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Integrated disperser freezing purification with extraction using fatty acid-based solidification of floating organic-droplet (IDFP-EFA-SFO) for triclosan and methyltriclosan determination in seawater, sediment and seafood

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ABSTRACT

A microextraction method for the determination of triclosan and methyltriclosan in marine environmental samples was developed. The disperser was first serves as a preliminary extractant for analytes, then as a frozen solvent to remove impurities at −20 °C, and finally as a disperser agent in the microextraction procedure. With the extractants solidified and float on the surface of the aqueous phase at low temperature, a separation was achieved to avoided use of specialized laboratory instruments. The method was optimized using Plackett-Burman design and central composite design as follows: 146 μL octanoic acid as extractant, 793 μL acetoneas disperser, 3.0 min centrifugation and 1.1 min vortex time. The limits of detection were 0.022–0.060 μg L^{-1} or μg kg^{-1} and recoveries were 83.3–103.5% for TCS and MTCS in seawater, sediments and seafood. The method has excellent prospects for sample pre-treatment and trace-level analysis of triclosan and methyltriclosan in marine environmental samples.

1. Introduction

Triclosan (TCS) is a broad spectrum antibacterial agent that has been widely used in soap, toothpaste, detergent and other personal care products since 1972 (Singer et al., 2002; Ying and Kookana, 2007). It is also used as a disinfectant for medical equipment and textiles and as an additive for toys and building materials (Bedoux et al., 2012; Chen et al., 2008; Dann and Hontela, 2011). Methyltriclosan (MTCS) is the primary microbial transformation product of TCS. TCS and MTCS are common contaminants in the environment originating from their widespread use and disposal in domestic sewage and industrial wastewaters. Reports indicate TCS and MTCS concentrations in the range of 0.08–2.3 μg L^{-1} in rivers, 11.4–130.7 μg kg^{-1} in sediments, and 3.4–596 μg kg^{-1} in aquatic animals (Agüera et al., 2003; Coogan et al., 2007; Fernandes et al., 2011; Kolpin et al., 2002; Leiker et al., 2009; Rüdel et al., 2013). TCS can affect the growth of aquatic plants and animals at μg L^{-1} concentrations (Foran et al., 2000; Fort et al., 2010). When tadpoles were exposed to 0.15 μg L^{-1} TCS for 4 days, decreased T3-mediated TRβ mRNA expression in the tadpole tail and increased hindlimb development were observed (Veldhoen et al., 2006). Thus, simultaneous quantification of TCS and MTCS in environmental matrices provides an important assessment tool for evaluating contamination and potential toxicological risks resulting from TCS use (Chen et al., 2012; Fernandes et al., 2011).

To date, detection of TCS and MTCS has mainly focused on freshwater environments (Leiker et al., 2009; Rüdel et al., 2013; Zhou et al., 2009) resulting in a paucity of data for marine environments that receive pollutant loads from river inputs (Agüera et al., 2003; Chau et al., 2008; Fernandes et al., 2011; Okumura and Nishikawa, 1996). The composition of seawater, sediments and seafood samples are complex and analytically challenging for accurate detection of trace-level analytes. For example, seafood is rich in fat and proteins that are difficult to purify by traditional pre-treatment methods, such as liquid-liquid extraction (LLE) (Chau et al., 2008), accelerated solvent extraction (ASE) (Boehmer et al., 2004) and solid phase extraction (SPE) (Chau et al., 2008; Gonzalo-Lumbreras et al., 2014). As a result, auxiliary purification methods are generally required for analysis of seafood (Kuban and Bocek, 2013). While traditional sample purification methods, such as gel permeation chromatography (GPC) provide good results (Peter S. Haglund et al., 1997), they require large amounts of harsh chemicals, organic solvents and specialized equipment. For example sulfuric acid can be used to destroy fat and denature proteins for sample purification,

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however, these multi-step methods are often very complicated and time-consuming, and potentially hazardous (Hartmann et al., 2007). Although hexane has good purification capabilities for fat (Wang et al., 2015), its strong toxicity to the human nervous system makes is potentially harmful to laboratory staff. To overcome the disadvantages of previous purification methods, (Liu et al., 2009) established a freezing-dispersive liquid-liquid microextraction (F-DLLME) combined with GC–MS to determine polybrominated diphenyl ethers (PBDEs) in aquatic animal tissues. This technique used acetone as an auxiliary purification solvent and achieved good purification for protein and lipophilic impurities in animal tissues at −80 °C. However, most traditional DLLME methods utilize chlorobenzene, toluene, carbon tetrachloride and other higher-than-water density organic solvents as extractants, which have high toxicity and are difficult to separate from the aqueous phase. In contrast, extractants with lower-than-water density are difficult to separate from the aqueous phase and require specialized, non-commercially available devices to isolate the extractant from the surface of the aqueous phase (Farajzadeh et al., 2016; Saleh et al., 2009). The need for specialized devices increases the operational complexity and cost of extraction, and are often difficult to clean for reuse.

Dispersive liquid-liquid microextraction-based on solidification of a floating organic-droplet (DLLME-SFO) was first reported by (Leong and Huang, 2008). This technique uses a low-density extractant with a room temperature melting point that solidifies and floats on top of the aqueous phase after centrifugation and placement in an ice bath. The organic droplet can be easily collected without a specialized apparatus and further reduces experimental error and improves extraction efficiency. However, suitable extractants for DLLME-SFO are limited, and only undecanol and dodecanol are commonly used (Wang et al., 2014; Wang et al., 2013). Both of these extractants are strongly toxic to aquatic organisms.

As an environmentally friendly solvent, medium-chain fatty acids have received increasing attention as a “green” solvent for sample pretreatment methods (Shih et al., 2015; Vakh et al., 2016). (Moghadam et al., 2017) used hexanoic acid to extract Ag (I) and Co (II) from cow milk, vitamin B12, orange juice, and tap water achieving high extraction efficiency, short extraction times and low organic solvent use. In this study, acetone was used as a preliminary extractant for analytes, a frozen solvent for purifying impurities, and a disperser for the microextraction procedure. The newly developed method reduced the amount of organic solvent required and eliminated the use of highly toxic organic solvents. In addition, the experimental operation was simple and rapid allowing for a high analytical throughput. The selected extractant octanoic acid was easily solidified and floated on the surface of aqueous phase at low temperature allowing for excellent separation efficiency that avoided the use of specialized laboratory apparatus and decreased operational errors. Therefore, for the first time, we combined the advantages of F-DLLME and DLLME-SFO, and established a microextraction IDFP-EFA-SFO method having excellent prospects for determination of trace-level concentrations of triclosan and methyltriclosan in seawater, sediment and seafood, as well as in a wide range of other environmental matrices.

2. Materials and methods

2.1. Reagents and materials

Analytical standards (99.9% purity) for triclosan (TCS) and methyltriclosan (MTCS) were purchased from Sigma-Aldrich, Shanghai, China. The chemical structure and molecular weight of TCS and MTCS are shown in Supplementary Fig. 1. Five medium-chain fatty acids (pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid and nonanoic acid) were purchased from J&K Chemical, Shanghai, China. HPLC-grade methanol, ethanol, ethylene glycol, acetone, acetonitrile, and dimethyl sulfoxide were purchased from Merck, Shanghai, China.

Stock standard solutions (1000 μg mL^{-1}) for TCS and MTCS were prepared by dissolving each chemical in methanol and stored at 4 °C until use. Working solutions were diluted with Milli-Q 18 MΩ ultrapure water (Millipore, Bedford, MA, USA) and methanol (v/v = 50:50) to prepare secondary mixed stock solutions. All working solutions were prepared fresh weekly and stored at 4 °C.

