Determination of Concentration Levels of Antifouling Booster Biocides in Port and Marinas of Gran Canaria Island Using SPE-HPLC.[1]

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Contents

[1] Determinación de los Niveles de Contaminación de Compuestos Antifloculantes en Puertos Deportivos y Pesqueros de La Isla de Gran Canaria mediante SPE-HPLC.

Resumen-Abstract

En el presente estudio se ha puesto en marcha una metodología analítica para la determinación de compuestos antifloculantes en muestras de agua procedentes de distintos puertos de la isla de Gran Canaria. Para la extracción de los analitos se ha utilizado la extracción en fase sólida (SPE) con cartuchos Envirelut Pesticide. Posteriormente el extracto ha sido analizado mediante cromatografía líquida de alta resolución acoplada a un detector de absorbancia UV (HPLC-DAD).

Las desviaciones standard relativas (RSDs) del método desarrollado son inferiores al 12 % para todos los compuestos en estudio. Las recuperaciones obtenidas son satisfactorias (superiores al 85%) y los límites de detección (LODs) hallados varian entre 0.007-0.4 μg/L. Finalmente el método ha sido aplicado a muestras de agua de mar procedente de diferentes puertos de la isla de Gran Canaria.

In order to study the concentration levels of the most important booster biocides in different ports of Gran Canaria island (Canary Islands), an analytical procedure was developed. The analytes studied were extracted form water samples by solid-phase extraction with an Envirelut Pesticide cartridge. After optimization of the extraction procedure, the biocides in the extract were directly determined by high performance liquid chromatography with diode array detector (HPLC-DAD).

The relative standard deviations (RSDs) of the developed procedure were lesser than 12 % for all studied compounds. Satisfactory recoveries (higher than 85 %) were obtained, and the limits of detection (LODs) varied between 0.007-0.4 μg/L for all antifouling agents. The method was applied to the analysis of seawater samples from ports and marinas of G.C. island.

Keywords: Solid Phase Extraction, Antifouling booster biocides,4-Chloro-3-methylphenol, Diuron, Chlorothalonil, Dichlofluanid, TCMTB, Thiram,Irgarol 1051, Gran Canaria Island, seawater, HPLC.

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1 Introduction

The function of antifouling paints is to avoid the growth of biofouling. The term biofouling make reference to the undesirable accumulation of microorganisms, plants and animals on artificial surfaces immersed in sea water. This accumulation causes some problems like major frictional resistance, deterioration of the coating, etc. with the consequent economic loss [1].

In the decade of 1970, Tributiltyn (TBT) was introduced in ship paint industry with a high efficiency. However, TBT can be considered as a persistent and toxic compound. TBT shows high toxicity in non target species like bivalves and gastropods, some of them of commercial interest, and slowly degradation in the environment with a half life in sediments of the order of months or even years [2]. Due to this, legislation in European Union (EU) countries prohibited the use of TBT-based paints in vessels < 25 m. in length [3]. More recently International Marine Organization (IMO) has banned make use of this kind of paints in ships independently of length, from 2003, and the hulls with TBT-based paints will has forbidden to enter in EU ports and harbours from 2008 [4].

Consequently, paint manufactures have developed new products having less environmental impact. These compounds are known like booster biocides, and are added to cooper oxides-based paints to improve the efficiency [5]. Moreover, these compounds inhibit photosynthesis and therefore are growth inhibitors of freshwater and marine algae [6].

Some of these compounds are frequently found in marine water samples at great concentrations, and their toxicological effect has been tested in non target species [7, 8]. For this reason, the concentrations of these new biocides need to be monitored in order to assess to risks to the environment.

One of the mayor problems with environmental samples is that conventional analytical techniques are seldom sufficient in terms of sensitivity, selectivity and reliability. Of fact, obtaining reliable data depends on the whole analytical procedure. Sample preparation includes several steps of which the most time-consuming and labor-consuming are extraction and clean-up of the extracts.

For the isolation of these antifouling from seawater samples different pre-treatment methods have been used, like Liquid-Liquid Extraction (LLE) [9] or Solid Phase Extraction (SPE) both

in on [10–12] and off-line mode [13, 14], Solid Phase Microextraction (SPME) [15–18], Solvent Microextraction [19] or by means of the use of Immunosorbents [20]. But due to the necessity of to obtain a great preconcentration and relative low time consuming analysis, the most frequent extractions for monitoring these compounds in sea water are SPE [21–25] and LLE [26–28].

In this work, we developed a method to evaluate the presence of seven booster biocides currently used in antifouling paints, in marina and harbour waters of Gran Canaria island (Spain) by SPE and HPLC-DAD.

For that purpose, we have optimized all conditions that affecting the extraction procedure by extracting the selected target compounds from spiked water samples. Finally, the method was tested for analysis of real water samples from different Gran Canaria island ports.

2 Experimental

2.1 Reagents

Booster biocides under study (Table 1, numbers identify the compounds in the tables and figures) were purchased from Dr. Ehrenstorfer (Germany) with purity in all cases greater than 98 %. Stock standard solutions were prepared at 1 g/L of each analyte in methanol and stored at 4°C.

Number	Compound	Retention Time (min.)	$\lambda_{abs.}$ (nm.)
1	Thiram	3.3	260
2	4-Chloro-3-methylphenol	6.7	280
3	Diuron	7.1	248
4	TCMTB (Busan)	8.9	280
5	Chlorothalonil	10.3	230
6	Diclofluanid	11.2	230
7	Irgarol 1051	12.4	230

Table 1: Peak identification numbers, retention times and absorbance wavelengths for the analytes under study.

SPE cartridges (6 mL) employed in this study were: Sep-Pak Vac C_{18} (500 mg) and Oasis HLB (200 mg) from Waters (Madrid, Spain), Mega BE-FL (1 g), Envirelut pesticide (500 mg) and Bond Elut-ENV (500 mg) from Varian (Madrid, Spain).

