Determination of booster biocides in sediments by focused ultrasound-assisted extraction and stir bar sorptive extraction~~–~~–thermal desorption~~–~~–gas chromatography~~–~~–mass spectrometry

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Abstract

Booster biocides present in natural waters may be captured by the sediments. Highly sensitive, robust, selective and reliable analytical methods are required to evaluate their presence in the sediments. A focused ultrasound assisted extraction method for the simultaneous determination of five organic booster biocides (Chlorothalonil, Dichlofluanid, Sea-Nine 211, Irgarol 1051 and TCMTB) in marine sediments is proposed. The isolation of the target compounds from 0.5 g of sediment was efficient using 2.5 mL of methanol as solvent, an output amplitude of 50 W, 100% duty cycle and an extraction time of 300 s. The extraction procedure was followed by solid-phase clean-up on non-polar sorbents (Oasis HLB). The compounds were sampled using stir bars (20 mm length, 0.5 mm film thickness) coated with polydimethylsiloxane (PDMS). The stir bars were stirred at 900 rpm for 3 h~~3 hours~~ at room temperature. Desorption was carried out by thermal desorption (TD), and the analytes were separated in the gas chromatography (GC). They were detected by triple quadrupole mass spectrometry (MS/MS) using the multiple reaction monitor (MRM) mode. The detection limits (DLs) of booster biocides were in the range of 0.02 to 2.2 ng g^{-1} dry weight. The regression coefficients were higher than 0.999. The average recovery was higher than 86% (R.S.D.: 7–11%). The analytical procedure was applied to determination of the booster biocides in port sediment samples from the southwestern Spain. Sea-Nine 211 was found in one sediment at a level of 2.1 ng g^{-1}. Irgarol was present in all the sediments at concentrations between 2 and 9 ng g^{-1}.

Keywords: Booster biocides; Sonication; Stir bar sorptive extraction; GC-MS/MS; Sediment analysis

1 Introduction

Booster biocides are organic compounds that in combination with copper are used in antifouling paints to prevent the colonization of boat hulls by algae and seaweed [1–3]. Some of the most used booster biocides are Irgarol 1051 (2-methythio-4-tert.-butylamin-6-cyclopropylamin-s-triazine), Clorothalonil, Dichlofluanid, Sea-Nine 211 (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one) and TCMTB (2thiocyanomethylthiobenzothiazole). Their physical and chemical nature are shown in Table S1 [4–7]. They present a water solubility between 0.6 and 125 mg L^{-1}, and octanol-water partition constants in the range of 2.8 and 3.9. The booster biocides have been employed as alternative to tributyltin (TBT) after global prohibition to their application in 2003 and absence in 2008 by International Maritime Organization (IMO) and the Directive 2002/62/EC [5,8–11]. The increased use of booster biocides resulted in higher concentrations in water column and, therefore, may be accumulated in marine sediments [12]. In addition, the binding of booster biocides to sediment particles retards their migration and increases their persistence, causing ecological as well as ecotoxicological damage in the aquatic environment [13].

In sediments, the concentration of booster biocides can be as low as ng g^{-1} range [14–26]. In addition, the sediments are complex matrix with a large number of interferences. Therefore, it is essential to develop simple, highly sensitive, robust and selective methods for determination of booster biocides in sediment samples. The methods include several steps as extraction, clean-up, pre-concentration, separation and detection of analytes.

Several extraction methods have been used for extraction of analytes from sediments. Some are based on soxhlet extraction [27], on mechanical shaking [3,14,15,23,28–31], on ultrasonic extraction (USE) [24–26,32–34], or both mechanical shaking and ultrasonic extraction [12,14]. These methods usually take long periods of time and large amount of organic solvent. There are other methods that require less time and solvent, such as accelerated solvent extraction (ASE) [20,35,36], microwave-assisted extraction (MAE) [21,37,38], surpercritical fluid extraction with CO_2 (SFE) [15] or vortex-assisted matrix solid phase extraction (VA-MSPD) [39]. However, a limited number of booster biocides were considered in most of these studies. New methods are needed to accelerate the extraction of a wide range of booster biocides from sediments with high reproducibility and efficiency. In this sense, the focused ultrasound assisted extraction (FUSE) has been used accelerate for the extraction of several organic compounds from soil samples [40], and it is a promising alternative method for the booster biocides extraction.

After booster biocides extraction, clean-up and pre-concentration steps must be performed to remove co-extractive interferences and concentrate the analytes prior to instrumental analysis. The interferences may be polar and non-polar in nature, and they have to be retained on polar and non-polar sorbents, respectively.

Some authors have used reversed solid-phase extractions (SPE) based on silica C18 [32,34] or polymeric materials, such as Oasis-HLB [41–44], for removing non-polar compounds. Others authors have employed Florisil [14,38,45], alumina [20,23], EnvirElut Pesticide [21], and ENVI-Carb [25,26] for removing polar interferences. Several procedures allowed the simultaneous clean-up and pre-concentration of the booster biocides in sediments. They were based on immunoaffinity chromatography [15], dispersive liquid–liquid microextraction (DLLME) [24], and solid phase microextraction (SPME) [33]. The use of the stir bar sorptive extraction (SBSE) coupled to GC lets sampling the analytes in a simple way with a high sensitivity. The extracted compounds are totally introduced in the chromatograph by using a thermal desorption injection system.

The separation of booster biocides has been accomplished using high performance liquid chromatography (HPLC, LC) [12,21,25,26,31,32,34,36,39] or gas chromatography (GC) [14,24,27–29,33,37,38,45,46]. The identification was carried out using several detectors, such as diode array (DAD) [32], electron capture (ECD) [14], flame photometric (FPD) [14], nitrogen and phosphor detector (NPD) [27], simple quadrupole mass spectrometry (MS) [12,14,26,33,35,37,46,47] and triple quadrupole mass spectrometry (MS/MS) [21,25,31,34,36,39,45]. MS/MS detector allows high sensitivity and selectivity, reducing the matrix interferences and increasing the signal to noise ratio, when is used in multiple reaction monitoring (MRM) mode for quantification.