2.2. Sample collection and processing

According to the state standard of the People's Republic of China (GB 17378.3-2007), seawater and sediment samples (0–20 cm) were collected in Laizhou Bay (Weifang, China) in August 2017. Seawater was filtered through a 0.45-μm membrane filter and stored at −4 °C. Sediment was air-dried at room temperature and stored in a cool, dry location after grinding and sieving through a 150-μm sieve. Fish (*Paralichthys olivaceus*), shrimp (*Fenneropenaeus chinensis*), shellfish (*Chlamys nobilis*) and squid (*Loligo chinensis*) were purchased from a local seafood market (Weifang, China). The scales and skin of fish, the shell and cephalothorax of shrimp, the shell and viscera of shellfish, the viscera and sepium of squid were removed. Then the meat of body on both sides of fish, the abdomen meat of shrimp, the belly meat of shellfish and squid were stored at −20 °C after grinding to pass a 500-μm sieve (JX-FSTPRP-24 grinder, Shanghai, China). All sample analyses were completed within one week.

2.3. Instrumentation

A Zorbax Eclipse-C_{18} column (250 mm × 4.6 mm, 5 μm particle size) was used with an Agilent-1260 HPLC-UV chromatography system (Agilent, Santa Clara, CA) under the following operating conditions: flow rate, 0.5 mL min^{-1}; column temperature, 40 ± 1 °C; mobile phase, acetonitrile-water (88:12, v/v); detection wavelength, 280 nm and injection volume, 100.0 μL.

2.4. Determining disperser recovery and purification capacities

An essential first step was to screen for an appropriate solvent to act as disperser. The optimum solvent characteristics included a high recovery capacity for TCS and MTCS and high purification capacity for impurities such as protein and fat in the seafood samples. Six dispersers were assessed for their recovery of TCS and MTCS: methanol, ethanol, ethylene glycol, acetone, acetonitrile, and dimethyl sulfoxide. Aliquots of sediment or homogenized seafood (1.0 g) were fortified with 50 μg kg^{-1} of TCS and MTCS. Then, 5 mL of the disperser was added and the solution was vortexed for 5 min at 3200 rpm (50 Hz, 115 w) (SI-0246, Scientific Industries, USA). After 5 min centrifugation at 3200*g* (TDL-50C, Anting Low Speed Centrifuge, Shanghai, China), the supernatant was collected and made up to 5 mL with additional disperser. TCS and MTCS concentrations were determined, and the recovery was calculated to judge the extraction capacity of each disperser. The symmetry of chromatographic peaks, the resolution and the effect of impurity peaks were examined for the various dispersing agents after traditional purification (concentrated H_2SO_4 and *n*-hexane) and using the newly developed method. Better symmetry, higher resolution and fewer impurity peaks indicated a higher purification efficacy.

2.5. Selection of extractant and disperser

Five medium-chain fatty acids (pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid and nonanoic acid) were chosen as extractants, and six organic solvents (methanol, ethanol, ethylene glycol, acetone, acetonitrile, and dimethyl sulfoxide) with high recovery and purification capacities were selected as dispersers. The extraction conditions were 150 μL of extractant, 800 μL of disperser, 3.0 min centrifugation time and 1.0 min vortex time. The fortification level was 50 μg L^{-1}/μg kg^{-1} for both TCS and MTCS.

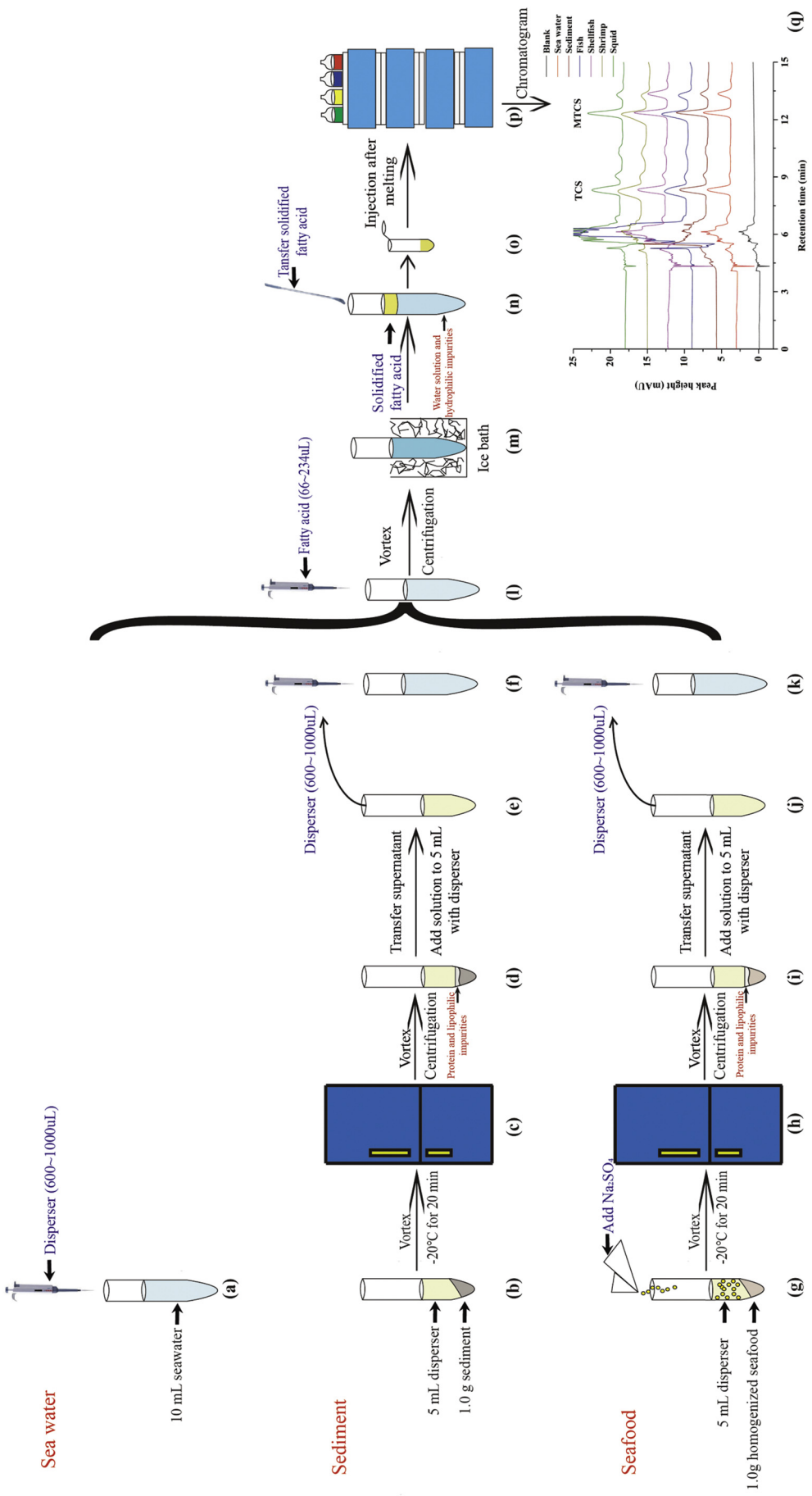

Fig. 1. Schematic representation of IDFP-EFA-SFO method.
Note: Each step in the IDFP-EFA-SFO procedure is described in the text.

2.6. IDFP-EFA-SFO procedures

A schematic diagram of the integrated IDFP-EFA-SFO procedure is shown in Fig. 1. As for seawater, 600–1000 μL disperser was added to 10 mL seawater sample as the first step (Fig. 1-a). For sediment, 1.0 g of sample was placed in a 10-mL high-density polyethylene (HDPE) conical centrifuge tube, and 5 mL of disperser was added (Fig. 1-b). Then, the sediment sample was vortex for 2 min and frozen for 20 min at −20 °C, before collecting the supernatant after vortexing for 2 min and centrifugation for 5 min (Fig. 1-c and d). An appropriate volume of disperser was added to the supernatant to obtain a final volume of 5 mL. Aliquots (600–1000 μL) of the above disperser solution were transferred into 10 mL ultrapure water (Fig. 1-e and f). As for seafood, 5 mL of disperser and 1.0 g of anhydrous sodium sulfate were added to 1.0 g of seafood (Fig. 1-g). The seafood samples were frozen for 20 min at −20 °C and the supernatant collected after vortexing for 2 min and centrifugation for 5 min (Fig. 1-c and d). Then, the sample solutions were made up to 5 mL with additional disperser and 600–1000 μL of the disperser solution added to 10 mL ultrapure water for subsequent microextraction using fatty acid-based solidification of a floating organic-droplet (Fig. 1-e and f).

