), and Toronto Research Chemicals (North York, Canada). HPLC grade solvents were obtained from Fisher Scientific (Hampton, NH, United States). Primary stock solutions of all individual analytes were prepared in methanol at 500 μg mL^{-1}. Multiple sub-stock solutions, mixtures of 21 native target analytes, were prepared from individual primary stock solutions in methanol at concentration range from 5 to 2500 ng mL^{-1}. One mixture solution of mass-labeled analogs was prepared from primary stock solutions at 100 μg mL^{-1}. Individual LC-MS calibration standards were prepared by adding 20 μL of the required mixture of native analytes and 20 μL of the mixture of mass labeled analogs into 940 μL of methanol or matrix. The concentration of calibration standards ranged from 0.1 ng mL^{-1} to 100 ng mL^{-1}. All samples were stored at −20 °C in the dark. Glassware was baked at 300 °C overnight, and rinsed with mobile phase before use.

2.2. Oyster samples

Forty-eight batches of oysters (*P. i. radiata*) were collected from 4 different sites along the coast of Qatar in 2017 and 2018. Soft tissues were removed from the shell and homogenized in a blender. The size was recorded for each organism and ranged from 2 to 7 cm in this study. Homogenized samples were then frozen at −20 °C, transported from Qatar to Canada (<0 °C), and frozen again at −20 °C prior to analysis.

2.3. Sample preparation and extraction

The extraction procedure of contaminants from oyster tissues was reported in a previous study (Bayen et al., 2015). In brief, 1.5 g of oyster tissues was mixed with 7 mL of acetonitrile/methanol (50:50 v/v) in a 50 mL polypropylene centrifuge tube and sonicated for 30 min at room temperature using a Branson 3510 sonicator (40 KHz). After sonication, the tubes were centrifuged for 10 min at 3000×*g* (25 °C), and the supernatant was filtrated with 0.22 μm PTFE syringe filter (Canadian Life Science, Canada) before introducing into the HPLC-Q-TOF MS.

2.4. Instrument analysis

Samples were analyzed with an Agilent 1290 Infinity II LC system coupled to the 6545 Q-TOF -MS (Agilent Technologies, Santa Clara, USA). The LC separation was conducted on a Poreshell120 EC-C18 analytical column (Agilent Technologies; 2.7 μm × 3 mm × 100 mm) connected with a Poreshell120 EC-C18 guard column (Agilent Technologies; 2.7 μm × 3 mm × 5 mm). The mobile phase A was HPLC water with 5 mM ammonium acetate and the mobile phase B was acetonitrile/methanol mixture (50:50 v/v) with 5 mM ammonium acetate. The HPLC and MS parameters were optimized separately for positive (ESI+) and negative ionization (ESI-) modes, based on the MS responses and separation of the 21

Table 1
Method performances (recoveries, MDLs, MQLs, matrix effects and precision) for the analysis of the 21 target analytes in *P. i. radiata*.

Target analytes	CAS number	Quantifier *m/z*	MDLs (ng/g ww)	MQLs (ng/g ww)	Mean absolute recoveries (%, n = 9)	Mean relative recoveries (%, n = 9)	Matrix effects (%, n = 3)	Intraday precision (RSD,%, n = 6)
ESI+								
Acetaminophen	103-90-2	152.0712	0.19	0.63	79 ± 17	106 ± 10	−6.1 ± 2.5	9.0
Atrazine	1912-24-9	216.1016	0.53	1.77	99 ± 5	110 ± 12	−8.0 ± 2.5	3.5
Caffeine	58-08-2	195.0882	0.35	1.17	87 ± 22	101 ± 18	−1.0 ± 2.1	6.8
Carbamazepine	298-46-4	237.1028	0.076	0.25	98 ± 3	112 ± 11	2.9 ± 1.1	2.6
Cotinine	486-56-6	177.1028	0.094	0.31	114 ± 41	110 ± 13	0.7 ± 2.5	2.9
Diuron	330-54-1	233.0249	0.11	0.37	102 ± 6	114 ± 12	7.8 ± 1.7	2.0
Fluoxetine	54910-89-3	310.1419	0.12	0.40	101 ± 2	118 ± 8	5.4 ± 1.2	2.4
Lincomycin	154-21-2	407.2216	0.17	0.57	93 ± 7	105 ± 10	18.8 ± 13.1	5.9
Monocrotophos	6923-22-4	224.0688	0.031	0.10	96 ± 12	112 ± 11	6.4 ± 4.1	2.9
Paroxetine	61869-08-7	330.1506	0.55	1.83	103 ± 1	123 ± 12	8.6 ± 1.2	2.8
Sulfadimethoxine	122-11-2	311.0814	0.070	0.23	99 ± 4	114 ± 20	10.7 ± 2.1	5.5
Sulfamethazine	57-68-1	279.0916	0.027	0.09	100 ± 3	117 ± 19	3.5 ± 2.5	3.9
Sulfamethoxazole	723-46-6	254.06	0.27	0.9	98 ± 6	110 ± 28	119.3 ± 4.1	4.4
Trimethoprim	738-70-5	291.1457	0.15	0.5	105 ± 16	121 ± 34	12.4 ± 2.6	3.2
Tylosin A	1401-69-0	916.5271	0.57	1.9	98 ± 4	111 ± 7	−24.5 ± 7.2	6.6
Tylosin B	11032-98-7	772.4484	0.49	1.63	97 ± 8	112 ± 18	91.1 ± 12.0	6.4
Venlafaxine	93413-69-5	278.212	0.048	0.16	98 ± 4	109 ± 10	4.7 ± 1.3	3.0
ESI-								
Bisphenol A	80-05-7	227.1072	0.68	2.27	91 ± 19	88 ± 10	−17.7 ± 13.4	7.0
Gemfibrozil	25812-30-0	249.1491	0.75	2.5	124 ± 1	109 ± 11	10.8 ± 2.7	6.1
Ibuprofen	15687-27-1	205.1229	0.82	2.73	119 ± 41	98 ± 16	−169.3 ± 13.5	6.5
Triclosan	3380-34-5	286.9433	0.26	0.87	111 ± 18	109 ± 10	−14.2 ± 0.3	1.7