The objective of this work was to develop an extraction procedure for the determination of Irgarol 1051, Clorothalonil, Dichlofluanid, Sea-Nine 211 and TCMTB in sediments using focused ultrasound assisted extraction (FUSE), and SBSE-TD-GC-MS/MS analysis. All the steps and parameters of the analytical procedure were optimized. Finally, the method was applied to three sediments with different characteristics collected from marinas in Southwestern Spain.

2 Experimental

2.1 Standards, chemicals and reagents

Triphenylphosphate (TPP), Chlorothalonil, Dichlofluanid and TCMTB were purchased from Supelco (Sigma-Aldrich, Gillingham, U.K.). Irgarol-d9 was supplied by LGC Standards (Barcelona, Spain). Irgarol 1051 and Sea-Nine 211 were kindly offered by Ciba-Geigy (Barcelona, Spain) and Rohm & Haas (Barcelona, Spain), respectively. In all cases, the purity level was higher than 98%.

Methanol (MeOH), n-pentane, i-octane, acetone and dichloromethane were pesticide grade and supplied by Romil (Loughborough, UK). Potassium chloride of analytical-reagent grade, Florisil (60–100 mesh), silica gel, neutral, basic and acidic alumina, and silica base C-18 cartridge were provided by Merck (Darmstadt, Germany). Oasis HLB cartridges, a copolymer of poly(divinylbenzene-co-N-vinylpyrrolidone (500 mg, 6 mL) was purchased from Waters (Milford, USA). Ultra-pure Milli-Q water (~~18~~ 18 MΩ cm) (Millipore, Bedford, MA, USA) was used throughout the process.

2.2 Preparation of sample

For optimization of the sonication extraction procedure in sediment, a spiked sample was prepared by mixing 200 mL of seawater containing the booster biocides at a concentration of 28, 100, 8, 10 and 420 μg L^{-1} for Chlorothalonil, Dichlofluanid, Sea-Nine 211, Irgarol 1051 and TCMTB, respectively, with 100 g of a sediment (2% organic matter content) free of booster biocides. It was taken from the shoreline at several km from any boats presence. The concentration used for each biocide was selected depending on its sensitivity in the chromatographic analysis. After mechanical shaking, the mixture was left to equilibrate for two hours in darkness, leaving both phases in contact. Then, the supernatant was collected for analysis [47]. The sediment was freeze-dried and stored at −~~20~~ 20 °C until analysis. The concentrations in the supernatant were 1.43~~±~~ ± 0.23, 5.54~~±~~ ± 0.09, 0.48~~±~~ ± 0.07, 0.55~~±~~ ± 0.02 and 21.3 ± 2.3 μg L^{-1}, for Chlorothalonil, Dichlofluanid, Sea-Nine 211, Irgarol 1051 and TCMTB, respectively, which represented a percentage lower than 5% for all the biocides).

2.3 Extraction of sediments

Aliquots of 1.0 g of sediment and solvent volumes in the range of 5~~-~~–15 mL were used for the extraction optimization. An ultrasonic homogenizer, model Sonopuls HD 2200 (Bandelin, GmbH & Co. KG) equipped with a converter UW 200, SH 213 horn as amplifier and sonotrode MS 73 (Ti microtip of 3 mm diameter) was used for sediment extraction. To avoid sample contamination, the probe tip was rinsed with water and dried with a lint-free tissue between sample runs. The extract was centrifuged at 5000 rpm for 10 min (Digiten 21 centrifuge, Ortoalresa, Spain). The residue was rinsed with 1.5 mL of solvent which was added to the extract, and analyzed by SBSE-TD-GC~~-~~–MS/MS.

Ten mL of three different volatile solvents, and covering a wide range of polarity (MeOH, dichloromethane and pentane) were first evaluated for the sediment extraction using a time of 100 s (duty cycle 50%, output amplitude 50 W with the probe placed 1 cm from the bottom of the polypropylene centrifuge tube of 50 mL used as extraction vessel) at room temperature. Then, a Box–Behnken experimental design (CSS Statistica, StatSoft Inc., Tulsa, OK) was used to evaluate the significance of the variables affecting the extraction, as well as the interactions between them. The variables investigated were output amplitude (X_1), duty cycle (X_2), solvent volume (X_3), and time (X_4). All variables were evaluated at three levels. This design involved 27 experiments which were performed in random order. The data were analyzed by multiple regression analysis through the least squares method to fit the following equation:

$$Y = A_0 + \Sigma A_i X_i + \Sigma A_{ii} X_i^2 + \Sigma A_{ij} X_i X_j$$

where Y is the predicted booster biocide signal, A_0, A_i, A_{ii}, A_{ij} are the constant and regression coefficients of the model, and X_i, X_j represent the coded level of independent variables i and j, respectively. Only the estimates of coefficients with significant levels higher than 95% (~~p~~ p ~~$<$~~ $\le$ 0.05) were included in the final models. The most influencing variables were further optimized by univariate experiments.

2.4 Clean-up procedure

The following procedure was used for extract clean-up after sonication. Polar and non-polar sorbents were evaluated for optimal solution to be analyzed.