Methanol HPLC grade was obtained from Panreac Química S.A. (Barcelona, Spain) and bidestilled water from Milli-Q purification unit (Millipore, USA).

2.2 Apparatus

The chromatograph system consists if a Varian pump fitted with a Varian Autosampler 410 with volume selector, a Column Valve Module with an internal oven and Varian PDA Detector. The System, acquisition and processing of a data were controlled by Star software 6.5 version (Varian Inc., Madrid, Spain). The stationary-phase column was a Waters Nova-Pak C_{18} 150 mm × 3.9 mm (4 μm of particle size) and ChromGuard C_{18} precolumn from Varian. The column was

placed in the column module and thermostated at 30 ± 0.2°C.

For SPE process was used a Vac Elut 20 SPE Manifold coupled a Sartorius vacuum pump.

2.3 Sampling

Seawater employed for optimization of the methodology was collected from Melenara beach, in the East of Gran Canaria island. Extractions over blank sample were carried out to check the ausence of analytes under study.

Sea water samples were collected from different ports and marinas around of Gran Canaria island. The samples were taken to 1 m of depth, then they were placed in 2.5 L amber glass bottles and stored at 4°C in the dark until extraction. Duplicate field samples were taken at each location and were filtered through 0.65 μm Durapore membrane (Millipore, USA).

2.4 Solid Phase Extraction Procedure

100 mL of sea water sample were spiked with a concentration of 50 μg/L of each analyte, except Dichlofluanid (150 μg/L). The spiked samples were then shaken in order to ensure the homogenous distribution of analytes in the matrix and then they were allowed to equilibrate for 10 min. prior to extraction. The cartridges were conditioned with 3 × 5 mL of methanol follows by 3 × 5 mL of bidistilled water. Subsequently the samples were passed through cartridges with a flow not greater than 12 mL/min. To remove the salts and other no target substances retained on the cartridges, a wash step was employed with an optimized composition (methanol / water) and then the samples were dried under vacuum during 5 min. To extract the analytes, an optimized volume of methanol was added and passed through the sorbent at low flow, approximately 1 mL/min.

2.5 Liquid-Liquid Extraction

For liquid-liquid extraction method, 1 L of sea water coming from Melenara's beach (previously analyzed to ensure the absence of analytes) was passed through of a filter of 0.65 μm pore size. Then it was spiked with the analytes to obtain a final concentration of 0.6 μg/L except

Dichlofluanid (2.4 μg/L). Finally was vigorously shaken and let it stand for 10 min. to ensure a homogenous distribution.

LLE was performed as follows: 1 L spiked sample was introduced in a 2 L separatory funnel, and was sequentially extracted with Dichloromethane (2 $\times$ 50mL), with a time of repose of 5 min. Subsequently combined extracts were dried and concentrated by a rotavapor, firstly in a 200 mL round bottom flask to approximately 5 mL and then the extract was passed to 10 mL round bottom flask where was completely dried. Finally the analytes were redissolved in 1 mL of methanol.

2.6 Chromatography Analysis

The analysis of extracted samples was carried out with high performance liquid chromatography with diode array detection (HPLC-DAD). 30 μL of sample was injected into chromatography system, and the absorbance was measured at maximum wavelength of each analyte. The retention times and wavelengths of analytes employed for their measurement are showed in Table 1. The mobile phase was methanol (50%) and bidistilled water (50%) for 3 min. up to proportion of 80% methanol-20% water for 14 min. at flow-rate 1 mL/min. A prudential time (5 min.) was employed to equilibrate the system and come back to initial conditions of gradient elution.

In real samples, to ensure the presence of the booster biocides, it was accomplished on the basis of their retention times and by comparison between the absorbance spectra in the sample and in the standards solutions.

The corresponding calibration curves were done in a concentration range between 0.05 and 4 μg/L. A linear relationship was obtained between peak areas and the analyte concentrations, with high correlation coefficients (R^2>0.99).

3 Results and Discussion

3.1 Solid Phase Extraction Optimization

Solid Phase Extraction is widely employed in environmental analysis. The basis of this extraction method is the selective retention of analytes from liquid samples over a solid sorbent and subsequent desorption with organic solvents.

The optimization of the extraction procedure include the type of the sorbent, sample volume, composition and volume of washed solution and volume of desorption.

For these studies we chose select conditions: 100 mL of seawater spiked samples, and 5 mL of methanol for desorption.

3.1.1 Type of Sorbent

The first parameter that was optimized was the type of cartridge. In this case, we have used five different cartridges: Sep-Pak Vac C_{18} (500 mg),Bond Elut-ENV (500 mg), Mega BE-FL (1 g), Oasis HLB (200 mg) and Envirelut pesticide (500 mg).

The results obtained (as peak areas) over the different SPE cartridge are shown in Figure 1 where it is observed that the efficiency of the extraction changes with the type of sorbent. Among types cartridge considered, C_{18} and Envirelut Pesticide provided higher responses for all compounds. Bond Elut-Env was not effective to retention of Thiram and TCMTB.

3.1.2 Wash Step Composition

Before desorption, a study was done for cleaning the SPE cartridge. When no wash step was employed, the salt retained in the cartridge was dragged on the desorption step and it was necessary to filter the solution. For the optimization of this step, we used 5 mL of wash solution with different water/methanol composition.

In Figure 2 we can see that the peak areas obtained decreases when for any methanol concentration used. The most satisfactory recoveries for all the compounds were achieved using only bidestilled water.