target analytes (17 and 4 analytes in ESI+ and ESI-, respectively). HPLC parameters were as follows: the flow rate is 0.4 mL min^{-1} for positive ion mode and 0.3 mL min^{-1} for negative ion mode. Since the MS response factor for the 4 analytes detected in ESI- was relatively lower than that for 17 analytes in ESI+, the injection volume was set to 6 μL and 10 μL for ESI+ and ESI-, respectively. The column temperature was set to 30 °C for both ionization modes. The mobile phase profile used for the run in ESI + mode was: 1% B (0–0.5 min), linear increase to 50% B (0.5–3 min), linear increase to 100% B (3–5 min), 100% B (5–10 min), decrease to 1% B (8–8.01 min) and finally 1% B (8.01–10 min). The mobile phase profile used for the run in ESI- mode was: 5% B (0–0.5 min), linear increase to 100% B (0.5–3 min), 100% B (3–7 min), decrease to 5% B (7–7.01 min) and finally 5% B (7.01–10 min). The mass spectrometer was equipped with a Dual AJS ESI ion source operating in both positive and negative ionization modes. Optimized MS conditions were as follows: for ESI+, the drying gas temperature was 150 °C, drying gas flow rate was 11 L/min, sheath gas temperature was 375 °C, sheath gas flow rate was 12 L/min, the pressure on the nebulizer was 30 psi, the capillary voltage was 4000 V, the fragmentor voltage was 125 V, the skimmer voltage was 45 V and the nozzle voltage was 1000 V; for ESI-, the drying gas temperature was 175 °C, the drying gas flow rate was 10 L/min, sheath gas temperature was 375 °C, sheath gas flow rate was 12 L/min, the pressure on the nebulizer was 30 psi, the capillary voltage was 4000 V, the fragmentor voltage was 125 V, the skimmer voltage was 50 V and the nozzle voltage was 2000 V. Full scan MS data were recorded between mass-to-charge ratios (*m/z*) 100 and 1100 at a scan rate of 2 spectra/s, and were collected at both centroid and profile mode. All Ions MS/MS data was collected between *m/z* 50 and 1100 at a scan rate of 2 spectra/s for four different collision energies (0, 10, 20, and 40 V). The first 1.5 and 3 min of elution were diverted to waste in ESI + and ESI- modes, respectively. Reference ions (*m/z* at 121.0508 and 922.0098 for ESI+, 112.9856 and 1033.9881 for ESI-) were used for automatic mass recalibration of each acquired spectrum. The average mass resolution was around 20000 within the *m/z* range of 50–1100 in both ESI+ and ESI- modes. Samples were kept at 4 °C in the multisampler compartment.

2.5. Targeted analysis of the 21 compounds

For the quantitative analysis of 21 target compounds, data treatment was conducted using Quantitative Analysis B.07.01 from Agilent MassHunter Workstation Software. For each target analytes, the most abundant isotope peak of either $[M+H]^+$ in ESI + or $[M-H]^-$ in ESI- was selected as the quantifier ion (Table 1). Mass extraction window (MEW) values of ±5, 10, 20 and 50 ppm were initially evaluated for the 21 targeted compounds over the investigated concentration range. A MEW of ±20 ppm resulted in better peak shape in the extracted ion chromatograms for all the targeted compounds over the entire calibration range, and was therefore used for the present quantitative analysis.