Florisil was selected as polar sorbent using solvents of increasing polarity. The glass column (150 × 10 mm) was packed with 2 g Florisil (60~~-~~–100 mesh) pre-purified with i-octane and activated at ~~360~~ 360 °C. The sorbent was topped with 1 cm of anhydrous sodium sulfate and conditioned with 5 mL of i-octane. 1 mL of i-octane was added to the sample extract to change the solvent. The volume was reduced to 1 mL under a nitrogen stream, and then loaded to the column. A volume of 20 mL of n-pentane/dichloromethane (50:50) mixture was first used to elute the least polar matrix components, which were discharged. It was followed by 15 mL of acetone/dichloromethane (10:90) mixture to elute the booster biocides at a flow of 1 mL min^{-1}. This solvent was changed to 5 mL of MeOH, spiked with 10 ng of TPP (as internal standard for the compensation of volume effects and signal variations) in ~~50~~ 50 μL of MeOH, and submitted to SBSE-TD-GC~~-~~–MS/MS.

Oasis HLB cartridge was selected as non-polar sorbent. It was conditioned with 10 mL of MeOH. The sample extract (5 mL of MeOH) was loaded onto the cartridge and eluted with 5 mL of MeOH. A volume of ~~50~~ 50 μL of MeOH spiked with 10 ng of TPP was added, and then analyzed by SBSE-TD-GC~~-~~–MS/MS.

2.5 Pre-concentration on PDMS~~pdms~~ stir bars

A stir bar (20 mm length × ~~x~~ 0.5 mm film thickness) coated with ~~47~~ 47 μL of PDMS from Gerstel (Mulheim an der Ruhr, Germany) was used to sample the booster biocides from the extract obtained from the sonication and sorbent clean-up treatment. The extract was reconstituted in 50 mL of water with 10 g KCl. PDMS coating is non-polar in nature, and suitable for the extraction of the booster biocides. Sampling was performed by stirring at 900 rpm (Gerstel magnetic stirrer, Mulheim an der Ruhr, Germany) for 90 min at room temperature. Prior to use, the stir bars were conditioned at ~~300~~ 300 °C for 15 min using helium at a desorption flow of 50 mL min^{-1}, and kept in new 2 mL vials.

2.6 TD-GC~~-~~–MS/MS analysis

TD-GC~~-~~–MS/MS analysis was performed using a commercial thermal desorption unit (TD-20, Shimadzu, Japan) equipped with an autosampler, and coupled to the gas chromatograph with a triple quadrupole mass spectrometer with inert electron-impact ion source (GCMS-QP6030 Ultra, Shimadzu, Japan). Separation conditions have been previously reported [47]. The acquisition mode was performed in multiple reaction monitoring (MRM) mode. Two transitions were monitored for each analyte, the first one for quantification and the second one for confirmation (Table 1). For control and data analysis, GCMS Postrun Analysis Shimadzu was used. The MS was tuned with perfluorotributylamine (PFTBA).

alt-text: Table 1

Table. 1

GC MS/MS system conditions for the determination of booster biocides.

Compound	t_R (min)	Quantitation Transition (m/z)	CE (eV)	Identification Transition (m/z)	CE (eV)
Chlorothalonil	14.6	266~~->~~>170	26	264~~->~~>168	25
Dichlofluanid	16.6	224~~->~~>123	9	167~~->~~>124	13
Sea-Nine 211	17.3	182~~->~~>155	15	264~~->~~>171	20
Irgarol 1051	17.4	182~~->~~>109	12	253~~->~~>196	17
TCMTB	18.0	180~~->~~>136	17	238~~->~~>180	8
Irgarol d9	17.4	183~~->~~>110	12		
Triphenyl Phosphate	19.7	326~~->~~>170	19		

Helium (purity of 99.9999%) and argon (purity of 99.999%) were used as carrier and collision gas, respectively. They were supplied by Abelló Linde S.A. (Barcelona, Spain).

Quantitative analysis was carried out adding 10 ng of TPP (as instrumental internal standard) to the extract and 4.5 ng of Irgarol-d9 (as recovery internal standard) to the sediment prior to its extraction. Response factors were obtained for each of the standards in a six-point calibration curve and compared to the response factors from the samples.

2.7 Statistical analysis

Analysis of variance (ANOVA) and *t*-test evaluated the experimental data using STATISTICA version 10.0 (StatSoft Inc., 2011). Prior to ANOVA analysis, all the data were tested for homogeneity of variance by the Levene tests. An α-value of 0.05 was adopted as the critical level giving a 95% confidence level.

3 Results and discussion

3.1 ~~o~~Optimization of the extraction step in sediment

Some preliminary experiments were performed in order to select the best extractant. Analysis was carried out using the SBSE-TD-GC~~-~~-MS method. A volume of 10 mL of MeOH, dichloromethane or n-pentane was used for the sonication of 1.0 g of sediment, at 50 W and 50% of duty cycle for 100 s. The highest recoveries were obtained with MeOH (Fig. S1), which was selected for further experiments.

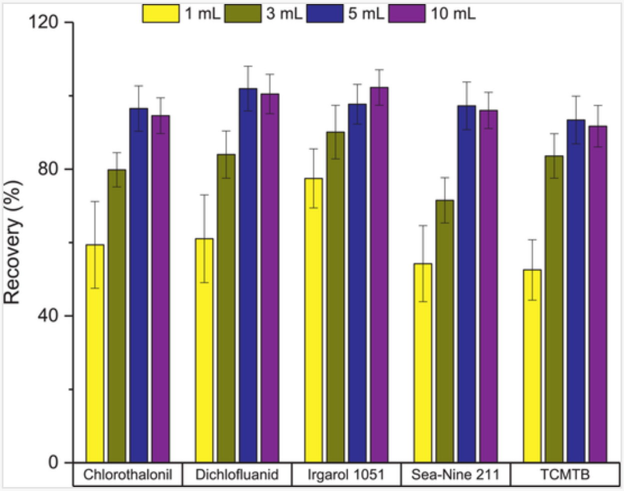
A multivariate design (Box−Behnken) was used to optimize the process variables, such as volume of MeOH (5–15 mL), ultrasound radiation amplitude (10–90 W), percentage of duty cycle of ultrasonic exposure (0–100%) and sonication time (60–600 s) (Table S2). The coefficients of multiple determination (R^2) were above 0.859 for all the booster biocides, indicating that the variables explained the data fairly well. The Pareto chart (Fig. S2) showed the influence of each variable on the response of each booster biocide, and no cross effects among these factors were noted. The conclusion of this screening study was that the duty cycle and MeOH volume had not influence on the biocides extraction in the ranges under study. A value of duty cycle of 100% was chosen for further experiments. The optimal ranges for the other variables, namely radiation amplitude and extraction time, were obtained from the three-dimension surface plots. The maximum response for all the biocides was obtained between 40–60 W for radiation amplitude, and 330–1000 s for sonication time. These ranges for both amplitude and time were studied by univariate experiments which were applied in triplicate. The MeOH volume to sediment weight ratio was also evaluated.