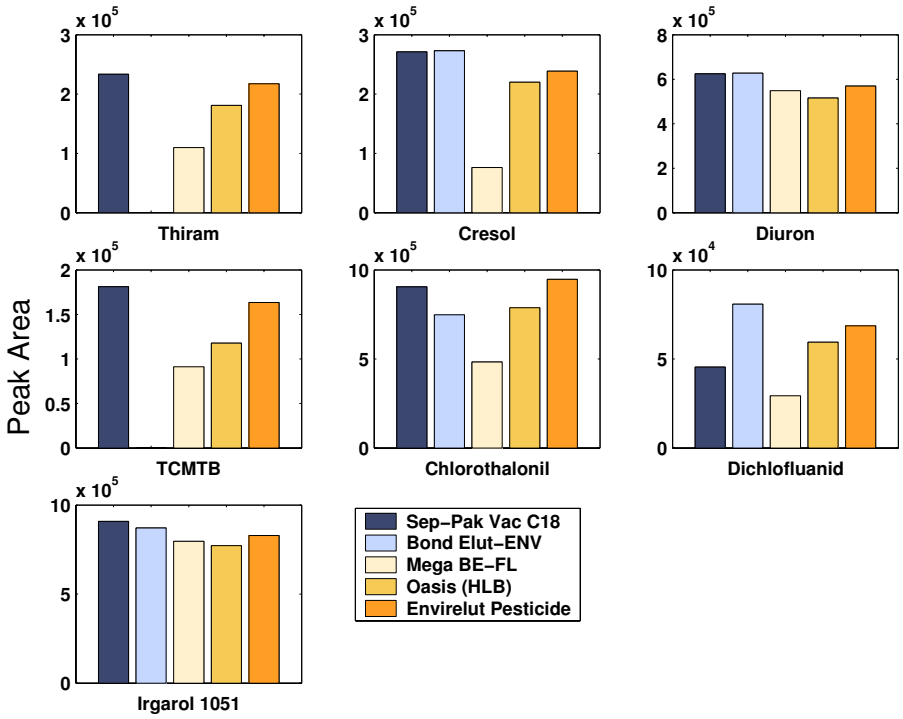

Figure 1: Comparison of peak areas obtained for extraction with different cartridges. A volume of 100 mL of seawater sample with 50 μg/L of each analyte except Dichlofluanid with 150 μg/L and 5 mL of methanol to desorption were employed.

Finally a wash step with 2 $\times$ 5 mL of bidestilled water was chosen in order to ensure the elimination of salts.

3.1.3 Desorption Volume

The desorption volume is an important factor in the SPE process. The desorption volume employed must be sufficient to ensure the total extraction of analytes, but not so higher than the time used in the desorption and posterior drying of the sample will be high. Normally with polymeric sorbents low volumes are used but with other types like Graphitized Carbon Black (GCB) it is necessary more desorption volumes due to its great adsorbing properties [29].

The solvent used for desorption was methanol. Firstly, it was compared the normalized peaks areas obtained for extractions with 2.5 mL and 2 $\times$ 2.5 mL (Table 2). It is observed that

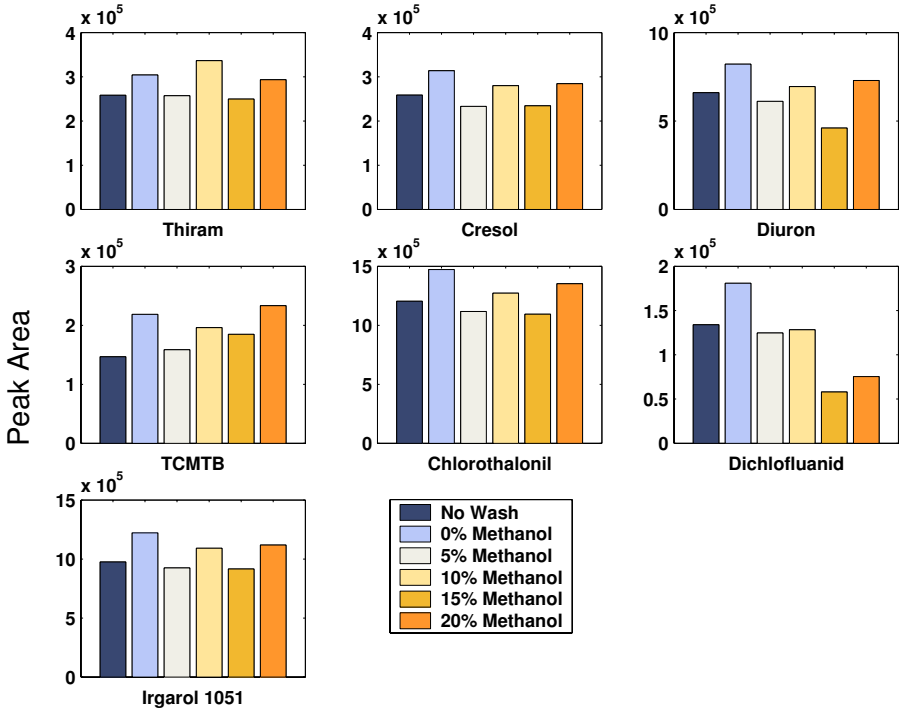

Figure 2: Peak areas obtained for extraction of 100 mL of sea water samples spiked with 50 μg/L for each analyte and 150 μg/L for Dichlofluanid, using different methanol proportion in wash step.

similar results are obtained and therefore desorption of analytes is effective with 2.5 mL for all compounds. For evaluation of required volume, the elution was carried out with 1, 1.5, 2 and 2.5 mL of methanol. In Figure 3 we can see that a volume of 1 mL was sufficient to desorb the target of analytes from SPE cartridge. With 1 mL desorption volume, the drying step can be avoided. This fact provides us great advantages: the time of extraction process is lower and prevents some compounds losses; for example TCMTB present a high vapour pressure and could degraded [30].

3.1.4 Sample Volume

In some case, the sample volume may be a important parameter for efficient extraction due to the breakthrough volume of the cartridge.