For the quantification of target analytes, the relative response (RR) or the relative factor (RF) were calculated for each analyte at each calibration point using the equations below, recommended by the US EPA for analysis in environmental matrices (US EPA, 2007).

When a labeled analog was available (isotope dilution), RR was calculated using Equation (1a):

$$RR = \frac{A_t/A_{is}}{C_t/C_{is}} \quad (1a)$$

where A_t and A_{is} were the peak area of target analyte and corresponded mass-labeled internal standards, respectively; and C_t and C_{is} were the concentration of target analyte and corresponded mass-labeled internal standards, respectively.

When a labeled analog was not available, a reference compound

was attributed as internal standard (See Table S2), and RF was calculated using Equation (1b):

$$RF = \frac{A_t/A_{is'}}{C_t/C_{is'}} \quad (1b)$$

where $A_{is'}$ and $C_{is'}$ represented the response and concentration of the reference analog, respectively.

Since a constant amount of mass-labeled compounds were spiked in all the samples, the concentration of each target compound in extracts was calculated at the following equations:

$$C_t = \frac{A_t/A_{is}}{RR} \times C_{is} \quad (2a)$$

$$C_t = \frac{A_t/A'_{is}}{RF} \times C_{is'} \quad (2b)$$

where, mean RR or RF values averaged from the whole calibration range was used for each target analyte.

Method validation also included the assessment of the linearity of the calibration response, instrument detection limits (IDLs signal-to-noise (S/N) of 3), method detection limits (MDLs; 3σ of the procedural blank signals (Currie, 1999),), the method quantitation limits (MQLs, 10σ of the procedural blank signals), precision, recovery, matrix effect and mass accuracy. Spiking tests were conducted at 3 levels in the oyster tissue samples to assess recoveries (11.6, 46.7 and 116.7 ng g^{-1} ww of oyster tissue, n = 3 for each level). Spiked oyster samples were homogenized using a vortex shaker and equilibrated for 30 min, followed by the same extraction method as described above. Absolute recovery (%) was calculated as the ratio of peak area for analyte spiked into an oyster sample prior to extraction and the one for analyte spiked into the extract at the same amount. Relative recovery (%) was calculated as the ratio of the area ratio A_t/A_{is} for target analyte and its internal standard spiked into an oyster sample prior to extraction, to the one for corresponded compounds spiked into an extract at the same amount. Matrix effects were assessed as the ratios of the slopes of matrix-matched calibration and the calibration in solvent.

2.6. Suspect screening

Suspect screening was performed using the software MassHunter Profinder B.08.00 from Agilent Technologies. Data treatment was performed in the "Targeted Feature Extraction" mode, which performs the peak alignment and of molecular feature extraction steps and compares the resulting MS information with a library (database). Data were extracted using the parameters listed in Table S4. The library *Environmental Water Screening PCDL* (Agilent Technologies) was used to screen. Among the tentatively identified compounds, only those with peak height above 300 were considered for the further data treatment.

3. Results and discussion

3.1. Method performances for the targeted analysis of 21 target CECs

Q-TOF-MS was applied in the present study for the analysis of 21 target analytes in oyster tissues. Extracted ion chromatograms (EICs) illustrating the signals obtained for all the target analytes spiked in an oyster extract at 2.5 ng mL^{-1} are presented in Fig. 1. The retention times were reproducible for all target analytes with relative standard deviations (RSD) ranging from 0.06% to 1.04% (Table S2). Mean mass measurement errors (Brenton and Godfrey, 2010) ranged from −1.2–2.5 ppm (0.03–1.0 mDa) for the compounds in a solvent (methanol/water), and from −5.9–3.7 ppm (0.1–1.8 mDa) for spiked oyster tissue extracts (Table S3, supporting information). Most of the absolute values were below 3 ppm in oyster tissue extracts. Kang et al. (2017) reported a measured mass error in the range of 0–12.8 ppm for the analysis of 100 veterinary drug residues using ion trap TOF-MS in food products including fish. Dasenaki et al. (2015) reported a maximum mass error of 2 mDa during the screening of 143 veterinary drugs and pharmaceuticals in fish tissue using LC-Q-TOF-MS, which is similar to the present mass measurement error for contaminants in oyster extracts. A small but significant difference ($p < 0.05$) shift in the mass measurement error was observed for 14 compounds for these two matrices. The small increase of mass measurement error in matrix is likely due to the interference of ions produced by other co-extracted compounds from the matrix. These ions could compete with the targeted ions during electrospray ionization and generate electrical repulsion to the targeted ions during ion accumulation, transmission and detection process, which could detrimentally affect the peak shape and statistic mass distribution of the targeted ions, eventually resulting in a decrease of the mass accuracy (Calbiani et al., 2006; Zhou et al., 2017).