Ultrasound radiation amplitudes of 40, 50 and 60 W were compared using 5 mL of MeOH and a sonication time of 300 s. No significant differences were found (ANOVA $p > 0.311$), and 50 W was used for the subsequent experiments.

Several MeOH volume to sediment weight ratios ranging from 1 to 10 were tested. We found that a ratio of 5 was adequate since a further increase of the ratio did not improve the extraction efficiencies to any great extent, and a decrease of the extraction was obtained for lower values (Fig. 1). Hence, a ratio of 5 was selected as optimal.

alt-text: Fig 1

Fig. 1

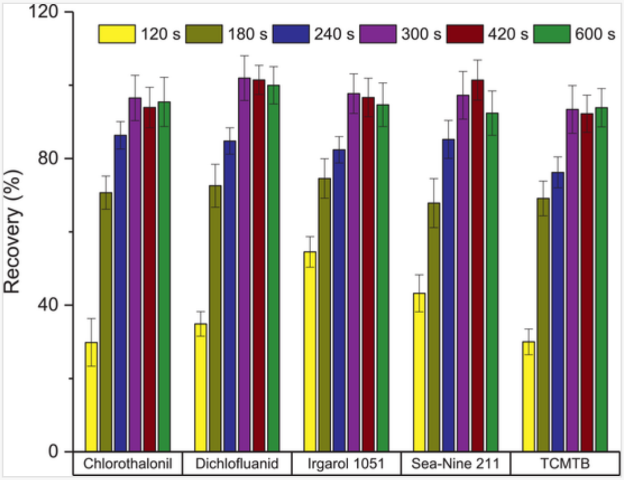


Effect of the MeOH volume on the recovery of booster biocides in marine sediment by ultrasound − assisted extraction using 1 g of sedime.

The effect of the sonication time on recoveries of booster biocides is shown in Fig. 2. The extraction efficiency increased with increasing sonication time from 120 to 300 s, and plateaued thereafter. A time of 300 s was selected for further experiments.

alt-text: Fig 2

Fig. 2



Effect of the extraction time on the recovery of booster biocides in marine sediment by ultrasound – assisted extraction.

Two consecutive extractions were performed on the same sample by extracting the residue obtained by centrifugation using the same volume of fresh MeOH under identical conditions. MeOH extracts from each extraction step were analyzed separately. The level of the booster biocides in the second one was below detection limits. Therefore, a single extraction step was used in the optimized procedure.

3.2 Optimization of the clean-up step

After sonication, the extracts were cleaned-up using polar (Florisil, silica gel, and neutral, basic and acidic alumina) or non-polar (C18 and Oasis HLB) sorbents.

Polar sorbents were conditioned with 5 mL of i-octane. Before sample loading, the sediment extract (free of booster biocides) was dried under a nitrogen stream and then 1 mL of i-octane spiked with 27, 98, 7, 9 and 400 ng of Chlorothalonil, Dichlofluanid, Sea-Nine 211, Irgarol 1051 and TCMTB, respectively, was added. The spiked extract was passed through the sorbent, and portions of 10 mL of solvents of increasing polarity were added. The elution pattern of the booster biocides was evaluated. The optimized conditions found for all the sorbents are shown in Table S3. Reproducibility data (~~*n*~~*n* ~~=~~= 5) are included in the Table S3. The recoveries were between 80~~-~~–98%. Therefore, all the polar sorbents studied can be used in the clean-up step.

Non-polar sorbents were conditioned with 10 mL of MeOH and then 5 mL of sediment extract spiked with 27, 98, 7, 9 and 400 ng of Chlorothalonil, Dichlofluanid, Sea-Nine 211, Irgarol 1051 and TCMTB, respectively, were passed through the sorbent. Several 5 mL portions of MeOH were used as eluent, and separately analyzed in order to select the minimum volume needed to elute quantitatively the booster biocides. Cartridge containing C18 showed poor elution efficiency for Chlorothalonil, Dichlofluanid and Sea-Nine 211, with elution recoveries less than 30% with 20 mL MeOH. However, a volume of 5 mL was only needed to elute quantitatively all the biocides from Oasis HLB, which was selected for further experiments.

3.3 Study of solvent removing

The solvents used for elution of the biocides from the polar sorbents are not suitable for the stir bar sorptive extraction, and must be removed. Moreover, MeOH used as eluent for non-polar sorbents must be removed or diluted with water [47]. A nitrogen stream was used to evaporate 5 mL of MeOH spiked with 27, 98, 7, 9 and 400 ng of Chlorothalonil, Dichlofluanid, Sea-Nine 211, Irgarol 1051 and TCMTB, respectively, to dryness, 0.1 and 1 mL. Then, they were analyzed by SBSE-TD-GC~~-~~–MS/MS. The results showed that the drying process affected all the analytes. The signal decreased between 50 and 95% for all the biocides except for Irgarol when solvent was removed to dryness, and between 30~~-~~–80% when was removed to 0.1 mL. No losses were observed when MeOH was removed to 1 mL.