Compound	1 × 2.5 mL	2 × 2.5 mL
Thiram	1532518	1431988
4-Chloro-3-methylphenol	1260464	1089570
Diuron	3140201	2977145
TCMTB	869994	790188
Chlorothalonil	5272106	5016663
Dichlofluanid	657185	535433
Irgarol 1051	4875729	4668835

Table 2: Normalized peaks areas for the extraction of 100 mL of sea water sample spiked with 50 μg/L and 150 μg/L for Dichlofluanid. Data are the mean of two measurements.

Different sample volumes of 100, 500, 800 and 1000 mL were spiked with 500 μL of standard solution with a concentration of 10000 μg/L for each analyte and 30000 μg/L for Dichlofluanid. The results of this study are shown in Figure 4, where it can be seen that comparable areas were obtained for the majority of compounds, except for 4-Chloro-3-methylphenol, where, the peak area increased with the volume of seawater. This can be due to a major efficient retention of low concentration of this analyte.

In order to obtain a great preconcentration as is possible, a volume of 1000 mL was chosen for extraction procedure.

3.2 Analytical Paramenters

Analytical parameters for SPE-HPLC procedure were obtained by analysis of different spiked seawater samples with booster biocides mixture on the range from 50 to 1000 μg/L, except for Dichlofluanid on the range 800 to 4000 μg/L. Linear relationship were found between peaks area and analyte concentrations, with high correlation coefficients, higher than 0.995 for all analytes except Dichlofluanid (R^2=0.992). In the optimum conditions recovery levels higher than 85 % were obtained for all biocides, except in the case of Dichlofluanid (68%) (Table 3).

Figure 5. shows a typical chromatogram obtain for extraction of a spiked sample. At the same time in Figure 6. is represented a chromatogram at λ_{abs}=248 nm. of a real sample coming

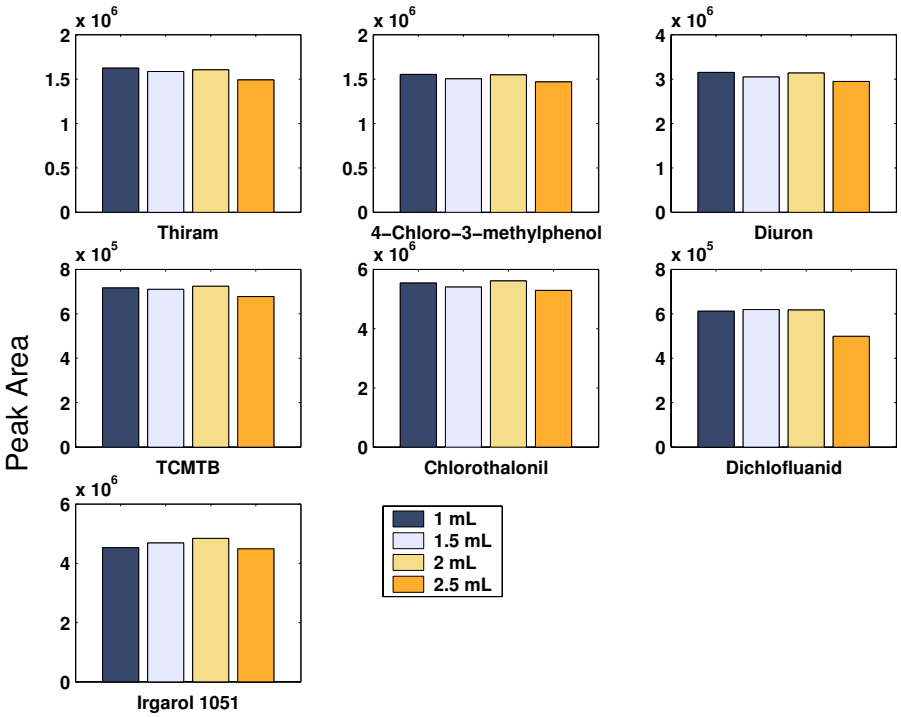

Figure 3: Normalized peak areas obtained with Envirelut pesticide cartridge for different analytes under study. A volume of 100 mL of sea water sample with 50 μg/L of each analyte except Dichlofluanid with 150 μg/l was employed.

from Puerto Rico marina (SW). In this figure also has been represented the absorbance spectra of the standard (dashed blue line) and real sample (black line).

The precision of the method was determinate by reproducibility studies expressed like relative standard deviation (% RSD). For this purpose, six spiked samples were analysed with optimized conditions. The results are summarized in Table 3. In all cases the reproducibilities are below of 10%, only Dichlofluanid shows a slowly superior reproducibility with 10.84%.

Limits of detection (LODs) for 1000 mL of sea water sample, expressed like three times the noise of each compound [31] were calculated and are showed in Table 3. It can be observed that LODs varied between 0.007 for TCMTB and 0.075 μg/L except for Dichlofluanid that showed a higher value (0.451 μg/L). These LOD are suitable for determinate the presence of booster biocides in real samples coming from Spanish coast, where can found concentrations over 0.1