Based on the recommendation by US EPA (2007), mean RR or RF were accepted for computing concentrations when the RSD amongst all the calibration standards was below 20% and 35% respectively. These criteria were achieved for all compounds, except for ibuprofen, which cannot get baseline separation with a neighbouring interference peak in the oyster extract. In this case, the concentration of ibuprofen was calculated using the linear fitting of the complete calibration curve (USEPA, 2007).

The IDLs were calculated as the amount (pg) of the analyte injected from a pure solvent resulting in a S/N ratio of 3 (Table S2). For 19 out of 21 targeted analytes, the IDLs were under 10 pg using the present method. Only the IDLs for tylosin B and bisphenol A were 10.0 and 18.1 pg injected, respectively. Overall, the IDLs for all the investigated compounds are comparable with reported data using LC-triple quadrupole MS (0.03–24 pg injected) (Bayen et al., 2015). MDLs and MQLs for tissues calculated using the procedure blank were generally below 1 ng g^{-1} and 3 ng.g^{-1}on the wet weight (ww) basis respectively (Table 1), and were in the range or in some cases lower than reported values from other seafood (mussels and clams) analyzed using triple quadrupole MS (Bayen et al., 2015).

The absolute recovery of all target analytes (Table 1) and internal standards (Table S1) ranged from 79% to 124%. The relative recoveries for the present method ranged from 88 to 123% for the target compounds (Table 1). The mean RSD (n = 6) on the relative recoveries for these 21 compounds was 13%, suggesting the method is overall reproducible. To further illustrate the importance of isotopically labeled analogs, matrix effects were assessed in oyster tissues, and ranged from 0.7 to 169%. Strong matrix suppression was observed for sulfamethoxazole and tylosin B, while strong matrix enhancement was noted for tylosin A and ibuprofen. Intraday precision (n = 6) was satisfactory with values consistently below 10% (Table 1).

3.2. Analysis of the 21 contaminants in pearl oyster samples collected in Qatar

The validated method was applied to oyster sample batches collected in the coastal waters of Qatar. None of the 21 compounds was detected in the oyster samples collected in 2017 and 2018. There are currently no comparative values for the pearl oyster reported in the literature.