Because removing solvents by evaporation was a slow process and analyte losses were also possible, Oasis HLB was selected for the clean-up step without MeOH evaporation.

3.4 Optimization of the stir bar sorptive extraction

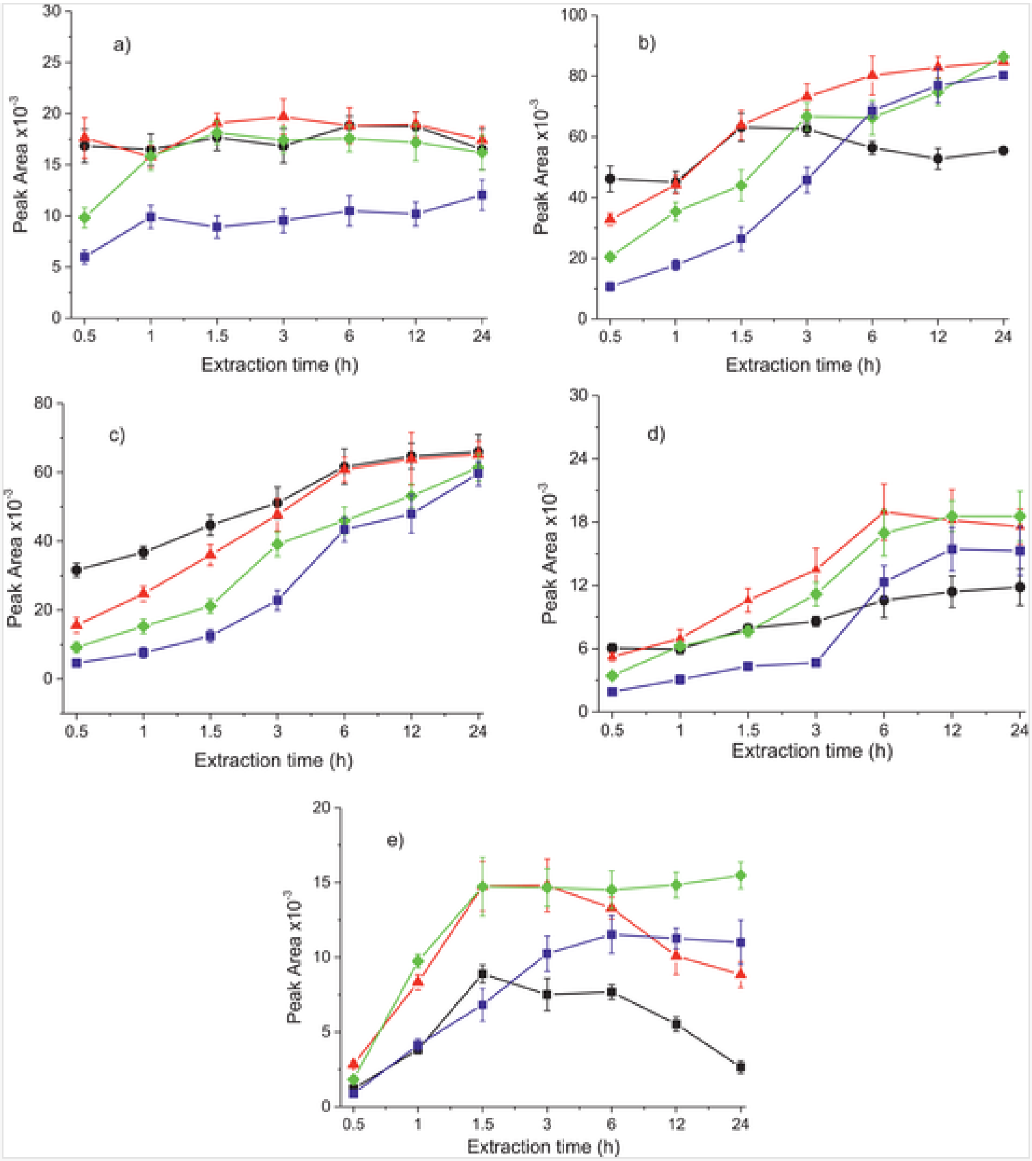
Several parameters as PDMS volume, extraction time, MeOH concentration, and simultaneous desorption from two stir bars in the glass desorption tube were tested. 20% KCl aqueous solutions spiked with 27, 98, 7, 9 and 400 ng of Chlorothalonil, Dichlofluanid, Sea-Nine 211, Irgarol 1051 and TCMTB, respectively, were used in the experiments.

Volumes of 24 µL (10 mm × ~~x~~ 0.5 mm), 47 µL (20 mm × ~~x~~ 0.5 mm), 63 µL (10 mm × ~~x~~ 1 mm) and 126 µL (20 mm × ~~x~~ 1 mm) of PDMS coated stir bars were studied. 5 mL of MeOH was added to 50 mL of sample, and the extraction was performed for 90 min at 900 rpm. The results showed a significant increase of sensitivity with 20 mm stir bars for all the analytes. No significant differences were found between 47 and ~~126~~ 126 µL of PDMS (ANOVA, $p > 0.465$). Therefore, a volume of ~~47~~ 47 µL of PDMS was selected for further experiments.