Following the above procedures, 66–234 μL of fatty acid was injected into the solution containing disperser and homogenized by vortexing for 1–5 min (Fig. 1-l). After 1–5 min centrifugation at 5000 rpm, the extraction agent, fatty acid was solidified in an ice bath for 10 min (Fig. 1-m). The solidified droplets were easily melted at room temperature, and a liquid aliquot (100 μL) was used for HPLC-UV quantification (Fig. 1-n-q).

2.7. Plackett-Burman design (PBD)

Significant operational factors were rapidly screened from several operational factors by a two-level PBD using 12 runs with no consideration of interactions. Based on a previous study (Ma et al., 2016), the following factors were investigated: volume of extractant (a), volume of disperser (b), pH (d), vortex time (e), and centrifugation time (g). In addition, systematic errors or unknown variables that affected the system were investigated by inclusion of three dummy factors (c, e and f) (Borges et al., 2016). Each factor was investigated at two levels, low (−1) and high (+1), as illustrated in Supplementary Table 1.

2.8. Central composite design (CCD)

After single-factor screening with a PBD, four key operational factors, volume of extractant (A), volume of disperser (B), centrifugation time (C) and vortex time (D), were selected for further optimization by CCD. The best extraction conditions determined by single factor optimization were 150 μL extractant, 800 μL disperser, 3.0 min centrifugation time and 1.0 min vortex time (Supplementary Fig. 2). A CCD with 22 treatments for four factors and five levels per factor ($-\alpha$, −1, 0, +1, $+\alpha$) was performed to investigate multi-factor interactions (Supplementary Table 2). We used two blocks to optimize values for each factor based on extraction recovery (ER) (Sereshti et al., 2012) (Table 1). The response of dependent variables on ERs of TCS and MTCS was assessed using a quadratic polynomial model:

$$Y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{ij=1(i\neq j)}^{6} b_{ij} x_i x_j + \sum_{i=1}^{4} b_{ii} x_i^2 \quad (1)$$

where Y is the dependent variable; x_i is the independent variable; b_0 is the intercept; b_i is the coefficient of linear effect; b_{ij} is the coefficient of interaction effect, and b_{ii} is the coefficient of the squared effect. The model determined by Design-Expert 8.0.5 (Minneapolis, USA) was evaluated by analysis of variance (ANOVA) and used to obtain response surfaces for factor optimization.

Table 1
Design matrix and responses for the CCD.

Run	Block	A: Volume of extractant (μL)	B: Volume of disperser (μL)	C: Centrifugation time (min)	D: Vortex time (min)	Recovery (%)
1	1	200	920	4.2	0.4	64.45
2	1	100	920	4.2	1.6	63.11
3	1	100	680	1.8	0.4	34.25
4	1	100	680	4.2	0.4	42.83
5	1	200	680	1.8	1.6	53.95
6	1	200	920	1.8	0.4	51.55
7	1	150	800	3.0	1.0	96.67
8	1	150	800	3.0	1.0	105.31
9	1	150	800	3.0	1.0	98.81
10	1	100	920	1.8	1.6	50.47
11	1	200	680	4.2	1.6	67.45
12	1	150	800	3.0	1.0	100.28
13	2	150	800	3.0	0.0	37.06
14	2	150	1000	3.0	1.0	82.46
15	2	150	800	1.0	1.0	64.47
16	2	150	600	3.0	1.0	45.92
17	2	66	800	3.0	1.0	44.97
18	2	150	800	3.0	1.0	94.56
19	2	150	800	3.0	1.0	106.17
20	2	150	800	5.0	1.0	86.12
21	2	234	800	3.0	1.0	90.06
22	2	150	800	3.0	2.0	92.67

2.9. Experimental evaluation

CCD evaluation: The significance level for the model factors and corresponding results was evaluated by ANOVA.

Methods evaluation: The method was evaluated by correlation coefficients (R^2), linear range (LR), limits of detection (LOD), limit of quantification (LOQ), precision and accuracy, and extraction recovery (ER) as determined by Eq. (2)

$$ER = \frac{C_{found} - C_{real}}{C_{added}} \times 100\% \quad (2)$$

where C_{found} was the concentration of analyte in the final sample solution, C_{real} was the concentration of analyte in the sample, and C_{added} was the concentration of the standard spiked into the sample. Calibration curves for TCS and MTCS were plotted using eight levels ranging from their respective LOQ values to 1000 μg L^{-1}/μg kg^{-1} (LOQ, 1, 5, 10, 50, 100, 500 and 1000 μg L^{-1}/μg kg^{-1}), and each calibration level was performed in triplicate.

Method stability evaluation: A precision study was carried out in six parallel experiments by determining the intra- and inter-day relative standard deviations (RSDs) at four fortification levels (10, 50, 100 and 1000 μg L^{-1}/μg kg^{-1}) of TCS and MTCS in seawater, sediments and seafood samples.

Analysis of samples: The optimized IDFP-EFA-SFO method was applied for determining TCS and MTCS in real-world seawater, sediment and four types of seafood at four fortification levels (10, 50, 100 and 1000 μg L^{-1}/μg kg^{-1}). The ER was used to assess the analytical performance of the optimized method.

3. Results and discussion

In this investigation, acetone was chosen as the preliminary enrichment and purification solvent, and for its ability to act as a simultaneous disperser in the microextraction procedure. Octanoic acid was selected as the extractant because of its high extraction efficiency for TCS and MTCS and its cold-induced solidification property in an ice bath. This extraction using a freezing-disperser and fatty acid extractant combined with solidification of a floating organic-droplet was successful for determination of trace-level concentrations of TCS and MTCS