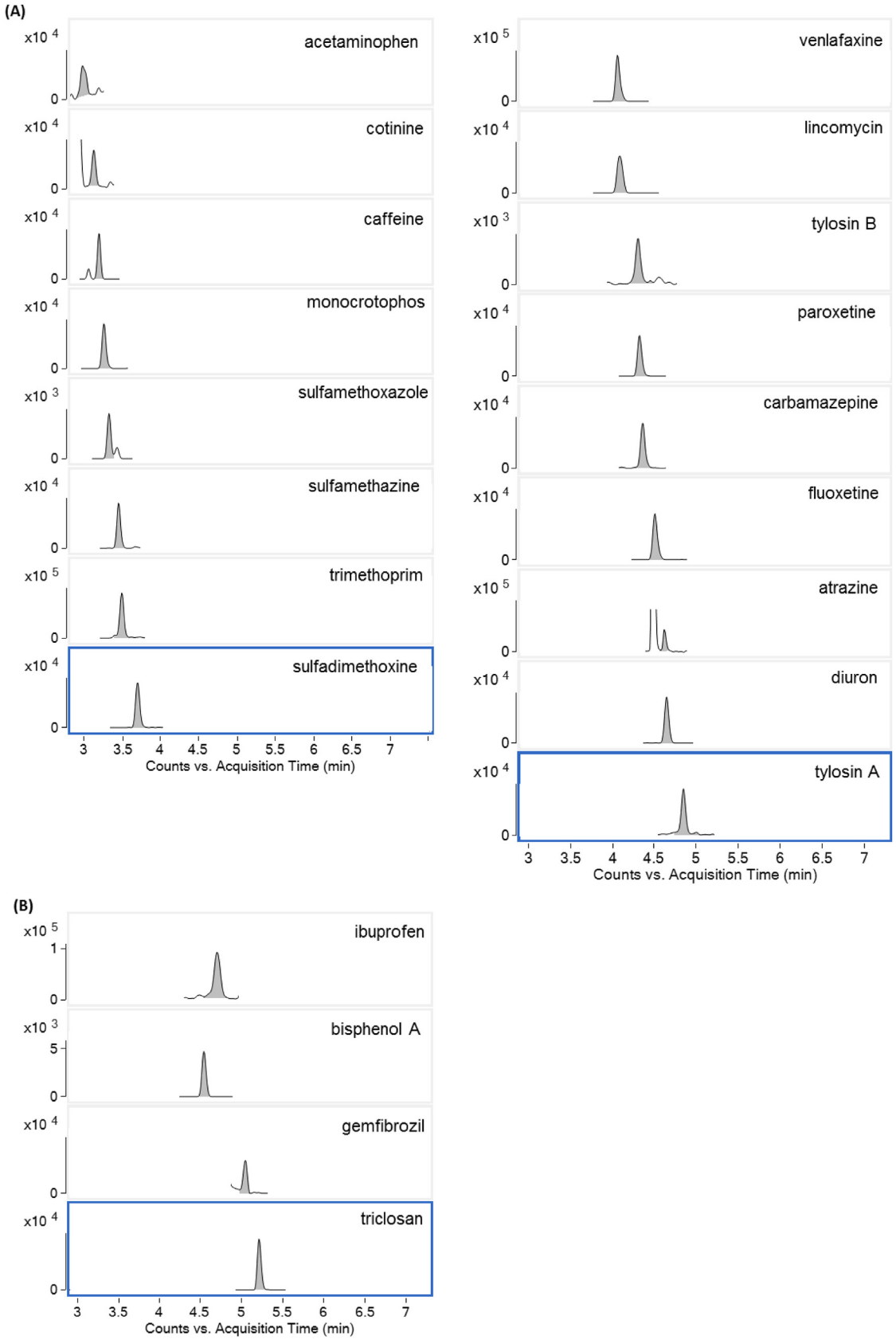

Fig. 1. Extracted ion chromatograms obtained for the 21 target analytes spiked in an oyster extract (2.5 ng mL^{-1}) in ESI+ (panel A) and ESI- (panel B) modes.

3.3. Suspect screening of contaminants in oyster tissues

Accurate mass measurement with HRMS can support the identification of unknowns, with the additional advantage of retrospective analysis (Hird et al., 2014). A second objective of this study was to assess the suspect screening capacity of this approach for trace contaminants in oyster tissues, using the data initially collected for targeted analysis.

First, the post-acquisition data treatment was challenged to identify the 21 targeted contaminants spiked at ten different concentrations (0.1–200 ng mL^{-1}) in the oyster extracts (See Table S5 for details). These spiking levels would correspond to approximately 0.5–930 ng g^{-1} fresh weight in oyster tissues. The "blind" screening of spiked model contaminants is an approach commonly used to validate the suspect screening capacity of this type of workflow (Tengstrand et al., 2013; Tian et al., 2019). After the LC-MS analysis of the complete batch (12 oyster tissue samples and 3 procedural blanks), data treatment was conducted using the "*Targeted Feature Extraction*" mode. This algorithm extracted all the features in the samples and compared the resulting mass and mass spectra information with a library, e.g. an in-house library of the 21 compounds in the present study.

The number of target compounds positively identified (out of a maximum of 21 target compounds) was plotted as a function of the concentration spiked in oyster extracts (Fig. 2). The concentration influenced the capacity of the suspect screening workflow to identify spiked contaminants. Eight compounds (atrazine, BPA, carbamazepine, diuron, ibuprofen, monocrotophos, sulfamethoxazole and triclosan) were positively identified even at the lowest level of spiking (0.1 ng mL^{-1}). Other compounds were positively identified only at a higher concentration in oyster extracts. For example, acetaminophen was successfully identified only when the concentration was greater than 25 ng mL^{-1}. Altogether, the existing workflow was able to identify >95% (20/21) of the 21 analytes when the compounds were present at a level above 5 ng mL^{-1}. The limit of identification (LOI) is defined as the lowest concentration level at which the library searchable mass spectra can be generated for a target compound in all samples (Dasenaki et al., 2015). The estimated LOI for the 21 individual contaminants ranged from 0.5 to 117 ng g^{-1} ww of oyster tissues (Table S4). These LOIs are comparable and sometimes lower than what has been reported for the non-targeted identification of veterinary drugs and pharmaceuticals in fish (Dasenaki et al., 2015). Several factors are known to influence the screening capacity of LC-HRMS based methods for contaminants and notably the matrix (Delatour et al., 2018). Kong et al. (2018) reported that the percentage of analytes (veterinary drugs) identified in marine species extracts using LC-Orbitrap increased with the spiked concentration increasing from 10 μg kg^{-1} to 50 μg kg^{-1}. Signal-to-noise ratios were calculated using Mass Hunter Workstation Software – Quantitative Analysis B.07.01 for each of the target compounds in the extracted chromatograms (extraction window values of ±20 ppm) for the spiked oyster extracts (Table S5). The mean S/N of the signals corresponding to the LOI was 7 ± 9 for the contaminants, and most of these S/N values were <5. The highest S/N (37) corresponding to a LOI was obtained for sulfadimethoxine, for which the poorest mass accuracy in the matrix had been recorded (Table S3). Tengstrand et al. (2013) reported that maxima with signal-to-noise ratios greater than 30 were accepted as peaks for the identification of 26 model contaminants in orange juice using UHPLC–TOF-MS. The two software packages used in the present study, MassHunter Profinder B.08.00 and Mass Hunter Workstation Software – Quantitative Analysis, do not apply the same algorithms to extract and quantify peaks. Even though, it would appear that the suspect screening workflow was able to detect the peak and attribute a formula (and therefore a possible structure through comparison with a database) to substances present in the extract at a level just above their limit of detection (S/N = 3) in LC-Q-TOF-MS (Table S5).