Furthermore, the effect of the extraction time (0.5~~-~~–24 h) and MeOH concentration (2.5~~-~~–20%, obtained by dilution of 5 mL of MeOH with different water volumes) on the extraction efficiency was evaluated. As shown in the Fig. 3~~-~~a, no significant differences were found for Chlorothalonil using extraction times longer than 1 h and MeOH concentrations higher than 2.5% (ANOVA, ~~*p*~~ *p* ~~=~~ = 0.708). For Dichlofluanid and Irgarol 1051 (Fig. 3b, d), the highest responses were obtained using 10% of MeOH and an extraction time higher than 3 h, (ANOVA, $p > 0.066$). For Sea-Nine 211 (Fig. 3~~-~~c) no significant differences were found for extraction times longer than 1.5 h and MeOH concentrations higher 10%, (ANOVA, ~~*p*~~ *p* ~~=~~ = 0.526). For TCMTB, there were no significant differences in the ranges of 5 to 10% MeOH concentration and 1.5 to 6 h (ANOVA, ~~*p*~~ *p* ~~=~~ = 0.687). Therefore, 10% of MeOH and 3 h duration were used for further experiments.

alt-text: Fig 3

Fig. 3



Effect of MeOH concentration and extraction time on the sampling of booster biocides by stir bar sorptive extraction: a) Chlorothalonil; b) Dichlofluanid; c) Sea-Nine 211; d) Irgarol 1051; e) TCMTB. ■ 2.5% MeOH ◆ 5% MeOH ▲ 10% MeOH ● 20% MeOH.

Finally, each one of the two 5 mL of MeOH eluates from the Oasis HLB clean-up step were sampled using two stir bars. In order to reduce the analysis time, the two stir bars were placed in a single glass desorption liner and simultaneously desorbed for GC-MS/MS analysis by multi-shot mode. The results were compared to those obtained using a single stir bar, and no significant differences were found (ANOVA, $p > 0.536$). Therefore, two stir bars of each sample were simultaneously desorbed and analyzed in further experiments.

3.5 Validation of the analytical method

Linearity, repeatability, reproducibility, detection and quantification limits of the method were checked under MRM mode (Table 2).

alt-text: Table 2

Table. 2

Analytical performance for the determination of booster biocides in sediment by FUSE-SPE-SBSE-TD-GC-–MS/MS.

Analyte	Time (min)	Linear range (ng)	R^2	DL (ng g^{-1})	QL (ng g^{-1})	RSD (%)
Chlorothalonil	14.6	0.05-–57	0.9996	0.1	0.3	9
Dichlofluanid	16.6	0.1-–200	0.9990	0.2	0.7	7
Sea-Nine 211	17.3	0.01-–15	0.9992	0.02	0.06	8
Irgarol 1051	17.4	0.01-–20	0.9997	0.02	0.06	7
TCMTB	18.0	1.1-–870	0.9992	2.2	7.2	10

The response was linear between DL and 57, 200, 15, 20 and 870 ng for Chlorothalonil, Dichlofluanid, Sea-Nine 211, Irgarol 1051 and TCMTB, respectively, with regression coefficients (R) above 0.999, using a six-point matrix-matched calibration and two internal standards (TPP and Irgarol-d9). The detection and quantification limits (DL and QL, respectively) were calculated with a signal to noise ratio of 3 (DL) and 10 (QL) [48]. DL and QL were in the range of 0.1 to 2.2 ng g^{-1} and 0.3 to 7.2 ng g^{-1} respectively, in sediment for all the booster biocides studied in this work.

DLs obtained by the proposed SBSE-TD-GC-–MS/MS method were much lower than those obtained by GC-–MS (Table S4) [14,33,38,45,46] and LC-MS [21,39] for all booster biocides, except for TCMTB. For TCMTB, DL of SBSE-TD-GC-–MS/MS was higher than that of LC-MS [21,41].

For repeatability, five replicates were analysed using the spiked sediment (Section 2.2-) on the same day, using the same equipment and carried out by the same analyst. In the case of reproducibility, five replicates were analysed on different days by different analysts. Repeatability and reproducibility R.S.D. values were lower than 5 and 10%, respectively, in all cases.

Three sediment samples with different physical characteristics (organic matter and fine fraction, Table S5) were used to evaluate matrix effects, the usefulness of the clean-up step and the method accuracy. The sediments were collected from Ayamonte Marina, El Rompido fishing port and Nuevo Portil Marina in September 2018.

For the estimation of the matrix effects, the same standard solutions were used with both standard addition and external calibration methods. The amounts of each compound were adjusted to add approximately 1, 2 and 4 times of the expected concentrations in the sample or QL. Concentrations in each sample were compared by both standard addition and matrix-matched calibration methods. The results are shown in Table 3. No significant differences were found in the slopes using both calibration methods (Test t, $p > 0.103$) for all the booster biocides. Irgarol 1051 was detected in the sediments from three ports with concentrations between 2.02 and 9 ng g^{-1}, whereas Sea-Nine 211 was only detected in the sediment from Nuevo Portil Marina, 2.1 ± 0.1 ng g^{-1}. No significant differences were found for Irgarol 1051 (Test t, $p > 0.300$) and Sea-Nine 211 (Test t, ~~*p*~~ *p* ~~=~~ = 0.973) concentrations for both calibration methods. Therefore, matrix-matched calibration methods can be used.

alt-text: Table 3

Table. 3

Slopes of the external calibration and standard addition calibration graphs and booster biocides concentrations in three sediment samples by FUSE-SPE-SBSE-GC~~-~~-MS/MS.