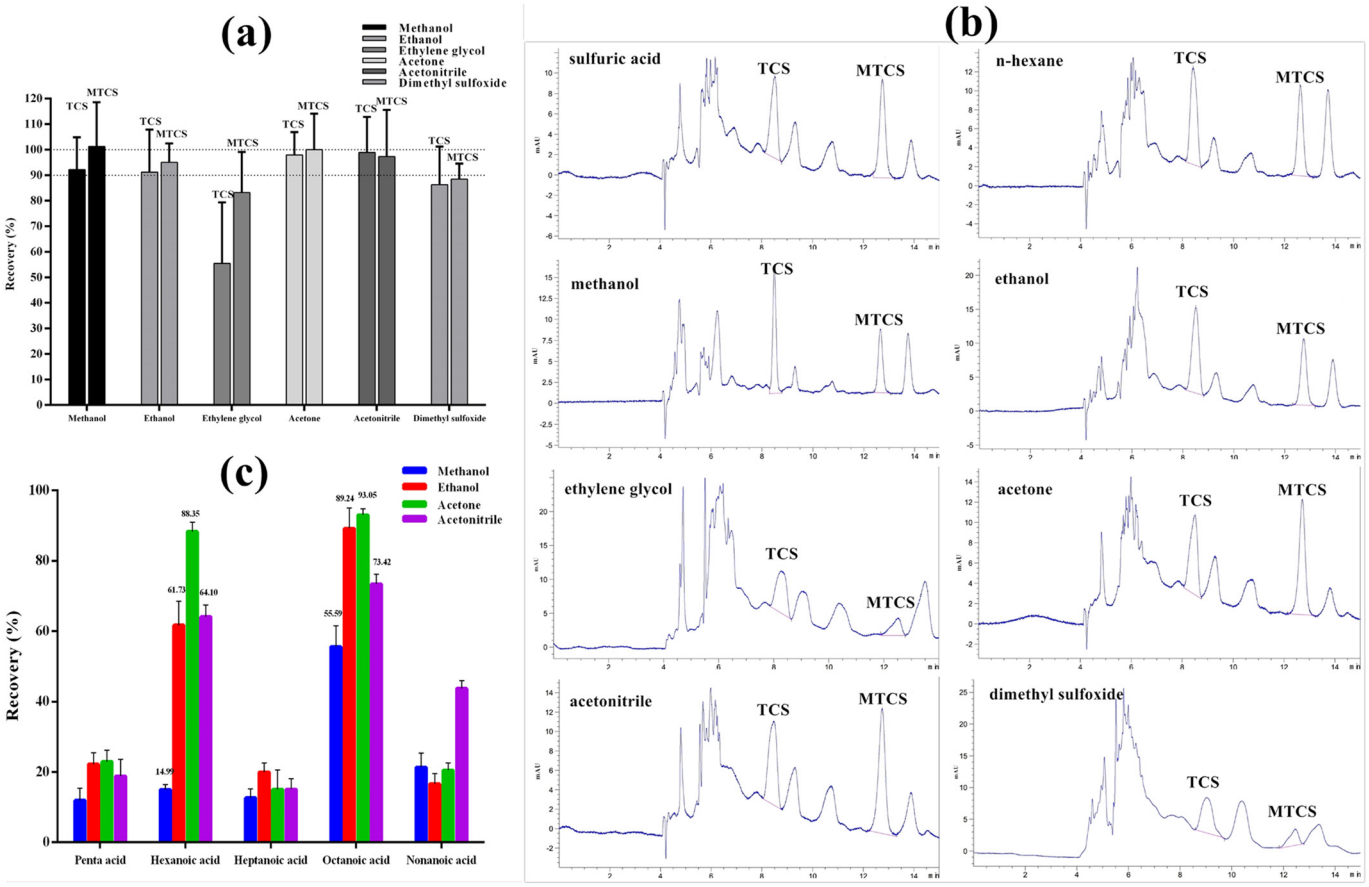

Fig. 2. (a) Enrichment ability of six disperser for TCS and MTCS, (b) Purification ability for marine environmental samples, (c) Extraction recovery of TCS and MTCS with different extractants and dispersers.

in marine samples.

3.1. Selection the enrichment and purification ability of disperser

The IDFP-EFA-SFO method is mainly divided into three steps: i) initial enrichment, ii) purification using a disperser, and iii) microextraction using an appropriate extractant. To simplify the procedure, the disperser solvent was selected for its simultaneous abilities as an extraction/purification solvent and as the disperser solvent in the DLLME procedure. The organic solvent was selected based on its enrichment and purification capacities for marine samples and as an appropriate disperser for the subsequent microextraction procedures. Six dispersers (methanol, ethanol, ethylene glycol, acetone, acetonitrile and dimethyl sulfoxide) were evaluated for their ability to enrich TCS and MTCS from marine samples. Enrichment recoveries ($n = 6$) for ethylene glycol and dimethyl sulfoxide were < 90% compared to recoveries > 90% for methanol, ethanol, acetone and acetonitrile (Fig. 2a). Although the enrichment recoveries for direct extraction were acceptable, the standard deviations (10–20%) were too high to facilitate quantitative accuracy. Additionally, there was concern for enrichment of impurities by the disperser that may not be completely removed by the microextraction procedure. These impurities have the potential to interfere with HPLC quantification of TCS and MTCS. Therefore, some purification is necessary after the enrichment step to enhance method performance.

Methanol, ethanol, acetone and acetonitrile had strong purification ability for the marine samples, which were similar in effectiveness to the traditional concentrated sulfuric acid and *n*-hexane purification method (Fig. 2b). The purification ability of ethylene glycol and dimethyl sulfoxide were poor resulting in chromatography peaks with low symmetry for TCS and MTCS and many interfering impurities peak. Thus, these two dispersers were not able to effectively remove fat, protein and other impurities in the samples. Furthermore, due to their melting point, ethylene glycol and dimethyl sulfoxide solidified in the freezing-purification step (−20 °C) rendering them incompatible with this purification approach. As a result ethylene glycol and dimethyl sulfoxide were eliminated from further consideration while methanol, ethanol, acetonitrile and acetone were selected for further evaluation.

3.2. Selection of disperser and extractant

In the microextraction procedure, selection of an appropriate disperser and extractant is crucial for optimizing the ERs of target analytes. Five medium-chain fatty acids (pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid and nonanoic acid) were chosen as potential extractants, and methanol, ethanol, acetonitrile and acetone as potential dispersers. Following the dispersive enrichment procedure, the supernatant was added to water resulting in the dissolution of hydrophilic impurities into the aqueous phase. The ERs for TCS and MTCS with different extractants and dispersers are shown in Fig. 2c. The ER with pentanoic acid, heptanoic acid and nonanoic were too low (< 40%) to be considered further. The highest extraction recoveries were obtained for octanoic acid (93.1%) and hexanoic acid (88.4%) as extractants and acetone as disperser. Because hexanoic acid and octanoic acid are both low-density solvents with a room temperature melting point, they solidify and float on top of the aqueous solution after centrifugation and ice bath making them candidates for use as the extractant in DLLME-SFO. Given the higher ER for octanoic acid, it was selected as the extractant for further optimization.

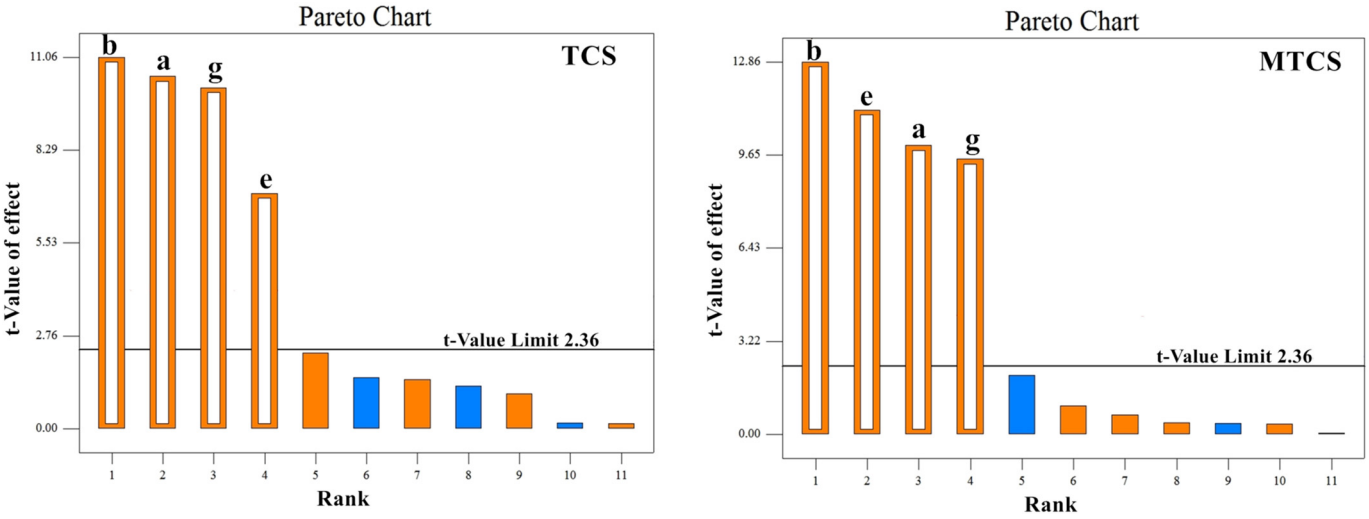

Fig. 3. Standardized Pareto charts ($p < 0.05$) for main operational factor effects.
Note: (A) volume of extractant; (B) volume of disperser; (E) vortex time; (G) centrifugation time.

3.3. Screening of variables by PBD

Standardized Pareto charts displaying the critical value representing statistically significant ($p < 0.05$) factors affecting the ERs for TCS and MTCS are shown in Fig. 3. Response for the three virtual factors, (c), (f) and (h), were not significant, indicating no significant unknown variables or systematic errors in the design. Four variables, extractant volume (a), disperser volume (b), vortex time (e) and centrifugation time (g), were identified as significant factors having positive effects (white hollow bar), while the other variables displayed either negative (solid bar) or no effect. For all the major variables evaluated, the extractant volume affected the extraction recovery directly, and the dispersive volume affected the formation of the emulsion system in the IDFP-EFA-SFO process. The vortex time affected the degree of homogeneity between extractant and extraction solution, and the centrifugation time affected the separation of the extractant from the extraction solution. Both vortex and centrifuge time affected the enrichment and extraction of TCS and MTCS.