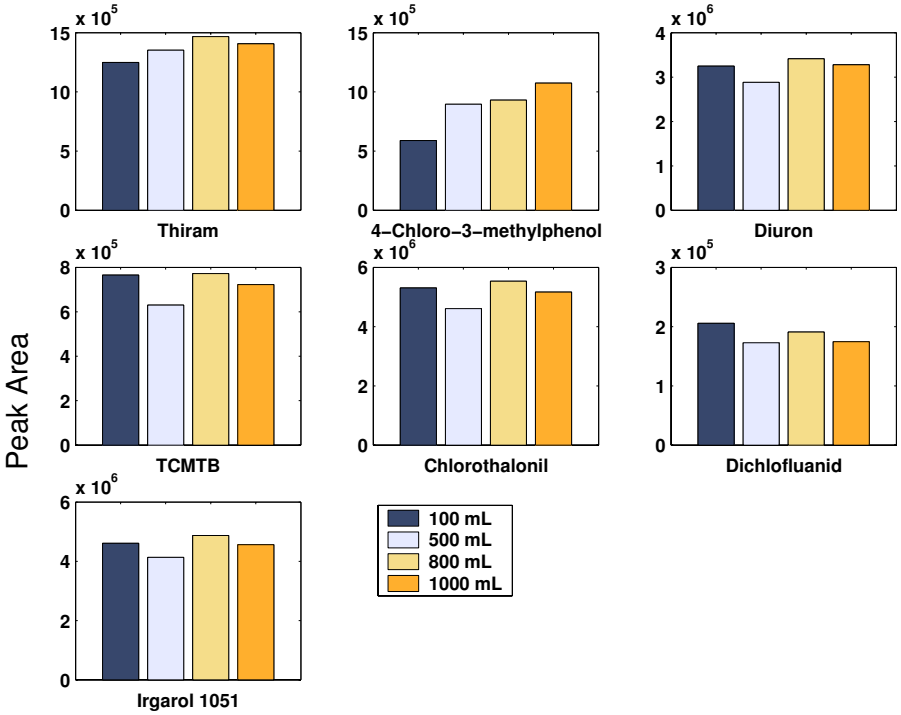

Figure 4: Peaks areas obtained for different volumes of sea water samples. A volume of 1 mL of methanol was employed for desortion of analytes.

μg/L, principally for Diuron and Irgarol 1051 [22].

In order to demonstrate the validity of the previously optimized method, it was compared with a conventional method, in this case Liquid-Liquid Extraction. A water sample of 1000 mL containing the seven booster biocides at a concentration of 0.6 μg/L and 2.4 μg/L was analyzed using Liquid-Liquid Extraction procedure [27,32] and HPLC-DAD determination. Figure 7 shows the results obtained, the results obtained for Chlorothalonil and Irgarol 1051 are similar in both procedures, however in the cases of Diuron and Dichlofluanid higher recoveries are obtained using SPE methodology. Finally we have note that Thiram, 4-Chloro-3-methylphenol and TCMTB could not be determined using LLE due to losses during evaporation step.

Compound	Linear Range [a](μg/L)	Recovery (%)	R.S.D.[b](%)	L.O.D.[a,c] (μg/L)
Thiram	0.1-1.00	96	6.59	0.022
4-Chloro-3-methylphenol	0.1-1.00	103	5.35	0.075
Diuron	0.05-1.00	101	3.49	0.038
TCMTB	0.1-0.80	85	4.70	0.007
Chlorothalonil	0.1-0.80	92	5.90	0.010
Dichlofluanid	0.8-4.00	68	10.8	0.415
Irgarol 1051	0.05-1.00	93	3.75	0.031

[a]Factor of preconcentration:1000

[b]Relative Standard Deviation

[c]Limit of Detection

Table 3: Analytical Paramenters

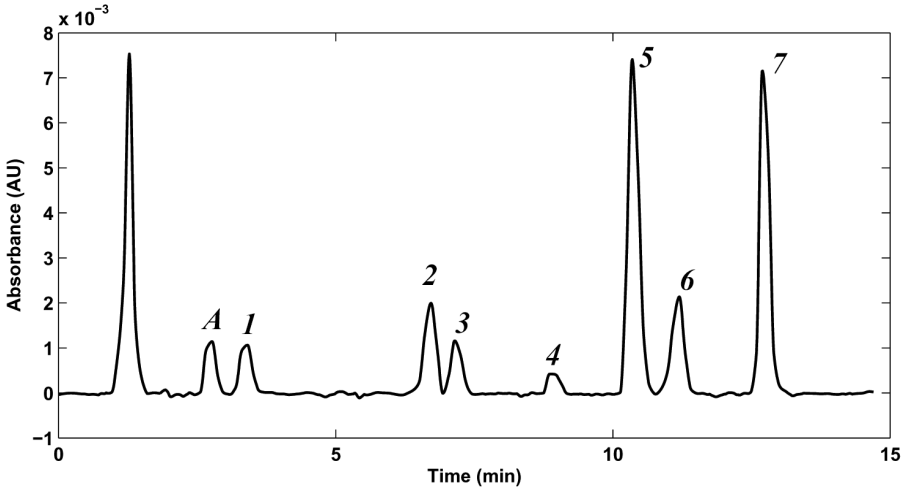

Figure 5: Chromatogram obtained for extraction of 100 mL of sea water spiked with a concentration of 3.5 μg/L for all compounds except Dichlofluanid, with 14 μg/L (λ_{abs}=230 nm.). 1: Thiram, 2: 4-Chloro-3-methylphenol, 3: Diuron, 4: TCMTB, 5: Chlorothalonil, 6: Dichlofluanid, 7: Irgarol 1051 and A: Dichlofluanid degradation product.

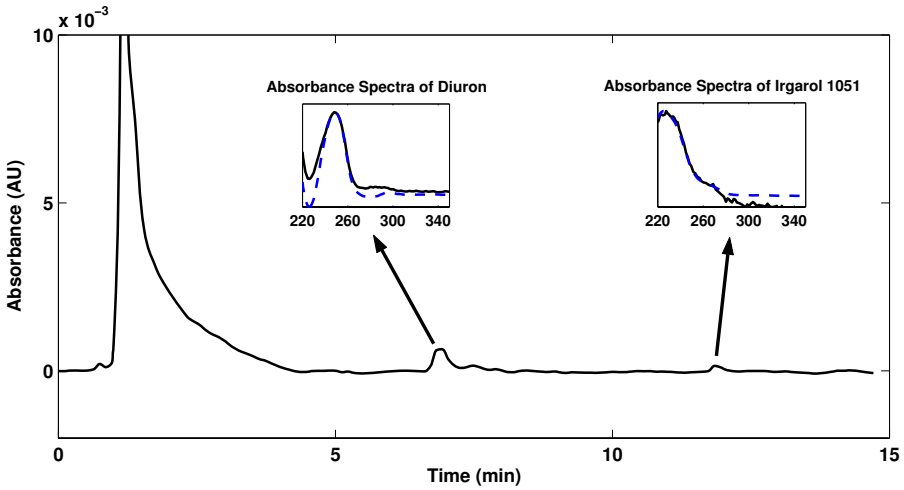

Figure 6: Chromatogram obtained by extraction of 1000 mL of a real seawater sample from Puerto Rico marina (point C2, λ_{abs}=248 nm). In small boxes it is shown the absorbance spectra of real sample (black line) and standards (dashed blue line).