	Ayamonte Marina		**El Rompido Fishing Port**		**Nuevo Portil Marina**	
	~~Slope~~ Slope ~~±~~ ± s (μg^{-1})					
	External calibration	Standard addition	External calibration	Standard addition	External calibration	Standard addition
Chlorothalonil	236 ± ~~±~~2	238 ± ~~±~~5	236 ± ~~±~~2	236 ± ~~±~~2	236 ± ~~±~~2	236 ± ~~±~~8
Dichlofluanid	71.8 ± 0.8	71.2 ± 0.5	71.8 ± 0.8	71.0 ± 1.7	71.8 ± 0.8	72.5 ± 1.7
Sea-Nine 211	548 ± ~~±~~5	542 ± ~~±~~11	548 ± ~~±~~5	540 ± ~~±~~11	548 ± ~~±~~5	548 ± ~~±~~9
Irgarol 1051	605 ± ~~±~~4	593 ± ~~±~~9	605 ± ~~±~~4	629 ± ~~±~~21	605 ± ~~±~~4	614 ± ~~±~~3
TCMTB	6.22 ± ~~±~~0.06	6.30 ± ~~±~~0.48	6.22 ± ~~±~~0.06	6.30 ± ~~±~~0.13	6.22 ± ~~±~~0.06	6.23 ± ~~±~~0.06
	Concentration ± ~~±~~ s (ng g^{-1})					
	External calibration	Standard addition	External calibration	Standard addition	External calibration	Standard addition
Sea-Nine 211	<DL	<DL	<DL	<DL	2.1 ± 0.1	2.1 ± 0.2
Irgarol 1051	2.~~02~~ 02 ± 0.19	2.13 ± ~~±~~0.16	~~9~~ 9 ~~±~~ ± 1	8.7 ± ~~±~~ 0.6	3.4 ~~±~~ ± 0.4	3.7 ± 0.2

The usefulness of the clean-up step was evaluated in the three sediments studied. All the sediments were spiked with 27, 98 and 400 ng of Chlorothalonil, Dichlofluanid, and TCMTB, respectively (natural levels were below detection limits). The sediments from Ayamonte Marina and El Rompido Fishing Port were also spiked with 7 ng of Sea-Nine 211. They were analyzed without clean-up and using both Florisil and Oasis HLB sorbents. Results are shown in Table 4. No effect of the clean-up step was noticed on the recoveries of the booster biocides for the Nuevo Portil Marina sample (ANOVA, *p*~~>~~ > 0.453). This sediment had the lowest organic matter content and the highest sand content. However, recoveries of the biocides obtained using Oasis

HLB or Florisil were significant higher than those obtained without clean-up for the Ayamonte and El Rompido sediment samples (ANOVA, p~~>~~ > 0.429), except for Irgarol. Probably, this compound was less influenced by the matrix components because of the use of Irgarol-d9 as recovery internal standard in the analytical method. No significant differences were found for the recoveries obtained using Oasis HLB and Florisil clean-up methods (ANOVA, p~~>~~ > 0.277).

alt-text: Table 4

Table. 4

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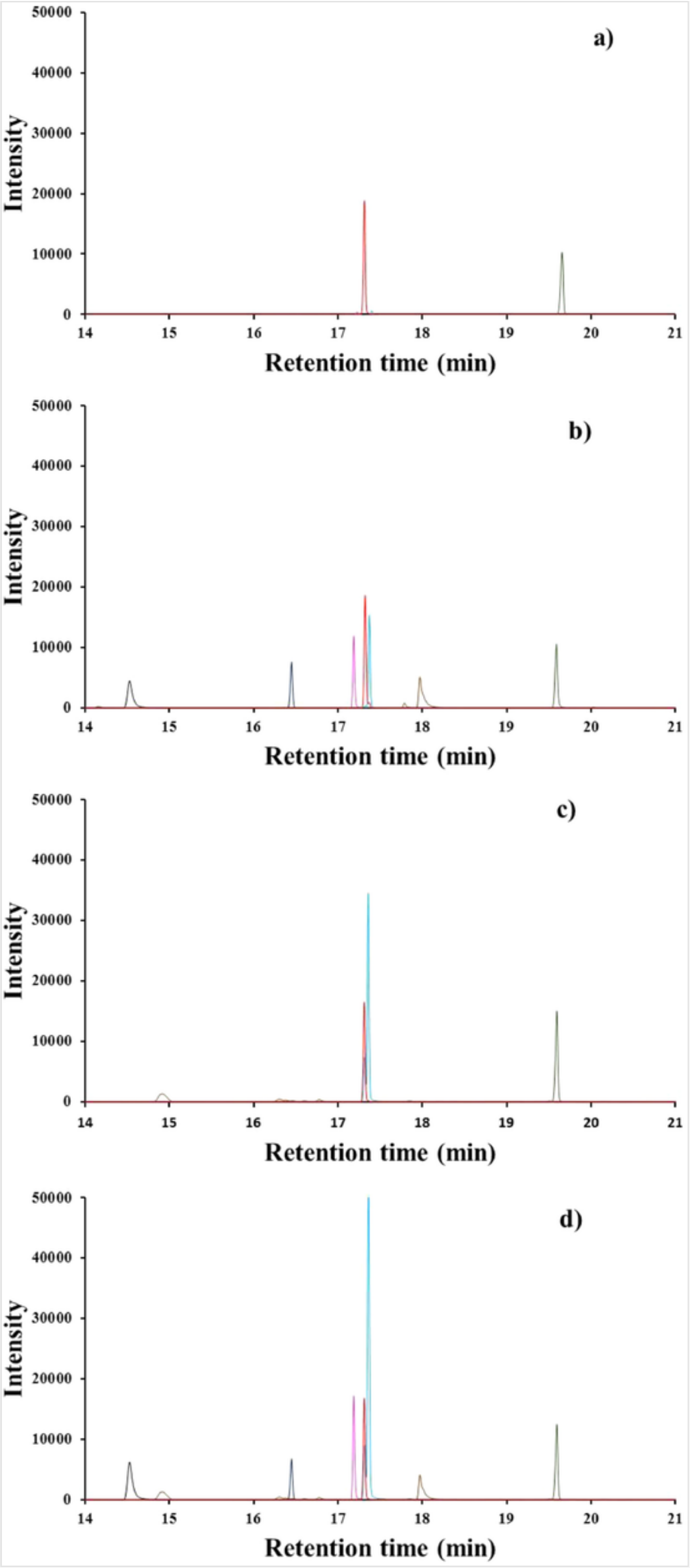
Booster biocides concentration ~~±~~ ± standard deviation (ng g^{-1}) using Florisil, Oasis and without clean-up methods in three sediment samples.