3.4. Optimization of IDFP-EFA-SFO procedures using CCD

We explored potential interactions among the four major operational factors identified by PBD to optimize ER for TCS and MTCS using CCD. CCD simultaneously optimizes all major factors and their interactions and thereby provides a more rigorous optimization approach than signal factor optimization alone. The CCD model equation and related terms were highly significant ($p < 0.001$), while the "lack of fit" was not significant ($p = 0.983$) indicating that other operational factors in this optimization had little effect on the overall model (Sereshti et al., 2012) (Supplementary Table 3). The goodness-of-fit for the polynomial model was assessed by the coefficient of determination (R^2 and adjusted-R^2). The R^2 (0.974) is a measure of the amount of variance explained by the model. The adjusted-R^2 (0.970) is the R^2 adjusted for the number of terms in the model, and it decreases as the number of terms in the model increases and those additional terms do not add value to the model (Sereshti et al., 2012). The *p*-values for A, B, C, D, AB, AD and BD were < 0.05 indicating that the extractant volume (A), dispersive volume (B), centrifugation time (C) and vortex time (D) and several of their interactions significantly affected ERs, consistent with PBD results. Eq. (3) illustrates the effect of all operational factors and their interactions on ER; Y is the ER, b_0 is the intercept and b_1 to b_{14} are parameter coefficients.

$$Y = bo + b_1A + b_2B + b_3C + b_4D + b_5AB + b_6AC + b_7AD + b_8BC + b_9BD + b_{10}CD + b_{11}A^2 + b_{12}B^2 + b_{13}C^2 + b_{14}D^2 \quad (3)$$

with $b_0 = 100.4$, $b_1 = 13.4$, $b_2 = 11.0$, $b_3 = 6.15$, $b_4 = 16.8$, $b_5 = 11.2$, $b_6 = 0.65$, $b_7 = 7.08$, $b_8 = 0.43$, $b_9 = 7.56$, $b_{10} = 0.58$, $b_{11} = -11.7$, $b_{12} = -13.1$, $b_{13} = -8.97$ and $b_{14} = -12.8$.

The "+" or "−" for each coefficient indicates the type of relationship (i.e., positive vs negative) between the related factor and the ER response. The absolute value of the coefficient indicates the strength of the relationship between the operational factor and the ER. The predicted versus actual data points were all located near the model regression line (Supplementary Fig. 3a) indicating a good model fit between operational factors and predicted ER values. Moreover, the residual plots were randomly distributed indicating that the variance of the experimental measurements was constant for all values of Y (Supplementary Fig. 3b).

The 3D response surfaces and contour lines of CCD were used to examine the relationship between analyte recovery (ER) and the four operational factors (Fig. 4). For example, Fig. 4a and b describe the 3D response surface and contour line for the effect of extractant volume and disperser volume on ER while holding centrifugation (3.0 min) and vortex (1.0 min) times constant. The ERs for TCS and MTCS increased with increasing extractant volume from 66 to 146 μL and disperser volume from 600 to 793 μL. However, further increases in extractant volume from 146 to 234 μL and disperser volume from 793 to 1000 μL resulted in lower ERs for TCS and MTCS. Fig. 4c and d depict the 3D response surface and contour line for the effect of extractant volume and vortex time on ER when the disperser volume and centrifugation time were set at 800 μL and 3.0 min, respectively. The maximum ER was obtained at 146 μL extractant and 1.1 min vortex time. With further increases in extractant volume (146–234 μL) and vortex time (1.1 to 2.0 min), the ERs sharply decreased. Fig. 4e and f illustrate the 3D response surface and contour line for the effect of disperser volume and vortex time on ER when the extractant volume and centrifugation time were set at 150 μL and 3.0 min, respectively. The maximum ER was observed at 793 μL disperser and 1.1 min vortex time. With further increases in these two factors, the ERs sharply decreased. Overall, though the calculation of software Design-Expert 8.0.5, the CCD optimization of the four operational parameters determined optimal conditions of 146 μL extractant, 793 μL disperser, 3.0 min centrifugation and 1.1 min vortex time.

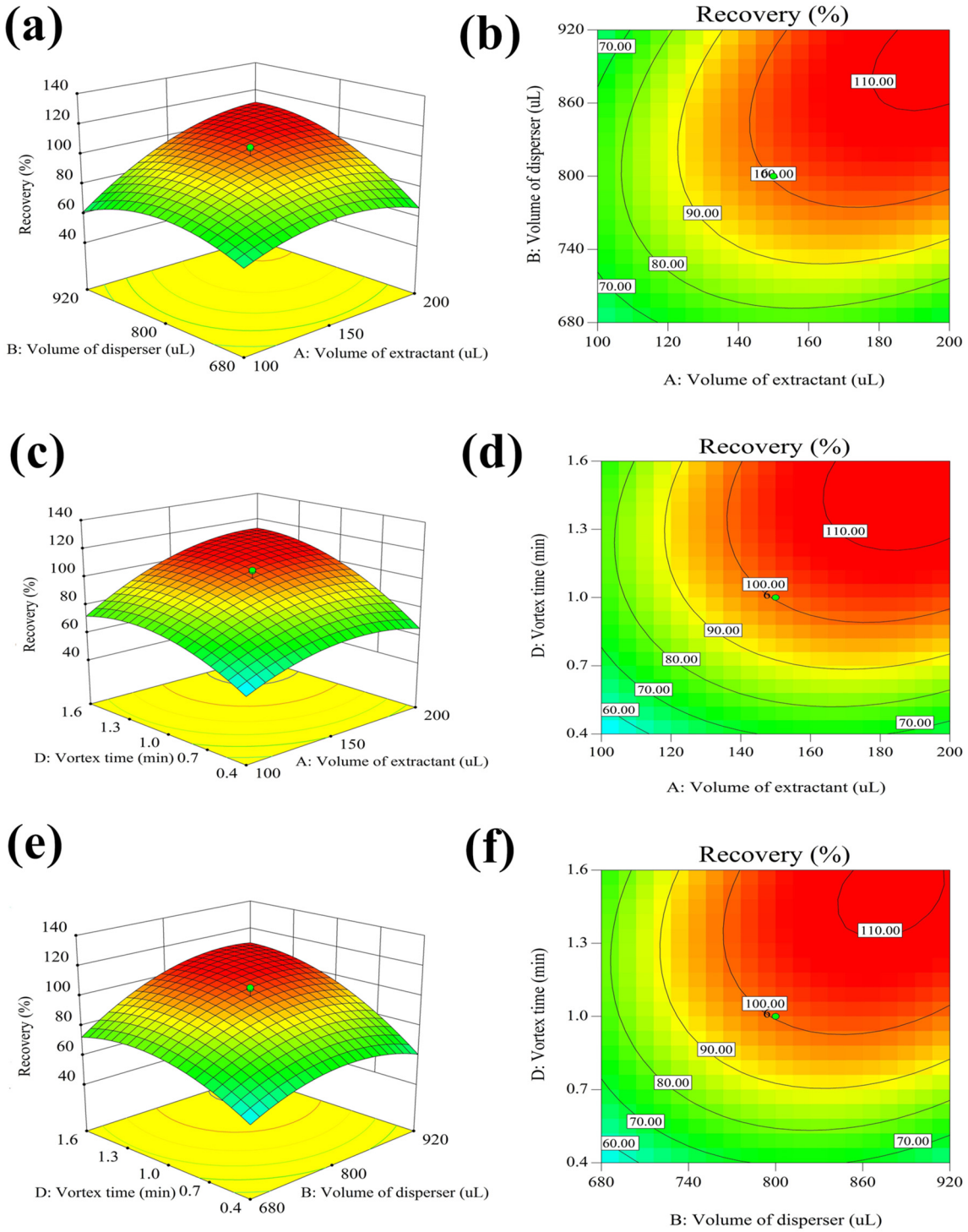

Fig. 4. (a) 3D response surface and (b) contour plot for extractant and disperser volume at 3.0 min centrifugation time and 2.5 min vortex time. Fig. 4 (c) 3D response surface and (d) contour plot for extractant volume and vortex time at 800 μL disperser and 3.0 min centrifugation time. Fig. 4 (e) 3D response surface and (f) contour plot for disperser volume and vortex time at 150 μL extractant and 3.0 min centrifugation time.