3.3 Determination of Antifouling Biocides in Real Seawater Samples

The method developed was utilized for determination of the analytes under study in real samples coming from different ports and marinas of Gran Canaria Island.

Canary Islands are a Spanish archipelago; it is localized in Northwest of African continent, between 27.37 ºN, 18.10 ºW and 29.25 ºN, 13.20 ºW. The archipelago is a significant point in the commercial ship routes between America, Africa and Europe; the marine traffic passes principally by major islands (Gran Canaria and Tenerife), being the port of Las Palmas of Gran Canaria one of most important. Also there is a great tourist activity. In Gran Canaria, the marinas are fundamentally localized in the South of the island.

Figure 8 shows the different points where were collected the samples. Within of area A (NE), is the port of Las Palmas of Gran Canaria and two marinas: 1-Club Náutico, which are under construction with low activity and 2- Muelle Deportivo, which are the most important marina in the island and present high yachting and sailing activity. The point B (SW) is Mogán dock with two zones, first (right of map) as marina and second as fishery harbour. The point C (SW) is Puerto Rico marina with high yachting and sporting activity associated to tourism and D (SSW)

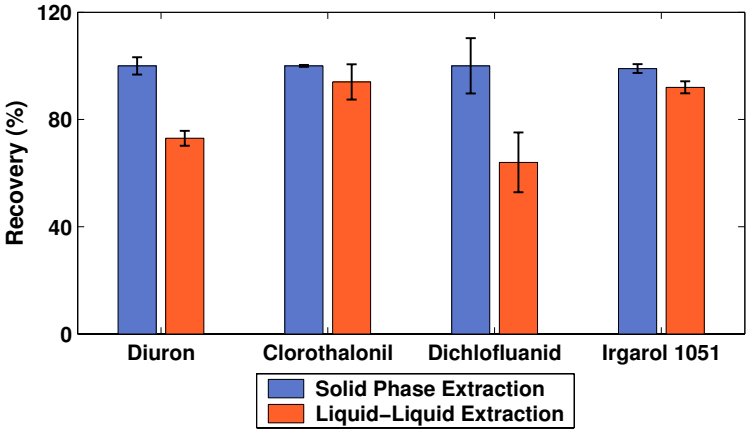

Figure 7: Recoveries obtained for four of booster biocides under study by optimized solid phase extraction and conventional liquid-liquid extraction.

is the Arguineguin fishery harbour.

Table 4 shows the results obtained for the different points analyzed. Diuron was the compound most frequently found, with concentrations in the range from 0.04 to 0.1 μg/L. Its common presence in water samples can be due to its extensive use and very low degradation [33]. Irgarol 1051 was detected only in the marina of Puerto Rico. Both Diuron and Irgarol 1051 are photosynthesis inhibitor and shown toxicological effects in non target species like invertebrates [8] and macrophytes [7].

Sample	Diuron(μg/l)[a]	Irgarol 1051(μg/l)[a]
A10	0.045 ± 0.003[b]	-
B1	0.049 ± 0.001[b]	-
B2	0.0050 ± 0.001[b]	-
B3	0.069 ± 0.001	-
B4	0.097 ± 0.003	-
C1	0.045 ± 0.001[b]	0.033 ± 0.001[b]
C2	0.078 ± 0.001	0.033 ± 0.003[b]
C3	-	0.046 ± 0.001[b]
C4	0.044 ± 0.002[b]	-
C5	0.060 ± 0.001	-
D1	0.071 ± 0.003	-
D2	0.100 ± 0.004	-

[a]Mean of two determinations ± Standard Deviation.

[b]Approximate concentrations, between limit of detection and limit of quantification.

Table 4: Real samples taken from Gran Canaria Island.