	Ayamonte Marina			**El Rompido Fishing Port**			**Nuevo Portil Marina**		
	with Florisil	with Oasis	without	with Florisil	with Oasis	without	with Florisil	with Oasis	without
Chlorothalonil	~~56~~ 56 ~~±~~ ± 3	~~54~~ 54 ~~±~~ ± 5	1.~~4~~ 4 ± 0.2	~~55~~ 55 ~~±~~ ± 5	~~54~~ 54 ~~±~~ ± 4	~~21~~ 21 ~~±~~ ± 2	~~53~~ 53 ~~±~~ ± 5	~~56~~ 56 ~~±~~ ± 4	~~52~~ 52 ~~±~~ ± 6
Dichlofluanid	~~184~~ 184 ~~±~~ ± 15	~~187~~ 187 ~~±~~ ± 18	~~33~~ 33 ~~±~~ ± 4	~~188~~ 188 ~~±~~ ± 19	~~186~~ 186 ~~±~~ ± 13	~~51~~ 51 ~~±~~ ± 5	~~192~~ 192 ~~±~~ ± 12	~~191~~ 191 ~~±~~ ± 10	~~205~~ 205 ~~±~~ ± 20
Sea-Nine 211	14.~~4~~ 4 ~~±~~ ± 1.2	13.~~7~~ 7 ~~±~~ ± 0.8	2.~~6~~ 6 ± 0.3	13.~~9~~ 9 ± 1.5	14.~~3~~ 3 ± 1.4	4.~~5~~ 5 ± 0.5	2.0 ± 0.2	2.1 ± 0.1	2.1 ± 0.2
Irgarol 1051	2.~~01~~ 01 ~~±~~ ± 0.10	2.~~02~~ 02 ~~±~~ ± 0.19	2.10 ± ~~- ±~~ 0.12	8.~~7~~ 7 ~~±~~ ± 0.6	~~9~~ 9 ~~±~~ ± 1	9.0 ± 0.6	3.2 ~~±~~ ± 0.2	3.~~4~~ 4 ~~±~~ ± 0.4	3.2 ± 0.2
TCMTB	~~754~~ 754 ~~±~~ ± 46	~~733~~ 733 ~~±~~ ± 5	~~467~~ 467 ~~±~~ ± 45	~~748~~ 748 ~~±~~ ± 62	~~759~~ 759 ~~±~~ ± 48	~~560~~ 560 ~~±~~ ± 56	~~832~~ 832 ~~±~~ ± 55	~~774~~ 774 ~~±~~ ± 58	~~835~~ 835 ~~±~~ ± 82

Certified reference materials of booster biocides in marine sediments do not exist for estimating the method accuracy. Therefore, the recovery of booster biocides from real sediments were evaluated by spiking at two concentration levels. Chromatograms of blank, standards (in the range of 2~~-~~–100 ng), sediment and spiked (in the range of 2~~-~~–100 ng) sediment from El Rompido Fishing Port are shown in Fig. 4. Table S6 shows that the recoveries were between 86 and 106%, and RSD lower than 10% for all booster biocides.

alt-text: Fig 4

Fig. 4



Chromatograms obtained by SBSE-TD-GC~~-~~–MS/MS under MRM mode (a) blank; (b) biocide standards at levels in a range of 2~~-~~–100 ng; (c) El Rompido Fishing Port; (d) El Rompido Fishing Port spiked with the five biocides at a level in a range of 2~~-~~–100 ng . — Chlorothalonil; — Dichlofluanid; — Sea-Nine 211; — Irgarol 1051; — TCMTB; — Irgarol-d9: — TPP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4 Conclusions

A focused ultrasound assisted extraction (FUSE) followed by a solid phase extraction (SPE) clean-up and a stir bar sorptive extraction (SBSE) pre-concentration step using thermal desorption system (TD) coupled to GC~~-~~-MS/MS was optimized to determine five booster biocides in marine sediments. A clean-up step must be included in the method for sediments with high organic matter and low sand contents. Polar and non- polar sorbents can been used in the clean-up step. However, non-polar sorbent method is much easier and less time consuming than the polar sorbent one. In addition, no clean-up may be needed if using deuterated recovery internal standard of each analyte. Therefore, the proposed method is simple, permits booster biocides determination in marine sediments to very low concentration level, and provides good recovery.

Uncited references~~:~~

~~[49]~~.

CRediT authorship contribution statement

E. García: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **I. Giráldez:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **M. Ruiz Montoya:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **E. Morales:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.microc.2019.104445.

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The corrections made in this section will be reviewed and approved by journal production editor.

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Graphical abstract

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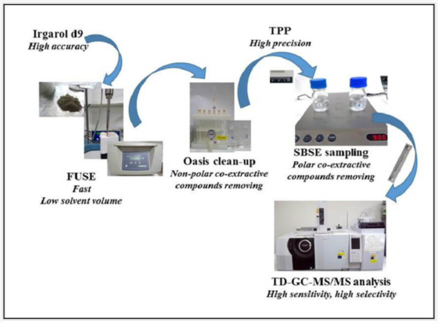

Highlights

- Optimization of FUSE-SPE-SBSE-TD-GC~~–~~-MS/MS for booster biocides in marine sediment.
- The use of SBSE for the pre-concentration of booster biocides in marine sediment.
- The use of FUSE for the extraction of booster biocides in marine sediment.
- Validation of FUSE-SPE-SBSE-TD-GC~~-~~-MS/MS for booster biocides in marine sediment.

Appendix Supplementary materials

Multimedia Component 1

alt-text: Image, application 1

Multimedia Component 2

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Multimedia Component 3

alt-text: Image, application 3

Multimedia Component 4

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Multimedia Component 5

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Multimedia Component 6

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Multimedia Component 7

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Multimedia Component 8

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Multimedia Component 9

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