3.5. IDFP-EFA-SFO method evaluation

Under optimized conditions (146 μL extractant, 793 μL disperser, 3.0 min centrifugation and 1.1 min vortex time), performance of the IDFP-EFA-SFO method was evaluated for linearity of standard curves (R^2), linear range (LR), limits of detection (LOD at S/N = 3) and limits of quantification (LOQ at S/N = 10) (Table 2). Coefficients of determination (R^2) for linearity of standard curves for TCS and MTCS were in the range of 0.9921–0.9995. The linear range (LR) was 0.073–1000 μg L^{-1}/μg kg^{-1} for TCS and 0.103–1000 μg L^{-1}/μg kg^{-1} for MTCS. The LODs of seawater, sediment and seafood were in the range of 0.022–0.045 μg L^{-1}/μg kg^{-1} for TCS and 0.031–0.060 μg L^{-1}/μg kg^{-1} for MTCS, and the LOQs of seawater, sediment and seafood were in the range of 0.073–0.149 μg L^{-1}/μg kg^{-1} for TCS and 0.103–0.201 μg L^{-1}/μg kg^{-1} for MTCS. The precision study was carried out in six parallel experiments by determining the intra- and inter-day RSDs (relative standard deviations) at four fortification levels for TCS and MTCS (10, 50, 100 and 1000 μg L^{-1}/μg kg^{-1}). The RSDs varied

Table 2
The analytical performance for the IDFP-EFA-SFO-HPLC-UV method.

Sample	Analytes	Regression equations	Correlation coefficients (R^2)	Linear range ($\mu g\,L^{-1}/\mu g\,kg^{-1}$)	LOD ($\mu g\,L^{-1}/\mu g\,kg^{-1}$)	LOQ ($\mu g\,L^{-1}/\mu g\,kg^{-1}$)
Sea water	TCS	y = 0.0369x + 1.80	0.9995	0.073–1000	0.022	0.073
	MTCS	y = 0.0418x + 0.83	0.9987	0.103–1000	0.031	0.103
Sediment	TCS	y = 0.0365x + 1.98	0.9976	0.076–1000	0.023	0.076
	MTCS	y = 0.0392x + 1.58	0.9985	0.110–1000	0.033	0.110
Fish	TCS	y = 0.0542x − 9.44	0.9928	0.149–1000	0.045	0.149
	MTCS	y = 0.0479x − 7.09	0.9927	0.201–1000	0.060	0.201
Shellfish	TCS	y = 0.0472x − 4.74	0.9922	0.148–1000	0.044	0.148
	MTCS	y = 0.0535x − 6.72	0.9922	0.190–1000	0.057	0.190
Shrimp	TCS	y = 0.0469x − 3.44	0.9921	0.144–1000	0.043	0.144
	MTCS	y = 0.0530x − 5.17	0.9921	0.186–1000	0.056	0.186
Squid	TCS	y = 0.0368x + 1.99	0.9994	0.145–1000	0.044	0.145
	MTCS	y = 0.0418x + 0.85	0.9984	0.187–1000	0.056	0.187

Table 3
Precision and accuracy data for the determination of TCS and MTCS in sea water, sediment and sea foods (6 days with 6 replicates).

Analytes	Compound	Concentration ($\mu g\,L^{-1}/\mu g\,kg^{-1}$)			RSD(%)	Concentration ($\mu g\,L^{-1}/\mu g\,kg^{-1}$)			RSD(%)
		Spiked	Detected	Recovery (%)	Intra-day	Spiked	Detected	Recovery (%)	Inter-day
Seawater	TCS	10	9.2 ± 0.5	92.0	5.4	10	9.5 ± 0.6	95.0	6.3
		50	48.5 ± 1.8	97.0	3.7	50	43.6 ± 2.7	87.2	6.2
		100	95.3 ± 4.6	95.3	4.8	100	98.2 ± 5.2	98.2	5.3
		1000	982.3 ± 13.2	98.2	1.3	1000	976.1 ± 12.1	97.6	1.2
	MTCS	10	9.9 ± 0.3	99.0	3.0	10	9.8 ± 0.3	98.0	3.1
		50	49.3 ± 2.2	98.6	4.5	50	46.8 ± 2.4	93.6	5.1
		100	99.7 ± 3.8	99.7	3.8	100	98.3 ± 4.6	98.3	4.7
		1000	1003.2 ± 18.6	100.3	1.9	1000	992.6 ± 20.7	99.3	2.1
Sediment	TCS	10	9.1 ± 0.6	91.0	6.6	10	9.3 ± 0.5	93.0	5.4
		50	47.8 ± 2.3	95.6	4.8	50	43.3 ± 1.8	86.6	4.2
		100	96.2 ± 3.3	96.2	3.4	100	98.5 ± 3.7	98.5	3.8
		1000	991.3 ± 8.2	99.1	0.8	1000	1008.3 ± 12.8	100.8	1.3
	MTCS	10	9.3 ± 0.4	93.0	4.3	10	9.5 ± 0.6	95.0	6.3
		50	48.8 ± 2.8	97.6	5.7	50	46.6 ± 3.1	93.2	6.7
		100	98.5 ± 4.1	98.5	4.2	100	102.2 ± 6.3	102.2	6.2
		1000	989.3 ± 9.8	98.9	1.0	1000	988.2 ± 17.7	98.8	1.8
Fish	TCS	10	9.2 ± 0.6	92.0	6.5	10	9.3 ± 0.5	93.0	5.4
		50	47.6 ± 3.3	95.2	6.9	50	46.5 ± 2.7	93.0	5.8
		100	96.3 ± 3.8	96.3	4.0	100	98.8 ± 3.3	98.8	3.3
		1000	978.5 ± 8.6	97.9	0.9	1000	982.4 ± 9.2	98.2	0.9
	MTCS	10	8.9 ± 0.4	89.0	4.5	10	9.2 ± 0.5	92.0	5.4
		50	50.8 ± 1.5	101.6	3.0	50	49.3 ± 2.5	98.6	5.1
		100	95.3 ± 3.8	95.3	4.0	100	99.6 ± 5.7	99.6	5.7
		1000	977.9 ± 10.1	97.8	1.0	1000	989.2 ± 23.8	98.9	2.4
Shellfish	TCS	10	9.6 ± 0.2	96.0	2.1	10	8.7 ± 0.7	87.0	8.1
		50	45.2 ± 2.7	90.4	6.0	50	43.2 ± 3.2	86.4	7.4
		100	90.5 ± 3.2	90.5	3.5	100	92.7 ± 3.9	92.7	4.2
		1000	928.8 ± 12.5	92.9	1.4	1000	934.2 ± 15.6	93.4	1.7
	MTCS	10	9.3 ± 0.3	93.0	3.2	10	8.9 ± 0.6	89.0	6.7
		50	43.8 ± 1.8	87.6	4.1	50	45.7 ± 2.3	91.4	5.0
		100	94.4 ± 4.4	94.4	4.7	100	95.8 ± 4.8	95.8	5.0
		1000	948.3 ± 8.3	94.8	0.9	1000	928.7 ± 18.4	92.9	2.0
Shrimp	TCS	10	9.3 ± 0.6	93.0	6.5	10	8.4 ± 0.8	84.0	9.5
		50	46.2 ± 1.7	92.4	3.7	50	43.4 ± 3.6	86.8	8.3
		100	101.4 ± 4.4	101.4	4.3	100	92.5 ± 5.5	92.5	6.0
		1000	978.8 ± 5.1	97.9	0.5	1000	966.5 ± 20.7	96.7	2.1
	MTCS	10	9.1 ± 0.5	91.0	5.5	10	9.3 ± 0.7	93.0	7.5
		50	48.6 ± 1.2	97.2	2.5	50	47.4 ± 2.8	94.8	5.9
		100	90.7 ± 3.6	90.7	4.0	100	92.4 ± 6.1	92.4	6.6
		1000	982.9 ± 6.2	98.3	0.6	1000	988.2 ± 19.2	98.8	2.0
Squid	TCS	10	8.8 ± 0.5	88.0	5.7	10	8.5 ± 0.6	85.0	7.1
		50	43.3 ± 2.3	86.6	5.3	50	48.4 ± 3.2	96.8	6.6
		100	92.1 ± 4.1	92.1	4.5	100	90.6 ± 5.1	90.6	5.6
		1000	972.2 ± 12.2	97.2	1.3	1000	1011.5 ± 24.5	101.1	2.4
	MTCS	10	9.2 ± 0.3	92.0	3.3	10	9.6 ± 0.5	96.0	5.2
		50	45.1 ± 3.1	90.2	6.9	50	51.3 ± 3.8	102.6	7.4
		100	90.8 ± 2.9	90.8	3.2	100	92.2 ± 4.2	92.2	4.6
		1000	976.3 ± 23.2	97.6	2.4	1000	988.6 ± 18.5	98.9	1.9

Note: recovery (relative extraction recovery).