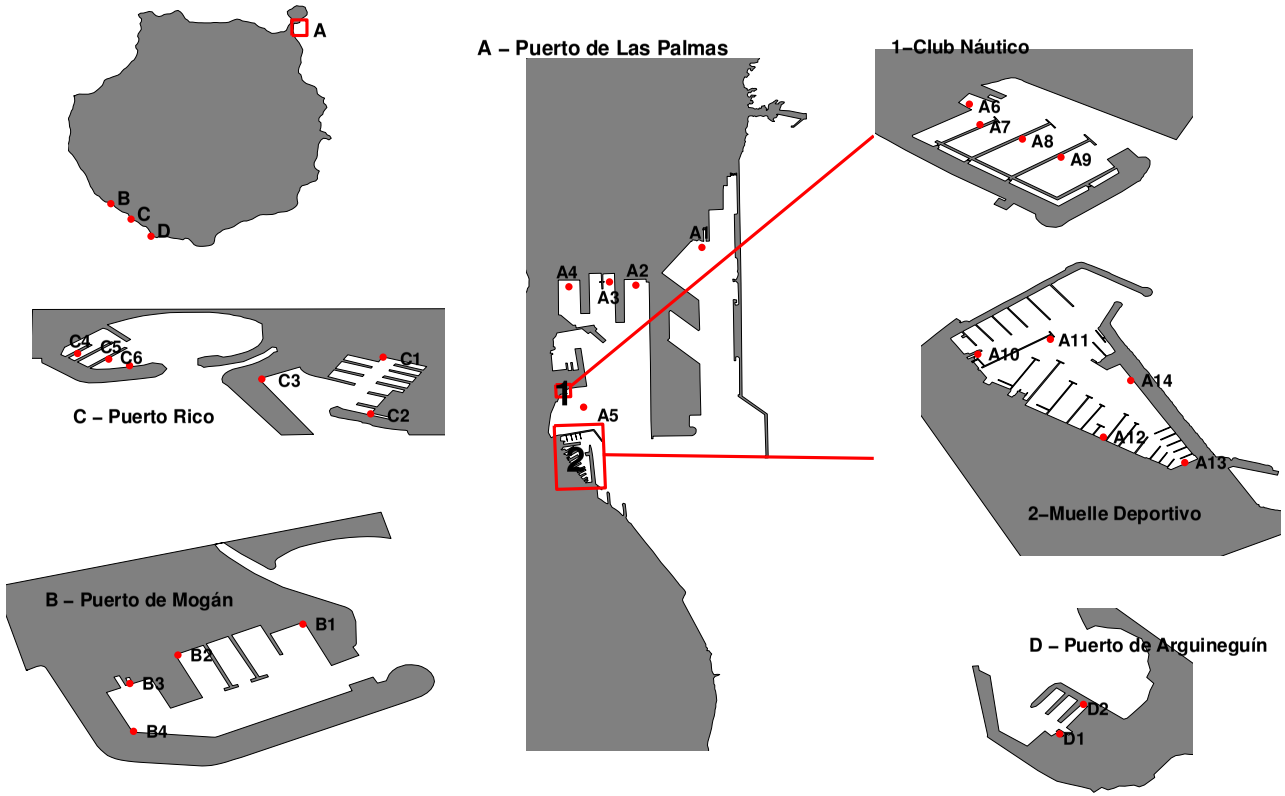

Figure 8: Points of sampling in ports and marinas around Gran Canaria Island.

4 Conclusions

An analytical method for the determination of booster biocides in seawater samples was optimized and developed. The proposed method includes SPE and HPLC-DAD detection and offers a combination of sensitivity and simplicity when is compared with other conventional sample-preparation methods.

The developed method was successfully applied to various aqueous samples obtained from different ports and marinas of Gran Canaria island.

Acknowledments

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A Congress Comunications and Publications

1. **OPTIMIZATION OF A METHOD FOR DETERMINATION OF ANTIFOULING PAINT BOOSTER BIOCIDES IN MARINE WATER SAMPLES.**

   International Symposium in Marine Sciences 2007 (ISMS 2007)

   March 2007. Valencia.

2. **AN APPROACH OF THE LEVELS OF CONTAMINATION BY THE PRESENCE OF ANTIFOULING BOOSTER BIOCIDES IN MARINE WATER SAMPLES COMING FROM PORTS AND MARINAS OF GRAN CANARIA ISLAND.**

   IX Internacional Symposium on Analytical Methodology in Environmental Field.

   October 2007. Mallorca.

3. **DETERMINATION OF CONCENTRATION LEVELS OF ANTIFOULING BOOSTER BIOCIDES IN THE PORT AND MARINAS OF GRAN CANARIA ISLAND USING SPE-HPLC.**

   Marine Pollution Bulletin (SUBMITTED)

OPTIMIZATION OF A METHOD FOR DETERMINATION OF ANTIFOULING PAINT BOOSTER BIOCIDES IN MARINE WATER SAMPLES

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INTRODUCTION

Due to toxic effect of Tributyltin in non target species (1) this compound has experimented a lot of restrictions like biocide in antifouling paints. To replace it, other compounds, booster biocides, are used actually in commercial formulations.

In this study we optimize a methodology to determinate seven booster biocides (Fig. 1) in marine water samples. Solid Phase Extraction (SPE), which has been used with good results in environmental samples (2), is utilized to extract and preconcentrate the analytes and High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) to separate and determinate all of compounds.

Fig. 1.- Compounds under study and chromatogram peak number.

1 **Thiram**

2 **4-chloro-3-methylphenol**

3 **Diuron**

4 **TCMTB**

5 **Chlorothalonil**

6 **Dichlofluanid**

7 **Irgarol 1051**

EXPERIMENTAL

Apparatus.-

- HPLC system from Varian equipped with Nova-Pak C18 column 3.9 × 150 mm (Waters) and ChromGuard C18 precolumn (Varian).
- SPE Manifold from Varian.

Chromatographic conditions.-

- The elution program used is:
  0 min:50% Methanol-50% Water/3.5 min: 50% Methanol-50% Water/ 14min: 80% Methanol-20% Water.
- Flow: 1 ml/min.
- Injection Volume: 30 µl.

RESULTS -Optimized variables for SPE

Variable	Set conditions	Optimun
Type of cartridge (6 ml)	Varian: Bond Elut Fl, Bond Elut Env, Envirelut Pesticide Waters: C18, Oasis (HLB)	Envirelut Pesticide (Varian)
Wash step	0, 5, 10,15 and 20% of methanol/water (v/v)	0 %
Desorption volume (ml)	1, 1.5, 2, 2.5, 5 and 8 of methanol	1 ml
Adsorption volume (ml)	100, 200, 500, 800 and 1000	1000 ml

Table 1.- Optimized variables for SPE.

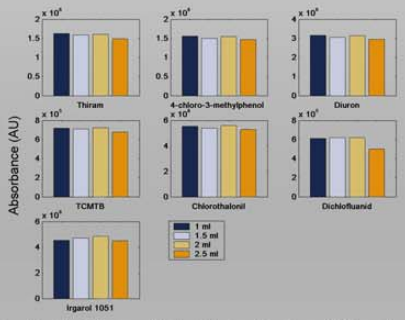

Fig. 2.- Absorbance obtained for extraction of 50 µg/l and 150 µg/l for Dichlofluanid from 100 ml of a sea water sample with different desorption volumes.