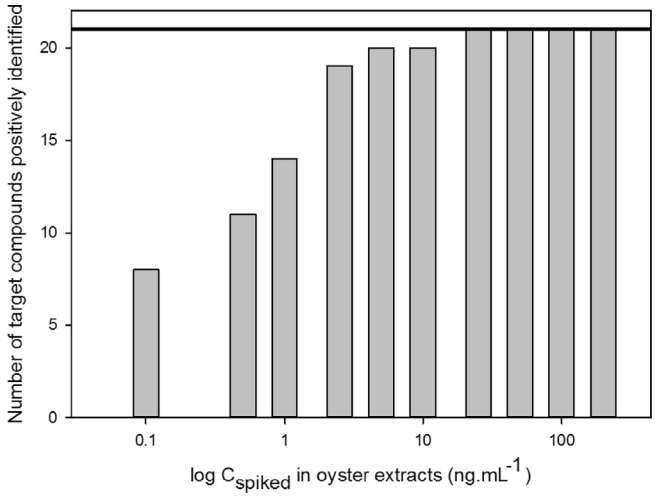

Fig. 2. Number of target compounds positively identified (out of 21 target compounds) through suspect screening as a function of the concentration spiked in oyster extracts (ng.mL^{-1}).

The isotope pattern of a molecular feature is key to generate a molecular formula. For each of the target compounds, the isotope ratios of the two most abundant isotopic peaks of $[M+H]^+$ in ESI+ (or $[M-H]^-$ in ESI-) were calculated in oyster extracts to evaluate if the matrix had any effect on the isotope distribution (Table S2). The actual isotopic ratios were in line, over the whole spike concentration range, with the theoretical ratios (Table S2), as calculated by the Isotope Distribution Calculator (http://www.sisweb.com/). Indeed, for all the compounds in oyster extracts, the % error between the measured and theoretical isotopic ratios was well below 10%, which contributed to the successful screening of these compounds.

After validation, the suspect screening workflow was applied to screen the oyster extracts for contaminants. Nonspiked samples and blanks were aligned and extracted by Agilent Profinder (B06.00) under "*Targeted Feature Extraction*" mode using the molecular database water screening PCDL for environmental contaminants (1452 compounds, with molecular weights up to 1400 Da). Notably, log K_{ow} was obtained for the compounds of this library with available LC retention time information (268 compounds in total; molecular weights ranging from 119 to 1100 Da). Log K_{ow} values ranged from −0.8 to 6 for this sub-group (Kim et al., 2019), which was overall similar to that of the 21 targeted analytes. This example supports that the selected library contained environmental contaminants falling within the applicability domain of the present method in terms of polarity. In total, 358 and 21 molecular features were identified with a matching score above 70% in ESI+ and ESI- modes, respectively, based on the matching of the *m/z* of molecular ions and the detection of at least 2 isotope/adduct ions.

False identification is an issue when applying non-targeted analyses to identify unknown or unexpected substances in real samples. In particular, tracking contaminants at trace levels is likely to increase the number of false positives (Delatour et al., 2018; Tengstrand et al., 2013; Kaufmann et al., 2011). Data treatment parameters such as the mass extraction window have a major impact on the rates of false identification. For example, a relatively wide mass extraction window has been reported to reduce the false negative during suspect screening at trace level (Turnipseed et al.,

2014). Examples of outcomes of the identification step were selected in the present study to illustrate the issue of false identification. The putative identity of four selected molecular features detected in oyster extracts based on the molecular database (labeled as F1− F4, Table 2) was further investigated using MS/MS information and pure analytical standards. Samples were re-run in LC-MS using the All Ions MS/MS mode at four collision energies (0, 10, 20 and 40 V).

Feature F1 was tentatively identified as tris(2-butoxyethyl) phosphate (TBOEP) in the molecular database. Interestingly, the RT of the feature F1 matched the one for the pure TBOEP standard. However, a thorough review of the All Ions MS/MS data vs those of the molecular database and those obtained from the pure analytical standard confirmed a false identification for F1.

Feature F2 was tentatively identified as sulfamethoxazole in the molecular database. Although, some fragments in the mass spectra matched with those in the molecular database, the analysis of the pure analytical standard confirmed a false identification for F2 (retention times not matching).