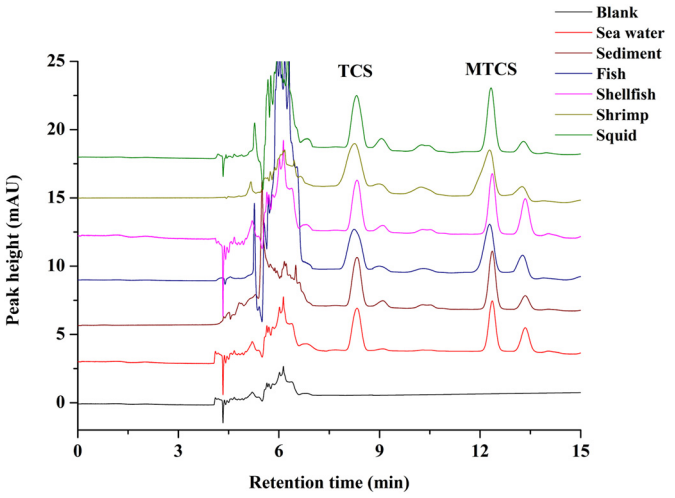

Fig. 5. Chromatogram of TCS and MTCS obtained by the newly developed IDFP-EFA-SFO method under optimized conditions.
Note: Experimental conditions: (1) samples were fortified with TCS and MTCS at 50 μg L^{-1} or μg kg^{-1}; (2) 146 μL extractant, 793 μL disperser, 3.0 min centrifugation and 1.1 min vortex time.

from 0.5 to 6.9% for intra-day analysis and from 0.9 to 9.5% for inter-day analysis in seawater, sediment and four seafood types (Table 3). The enrichment factors (EFs) were ranged from 57.1–70.9 for seawater, sediment and seafood samples, demonstrating a high enrichment capacity for TCS and MTCS by this newly developed IDFP-EFA-SFO method.

3.6. Analysis of real-world seawater, sediment and seafood samples

Fig. 5 illustrates typical chromatograms for seawater, sediment and seafood samples (fish, shellfish, shrimp and squid) at a fortification level of 50 μg kg^{-1} for TCS and MTCS using the optimized IDFP-EFA-SFO method ($n = 6$). At the four spiked levels (10, 50, 100 and 1000 μg L^{-1}/μg kg^{-1}), the ERs (n = 6) for TCS and MTCS were in the range of 89.2–103.5% and 83.3–101.1%, respectively, in seawater, sediment and seafood samples (Table 4). Concentrations of TCS and MTCS in non-fortified samples were all below their respective LODs in seafood (fish, shellfish, shrimp and squid). However, TCS was detected at 0.10 μg L^{-1} and 0.09 μg kg^{-1} in seawater and sediment, respectively, and MTCS was detected at 0.12 μg L^{-1} in seawater. In total, these results demonstrate that the newly developed IDFP-EFA-SFO method has excellent prospects for analyzing trace levels of TCS and MTCS in marine samples with high precision and accuracy.

3.7. Comparison of the IDFP-EFA-SFO method with the traditional DLLME-SFO method

Following optimization, a performance comparison between the newly developed IDFP-EFA-SFO and traditional DLLME-SFO without disperser for enrichment and freezing purification was conducted for TCS and MTCS quantification in marine samples (Supplementary Fig. 4). Many impurity peaks interfered with chromatograms in the traditional DLLME-SFO method rendering the chromatographic profiles inadequate for accurate and sensitive detection of TCS and MTCS (Supplementary Fig. 4a). Additionally, the highest extraction yield was obtained by the IDFP-EFA-SFO technique (Supplementary Fig. 4b). Thus, the IDFP step before microextraction analysis enriched target analytes and removed impurities that caused chromatographic interferences.

3.8. Comparison of the IDFP-EFA-SFO method with others methodologies

To verify the efficacy of the newly developed IDFP-EFA-SFO method, the optimized method was applied for the determination of TCS and MTCS in seawater, sediment and four types of seafood. Typical chromatograms for the marine samples at a fortification levels of 50 μg L^{-1}/μg kg^{-1} displayed good peak symmetry, separation and sensitivity indicating that the method was effective for accurate, trace-level quantification of TCS and MTCS (Fig. 5). The newly developed IDFP-EFA-SFO method was also compared with other methods reported in the literature to evaluate its relative efficacy: DLLME (Chau et al., 2008; Okumura and Nishikawa, 1996), SPE (Chau et al., 2008; Gonzalo-Lumbreras et al., 2014), pressurized liquid extraction-solid phase extraction (PLE-SPE) (Agüera et al., 2003), matrix solid phase dispersion (MSPD) (Escarrone et al., 2014; Gonzálezmariño et al., 2010), and accelerated solvent extraction (ASE) (Boehmer et al., 2004) (Table 5). The LODs for IDFP-EFA-SFO were in the range of 0.022–0.060 μg L^{-1}/μg kg^{-1}, which approached the LODs for LLE, SPE (Chau et al., 2008) and ASE (Boehmer et al., 2004), and were lower than other pre-treatment methods. The ERs for IDFP-EFA-SFO were in the range of 83.3–103.5%, which were similar to other pre-treatment methods and fully suitable for determining TCS and MTCS in seawater, sediment and

Table 4
The fortified recoveries of TCS and MTC by the IDFP-EFA-SFO method in sea water, sediment and sea food.

Analytes		TCS				MTCS			
Added (μg L^{-1}/μg kg^{-1})		10	50	100	1000	10	50	100	1000
Sea water (mean ± SD, n = 3)	Blank (μg L^{-1})	0.10 ± 0.01				0.12 ± 0.01			
	Detected (μg L^{-1})	9.3 ± 0.5	51.7 ± 1.5	99.7 ± 1.3	982.7 ± 2.2	9.8 ± 1.3	48.4 ± 1.7	99.7 ± 1.9	993.7 ± 3.3
	ER (%)	92.0	103.2	99.6	98.3	96.8	96.7	99.6	99.4
Sediment (mean ± SD, n = 3)	Blank (μg kg^{-1})	0.09 ± 0.02				ND			
	Detected (μg kg^{-1})	10.0 ± 0.7	50.3 ± 1.4	98.4 ± 1.7	991.3 ± 4.6	9.6 ± 1.0	49.1 ± 1.4	101.1 ± 2.8	979.2 ± 6.5
	ER (%)	98.8	100.4	98.3	99.1	95.7	98.2	101.1	97.9
Fish (mean ± SD, n = 3)	Blank (μg kg^{-1})	ND				ND			
	Detected (μg kg^{-1})	9.0 ± 1.3	48.4 ± 2.0	93.1 ± 2.6	958.2 ± 3.5	8.7 ± 0.7	48.1 ± 2.0	95.2 ± 1.5	948.4 ± 4.9
	ER (%)	90.2	96.7	93.1	95.8	86.8	96.1	95.2	94.8
Shellfish (mean ± SD, n = 3)	Blank (μg kg^{-1})	ND				ND			
	Detected (μg kg^{-1})	9.4 ± 0.5	48.6 ± 0.9	95.3 ± 1.4	972.2 ± 1.9	9.4 ± 0.6	50.2 ± 1.0	97.2 ± 1.5	997.6 ± 2.3
	ER (%)	94.2	97.3	95.3	97.2	93.7	100.4	97.2	99.8
Shrimp (mean ± SD, n = 3)	Blank (μg kg^{-1})	ND				ND			
	Detected (μg kg^{-1})	8.9 ± 0.3	51.1 ± 1.4	96.4 ± 2.2	988.4 ± 3.7	8.3 ± 1.0	49.2 ± 1.3	92.6 ± 1.9	965.1 ± 4.6
	ER (%)	89.2	102.1	96.4	98.8	83.3	98.3	92.6	96.5
Squid (mean ± SD, n = 3)	Blank (μg kg^{-1})	ND				ND			
	Detected (μg kg^{-1})	9.7 ± 0.3	56.7 ± 3.6	98.6 ± 1.9	1003.6 ± 2.2	9.1 ± 0.8	49.1 ± 1.4	99.1 ± 2.2	988.8 ± 3.3
	ER (%)	100.22	103.5	98.6	100.4	91.3	98.2	99.1	98.9

Note: (1) ER indicates extraction recovery; (2) Each treatment includes three replicates; (3) Each detected value is mean ± SD (standard deviation).