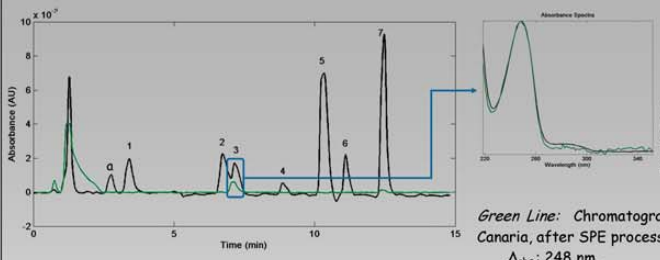

Fig. 3.- *Black Line:* Chromatogram of a spiked sea water sample (100 ml) with all analytes, after SPE process. Conc.: 5 µg/l, except for Dichlofluanid, 20 µg/l. Peak "a" is a Dichlofluanid degradation product.
$\Lambda_{abs.}$: 230 nm.

Green Line: Chromatogram of a real sea water sample from Marina of Las Palmas of Gran Canaria, after SPE process.
$\Lambda_{abs.}$: 248 nm.

Right: Absorbance Diuron spectrum of standard (black) and found in real sample (green).

CONCLUSION

The methodology optimized in the present study shows good reproducibility and very satisfactory limits of detection. It can be employed for determinate the presence of these compounds in real samples taken from polluted areas.

FUTURE WORKS

In the future this methodology will be used for temporal monitoring of these compounds in real samples coming from different ports and marinas of Gran Canaria Island.

Compound	Linear Range[a] (ng/l)	R^2	R.S.D.[b] (%)	L.O.D.[a,c] (ng/l)
1	100-1000	0.9974	6.59	21.89
2	100-1000	0.9964	5.35	74.81
3	50-1000	0.9971	3.49	37.45
4	100-800	0.9961	4.70	7.39
5	100-800	0.9987	5.90	9.52
6	800-4000	0.9917	10.8	415.4
7	50-1000	0.9975	3.75	31.29

Table 2.- Analytical parameters.
a: Factor of preconcentration: 1000.
b: Relative Standard Deviation (n = 6).
c: Limit of Detection.

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AN APPROACH OF THE LEVELS OF CONTAMINATION BY THE PRESENCE OF ANTIFOULING BOOSTER BIOCIDES IN MARINE WATER SAMPLES COMING FROM PORTS AND MARINAS OF GRAN CANARIA ISLAND

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INTRODUCTION

Tributyltin (TBT) has been used in antifouling paints formulations since, 1970. This compound has showed toxicological effects in no target species [1]. Actually, others compounds known like antifouling booster biocides are been utilized. The majority of them has been employed previously like herbicides or fungicides and also presents a high toxicity in non target species like macrophytes [2] and invertebrates [3].

In this study, a previously optimized SPE-HPLC method for extraction and determination of seven common booster biocides (Table 1) of marine water samples has been employed. Real samples are taken from different ports and marinas of Gran Canaria Island (Figure 1).

EXPERIMENTAL

The best conditions obtain for the optimized method were:

- Sample volume: 1000 ml
- Volume desorption: 1 ml of methanol
- Cartridge: Envirelut Pesticide 6ml (Varian)
- Wash step: 10 ml of Bidestilled water.

For the determination was employed HPLC with a Diode Array Detector (DAD).

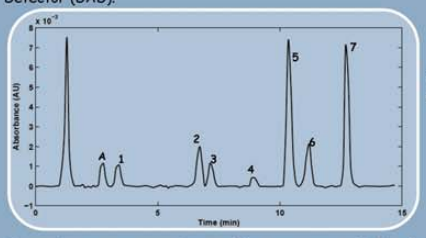


Figure 2.- Cromatogram obtained for extraction of 100 ml of sea water spiked with a concentration of 3.5 µg/l for all compounds except Dichlofluanid with 14 µg/l (λ_{abs}= 230 nm). Peak A is a degradation product of Dichlofluanid.

Figure 3.- Chromatograms of real samples:

Green Line: Chromatogram at λ_{abs}= 248 nm of C5 sample (July).

Red Line: Chromatogram at λ_{abs}= 230 nm of C6 sample (July).

Blue Line: Chromatogram at λ_{abs}= 230 nm of C2 sample (May).

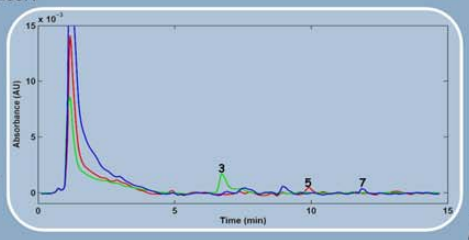
Nº	Compound
1	Thiram
2	4-chloro-3-methylphenol
3	Diuron
4	TCMTB
5	Chlorothalonil
6	Dichlofluanid
7	Irgarol 1051

Table 1.- Antifouling Booster Biocides under study.

Figure 1.- Sample points.

RESULTS

The previously optimized method was employed for the study of, in a first approximation, the presence of antifouling booster biocides in some Gran Canaria's ports and marinas between months of May and July of 2007.



Samples	May (ng/l)ª	July (ng/l)ª
A11	–	69.6 ± 0.4
A12	–	61.7 ± 0.2
A13	–	65.5 ± 3.4
B3	69.3 ± 0.7	61.0 ± 1.9
B4	96.7 ± 3.1	–
C2	78.3 ± 1.0	50.6 ± 1.2
C4	–	61.0 ± 1.0
C5	60.4 ± 1.0	207.3 ± 2.6
D1	71.1 ± 0.3	–
D2	100.1 ± 3.5	61.0 ± 0.3

Table 2.- Concentrations of Diuron found between May and July.
a: Mean of two extractions ± standard deviation.

CONCLUSIONS

- The method employed is useful to determination of the analytes in real samples.
- The compound most commonly found in analyzed samples was Diuron. This can be due to its low degradation and extensive use.
- Other compounds found in real samples were Irgarol 1051 and Chlorothalonil.

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