Feature F3 was tentatively identified as dibutyl phthalate (DBP) in the molecular database. The RT of the feature F3 matched with the one for the pure DBP standard. A thorough review of the All Ions MS/MS data vs those of the molecular database and those obtained from the pure analytical standard confirmed the correct identification of F3. However, the quantification of the signals indicated there was no statistical difference between the levels of DBP in the samples and the procedural blanks. In other words, this feature corresponded to method contamination.

Finally, feature F4 was identified as di(2-ethylhexyl) phthalate (DEHP) during the suspect screening step, with peak abundances significantly greater in oyster samples collected in 2018 than that in procedure blanks. The mass accuracy and the isotope fidelity for feature F4 (Fig. 3C) matched with both the theoretical values and those obtained for a pure DEHP standard (Fig. 3D). In addition, multiple ion fragments were obtained with a similar abundance distribution in the All Ions MS/MS data of feature F4 in the oyster samples (Fig. 3E) and DEHP (see Fig. 3F and Table S7). The RT (7.01 min, Fig. 3A) of the feature F4 was greater than those of the above 21 target CECs, which was compatible with the fact that DEHP is relatively more nonpolar (log $K_{ow} = 7.6$). In the end, the correct identification of F4 was fully confirmed using the pure DEHP standard (RT = 7.02 min, Fig. 3B).

Taken together, accurate mass alone is insufficient to confirm the identification of the correct molecular formula (Knolhoff and Croley, 2016). The "identification level" and confidence in identification for unknown compounds have been previously defined by Schymanski et al. (2014). In this classification, the level-1 identification ("Confirmed structure") is achieved when appropriate measurement of a reference standard with MS, MS/MS and retention time matching are combined together. The present data confirmed that both MS/MS and RT matching are necessary to confirm the identity of contaminants in oyster tissues, as some false identification could be obtained if only one of these two parameters was used.

4. Conclusions

In this study, a simple method, based on solvent extraction followed by direct injection in LC-QTOF-MS, was developed for the targeted analysis of 21 contaminants in pearl oyster tissues and for the suspect screening of other contaminants using the same data. Detection limits, quantification limits, recoveries, precision, retention time stability, mass accuracy and matrix effects were evaluated for targeted analysis with satisfactory performances for all 21 targeted compounds. None of the 21 targeted compounds was detected in oyster samples collected around the Qatari coast in 2017/2018.

Since LC-HRMS data were recorded during the targeted analysis of the 21 contaminants, the accurate mass analysis was then used as a tool for mining other contaminants (suspect screening) and a list of molecular features was generated for the oyster tissues from ESI-MS full scan data. In total, 379 molecular features were identified as possible environmental contaminants in oyster tissues through suspect screening. Four suspect compounds were selected for confirmation using pure standards. A phthalate, DEHP, was positively detected in some oyster samples collected in 2018. However, other three suspect compounds were confirmed as either false positive or method contamination.

The present results showed that datasets acquired via accurate mass HRMS for targeted analysis can indeed be exploited through suspect screening workflows, to identify contaminants in the tissues of bioindicator mollusks. Accurate mass, isotope pattern, fragments information and retention time need to be combined together to avoid false identification. Overall, the high sensitivity of Q-TOF-MS, its relatively higher resolution and mass accuracy compared to triple quadrupole-MS, or its faster scan speed and lower instrument cost compared to some other HRMS instruments, make it quite competitive for the combined targeted and non-targeted analyses of trace contaminants. More work will be carried to investigate the number of "confirmed structures" and the success rate of the identification step for new contaminants in complex matrices. For example, adding retention time predictions has been shown to improve the identification rate of emerging contaminants and biocides in wastewater and sludge (Aalizadeh

Table 2
Comparison of the identification outcomes for four selected molecular features detected in oyster extracts.

Feature	F1	F2	F3	F4
ESI mode	ESI+	ESI +	ESI +	ESI +
Detected *m/z* for $[M+H]^+$	399.2508	254.0575	279.1575	391.2857
RT (min)	5.51	3.94	5.45	7.01
Putative identity based on molecular database	Tris(2-butoxyethyl) phosphate	Sulfamethoxazole	Dibutyl phthalate	di(2-ethylhexyl) phthalate
Calculated *m/z* for $[M+H]^+$ of putative identity	399.2511	254.0600	279.1596	391.2848
MS/MS fragments vs. literature or compound library (Confidence Lvl 2)	✗[a]	✓	✓	✓
RT comparison with pure standards	✓	✗	✓	✓
MS/MS fragments vs. pure standards	✗	n.a.	✓	✓
Samples vs. procedural blanks[b]	samples > blanks ($p < 0.05$)	samples > blanks ($p < 0.05$)	no difference ($p > 0.05$)	samples > blanks ($p < 0.05$)

n.a.: not applicable.