Table 5
Comparison of the proposed method with others for determination of TCS and MTCS.

Method	Matrices	Contaminant	LOD ($\mu g\ L^{-1}$/$\mu g\ kg^{-1}$)	RR (%)	Extraction time (min)	Organic solvents	References
LLE-GC-MS	Seawater	TCS	0.030–0.059	91–119	> 60	200 mL *n*-hexane	(Okumura and Nishikawa, 1996)
	Sediment		1.7–4.6	83–117	> 80	50 mL *n*-hexane	
	Fish		0.89–2.5	85–119	> 80	50 mL acetonitrile,20 mL acetone,100 mL *n*-hexane	
PLE-SPE-GC-NCI	Sediment	TCS	0.09	87–114 82.3–112.7	5	160 °C,2500 psi	(Agüera et al., 2003)
PLE-SPE -GC-EI			5.00	81–99			
PLE-SPE -LC-MS			3.5				
ASE-GC-MS/MS	Fish	TCS, MTCS	0.030–0.084		> 60	0.2 mL (dichloromethane/cyclohexane = 50/50), 1.0 mL *n*-hexane	(Boehmer et al., 2004)
SPE-GC-MS	Seawater	TCS	0.002	85–91	–	9 mL ethyl acetate, 5 mL methanol	(Chau et al., 2008)
LLE-GC-MS	Seawater	TCS	0.002	84–90	> 31.5	60 mL dichloromethane, 100 μL acetidin	
MSPD-GC-MS	Sediment	TCS, MTCS	1.80–2.10	86–113	> 5	5 mL *n*-hexane, 10 mL dichloromethane, 1 mL ethyl acetate	(Gonzálezmariño et al., 2010)
MSPD-LC-MS/MS	Fish	TCS	16.6	79–108	16	5 mL acetonitrile	(Escarrone et al., 2014)
SPE-GC-MS	Fish	TCS, MTCS	2.0–2.1	97	> 30	1.45 mL ethyl acetate, 80 μL isooctane, 70 °C	(Gonzalo-Lumbreras et al., 2014)
IDFP-EFA-SFO-HPLC-UV	Seawater	TCS, MTCS	0.022–0.031	93.1–103.3	41.1	146 μL octanic acid, 793 μL acetone	This work
	Sediment		0.023–0.033	95.7–100.5			
	Fish		0.045–0.060	86.8–96.7			
	Shellfish		0.044–0.057	93.7–100.4			
	Shrimp		0.043–0.056	83.3–102.1			
	Squid		0.044–0.056	91.3–103.5			

Note: (1) LLE-GC-MS indicates liquid-liquid extraction combined with gas chromatography-tandem mass spectrometry detector; (2) PLE-SPE-GC-NCI indicates pressurized liquid extraction-solid phase extraction combined with gas chromatography-negative chemical ionization detector; (3) PLE-SPE-GC-EI indicates pressurized liquid extraction-solid phase extraction combined with gas chromatography-electron impact ionization detector; (4) PLE-SPE-LC-MS indicates pressurized liquid extraction-solid phase extraction combined with liquid chromatography-tandem mass spectrometry detector; (5) ASE-GC-MS/MS indicates accelerated solvent extraction combined with gas chromatography-tandem mass spectrometry detector; (6) SPE-GC-MS indicates solid-phase extraction combined with gas chromatography-tandem mass spectrometry detector; (7) MSPD-GC-MS indicates matrix solid phase dispersion combined with gas chromatography-tandem mass spectrometry detector; (8) MSPD-LC-MS/MS indicates matrix solid phase dispersion combined with liquid chromatography-tandem mass spectrometry detector; (9) IDFP-EFA-SFO-HPLC-UV indicates integrated disperser freezing purification extraction using fatty acid-based solidification of floating organic-droplet combined with high-performance liquid chromatography-ultraviolet detector.

seafood.

The newly developed method used acetone as the enrichment solution, purification solution for protein and fat, and dispersive agent for IDFP-EFA-SFO. Firstly, the acetone was used to enrich TCS and MTCS from samples, but it also concentrates some impurities as part of the process (Fig. 1-b and g). Secondly, hydrophobic impurities (e.g., proteins and fats) were precipitated at −20 °C for 20 min and removed, while the hydrophilic impurities were re-dissolved into the aqueous phase with the acetone used as the dispersive agent (Fig. 1-e to f; h to k). Finally, TCS and MTCS, which have strong hydrophobic properties, were concentrated by octanoic acid (extractant) from the aqueous phase (Fig. 1-n). This method further reduced error by facilitating easy and effective collection of the fatty acid, which improves extraction recovery (Shih et al., 2015; Vakh et al., 2016). LLE requires a great deal of time for the ultrasound, centrifugation and rotary evaporation steps, while ASE requires derivatization at 60 °C for 1 h. All of these additional processing steps increase the extraction time and thereby limit sample throughput. Although the pre-treatment time for PLE-SPE (5 min) and MSPD (5 min) are short, PLE-SPE requires special reaction conditions (160 °C, 2500 psi) and MSPD consumes large amounts of organic solvents (Agüera et al., 2003; Gonzálezmariño et al., 2010). For IDFP-EFA-SFO, only 145 μL octanoic acid and 793 μL acetone were used IDFP, which is much less than required for LLE (60 mL of dichloromethane) (Chau et al., 2008); or 50–200 mL n-hexane, (Okumura and Nishikawa, 1996), SPE (9 mL of ethyl acetate and 5 mL methanol) (Chau et al., 2008) and MSPD (5 mL of acetonitrile) (Escarrone et al., 2014). In contrast, PLE-SPE (Agüera et al., 2003) does not require organic solvents and ASE requires only 1 mL of n-hexane (Boehmer et al., 2004), but both of these techniques require specialized instruments to achieve the extraction conditions of high temperature and high pressure, which increase procedural complexity and cost of operation.

In the newly developed IDFP-EFA-SFO method, acetone was simultaneously used as the enrichment solution, purification solution and dispersive agent. Further, the solidification of octanoic acid in an ice bath reduces experimental error by facilitating rapid and effective collection of the extraction agent without the need for a specialized extraction device, which simplifies the operation and reduces cost. Finally, the IDFP-EFA-SFO method reduces the influence of matrix effects and impurities on analyte recoveries (Shih et al., 2015; Vakh et al., 2016), which further enhances the accuracy and recovery of the extraction.

4. Conclusion

This investigation developed a new method utilizing acetone-based freezing purification and solidification of a floating organic-droplet for trace-level quantification of TCS and MTCS in seawater, sediment and seafood. The operational factors of the method were optimized by Plackett-Burman design and Central Composite Design. The prominent advantages of the newly developed IDFP-EFA-SFO method are reduced matrix interferences, improved detection sensitivity, and simultaneous detection of trace-level concentrations of TCS and MTCs in complex marine matrices. In sum, the new method possesses a low detection limit, wide linear detection range, very good precision and relatively low environmental impact from harsh chemicals and solvents. As a result, this new method has excellent prospects for determination of triclosan and methyltriclosan in several marine matrices, as well as in a wide range of other environmental matrices.

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