[a] The mark "✗" indicates that the data obtained for the oyster samples did not match the literature or data from the pure analytical standard; "✓" indicates a match between the data obtained for the oyster samples and either the literature or data from the pure analytical standard.

[b] Comparison of the peak intensities for oyster extracts and procedural blanks.

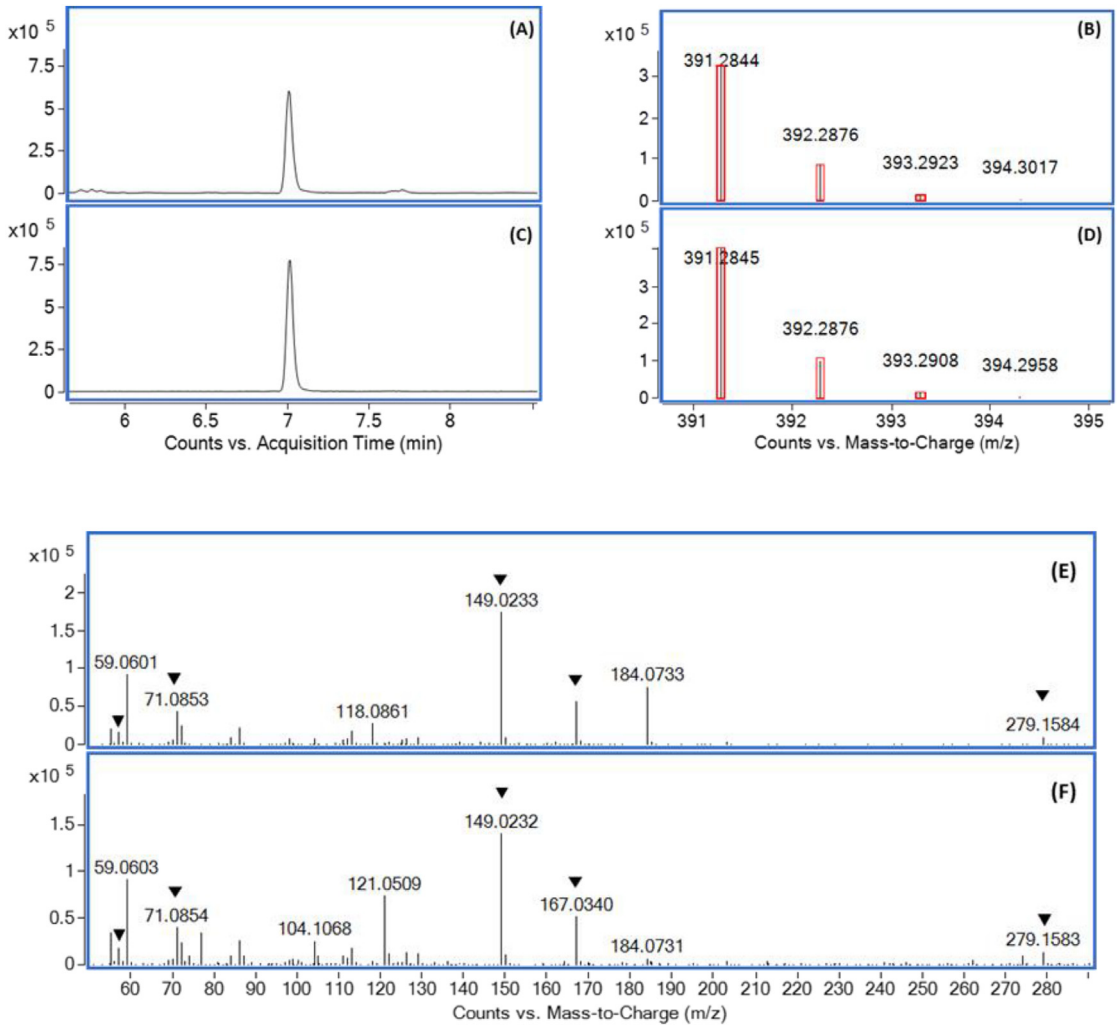

Fig. 3. Extracted ion chromatograms at *m/z* 391.2857 for an oyster extract (Panel A) and for a DEHP pure standard in solvent (Panel C). Mass spectra around *m/z* 391.2857 (RT = 7.01 min) illustrating the isotope distribution for molecular feature F4 in an oyster extract (Panel B) and for a DEHP pure standard in solvent (Panel D; red rectangles indicate the theoretical isotope pattern of DEHP). All Ions MS/MS spectra for molecular feature F4 in oyster extracts (Panel E) and in a DEHP pure standard in solvent (Panel F; the inverted black triangles indicate characteristic ion fragments of DEHP). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

et al., 2019). Retention time predictions will be used in future studies to improve the screening efficiency of contaminants in the tissues of bioindicators species such as oysters.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.07.047.

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